Proteolysis-PrEP (Lysozyme): Effect of Pressure Cycling on Lysozyme Activity

Introduction

Pressure cycling technology (PCT) has been proven to accelerate enzymatic protein digestion. For example, the effect of PCT on trypsin digestion has been demonstrated by several laboratories. They report that digestion times can be reduced from hours to minutes [1, 2]. Not only has PCT been shown to accelerate and improve protein digestion in solution, but it also can accelerate the digestion by trypsin of proteins in polyacrylamide gel slices [3]. Additionally, the enhancing effect of PCT on the activity of several other enzymes, including Proteinase K, PNGase F, and Lys-C, has been reported [4, 5, 6]. It is thought that PCT may act synergistically with other protein denaturants, such as organic solvents and elevated temperature, to help maintain substrates in a denatured state leading to more exposure of enzyme target sites which results in better cleavage,. Here we report the enhanced effect by PCT on the activity of the enzyme lysozyme. Lysozyme acts to hydrolyze peptidoglycans found in bacterial cell walls. This enzyme is frequently used for bacterial cell lysis prior to extracting DNA or proteins from bacteria. We also propose an alternate or additional mechanism by which PCT may enhance the activity of lysozyme.

PCT Sample Preparation System (PCT SPS)

The Pressure Cycling Technology Sample Preparation System (PCT SPS) uses rapid cycles of hydrostatic pressure between ambient and ultra high levels to control biomolecular interactions. The PCT SPS can be used to disrupt tissues, cells, and cellular structures to extract proteins and nucleic acids [7]. In addition, the PCT SPS can be used to accelerate enzymatic reactions such as trypsin and proteinase K digestion. The PCT SPS uses a small, semi-automated bench-top instrument (Barocycler NEP3229 or the NEP2320) in combination with PCT MicroTubes or FT500-ND PULSE Tubes. The specially designed PCT MicroTubes are single-use sample processing containers designed to hold 50-150 µL. For larger reaction volumes, the FT500-ND PULSE Tubes hold up to 1.4 mL. The combination of PCT SPS employs rapid pressure changes, chemistry, temperature and other biophysical mechanisms, to accelerate trypsin digestion as well as many other enzymatic reactions.

Materials and Methods

Hen egg white lysozyme and fluorogenic substrate (4-Methylumbelliferyl-β-D-N,N′,N″-triacetylchitotriose) were purchased from Calbiochem [8]. Lysozyme activity was assayed at different pressures and temperatures in 50 mM sodium citrate (pH 5.5). In all experiments, the substrate concentration was 25 µM and the enzyme was 2.0 mg/mL.

Pressure cycling was performed in FT500-ND PULSE Tubes at the indicated pressure using one minute cycling parameters (50 seconds at high pressure and 10 seconds at ambient pressure per cycle) for 5, 30 or 120 minutes. Control reactions were also incubated in the FT500-ND tubes. For reactions performed at 50˚C, the Barocycler was heated using an external circulating water bath.

Results and Discussion

It has been reported that thermodynamic treatments, such as High Intensity Focused Ultrasound (HIFU) [9], microwave radiation [10] and high pressure [11, 12], can accelerate digestion by trypsin and other enzymes. Pressure cycling technology (PCT) has also been reported to effectively accelerate protein digestion by trypsin and other enzymes. Here we report the effect of pressure, a thermodynamic process, on lysozyme from hen egg white.

The effects of different levels of pressure on the lysozyme reaction were assayed at room temperature and 50˚C. Data show that not only is lysozyme stable at high pressure, but that its activity is significantly enhanced at pressures as high as 40,000 psi (276 MPa) (Figure 1). Furthermore, the improved activity was more pronounced when the reactions were carried out at 50˚C compared to reactions at room temperature (Figure 2).

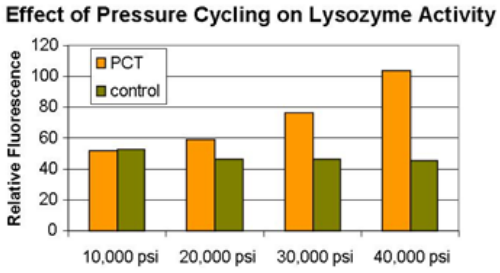

Figure 1: PCT-associated acceleration of enzyme activity increases with increasing pressure. PCT and control reactions were carried out for 30 minutes at 50˚C. The legend on the X-axis indicates the pressure at which PCT was performed (orange bars). For each PCT reaction, a simultaneous control reaction, performed at atmospheric pressure, was assayed (green bars). Note that the control values show little variability from experiment to experiment.

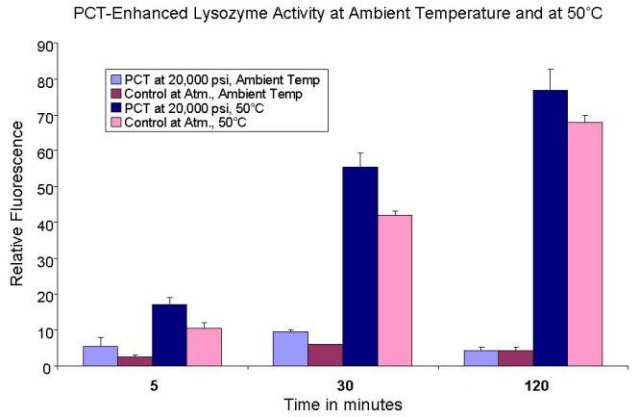

Figure 2. Effect of PCT on lysozyme activity at ambient temperature and at 50˚C. PCT was performed at 20,000psi. Modest PCT-associated acceleration of enzyme activity was observed at both temperatures.

Other enzymes, most notably trypsin, have been shown to be enhanced by pressure. The increase in apparent activity is more likely due more to pressure-induced denaturation of the substrate protein rather than by affecting the kinetics of the enzyme itself. By helping to maintain substrates in a denatured state, PCT effectively makes them "better" substrates for the enzyme.

In contrast, our data may suggest an alternate mechanism by which pressure accelerates lysozme reactions. Specifically, the effect of PCT on the activity of lysozyme appears to be a direct result of increased enzymatic activity. Since the relatively small synthetic fluorogenic substrate (as compared to a protein) is not likely to be affected by pressure cycling under these conditions (see structure in Figure 3), it is possible that pressure is acting on the enzyme itself.

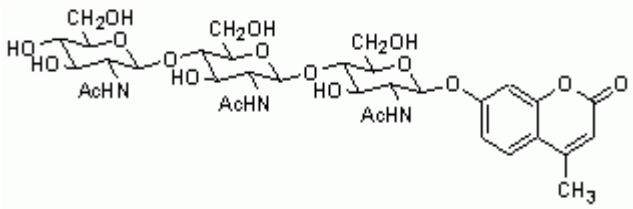

Figure 3: 4-Methylumbelliferyl-β-D-N,N′,N″-triacetylchitotriose, molar mass 785.8 from Calbiochem EMD4Biosciences

The PCT conditions used in these experiments should be considered a starting point for optimization of the lysozyme reaction and for determination of the mechanism of action. Standard enzyme kinetic studies still need to be conducted to fully understand the effects of PCT on enzyme activity.

References

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