NOTES

DSC Analysis of Select Diagnostic Enzymes

INTRODUCTION

Enzymes are marvelously designed though very specific catalysts. They are derived from plant and animal tissues; however, fermentation is currently the popular production method. Enzymes are extensively used in diagnostics, immunodiagnostics, and biosensors to measure or amplify signals of many, but specific, metabolites. Preparation, specifications, properties, applications, and assays of industrially important enzymes can be found in Refs. 1 and 2.

Enzymes are employed in both wet and dry chemistries. In dry chemistry, suitable reactants, namely, enzymes and indicators, are coated as thin films onto a plastic base employing polymeric binders (see Ref. 3 for a comprehensive review on dry chemistries).

Biochemical reactions for glucose and cholesterol analysis are schematically presented below:

Biochemical Reactions in Glucose Analysis

$$\text{D-Glucose} + H_2O + O_2 \xrightarrow{\text{glucose oxidase (GOD)}} \text{D-Glucono-1,4-lactone} + H_2O_2$$

$$\text{D-Glucono-1,4-lactone} + H_2O \rightarrow \text{gluconic acid}$$

$$H_2O_2 + \text{Indicator}_{(\text{Reduced})} \xrightarrow{\text{Peroxidase (POD)}} \text{Indicator}_{(\text{Oxidized})} + 2H_2O$$

Biochemical Reactions in Cholesterol Analysis

$$\text{Cholesterol–fatty acid–lipoprotein complex} + H_2O \xrightarrow{\text{Cholesterol esterase (CHE)}} \textit{cholesterol} + \textit{fatty acid} + \textit{protein}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase (CHO)}} \text{cholest-4en-3one} + H_2O_2$$

$$\text{4-Aminoantipyrine} + \text{phenol} + 2H_2O_2 \xrightarrow{\text{POD}} \text{quinoneimine dye} + 4H_2O$$

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In general, most enzymes are very fragile and sensitive to pH, solvent, and elevated temperatures. The catalytic activity of most enzymes is reduced dramatically as the temperature is increased. Typically used enzymes in diagnostics, e.g., GOD and POD, are almost completely deactivated around 65°C in solid form or aqueous solution. Despite wide and continued use of such enzymes in diagnostics for more than 30 years, limited or no thermal analysis work on these biopolymers has been reported in the literature. This being the case, we report herein differential scanning calorimeter (DSC) analysis of select enzymes. We have also been successful in maintaining the thermal stability of such enzymes in a polymeric compound. A speculative mechanism has been proposed for the added stability.

EXPERIMENTAL

Materials

GOD, POD, CHO, and CHE enzymes were obtained from Boehringer Mannheim Biochemicals and used in the form obtained. Polyhydroxyethyl methacrylate (PHEMA) was made by mass polymerization using 1% benzoyl peroxide at 125°C/16 h. PHEMA was crushed, pulverized, and finally ground in a ball mill to −80 mesh (less than 177 μm).

Methods

DSC runs on enzymes were performed on Perkin-Elmer DSC-7. Compression moldings in forms of disc, strips ($\frac{1}{2} \times 1\frac{1}{2} \times \frac{1}{16}$ in.) were done on a Carver Press.

RESULTS AND DISCUSSION

Results of DSC analysis indicating glass transition temperature (T_g), melting temperature (T_m), and decomposition temperature (T_d) are shown in Table I.

A knowledge of the stability of enzymes in solid phase dry chemistries is very important (shelf life, heat excursions encountered during transportation and storage). Stability of dry chemistries and how to predict lifetime have been recently discussed by Azhar et al.[4] Below the

Table I Thermal Analysis of Enzymes

Enzyme	Source	T_g (°C)	T_m (°C)	T_d (°C)
Cholesterol oxidase	Nocardia	50	98	210
Cholesterol oxidase	Streptomyces	51	102	250
Cholesterol esterase	Pseudomonas	43	88	162
Glucose oxidase	Aspergillus	50	105	220
Peroxidase	Horseradish	50	100	225

glass transition temperature (T_g), the enzymes are in a glassy state and should be thermally stable. Around T_g, onset of the rubbery state begins and the enzyme becomes prone to thermal instability. When the enzyme melts around T_m, all the tertiary structures are destroyed, thus making the enzyme completely inactive. The presence of chemicals can considerably influence enzyme stability.

An enzymatic compound containing GOD, POD, tetramethyl benzidine (TMB) indicator, linear alkylbenzene sulfonate, and PHEMA with wt % composition similar to a commercial dry reagent chemistry was ball-milled for 48 h. The prepared molding compound was compression-molded onto a thermoplastic film at 105, 125, 150, and 200°C. The dwell time for the compound to see heat was 1 min.

The plastic strip molded at 105°C responded quickly (less than 15 s) to glucose solution in the 10–800 mg/dL glucose range with a good dose response. This indicates that the enzymes in sufficient amounts survived the elevated temperature of 105°C without any decrease in activity. An increase of the dwell time to 5 min even produced the blue color. Moldings made at 125 and 150°C retained much of the enzyme activity to produce the blue-color reactions. However, moldings done at 200°C did not give any blue color even at 800 mg/dL glucose concentration. The enzymes have thus been completely inactivated, i.e., denatured at 200°C.

Why should the enzymes be stable in polymer melt whereas they quickly deactivate in water or dry form? We speculate that the enzyme is surrounded very tightly by coils of PHEMA at room and elevated temperatures. Temporary melting of the crystalline regions of the protein, up to 150°C, does not disturb the conformation due to PHEMA chains. When the molding is cooled, the protein recrystallizes in the same form and conformation as it was before at room temperature. Therefore, the molded strip responds to glucose. At 200°C, the viscosity of the melt blend is lower with increased mobility. The conformation changes at 200°C and the cooled strip therefore becomes inactive. Certainly, the stability of the enzymes will depend on the concentration of PHEMA and the melt viscosity of the enzymatic compound. These parameters have not been studied by us so far.

We do not envision melt blending of enzymes and polymers followed by molding to make molded dry chemistries. Dry blending followed by size reduction using ball milling can produce enzymatic compounds that can be sintered or molded. Furthermore, it is distinctly possible to develop low molecular weight precursors that may lend to reaction injection molding. For example, enzyme-containing polymeric isocyanate and polyol will give polyurethane or caprolactam and polyol will produce nylon block copolymer RIM strips.

References

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