Differential Scanning Flourimetry of Glucose Oxidase at High Hydrostatic Pressure

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Most efforts associated with high pressure processing (HPP) of foods have focused on killing pathogens and spoilage microorganisms as well as on the inactivation of intrinsic, deleterious enzymes. However, it is well-known that high hydrostatic pressure (HHP) stabilizes and activates several enzymes. Processing enzymes play an important role in the production of flavors and other added-value food products. We and others have researched the potential application of HHP to enzyme-catalyzed processes. However, a fundamental understanding of the relationship between enzyme structure and the effect of pressure is still missing. Here we report the use of high-pressure differential scanning fluorimetry to characterize the stabilizing effect of high hydrostatic pressure on glucose oxidase (GOx). Glucose oxidase solutions were prepared in phosphate buffer pH 5.1. Approximately 0.5 mL of GOx were pipetted into a quartz cylindrical cuvette caped with Teflon plungers and inserted into a high-pressure reactor with sapphire windows. The high-pressure reactor was placed in a fluorescence spectrometer. The cell was then pressurized and then heated at 1 ?C/min from 20 ?C to 90 ?C. The sample was excited at 280 nm and fluorescence at 350 nm was continuously measured. The first derivative of the fluorescence was calculated. The melting temperature Tm increased from 69.7 ?C at 0.1 MPa to 80.6 ?C at 300 MPa. The results are consistent with measurements of residual activity experiments after thermal treatment at HHP. A decrease in intrinsic fluorescence upon pressurization suggests that HHP folds the protein burying deeper hydrophobic tryptophan residues. Then thermally induced denaturation is revealed by the increase in fluorescence with the increase in temperature.