High Pressure Optical Spectroscopy (HiPOS) and Real-Time Analysis of Enzymes Kinetics at Elevated Hydrostatic Pressure

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Objectives: Many biochemical reactions are influenced by high hydrostatic pressure. Pressure lowers the activation volume of reactions via sample compression, hence concentrating enzymes, substrates, and their reaction products. High pressure disrupts protein secondary and tertiary structure, which can have either positive or negative impact on protein functionality. High pressure has been shown to enhance the proteolytic digestion of proteins for mass spectrometry, resulting in improved sequence coverage, higher recovery, and significantly reduced digestion times. The current study uses high pressure spectroscopy (HiPOS) to investigate the synergy of pressure and temperature on the catalytic activity of several proteases.

Methods: Using a high pressure optical cell and programmable pressure generator, real time enzyme kinetics based on the conversion of synthetic substrates were measured from atmospheric pressure to 4.1 kilobar and at temperatures ranging from 23°C to 55°C.

Results: The synergy between elevated pressure and temperature was most pronounced with lysyl endopeptidase (Lys-C) where the rate of substrate conversion was increased more than 20-fold at 55°C at 4.1 kilobar, relative to room temperature at atmospheric pressure. Substrate conversion by papainase was accelerated nearly 10 times at 4.1 kilobar and 60°C. Trypsin and chymotrypsin reaction rates were increased maximally at 55°C at 2.8 and 3.1 kilobar, respectively. Other enzymes such as horseradish peroxidase and alkaline phosphatase were progressively inactivated with increased pressure.

Conclusions: High pressure can be used to accelerate or inactivate enzymes. HiPOS enables real-time analysis of biochemical reactions at elevated pressure, while facilitating rapid optimization of reaction conditions.

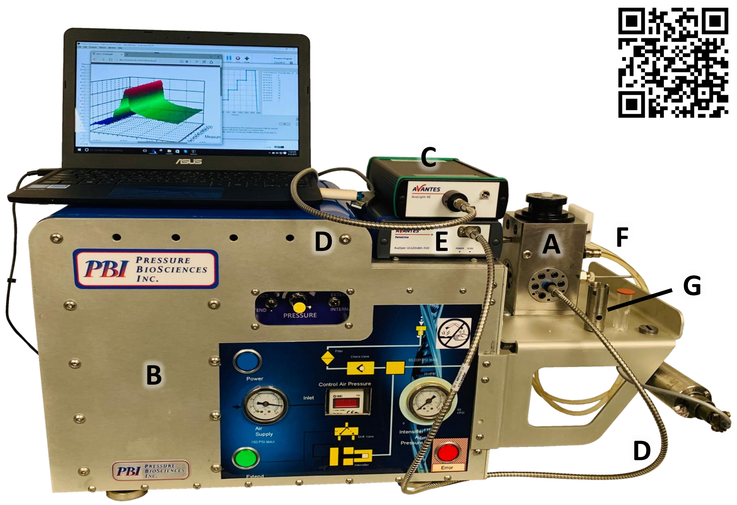

FIGURE 1. Components of the fiber optics-based HiPOS system. **(A)** the high pressure optical cell interfaces with **(B)** the programmable PBI HUB440 pressure generator. **(C)** The xenon light source is coupled to the optical cell via **(D)** fiber optics, which in turn, is coupled to the **(E)** AvaSpec spectrophotometer. **(F)** Operating temperature in the optical cell is controlled by a peripheral circulating water bath. **(G)** shows cuvette holder, semi-micro volume cuvette, and silicon seal.

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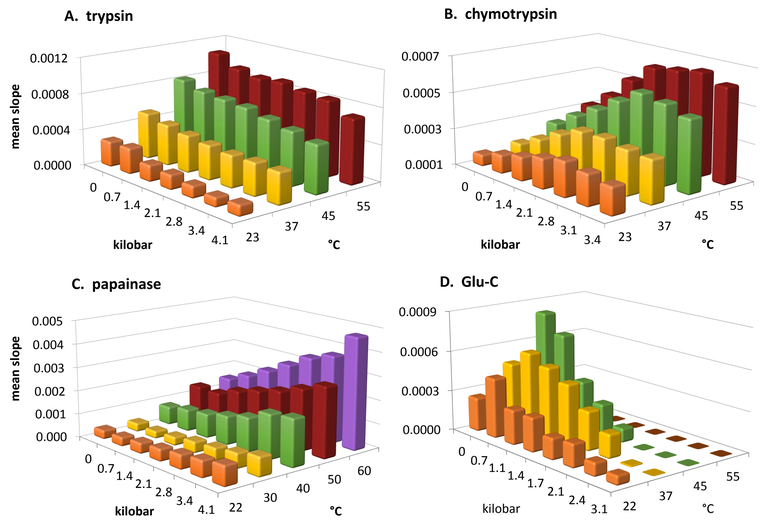

FIGURE 2. Synergistic effects of pressure and temperature on the activity of several proteases. **(A)** Trypsin and **(B)** chymotrypsin reaction rates were increased maximally at 55°C at 2.8 and 3.1 kilobar, respectively. Substrate conversion by **(C)** papainase was accelerated nearly 10 times at 4.1 kilobar and 60°C. Conversion of **(D)** Glu-C substrate was maximal at 0.7 kilobar at 37°C, but decreased at higher pressures. Glu-C was rapidly inactivated at 45°C and 55°C.

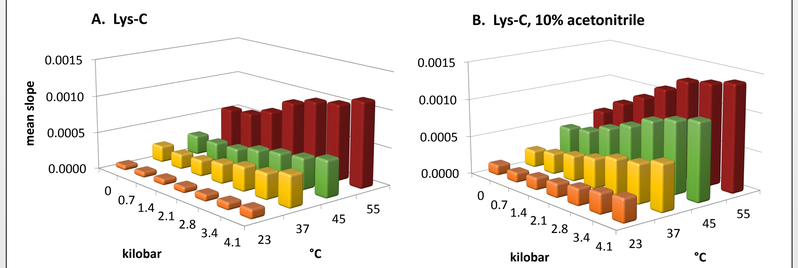

FIGURE 3. Application of HiPOS to buffer optimization. Lys-C reactions in **(A)** 100 mM ammonium bicarbonate or **(B)** 100 mM ammonium bicarbonate with 10% acetoniltrile. The rate of substrate conversion was increased more than 20-fold at 55°C at 4.1 kilobar, relative to room temperature at atmospheric pressure.

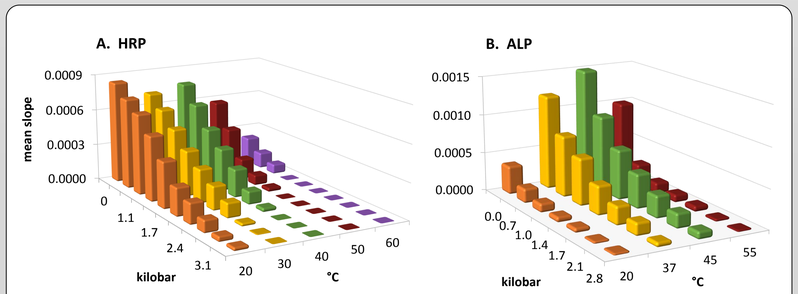

FIGURE 4. Inactivation of enzymes at high pressure. Progressive inactivation of **(A)** Horseradish peroxidase (HRP) and **(B)** alkaline phosphatase (ALP) at elevated pressure and temperature. ALP regained partial activity upon return to atmospheric pressure.

SUMMARY

- HiPOS enables real-time analysis of enzyme kinetics at high pressure and facilitates rapid optimization of reaction conditions for analytical and large-scale applications.
- High pressure accelerates some enzymes, while inactivating others. Lys-C activity was increased 20 times at 4.1 kilobar and may remain active at even higher pressures.
- HiPOS can be used to determine the pressure and time at high pressure require to inactivate enzymes.