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Research review paper

Development of high hydrostatic pressure in biosciences: Pressure effect on biological structures and potential applications in Biotechnologies ☆

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ABSTRACT

Compared to temperature, the development of pressure as a tool in the research field has emerged only recently (at the end of the XIXth century). Following several developments in Physics and Chemistry during the first half of the XXth century (in particular the synthesis of diamond in 1953–1954), high pressures were applied in Food Science, especially in Japan. The main objective was then to achieve the decontamination of foods while preserving their organoleptic properties. Now, a new step is engaged: the biological applications of high pressures, from food to pharmaceuticals and biomedical applications. This paper will focus on three main points: (i) a brief presentation of the pressure parameter and its characteristics, (ii) a description of the pressure effects on biological constituents from simple to more complex structures and (iii) a review of the different domains for which the application of high pressures is able to initiate potential developments in Biotechnologies.

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Contents

1. High hydrostatic pressure (HHP): position of this thermodynamical parameter in the universe, main factors characterizing its action and different applications . . . 660
   1.1. Pressure as a thermodynamical parameter . . . 660
      1.1.1. Definition of pressure . . . 660
      1.1.2. Different types of pressure . . . 660
   1.2. Pressure, the Earth and the universe . . . 660
   1.3. Main factors characterizing pressure . . . 661
   1.4. First developments of high pressure in different scientific areas . . . 661
   1.5. Development of high pressure in Biosciences . . . 661
2. Pressure effects on various components of biological systems . . . 662
   2.1. Pressure effects on proteins . . . 663
   2.2. Pressure effects on lipids and biomembranes . . . 663
   2.3. Pressure effects on nucleic acids . . . 663
3. Pressure effects on more complex living systems . . . 664
   3.1. Pressure effects on mammalian cells . . . 664
   3.2. Pressure effects on pathogens . . . 664
      3.2.1. Bacteria . . . 664
      3.2.2. Yeasts and molds . . . 664
      3.2.3. Bacterial spores . . . 665

☆ This paper is dedicated to Jacques BASSET, on the occasion of his 90th birthday, and his father James BASSET who were pioneers in high pressure studies involving Physics, Chemistry and Biology. The scientific activity of Jacques and James BASSET, conducted between 1930 and 1950 on Biology under high pressure, is an excellent illustration of the various potentialities of high pressure applications in Biosciences.

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3.2.4. Viruses . . . 665
3.2.5. Parasites . . . 665
3.2.6. Infectious prion protein: "*a new pathogen type*" . . . 665
4. Biological applications of high hydrostatic pressure . . . 666
4.1. Food industry . . . 666
4.2. Proteins from extremophiles as stable tools for biotechnological applications . . . 666
4.3. High pressure effects on allergenicity and digestibility . . . 666
4.4. Disinfection of biomaterials . . . 666
4.5. Modulation of enzymatic activities . . . 667
4.6. Stabilization of protein intermediates . . . 667
4.7. Dissociation of protein complexes . . . 667
4.8. Protein-DNA interactions . . . 667
4.9. Vaccine development . . . 668
4.10. Preparation of viral vectors . . . 668
4.11. Genetic transformation . . . 668
4.12. Cell extraction . . . 668
4.13. Pressure-assisted cryopreservation . . . 668
4.14. Applications in oncology . . . 668
5. General conclusions . . . 669
References . . . 669

The use of pressure as a thermodynamical parameter was firstly introduced into the Biology field at the end of the XIXth century by Regnard (Regnard, 1884b), Royer (Royer, 1895) and Hite (Hite, 1899). Then, over the period 1910–1955, two main teams, the one of Bridgman in the USA (Bridgman, 1914, 1949) and the one of Basset in France (Basset, 1927; Basset and Macheboeuf, 1932; Basset et al., 1956) had focused their works on this new research area, contributing to its development.

After a long period during which high pressure had been mainly developed in other domains (Geology, Geochemistry, Chemistry, and Physics), it was rediscovered in Japan in 1980–1990, in particular for food processing applications. The purpose was then to find an alternative to radiation processes. During the last twenty years, an increase of interest for researches dealing with Biology under high pressure have permitted to initiate a large range of biological applications, from food processing to potential developments in medicine.

The purpose of this review is to recall this evolution, from the first approaches to the most recent advances on the high pressure effects on biological structures and their applications in health and medicine.

1. High hydrostatic pressure (HHP): position of this thermodynamical parameter in the universe, main factors characterizing its action and different applications

1.1. Pressure as a thermodynamical parameter

1.1.1. Definition of pressure

Pressure is defined as the force per unit area applied on a surface in a direction perpendicular to this surface: mathematically:

$$P = F / A \quad (1)$$

in which P is the pressure, F is the normal force applied to the surface and A is the area of the surface. The official pressure unit is the Pascal (Pa) (1 Pa = 1 N/1 m^2 = 10^{-5} bar). The Newton representing a small force and 1 m^2 corresponding to a large surface, the Pascal unit is a very small pressure unit. Consequently, the Megapascal (MPa) [1 MPa = 10^6 Pa] is the pressure unit commonly used in high pressure studies.

The conversion from MPa to other pressure units is given in the Table 1.

1.1.2. Different types of pressure

Two types of pressures can be considered: static and dynamic pressures.

Static pressures are used in treatments where the pressure value can be maintained over a long time. Two different categories of static pressure can be defined:

- *Isostatic pressure*, where the pressure value is the same in all the directions of the space. This is in particular the case in water (hydrostatic pressure),
- *Non-isostatic pressure,* where a pressure gradient is induced versus the structure of the equipment generating the pressure or versus the non-homogeneous compressibility of the medium (in particular in the case of solids with an anisotropic structure).

Dynamic pressures concern super-high pressures developed over a short length of time and usually associated with temperature. Shock-waves are mainly used to generate such pressures (Fortov, 2007; Nellis, 2006).

1.2. Pressure, the Earth and the universe

HHP is a parameter characteristic of the Biosphere considering the volumes occupied respectively by its major terrestrial (land) and aquatic components. Terrestrial habitats, where pressure value is close to one bar or lower, account for less than 1% of the total volume of the biosphere. The oceans, which cover approximately 70% of the surface of the Earth, have an average depth of 3800 m and consequently an average pressure of 381 atm (38.5 MPa). Approximately 79% of the volume of the marine component of the Biosphere lies below 1000 m. The greatest depth in the oceans, the "Challenger Deep" in the Marianas Trough, is near 11,000 m (Somero, 1992).

Consequently, pressure appears to be an important parameter at/or near the surface of the Earth. Taking into account that the maximum pressure value at the centre of the Earth is evaluated to be 4 Mbar (400 GPa) and that such a value is higher for the Giant Planets,

Table 1
Conversion of the different units used for pressure.

	Atmosphere	Bar	kg/cm^2	MPa	P.S.I. (pounds/inch^2)
Atmosphere	1	0.987	0.968	9.901	0.068
Bar	1.013	1	0.981	10.000	0.069
kg/cm^2	1.033	1.021	1	10.228	0.070
MPa	0.101	0.1	0.098	1	0.00689
P.S.I.	14.696	14.504	14.223	145.038	1

approximately 90% of the Universe is submitted to a pressure higher than 100 kbar (10 GPa) (Jayaraman, 1984).

Furthermore, the discovery of piezophile (or barophile) and thermophile micro-organisms has led some recent studies to consider that pressure, and in particular hydrothermal processes, could be at the origin of life (Daniel et al., 2006; Di Giulio, 2003; Hazen et al., 2002; Pradillon and Gaill, 2007).

1.3. Main factors characterizing pressure

Roughly, three main factors can characterize the pressure effects: the energy, the densification effect and the chemical reactivity.

(i) The energy

When the energy conveyed by high pressure is compared to the average value of the energy of chemical bonds, it can be underlined that energy developed by high pressure is quite low. Consequently high pressure will only affect weak chemical bonds. Table 2 shows a comparison of the energy conveyed by pressure in different media (gas, liquid and solid) and the average energy of a chemical bond. This demonstrates that the energy is directly correlated to the compressibility of the medium but in all cases its value is small (even in the gases) compared to the one of a chemical bond.

Table 3 gives a comparison of the energy conveyed by pressure and temperature in the same medium: water. Such a comparison underlines that the energy developed by pressure is very small compared to that developed by temperature; consequently the phenomena induced by both parameters in Biosciences will be completely different.

(ii) The densification effect

Due to compressibility, the difference between final and initial volumes under high pressure (ΔV value) is always negative.

This factor induces different phenomena such as:

- the formation of new structural forms (such as different structural spatial forms observed, for example, in Materials Chemistry during the high pressure direct conversion from graphite to diamond (Bundy, 1963) or in Biosciences with the high pressure effects on food biopolymers) (Knorr et al., 2006).
- the modification of the equilibria, for example the dissociation of water. Pressure increases the dissociation of water and consequently the k_e value is improved ($k_e = [H^+][OH^-]$). Such a phenomenon is the result of two aspects: (a) the negative ΔV value ($\Delta V = -22$ ml/mole for the dissociated water) (Heremans et al., 1996), (b) the electrostriction phenomenon (the positive or negative charges being rearranged in a more compact structure around the ions) (Van Eldik et al., 1989). Under very high pressure conditions ($P \approx 5$ GPa), water can be considered as a melt-salt due to the displacement of the dissociation equilibrium.

Taking into account these two factors (low energy and $\Delta V < 0$), which are characteristic of the thermodynamical parameter pressure, only the weak chemical bonds leading to a negative ΔV value will be affected by high pressure.

Table 2
Energy developed by compression versus the nature of medium compared to the average energy of a chemical reaction (Demazeau, 2006; Wentorf, 1961), adapted by Demazeau.

Pressure	Medium	Energy (cal/mol)
100 MPa	Gas	3000
100 MPa	Solid	1
1 GPa	Solid	5
10 GPa	Iron	20
10 GPa	H_2O	1000
0.1 MPa	Chemical reaction	20,000

Table 3
Evaluation, for 1 l of water, of the energy developed by compression (from 0.1 MPa to 400 MPa) with the one corresponding to heating (from 20 °C to 25 °C). Adapted from (Mertens, 1995).

	1 l of H_2O	
Temperature	$P = 0.1$ MPa 20 °C → 25 °C	$E \approx 20.9$ kJ
Pressure	$T = 20$ °C 0.1 MPa → 400 MPa	$E \approx 19.2$ kJ

(iii) The chemical reactivity

Temperature and pressure are used to shift reaction equilibria. In particular, considering an equilibrium between a solid and a liquid, pressure usually improves the solubility and consequently the concentration of the solvated species is increased. In addition, due to the compressibility of the solution, the average distance between the solvated species is reduced (Schettino and Bini, 2007). All these phenomena lead to an improvement of the chemical reactivity, inducing an increase of the kinetics. Such kinetical effect has been underlined, for example, for the stabilization of metastable materials using solvothermal processes (Demazeau, 2008) and the investigations of the diffusion/impregnation of saccharose and NaCl under HHP (Lambert et al., 1997).

1.4. First developments of high pressure in different scientific areas

The applications of high pressure in different scientific domains were strongly dependent on the development of the associated technologies.

High pressure was, at the beginning, mainly applied in Physics and Chemistry. During the nineteenth century, pressure (from about ten MPa to several hundred MPa) was developed for studying the compressibility of gases and liquids (Amagat, 1893; Andrews, 1861; Basset, 1927; Bridgman, 1949; Raoult, 1890).

During the beginning of the XXth century, in chemistry, high pressure was mainly developed towards three main directions (i) to reproduce synthesis of minerals (Eugster, 1986), (ii) to use hydrometallurgy-extracting metals from ores (Habashi, 1971), and (iii) to improve the crystal growth of functional materials such as α-quartz due to its piezoelectric properties (Iwasaki and Iwasaki, 2002).

The main success was, in the middle of the XXth century, the synthesis of diamond either by A.S.E.A. (Sweden) or General Electric (USA) (Bovenkerk et al., 1959; Liander and Lundblad, 1960).

The first industrial application of high pressure was the synthesis of NH_3 (because of the negative ΔV value characterizing the reaction: $N_2 + 3H_2 \rightarrow 3NH_3$) (Travis, 1998). Different processes were then developed in Materials Science, such as, for example, polymerization (Ipatiev and Rutala, 1913), hydrothermal crystal growth of materials (Spezia, 1898), synthesis and crystal growth of diamond for the development of machining or cutting processes for super-hard alloys (Sung, 2001), elaboration of CrO_2 as fine particles well defined in size and morphology for magnetic recording applications (Demazeau et al., 1980), high pressure sintering of dense ceramics (Akimov, 1998).

These first successes and the technological developments associated with high pressure led to an increase of the use of this thermodynamical parameter in different scientific domains such as Physics (Bridgman, 1949), Chemistry (Materials chemistry (Demazeau, 1985) or organic chemistry (Klarner and Wurche, 2000)), Geosciences (Schreyer, 1982), and Biosciences (Balny et al., 1992a).

1.5. Development of high pressure in Biosciences

The effect of high pressure on living systems was first investigated by Regnard (Regnard, 1884a,b). Three different approaches were

developed to study high pressure effects in Biosciences: (i) the discovery of piezophile (or barophile) micro-organisms in the deep marine environments with the studies involving the pressure adaptation and in particular the role of high pressure in the origin of life (Daniel et al., 2006; Hazen et al., 2002; Pradillon and Gaill, 2007), (ii) the decontamination of food products (Tauscher, 1995) and (iii) the investigations dealing with the high pressure effects on biological systems and their constituents (Masson, 1999).

High pressure treatment to kill bacteria such as *Escherichia coli* and *Staphylococcus aureus* was first described in 1895 by Royer (Royer, 1895). Hite studied the preservation of milk under high pressure (Hite, 1899). Bridgman (1914) investigated the coagulation of albumen under pressure (Bridgman, 1914) but this phenomenon was elucidated by Grant et al (1941) as the protein denaturation phenomenon (Grant et al., 1941).

Hite et al. (1914) developed hydrostatic pressure for the inactivation of some micro-organisms in order to preserve fruits and vegetables (Hite et al., 1914).

Between 1932 and 1952, James and Jacques Basset et al. investigated high pressure effects in order to inactivate different micro-organisms for either food processing development or biological applications. For example, they studied the pressure resistance of bacteria (Basset and Macheboeuf, 1932) and viruses (Atanasiu et al., 1951; Basset et al., 1956; Basset and Macheboeuf, 1933a; Basset et al., 1935a), the pressure effects on antigens and antibodies and their influence on the immunogenicity (Basset and Macheboeuf, 1933b), the role of high pressures on tumors (Basset et al., 1935b). These studies led to the evaluation of the antigenic power and vaccinal properties of bacteria killed under high pressure (Vignais et al., 1952).

Over the sixties and the seventies, such research activity was maintained in different scientific groups involved in food aspects such as Timson and Short's (1965) research works on pressure effects on micro-organisms in raw milks, Gould and Sale's (1970) research work involving the germination of spores under hydrostatic pressure, Wilson's works (1974) on the sterilization of low-acidic foods using pressure and pasteurization temperatures, Elgasim and Kennick's (1980) research activities concerning the pressure effects on beef proteins, Charm et al.'s (1977) studies on the use of pressure for long-term refrigerated storage of foods, and Marquis (1976), and Marquis and Matsumara's (1978) works on the response of biological systems to hydrostatic pressures up to 110 MPa.

During the eighties, research involving High Pressure and Food Processing had evolved with the works of Morild (1981) on the high pressure effects on enzymes , Heremans (1982) on the high pressure effects on proteins and other biomolecules, Popper and Knorr (1990) on the applications of high pressure homogenization for food preservation, Hoover et al. (1989) on the biological effects of high hydrostatic pressure on food micro-organisms, Farr (1990) on the high pressure technology in food industry.

Strong efforts for setting up new food processes were conducted in Japan in particular (Hayashi, 1989, 1990; Horie et al., 1991; Ogawa et al., 1990; Tanaka and Hatanaka, 1992). Such developments in Japan can be explained by different factors: (i) the difficulty to use ionizing treatments, (ii) the preservation of the organoleptic properties of the raw material in agreement with the Japanese cooking culture, and (iii) the development of original processes supported by a specific technique (high pressure). Such research activity led to: (i) a strong interest, in basic research, to explain the mechanisms of micro-organisms inactivation under high pressure (Balny et al., 1992a; Cheftel, 1995; Cheftel and Culioli, 1997; Demazeau, 1993; Hoover, 1993; Knorr, 1993; Tauscher, 1995; Tonello et al., 1993), and (ii) new industrial processes for food preservation (the first food product stabilized under high pressure reached the Japanese market in 1993).

Over the last fifteen years high pressure technology in food processing has steadily increased.

Several products are now available on the market in different countries: fruit juices, jam, tofu, ham, shellfish, and biopolymers (such as proteins or starches).

The main application of high pressure in the food industry is for the elimination of microbial pathogens and the extension of shelf-life. The viability of vegetative micro-organisms is affected by inducing structural changes in their cell membrane or by the inactivation of enzyme systems responsible for the control of metabolic reactions (Knorr et al., 2006; Matser et al., 2004; Rastogi et al., 2007; San Martin et al., 2002).

At the present time, approximately 90 industrial installations are in operation in the world, the volumes of the High Hydrostatic Pressure (HHP) equipments varying from 35 to 3601 (Tonello-Samson, 2007). In addition, different physico-chemical parameters were added to HHP treatments: low temperature (Dumay et al., 2006; Kalichevsky et al., 1995; LeBail et al., 2002; Luscher et al., 2004; Urrutia Benet et al., 2004), the use of carbon dioxide (Garcia-Gonzalez et al., 2007; Parton et al., 2007a,b).

General papers dealing with the development of pressure indicators for HHP processing of foods (Minerich and Labuza, 2003), food safety (Fonberg-Broczek et al., 2005) or “commercial opportunities and research challenges in high pressure processing of foods” (Torres and Velazquez, 2005) underline the strong interest of such non-thermal treatment.

In parallel, marine medium has been used as a model in different cases for the study of micro-organisms (Zobell, 1964, 1970; Zobell and Cobet, 1964; Zobell and Johnson, 1949). The discovery of piezophiles has prompted researchers to investigate the survival strategies developed by these micro-organisms for their adaptation to high pressure environments (associated in some cases with high temperature) (Abe et al., 1999; Bartlett, 2002; Simonato et al., 2006; Yayanos, 1986, 1995).

Such a scientific domain has led to works on the adaptation, according to the pressure value, of different components of the living systems such as lipids and biological membranes (Braganza and Worcester, 1986; Delong and Yayanos, 1985) or proteins (Gross and Jaenicke, 1994; Kato and Bartlett, 1997; Nakasone et al., 1998; Prieur, 1992).

In parallel, over these last years, HHP has been investigated for biotechnological applications (Aertsen et al., 2009; Balny et al., 1992b; Mozhaev et al., 1994; Rigaldie and Demazeau, 2004; Rigaldie et al., 2001; Silva et al., 2004).

2. Pressure effects on various components of biological systems

For various reasons, pressure appears as an important tool for the investigation of biological systems (Winter and Dzwolak, 2005).

- The studies versus temperature of a biological system at ambient pressure induce changes simultaneously in thermal energy and volume. Consequently, in order to discriminate both effects (thermal and volumetric), high pressure parameter appears as an appropriate tool.
- Non covalent interactions play an important role in the stabilization of biological systems. Due to the low energy developed by pressure, high pressure alteration of weak bonds (in particular weak bonds characterized by a negative ΔV value) can play a crucial role in the investigation of the mechanisms of this stabilization. Pressure affects also chemical equilibria and reaction rates but all these parameters are governed by the Le Chatelier's rule leading to the stabilization of the state corresponding to the smallest volume.
- Due to the existence of hydrogen bonds, the specific pressure-temperature diagram of water (the most common solvent in biological systems) favors the liquid state until −20 °C if the pressure value is high enough (−20 °C, 200 MPa). Consequently, experiments at subzero temperature in liquid phase are possible.
- In addition, the role of the negative value of ΔV in the stabilization of novel phases can open the route to the investigation of novel phases unknown at ambient pressure.

2.1. Pressure effects on proteins

Denaturation of proteins is induced by different factors: (i) heat, (ii) chemicals, and (iii) pressure. Temperature and/or chemicals lead to protein denaturation and often irreversibly unfold the complete protein because of covalent bond breaking and/or aggregation of the molecule. Indeed, works of Zhang et al. demonstrated, for example, that pressure denatured ribonuclease A preserved some partial secondary structure contrary to the heat unfolded one. The preservation of some β-like structures was also observed for pressure denatured staphylococcal nuclease (Winter and Dzwolak, 2005; Smeller, 2002; Zhang et al., 1995).

High pressure can maintain some parts of the molecule unchanged due to the fact that only weak bonds are affected (and only weak bonds characterized by a negative ΔV value). Consequently, the denaturation mechanisms induced by pressure are different from the ones observed using temperature or chemicals.

For any chemical reactions the equilibrium constant K is related to the Gibbs free energy ΔG, according to the equation 2:

$$\Delta G = -RT \ln K \quad (2)$$

The pressure dependence P (MPa) of the Gibbs energy is given by the following equation:

$$(\delta(\Delta G)/\ \delta P)_T = \Delta V \quad (3)$$

in which ΔV is the reaction volume change of the system in cm^3/mol.

$$\Delta V = V\ (products) - V\ (reactants) \quad (4)$$

These partial volumes include both parts: intrinsic (van der Waals volumes) and solvational (contraction of the solvation shell and change in volume of the cavities).

$$Consequently: \ (\delta(\Delta G)/\ \delta P)_T = -\Delta V / RT \quad (5)$$

The main effect of pressure is therefore to displace the equilibrium towards the state characterized by the smallest volume (Royer, 2005).

Among the weak interactions stabilizing the protein conformation, hydrophobic interactions are the ones characterized by the most negative ΔV value and therefore the most pressure sensitive. These interactions play a major role in the stabilization of the tertiary structure and in protein–protein interactions. Columbic interactions are not favored by pressure and hydrophobic interactions are destabilized by pressure (Table 4) (Balny et al., 2002; Boonyaratanakornkit et al., 2002; Mozhaev et al., 1994; Mozhaev et al., 1996a).

The entry of water molecules inside the protein matrix seems to play a major role in the high pressure denaturation process. Therefore, the stability of proteins under high pressure mainly depends on their conformational stability to compensate the loss of weak interactions but also on the size of the cavities within which water molecules can penetrate. Structural transitions of proteins under high pressure are often driven by the hydration changes that accompany protein conformational transitions. This modification in the hydration degree can be explained by two main contributions. First, the opening up of the cavities allows a solvent to occupy an internal volume that was previously excluded from interactions with this solvent. Second, the surface area in contact with the solvent is larger for unfolded proteins than for native ones (Silva and Foguel, 2009; Heremans, 2005; Royer, 2005; Winter and Dzwolak, 2005; Smeller, 2002).

Taking into account all the molecular interactions and pressure effects, a pressure/temperature transition diagram for protein denaturation can be drawn (Fig. 1). This phase diagram of protein unfolding is elliptical. The major role of water in protein denaturation under pressure is comforted by the fact that this elliptic diagram can only be observed for protein solutions whereas proteins in the dry state are very stable against pressure (Smeller, 2002; Balny et al., 1997).

Table 4
Susceptibility to high pressure of chemical interactions. Adapted from (Federighi et al., 1995).

Type of interaction	$\Delta V_{dissociation}$ (ml mol^{-1})	Pressure effect
Covalent	+10	Stabilization
Ionic	−10	Destabilization
Hydrogen	+3 to −1	Stabilization or low destabilization
Hydrophobic	<0 (−10 to −20)	Destabilization

2.2. Pressure effects on lipids and biomembranes

Lipid systems are the most pressure sensitive biological components. Lyotropic lipid mesophases are organized in biomolecular systems composed of amphiphilic molecules (mostly phospholipids) in the presence of water (Winter and Dzwolak, 2005). They exhibit a large structural polymorphism depending on different parameters: their molecular structure, water content, pH, ionic strength, temperature and pressure (Cevc, 1993; Lipowsky and Sackmann, 1995; Winter and Czeslik, 2000; Winter and Jonas, 1999; Wong and Mantsch, 1985).

The basic structural element of biological membranes consists of a lamellar phospholipids bilayer matrix (Winter and Dzwolak, 2005). When saturated phospholipids are placed into water, two phase transitions take place: a gel-to-gel pretransition ($L_{\beta'}$–$P_{\beta'}$) and a gel-to-liquid-crystalline ($P_{\beta'}$–L_α) main transition. The compression of the phospholipidic bilayer is anisotropic. Under high pressure conditions, the acyl chains straighten which result in a lateral shrinking and an increase in thickness. This phenomenon is also accompanied by a phase transition from the liquid–crystalline to the gel phase (Winter and Czeslik, 2000; Winter and Jeworrek, 2009).

Barophilic organisms display membranes that are more fluid, and this is partly due to an increase of the unsaturated to saturated lipid ratio. Because of the sensitivity of lipids against pressure, these biological components are often considered as the main target in the pressure inactivation of micro-organisms (Winter and Jeworrek, 2009).

2.3. Pressure effects on nucleic acids

Due to the stabilizing effect of high pressure on hydrogen bonds and in particular DNA hydrogen bonds, the duplex to single strand

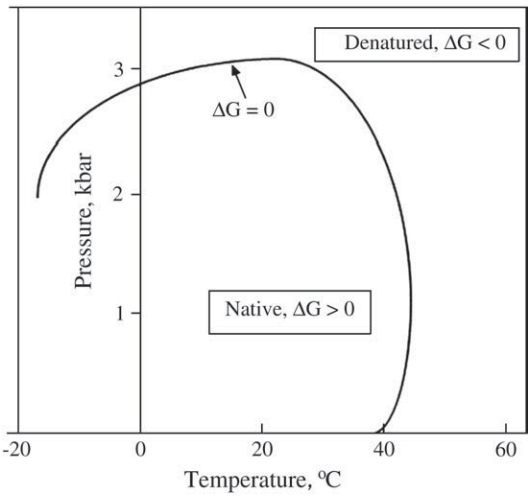

Fig. 1. Pressure–temperature transition diagram for protein denaturation (Balny et al., 1997).

transition temperature (melting temperature T_M) is increased by pressure (Mentré and Hui Bon Hoa, 2001).

3. Pressure effects on more complex living systems

3.1. Pressure effects on mammalian cells

High pressure treatment of mammalian cells usually leads to cell death, and the mechanism of this death mainly depends on the pressure level:

- for pressures around 200 MPa, cell death is the result of apoptosis,
- for pressures higher than 300 MPa, cell death occurs through a necrotic-like pathway (Aertsen et al., 2009; Frey et al., 2008; Yamaguchi et al., 2008).

Apoptosis usually happens after a high pressure treatment around 100 MPa. This phenomenon was observed on many cell lines, including MEL cells, human lymphoblasts, B35, PC12 and retinal ganglion cell lines (Agar et al., 2006; Takano et al., 1997; Yamaguchi et al., 2008). High pressure-induced apoptosis occurs through the activation of caspase-3, via both extrinsic and intrinsic pathways. The extrinsic pathway is characterized by the binding of the Fas ligand to the cell surface cell death receptor Fas. When the intrinsic pathway is activated, cytochrome c is often released from mitochondria into the cytosol. The apoptotic stimulus, however, is still undefined (Yamaguchi et al., 2008). Apoptosis results in cell shrinkage, condensation of chromatin, loss of microvilli that lead to cell death (Takano et al., 1997; Yamaguchi et al., 2008).

Necrosis occurs in mammalian cells exposed to a pressure over 300 MPa (Frey et al., 2008; Takano et al., 1997). This was observed on human lymphoblasts immediately after decompression. (Takano et al., 1997) Necrosis is not dependent on caspase activation and leads to cellular swelling, organelle degradation, in particular irreversible damage to mitochondria, altered ionic concentrations within cells. This finally results in the disruption of the cell membrane and release of cellular contents that provokes inflammation (Takano et al., 1997; Yamaguchi et al., 2008).

3.2. Pressure effects on pathogens

3.2.1. Bacteria

High pressure inactivation of bacteria was the main objective of the first experiments conducted under high pressures (Hite, 1899). Since then, many advances have been made in this field, especially in the comprehension of the mechanisms involved in the bacterial inactivation processes.

Cell membrane is often considered as the first site of injury in pressure-inactivated bacteria. Indeed, scanning electron microscopy observations show some bud scars on the surface of pressurized cells, suggesting that the cellular wall or membrane may be one of the targets of high pressures (Ritz et al., 2002). However, membrane damage in some bacteria strains (assayed with Propodium Iodide staining) appears later than cell death (Ananta and Knorr, 2009; Ritz et al., 2002) and disruption of this membrane cannot be observed even at the highest pressure treatments (Tholozan et al., 2000). So, the action of high pressures on cell membrane may involve some other mechanisms.

It is known that membrane fluidity decreases when pressure increases, implying that micro-organisms with less fluid membranes are more pressure sensitive (Yaldagard et al., 2008). This results in alterations of membrane bound enzyme functionality (Kato and Hayashi, 1999). For example, cell death of *Lactobacillus rhamnosus* can be correlated with inactivation of ATP-mediated dye exclusion system, which is localized on the cell membrane. The degradation of this system impairs the ability of cells to reproduce and develop colonies on nonselective media (Ananta and Knorr, 2009). Furthermore, analysis of scanning electron microscopy images of *S. aureus* and *E. coli* demonstrates that following pressure inactivation the average cell view area and volume increases. It is proposed that these observations could be associated to modifications of membrane properties, such as the denaturation of membrane bound-proteins or phase transition of the membrane lipid bilayer (Pilavtepe-Çelik et al., 2008). An other evidence that membrane bound-proteins may be affected by high pressure treatment is the decrease in cellular ATP content and membrane potential, which could be associated respectively with a dysfunction of the ATPase and the incapacity of the cell to maintain its homeostasis, process that involve several transmembrane proteins (Ritz et al., 2002; Tholozan et al., 2000).

As explained earlier, the loss of the integrity of the cell membrane is not enough to explain the pressure inactivation process of bacteria. After high pressure treatment of *E. coli*, the internal structure of the cells is also modified, as shown by the condensation of nucleoids and the aggregation of cellular proteins. However, no absolute correlation between these phenomena and cell inactivation can be defined. The condensation of nucleoids could be due to the direct DNA packing effect in the cells (which is not associated with DNA dysfunction) or by the action of pressure on protein–DNA complexes. The aggregation of cellular proteins could be caused by their denaturation under pressure. It is interesting to underline that these two effects could also be partially explained by cell membrane damage. Indeed, protein–DNA complexes as well as intracellular proteins such as ribosomes necessitate divalent metal ions for their stabilization. Upon membrane cell damage, leakage of such ions from the cell is highly probable, resulting in destabilization of these entities (Moussa et al., 2007).

It is worth noting, however, that some works demonstrated that the stability diagram of some bacteria such as *E. coli* displays an elliptic shape. This type of diagram is similar to the one observed for proteins but not for membranes or nucleic acids. Therefore, this suggests that proteins are the primary target of the pressure inactivation of micro-organisms (Heremans, 2005; Smeller, 2002; Ludwig et al., 1996).

The efficacy of high pressure inactivation of bacteria is dependant of many parameters, including the cell itself, the water activity of the system and the temperature used for the high pressure treatment.

Gram positive bacteria appear to be more pressure resistant than Gram negative bacteria. It is assumed that this difference of sensitivity to high pressure could be associated with difference of structures of the cell envelope. Indeed, the cell envelope of Gram negative bacteria is composed of an additional layer: the outer membrane which is more pressure sensitive than the cytoplasmic membrane (Pilavtepe-Çelik et al., 2008).

The water activity (a_w) of the system is also an important factor for the inactivation of bacteria under high pressure. It appears that as a_w decreases, bacteria become more resistant to the pressure effect. It is assumed that by decreasing the a_w of the cell cytoplasm, this latter become less compressible, and thus the area/volume ratio, which seems to be involved in cell inactivation, is modified (Moussa et al., 2006).

The inactivation efficiency of high pressure treatments on bacteria, as *E. coli* for example, is increased when applied at subzero temperatures (Moussa et al., 2006). Under these conditions, high pressures appear to be more effective when they induce phase transition between ice I and ice III, and this could be associated with the stress caused by the phase transition itself (Luscher et al., 2004). It is suggested that when high pressure treatment is applied at subzero temperature, the inactivation process occurs mainly during the transiently permeabilized state (Moussa et al., 2007).

3.2.2. Yeasts and molds

In general, yeasts and fungi are more sensitive to high pressure than vegetative bacteria.

The inactivation mechanism for yeasts by high pressure is close to the one for bacteria, in that high pressure affects the cell membrane

permeability and cellular structures, is responsible for protein denaturation (Black et al., 2007; Perrier-Cornet et al., 1999). Indeed, a mild high pressure treatment (300 MPa, 15 min, 25 °C) modifies cell walls and plasma membrane of *Saccharomyces cerevisiae*, but it seems that the intracellular membrane is the first target in the inactivation process (Brul et al., 2000). The idea that membrane permeability is affected by high pressure is reinforced by the cell volume decrease observed after treatment. This implies a water flux which is possible if the cell membrane becomes more permeable and allows internal molecules to exit (Perrier-Cornet et al., 1999).

Contrary to bacteria, yeasts are eukaryotic cells and thus possess mitochondria. It appears that mitochondria could be one of the elements altered over the pressure inactivation process. High pressures may cause the release of cytochrome c from mitochondria, which is presumed to be a key signalling step in the apoptosis process, leading to cell death (Brul et al., 2000).

Ascospores of heat-resistant molds appear to be more pressure resistant and many of them are not inactivated in the pressure range of 300 to 800 MPa. Their vegetative counterparts (conidia) are much more pressure sensitive, with a sensitivity similar to yeasts (Ludwig, 2003). It is hypothesized that high pressure acts on the permeability and rigidity of the ascospores wall that, in turn, increases their permeability to water. This rehydration is the first step in the course of spore germination (Black et al., 2007).

3.2.3. Bacterial spores

Bacterial spores are the environmentally resistant form of some Gram positive bacteria. This process of sporulation is initiated when there is a lack of nutrients. The high resistance of spores to numerous stresses, such as heat, radiation, desiccation, chemicals, is mainly due to their particular structure made up of a number of layers. The cortex, which is responsible for the establishment and maintenance of the low water content in the spore core, is probably the main origin of the high pressure resistance of spores (Black et al., 2007; Setlow, 2008). The direct inactivation of spores by high pressure necessitates the application of very high pressures, as high as 827 MPa for 30 min at 75 °C (Delacour et al., 2002; Reddy et al., 2006).

In order to kill spores using high pressure, the process is often divided in two steps: one at lower pressures (50 to 300 MPa) which initiates the germination process, and one at higher pressures (>400 MPa) that inactivates the germinated spores obtained at the end of the first step (Setlow, 2008). Indeed, moderately high pressures (mHP) trigger the germination process via the activation of nutrient germinant receptors present at the surface of the spore's inner membrane. However, how mHP activates these receptors is still a matter of debate. They could act directly on the receptors themselves, causing some structural changes, or on the inner membrane in which the receptors reside. Following the activation step, mHP germination follows the same pathway as nutrient germination (Black et al., 2007; Paidhungat et al., 2002; Setlow, 2008).

3.2.4. Viruses

The pressure resistance of viruses varies greatly among virus strains (Smelt, 1998). Viruses are generally classified in two groups: the enveloped and the non enveloped viruses, according to their structure which is either a membrane enveloped or non enveloped protein shell and nucleic acid (Oliveira et al., 2008).

Most of the studies have been conducted on enveloped viruses. In this case, high pressure can affect three types of interactions: protein–lipid, protein–protein, and protein–nucleic acid. This later seems to remain intact under high pressure (Gaspar et al., 2002).

After high pressure treatment, the overall structure of the virus is not altered, and the only noticeable difference with electron microscopy observations is the presence of a bulge in the surface, which can be explained by a displacement of the capsid subunits retained under the lipid and protein membrane (Gaspar et al., 2008; Silva et al., 1992). These subtle conformational changes in the viral coat proteins and/or its envelope glycoproteins, induced by high pressure, mimic the binding process of the viral particles to the host cellular receptors, the so-called "fusion-active state" (Aertsen et al., 2009; Gaspar et al., 2002). The transition to the fusogenic state impedes the binding of the virus to its cellular receptors and thus prevents endocytosis and virus infection (Oliveira et al., 2008).

Non enveloped viruses are usually more pressure resistant than enveloped viruses. The fusogenic state described for enveloped viruses can also be found in pressure-inactivated non enveloped viruses. It is often proposed that under pressure, the capsid disassembles and when pressure is released, there is reassociation to a non-infectious particle, which resembles the fusion intermediate state described for enveloped viruses (Oliveira et al., 2008; Tian et al., 2000).

Their inactivation is often enhanced when high pressure treatment is applied at subzero temperatures. Under these conditions, proteins can undergo cold denaturation due to a synergistic destabilization of hydrogen bonds and hydration of hydrophobic groups, leading to the loss of quaternary and tertiary structures (Buckow et al., 2008; Kingsley et al., 2007).

3.2.5. Parasites

Only little information is available on the effect of high hydrostatic pressure on parasites. Until now, studies have been conducted on two types of parasites: protozoan parasites and nematodes.

Oocysts are the resistant form of protozoan parasites. Oocysts from parasites such as *Cryptosporidium parvum* or *Toxoplasma gondii* can be easily inactivated by a pressure comprised between 340 MPa and 550 MPa applied for a short time (≤3 min) (Collins et al., 2005; Lindsay et al., 2008; Slifko et al., 2000). The viability of oocysts is usually assayed by excystation, which is based upon the ability of oocysts to release sporozoites when exposed to a solution containing bile salts and trypsin (Slifko et al., 2000). After high pressure treatment, oocysts present no morphological modifications as seen with light microscopy or transmission electron microscopy (Lindsay et al., 2005; Shearer et al., 2007). The permeability and fragility of their wall is unchanged. It seems, however, that high pressure affects sporozoites integrity since their recovery and morphology are modified (Shearer et al., 2007).

Studies on nematodes such as *Ascaris suum* or *Anisakis simplex* demonstrated that a relatively low pressure (≈200 MPa) applied for a short time (≤10 min) is sufficient to inactivate these parasites (Brutti et al., 2010; Dong et al., 2003; Molina-Garcia and Sanz, 2002; Rosypal et al., 2007). Observations of larvae with light microscopy show no morphological alterations of the nematode after high pressure treatment (Molina-Garcia and Sanz, 2002; Rosypal et al., 2007). Cryo-Scanning Electron Microscopy for pressure-dead larvae also does not reveal any external damage to the cuticles (Molina-Garcia and Sanz, 2002). Histochemical observations using blue staining with Azan demonstrated a change of coloration in the dead larvae. The authors hypothesized that high pressure may trigger the transition of tissues from acidophilic to basophilic. The results with periodic acid-Schiff stain also suggest that pressure promotes the decomposition of glycogen and/or glycoproteins (Ohnishi et al., 1994).

3.2.6. Infectious prion protein: "a new pathogen type"

Prions are generally defined as infectious proteins, able to transmit their special conformation to normal molecules of the same proteins via protein–protein interactions, leading to neurodegenerative diseases in animals and humans. The extra- or intracellular accumulation of infectious proteins under the form of fibrillar protein aggregates is characteristic of these diseases. The normal form of prion protein is usually designated as PrP^{C} while the pathological form is denominated PrP^{Sc}. These two forms share the same amino acid sequence and differ only in their conformation, PrP^{Sc} containing more β-structures than PrP^{C}. This difference confers to PrP^{Sc} resistance to proteinase K

(PK) activity (Torrent et al., 2006a,b; Shkundina and Ter-Avanesyan, 2007; Wilson and Nixon, 2009).

High pressure inactivation of infectious prion, such as 263K scrapie, requires very drastic conditions, with the application of pressures up to 1200 MPa for 10 min at a temperature up to 135 °C (Cardone et al., 2006). Works on Ure2 protein from *S. cerevisiae* also demonstrated that a pressure up to 600 MPa was insufficient to unfold the protein. This protein is often used as a model for prion because it can undergo a conformational change to an aggregated state (Lian et al., 2006).

Pressure-inactivated prion proteins lose their PK resistance, which is probably due to some conformational changes (Heindl et al., 2006). High pressure/high temperature conditions might be sufficient to dissociate highly aggregated PrP^{Sc} irreversibly. Hydrophobic and electrostatic interactions are probably the main stabilizing parts of such aggregates, which are also characterized by the presence of voids due to weak packing. High pressures will favour the state of smaller volume and so the disruption of these types of interactions and the loss of internal cavities, leading to the dissociation of aggregates (Heindl et al., 2008).

Conformation of pathological prion proteins, however, appears to be a main parameter in their sensitivity to high pressure. Indeed, depending on their origin: native prion proteins from crude brain homogenates of infected hamsters or purified prion proteins, PrP^{Sc} molecules show difference in pressure sensitivity, the second being more pressure resistant. This suggests that the purification process may cause conformational modifications. These results reinforce the idea that the native PrP^{Sc} exist under two forms: one containing pressure sensitive β-structures, and one composed of very high pressure resistant β-structures. This shows the importance of the choice of the prion protein origin for inactivation studies (Heindl et al., 2006).

4. Biological applications of high hydrostatic pressure

4.1. Food industry

The potential of high pressure to inactivate many pathogens while keeping intact most of the organoleptic properties of food products is of great interest for the food industry. Indeed, the application of high pressure processing presents many advantages, including the preservation of vitamin and flavour compounds, its uniform and instantaneous application which is independent to the size and shape of the treated product, the low energy required for its running (Yaldagard et al., 2008).

High pressure processing is already used in the food industry because of its capacity to diminish the microbial load while preserving most of the sensory, nutritional and functional properties of the treated products. That's why such processing has already been developed for the production of fruit juices, jams, guacamole… (Manas and Pagan, 2005; Yaldagard et al., 2008).

Since the first approaches of high pressure processes in food technology (Cheftel, 1992), the research of indicator systems (physical and biological) in order to evaluate the impact of high pressure treatment of foods (pascalisation) compared to the conventional food safety treatments (pasteurization, and sterilization) is an important key for industrial developments (Van der Plancken et al., 2008).

4.2. Proteins from extremophiles as stable tools for biotechnological applications

Proteins and enzymes isolated from extremophiles, in particular piezophile and thermophile micro-organisms, open the way to new applications in different domains: very sensitive to pressure and temperature parameters in clinical, environmental and food analysis, the construction of nanosensors (Abe, 2007; De Champdoré et al., 2007).

4.3. High pressure effects on allergenicity and digestibility

It is often proposed that partial proteolysis under high pressure of food products (in particular milk proteins) increases their digestibility and reduces their allergenicity (Chicon et al., 2008; Masson et al., 2001; Zeece et al., 2008).

Whey proteins are commonly used in the food industry. For hypoallergenic products, they are often replaced by whey protein hydrolysates obtained through enzymatic digestion because they present low immunoglobulin binding. The proteolysis process, however, also reduces the functional properties of whey proteins, in particular their emulsifying properties. When enzymatic proteolysis is conducted under high pressure, proteins such as beta-lactoglobulin become more susceptible to enzymes like pepsin, trypsin, chymotrypsin, pronase, and thermolysin. The resulting hydrolysates show reduced allergenicity with low IgG- and human IgE-binding properties, and keep acceptable functional properties, with improved heat stability and an emulsifying activity index comparable to the whey protein isolate (Chicón et al., 2009; Zeece et al., 2008).

High pressure processing can also reduce the allergenic activity of foods other than milk-based foods, such as rice (Masson et al., 2001). Indeed, endosperm cells present in rice grains are partially destroyed under high pressure, and become permeable leading to the release of major rice allergens (Aertsen et al., 2009).

4.4. Disinfection of biomaterials

Since high pressure processing has proven its efficiency in the food industry, it has been proposed that such treatment could be used for the disinfection of biomaterials of both artificial and natural.

Synthetic biomaterials include prosthesis, bone plates, artificial ligaments and tendons. For example, it was demonstrated that a high pressure treatment of 300 MPa at 4 °C for 30 min is sufficient to sterilize standard screws contaminated with *S. aureus* (Gollwitzer et al., 2009). Bone substitute calcium phosphate materials can also be sterilized by high pressure treatment, since a treatment by two cycles, spaced at a 24-hour interval, of 5 min each at 140 MPa rendered calcium-deficient apatite, previously contaminated with *S. aureus*, totally sterile (Brouillet et al., 2009).

Natural biomaterials used for autograft or allograft procedures include bone segments, tendons, and cartilage.

In the case of autografts, the need of disinfection can come from an infection or the development of tumor cells. For this procedure, that mainly concerns bone segments and tendons, the bone fragment is resected, then disinfected, and reimplanted. To date, the devitalisation is conducted by extracorporeal irradiation, autoclaving, heat, or chemical detergents, but this leads to loss of biomechanical and biological integrity of the bone (Diehl et al., 2005; Diehl et al., 2006; Gollwitzer et al., 2009). It was proven that the main protein present in bone tissue (fibronectin, vitronectin, type I collagen) were not altered after high pressure treatment up to 600 MPa (Diehl et al., 2005). Furthermore, high pressure-treated Achilles tendons and trabecular bone (600 MPa) show no loss of their biomechanical properties, as determined by their Young's modulus and tensile strength values (Diehl et al., 2006; Steinhauser et al., 2006). At such pressure values, tumor cells are inactivated, so that such procedure would allow immediate reimplantation of the treated grafts without important loss of their biomechanical properties (Steinhauser et al., 2006).

For allografts, the transplant comes from an individual from the same species. Because the donor may have developed some disease, the graft may be contaminated, for example with bacteria. High pressure processing is an interesting new method for the disinfection of such grafts, in particular bone, tendon, ligament, and cartilage. For

example, it was proven that bovine menisci can be exposed to 600 MPa with no modification in collagen type I, II, III, proteoglycans versican, aggrecan and for link-protein, as observed with immunohistochemical analysis (Naal et al., 2008).

4.5. Modulation of enzymatic activities

Enzyme activity, such as the specificity of proteases, can be modulated by pressure. This is possible because at pressure lower than 200 MPa, the stability and functionality of most enzymes are not altered (Masson et al., 2001).

Some enzymes lose their activity with a pressure increase. For example, it was shown that the catalytic activity of horse liver alcohol dehydrogenase (HLADH) strongly decreased in the pressure range between 100 and 400 MPa and this was correlated to conformational changes as followed by fluorescence and FTIR spectroscopy (Trovaslet et al., 2003).

On the contrary, if the volume change induced by the catalytic reaction is negative, then high pressure can enhance the associated enzymatic activity. For example, the enzymatic activity of alpha-chymotrypsin at 470 MPa is 6.5 fold higher than the one at atmospheric pressure (Aertsen et al., 2009; Balny, 1996; Mozhaev et al., 1996b). This effect was also observed on thermolysin, its activity increasing by 45-fold at 200 MPa compared to the one at ambient pressure. However, when pressure reached values above 200 MPa, this enzyme rapidly lost its activity through structural deformation, canceling the activity increase promoted by pressure (Kunugi et al., 1997).

It has also been established that thermal resistance of protein can be increased under mild high pressure conditions. It is known that many enzymes present enhanced enzymatic reaction rate when temperature is raised. The use of mild heat-mild high pressure processing could define new optimum reaction conditions for which an enzyme, thermostabilized by high pressure, shows improved reaction rate, leading to higher production yields. For example, the enzymatic activity of alpha-chymotrypsin at 50 °C is increased by 30 times when the reaction takes place at a pressure of 360 MPa (Aertsen et al., 2009; Balny, 1996; Mozhaev et al., 1996b).

4.6. Stabilization of protein intermediates

The protein folding pathway is still poorly understood because of the difficulty to isolate and describe the intermediate structures. Under pressure, it is possible to reach these intermediates, so that their structures can be studied using different physical techniques (fluorescence, NMR, and light scattering). Reactions leading to protein folding and associations show similarities, in that they are associated with increases in entropy and volume of the protein system. Contrary to the use of common perturbing agents, such as temperature, urea, and guanidine that cause drastic modifications in protein structures, high pressure only affects non-polar interactions that are essential in protein folding if this leads to a decrease in volume. Consequently, high pressure denaturation of proteins mainly depends on their tertiary and quaternary structures (Aertsen et al., 2009; Balny, 1996; Silva et al., 1996, 2004).

For many proteins systems, the use of high pressure was very useful to assess their molten globule state (partially folded state). Indeed, the equilibrium is shifted toward the molten globule state under pressure, demonstrating the presence of many partially folded conformations between the completely unfolded and the fully folded states with in particular the aggregation and amyloidogenesis. The isolation of folding intermediates is crucial to the understanding of protein misfolding and protein aggregation either in biotechnology industry (for example: expression of inclusion bodies in bacteria) and because this tool can help identify the mechanisms behind amyloidogenic diseases, and so develop therapies (Balny, 2006; Meersman and Dobson, 2006; Silva et al., 1996, 2004; Torrent et al., 2004, 2006a,b). For example, using the truncated form of a hamster prion protein, several distinct routes of misfolding were revealed by varying the experimental conditions (pressure, temperature, pH, protein concentration and time). The authors suggested that some of these structural forms may be localized on the pathogenic routes (Torrent et al., 2006a,b).

In addition some cancers have been found to be caused by protein misfolding (Caughey and Lansbury, 2003; De Bernardez, 2001; Dobson, 1999; Horwich, 2002; Sacchettini and Kelly, 2002).

Consequently high pressure appears as a promising tool for developing research activities in Biotechnological and Pharmaceutical applications associated with protein misfolding (Silva et al., 2004).

4.7. Dissociation of protein complexes

Because high pressures lower than 200 MPa are able to dissociate oligomeric proteins, they can be used to dissociate protein aggregates or inclusion bodies. When proteins are expressed in bacteria, they often aggregate into inclusion bodies which makes difficult to obtain fully functional bioactive compound. The application of high pressure leads to accurate refolding of the constituting proteins, and thus allows simultaneous solubilization and refolding (Aertsen et al., 2009; Balny, 1996, 2006; Crisman and Randolph, 2009; Phelps and Hesterberg, 2007; Silva et al., 2004).

In the process of the preparation of bioactive molecules, one of the main challenges is the purification of the product of interest from its production medium. One way to achieve this step is the immunoaffinity based separation that uses the steric recognition between the molecule of interest (considered as the antigen) and its corresponding antibody, linked to some matrix. The critical step is then the dissociation of the antigen-antibody complex, which often requires drastic elution conditions, for the recovery of the eluted antigen in its active form, and without any important loss of the properties of the immunoadsorbent (Gavalda et al., 1996; Lemay, 2002).

Because formation of antigen-antibody complexes is often characterized by an increase in molecular volume, it has been proposed that high pressure treatment, which is always associated with a volume decrease, could serve as an alternative for immunodesorption. It was demonstrated on several antigen-antibody complexes (for example beta-galactosidase — anti-beta-galactosidase and prostate specific antigen (PSA) — anti-PSA systems), that high pressure treatment leads to the recovery of the bioactive compound and a greater stability of the affinity matrix than after low pH treatment (Aertsen et al., 2009; Balny, 1996; Cheung et al., 1998; Gavalda et al., 1996; Lemay, 2002).

Biopurification under high pressure could also be considered for other purification techniques based on the weak interactions between a bioactive compound and a ligand linked to a matrix (Lemay, 2002). For example, high pressure can be used to dissociate amphiphilic biomolecules that are purified through a fixed bed adsorption set up. (Niemeyer and Jansen, 2007).

Cell adhesion is an important step in many biological processes, such as cell differentiation or proliferation. It mainly depends on protein interactions, either between cell surface proteins, in the case of cell–cell adhesion, or between cell surface proteins and extracellular matrix proteins. Because high pressure can perturb protein interactions, it can be used as an interesting tool to study cell adhesion (Hashiguchi et al., 1999).

4.8. Protein-DNA interactions

Regulation of many cellular functions, such as transcription, replication, translation, gene regulation… is dependent on protein–DNA interactions. These interactions rely upon van Der Vaals, hydrogen, and electrostatic bonds. Because high hydrostatic pressure stabilizes only hydrogen bonds, it allows one to discriminate the

contribution of each type of interactions implied in the recognition process (Lima et al., 2000; Silva et al., 1996).

Pressure studies reveal that a gradient of partially folded (molten globule) conformations is present between the unfolded and fully folded structures of several bacteria, plant and mammalian viruses. For example, a ribonucleoprotein intermediate was detected under high pressure, in which the coat protein was partially unfolded but still bound to RNA. These types of intermediates are potential targets for antiviral compounds and biotechnological applications (Silva et al., 2002).

4.9. Vaccine development

It is often hypothesized that pressure-inactivated pathogens would be able to stimulate the immune system and thus could be used as a vaccine. Indeed, the unfolding of proteins under high pressure unmasks antigenic sites and may increase immunogenic properties of pressure-treated proteins, killed viruses and micro-organisms (Masson et al., 2001; Shearer and Kniel, 2009).

First assays on the ability of pressurized parasites to elicit an immune response were conducted on a protozoan parasite: *Eimeria acervulina*. It was demonstrated that partial immunity could be developed as, upon challenge with non-treated parasites, lesions in chickens that had been inoculated with pressure-treated oocysts is less severe (Shearer et al., 2007).

The effect of high pressures on the immunogenicity of bacteria was first reported by C. C. M. Silva. Pressure-inactivated *Leptospira interrogans* serovar *hardjo*, when injected in rabbits, elicit an important immune response, as measured by antibody titer. This response, which probably implies the apparition of new epitopes, could be explained by the partial extrusion of membrane proteins or the dissociation of oligomeric integral membrane proteins. These results show promises in the potential development of pressure-inactivated bacteria based vaccines (Silva et al., 2001).

Another vaccine strategy is the use of bacterial ghosts instead of killed or attenuated micro-organisms. Bacterial ghosts are usually obtained through the expression of a lysis gene that leads to the formation of a transmembrane tunnel structure and subsequent cell constituent leakage. These entities retain their immunogenic properties since their cell surface remains intact. High pressure treatment of bacteria could constitute an interesting alternative in the production of bacterial ghosts. Indeed, it had been demonstrated that the overexpression of *E. coli* K12 Mrr protein rendered the cell hypersensitive to high pressure treatment, these cells being totally inactivated after a treatment of 100 MPa for 15 min at 20 °C. Furthermore, these Mrr-assisted high pressure killed bacteria retain their general cellular structure and are not lysed or permeabilized, contrary to bacterial ghosts prepared via the expression of a lysis gene. This last characteristic presents the advantage that pressure-prepared bacterial ghosts could be used as delivery vehicles for subunit or DNA vaccines without the need to artificially attach them to the membrane, as when bacterial ghosts are used (Vanlint et al., 2008).

The development of vaccines has attracted most interest in the field of viruses. It has been demonstrated that pressure-treated viruses lose their infectivity but still retain their ability to elicit neutralizing antibodies. Indeed, it is generally hypothesized that under high pressure, viral capsid proteins dissociate, and when pressure is released, they reassociate, leading to non-infectious particles that still retain their immunogenic properties (Aertsen et al., 2009; Gaspar et al., 2008, 2002; Oliveira et al., 2008; Patterson, 2005; Silva et al., 1992). Furthermore, previously hidden epitopes can be exposed, increasing the immunogenicity of the pressure-inactivated viruses (Oliveira et al., 2008). Compared to traditional vaccine preparations, high pressure production of vaccines presents many advantages, including a lower risk of developing the disease when compared to attenuated vaccines, and a probable higher immunogenicity than isolated subunits (Silva et al., 1992).

4.10. Preparation of viral vectors

Adeno-associated viruses (AAV) present great potential in the development of gene delivery vectors. AAV production necessitates helper virus, such as adenovirus, which need to be inactivated and removed from the preparation in a second step to recover AAV only.

Leonard et al. showed that AAV serotypes 2 and 5 are more pressure resistant than human adenovirus serotype 5. This difference in pressure sensitivity could lead to a novel method of preparation of AAV gene delivery systems (Leonard et al., 2007; Schaffer and Leonard, 2009).

4.11. Genetic transformation

Genetic transformation of cells is a common procedure in bioengineering development, but is often limited by the efficiency of transformation as only few cells take up the plasmid of interest. It was demonstrated that high pressure-treated plasmids (pUC18 and pBR322) exposed to high pressure treatment (200 and 400 MPa respectively) present an increased capacity to transform competent cells. This observation can be explained by the stabilization of hydrogen bonds under high pressure conditions, as many properties of the plasmids, such as their mobility and their ethidium bromide binding efficiency, are modified after high pressure treatment (Sharma et al., 1997).

4.12. Cell extraction

A new method of cell extraction has been developed by Pessure Biosciences Inc., using the combination of pressure cycling technology (PCT) and extraction solvents that allow dissolution and partition of each type of molecules into separate fractions (Tao et al., 2009). The use of this technology followed by centrifugation leads to good recovery of proteins and lipids, high yields of intact DNA or RNA, without any further purification steps (Gross et al., 2008a; Tao et al., 2003). It is also possible to adjust the PCT conditions to recover intact organelles such as mitochondria (Gross et al., 2008b).

4.13. Pressure-assisted cryopreservation

After high pressure treatment, mammalian cells appear to be more resistant to cryopreservation (Aertsen et al., 2009). For example, fresh bull semen treated under high pressure (40 MPa for 90–120 min) followed by freezing shows more viability, motility and fertility than semen directly freezed (Pribenszky et al., 2007). Post-thaw survival of frozen mouse and bovine blastocysts, and pig oocysts, is also enhanced after high pressure treatment. It is suggested that this increase of freezing resistance may be due to the production of "shock proteins" (Pribenszky et al., 2008, 2005a,b).

4.14. Applications in oncology

High pressure processing shows promising perspectives in the oncology field, with two main objectives:

- the first one is the production of whole-cell-based tumor vaccine,
- the second one is specific to orthopaedics and concerns the disinfection of infectious or tumor-afflicted bone segments before their reimplantation (see Section 4.4) (Aertsen et al., 2009).

To support the idea of the development of whole-cell-based tumor vaccines, it has already been proven that some cancerous cells, such as

EL-4-leukemia cells, treated under mild high pressure conditions (120–150 MPa — 15 min) are more immunogenic. Mice injected with such high pressure-treated cells show delays in the development of tumor and a higher survival rate upon challenge with untreated EL-4 cells (Richert et al., 1986).

Since this discovery, the procedure for the development of high pressure tumor vaccine has evolved. Tumor cells are exposed to high pressure treatment (120 MPa) in the presence of a biocompatible cross-linker (CL), 2′–3′-adenosine dialdehyde. The resulting cells are potent immunogens because this treatment increases their antigenic presentation by rearranging cell surface proteins into immunogenic clusters (Eisenthal et al., 1996).

To improve the vaccine properties of PCL-cells, high pressure treatment of cancerous cells in the presence of a cross-linker was followed by the reduction of surface protein disulfides with N-acetyl-L-cysteine (NAC). The combination of the injection of such cells at the tumor site and intravenous delivery of NAC developed an anti-tumor response, capable of eradicating established metastases (Goldman et al., 2000; Goldman and Shinitzky, 2000; Shinitzky and Goldman, 2000).

5. General conclusions

High pressure can be a useful tool either in fundamental research or in the development of new biotechnological applications. Because of the low energy conveyed by high pressure, this parameter can modify different low energy equilibriums involved in most of the physiological mechanisms in living systems. Indeed, it has proven to be helpful in the understanding of some biological processes, such as protein misfolding because of its capacity to discriminate thermal and volumetric effects. Furthermore, high pressure processing shows great potential for different industrial fields, from food to pharmaceutical industries from the decontamination of various products or biomaterials to the production of new types of vaccines.

In all the possible biological applications of high pressure described in this article, high pressure processing can always be thought as a subtle equilibrium between the wanted effect (for example inactivation of pathogens) and the preservation of the specific properties of the treated product. In the future, the development of high pressure research in Biology will probably be correlated to contributions from other research fields (Physics, and Chemistry), enlarging the potentialities of this parameter.

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