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Effect of Polyols on the Conformational Stability and Biological Activity of a Model Protein Lysozyme

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ABSTRACT

The purpose of this study was to investigate the stabilizing action of polyols against various protein degradation mechanisms (eg, aggregation, deamidation, oxidation), using a model protein lysozyme. Differential scanning calorimeter (DSC) was used to measure the thermodynamic parameters, mid point transition temperature and calorimetric enthalpy, in order to evaluate conformational stability. Enzyme activity assay was used to corroborate the DSC results. Mannitol, sucrose, lactose, glycerol, and propylene glycol were used as polyols to stabilize lysozyme against aggregation, deamidation, and oxidation. Mannitol was found to stabilize lysozyme against aggregation, sucrose against deamidation both at neutral pH and at acidic pH, and lactose against oxidation. Stabilizers that provided greater conformational stability of lysozyme against various degradation mechanisms also protected specific enzyme activity to a greater extent. It was concluded that DSC and bioassay could be valuable tools for screening stabilizers in protein formulations.

KEYWORDS: differential scanning calorimeter, protein conformational stability, polyols, enzyme activity assay, lysozyme

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INTRODUCTION

The tremendous growth in biotechnology and the completion of human genome sequencing have made large-scale production of therapeutic protein a reality.[1] These macromolecules perform the function of their natural blueprints in soliciting desired responses from the body.[2] Unfortunately, proteins possess unique physical and chemical properties, which create difficulties in formulation and delivery.

Many differences exist between protein/polypeptide and conventional small molecular weight compounds, which may affect their predicted stability profiles. Polypeptides consist of a regularly repeating backbone with distinctive side chains that interact with each other to contribute to the 3-dimensional structure of the protein. Generally, small molecular weight compounds are either linear or cyclical in nature, and their size prohibits extensive intramolecular bonding. Thus, the majority of conventional small molecular weight compounds do not exhibit higher level structures found in polypeptide molecules.[3] Many such molecules are quickly broken down and processed in vivo within presystemic and systemic regulatory mechanisms. In environments other than their physiologic ones, therapeutic proteins may also rapidly denature or easily lose their biological activity.[2] Currently, there are more than a hundred recombinant proteins in phase 1 clinical trials or beyond and several dozens have received Food and Drug Administration (FDA) approval. Hence, the formulation design, which ensures an efficient and safe delivery of protein and peptide in a conformationally stable and biologically active form, is the key to commercial success and the demonstration of efficacy in current and future biotechnology products.[4]

Physical and chemical instabilities of proteins are the most daunting and challenging task in the development of a suitable protein formulation. The most common physical instability is protein aggregation and its macroscopic equivalent precipitation. Aggregation differs from association in the sense that the former involves

the irreversible interaction of 2 or more denatured protein molecules, while the latter involves the reversible interaction of 2 or more native protein molecules often resulting in reversible precipitation of protein.[5] Aggregation may result in no or reduced activity, reduced solubility, and altered immunogenicity.[6]

Deamidation appears to be the most common degradation mechanism in protein pharmaceuticals. Asn and Gln are the 2 amino acids susceptible to deamidation in proteins, and Asn is more labile.[7] Deamidation of Asn in proteins and peptides in an aqueous solution can proceed at a much higher rate than hydrolysis of a peptide bond.[8]

Oxidation, one of the major degradation pathways in proteins and peptides, can occur during all steps of processing, from protein isolation to purification and storage.[9] An extensive listing of the proteins liable to oxidation, the primary amino acids involved, and the biological activity of the products has been reported.[5] Oxidation of a critical residue at or near the enzyme active site or receptor binding site, or a dramatic change in the structure of the protein upon oxidation may be the molecular basis for the altered bioactivity in the oxidation products of protein.[10]

In this study, the stabilizing effect of polyols on a model protein lysozyme was investigated against various degradation mechanisms (aggregation/ precipitation, deamidation, and oxidation) using differential scanning calorimeter (DSC) and biological activity assay.

MATERIALS AND METHODS

Materials

Lysozyme (EC 3.2.1.17) from chicken egg white, Micrococcus Lysodeikticus (*Micrococcus luteus*), mannitol, sucrose, lactose, glycerol, and propylene glycol were purchased from Sigma Chemical Company, St Louis, MO. Micro BCA protein assay reagent kit was purchased from Pierce Biotechnology, Inc, Rockford, IL. All other chemicals used were of analytical grade.

Sample Preparation

Citrate phosphate (CP) buffers of different pH were prepared by mixing the different proportions of 100mM citrate and 200mM phosphate stock solutions.[11] Lysozyme was dissolved at a concentration of 2 mg/mL (0.14mM) in the CP buffer (pH 4.4, 72.2mM) and centrifuged (4229 × g) for 20 minutes to remove any insoluble material. Supernatant was filtered through 0.1 μm polytetrafluoroethylene (PTFE) filter (Millipore Corp., Bedford, MA) before filling in DSC cell. All pH measurements were done using a VWR Scientific model 8010 pH meter (VWR Scientific Products, Batavia, IL).

Aggregation/Precipitation

Lysozyme solutions were prepared containing 0.05M, 0.06M, 0.12M, 0.17M, 0.50M, and 0.83M NaCl and were kept at room temperature for 1 hour and centrifuged. Supernatant was filtered and used for the determination of conformational stability and biological activity of lysozyme. Sodium chloride (0.83M) was added to lysozyme solution containing polyols (10% wt/vol mannitol, sucrose, lactose, glycerol, or propylene glycol). Increase in the conformational stability and biological activity of lysozyme from these solutions in comparison with the control (containing 0.83M NaCl but no polyol) was considered as protection provided by polyols against aggregation/precipitation.

Deamidation

Lysozyme solution (0.14mM) in CP buffer (pH 7.4, 62.2mM) was kept at 60°C for 24 hours with or without 10% wt/vol polyols (mannitol, sucrose, lactose, glycerol, or propylene glycol) for studying the deamidation. For studying deamidation by direct acid hydrolysis of the amide side chain, lysozyme (0.14mM) in CP buffer (pH 2.0, 62.2mM) was kept at 60°C for 24 hours with or without 10% wt/vol polyols. Lysozyme without any polyol was used as a control.

Oxidation

Lysozyme solutions (0.14mM) in CP buffer (pH 4.4, 62.2mM) with or without 10% wt/vol polyols (mannitol, sucrose, lactose, glycerol, or propylene glycol) were mixed with hydrogen peroxide (50% vol/vol) and kept at room temperature for 1 hour and then centrifuged. Supernatant was filtered and used for the determination of conformational stability and biological activity of lysozyme. Lysozyme solution without polyol but mixed with hydrogen peroxide was used as a control.

Determination of Conformational Stability by Differential Scanning Calorimeter

Conformational stability of lysozyme in samples was evaluated by using an ultra-sensitive DSC (VP-DSC, MicroCal, Northampton, MA). All samples were centrifuged, and supernatants were filtered through a 0.1 μm filter. These samples and buffer were degassed by stirring under vacuum before loading into the DSC sample and reference cells, respectively. The heat flow required to keep the sample cell and reference cell at the same temperature was recorded at a temperature range of 15°C to 95°C and a scan rate of 1.5°C/min. To ensure that the heat transition during protein conformational alterations is the only source of thermal difference between sample cell and reference cell, a baseline thermogram was obtained by loading the buffer in both—sample cell and reference cell. This baseline was subtracted from the sample thermogram during data analysis. Midpoint transition temperature (Tm) and calorimetric enthalpy (ΔH) were used as conformational stability indicating thermodynamic parameters. Increase in ΔH and Tm of the lysozyme was interpreted as an indication of stabilizing effect provided by different polyols. All data manipulations were performed by using Origin software (MicroCal) provided with the DSC.

Biological Activity of Lysozyme

A portion of vigorously shaken *Micrococcus luteus* stock suspension (0.01% wt/vol) was diluted with phosphate buffer (0.66mM, pH 6.24), so that it had an A450 between 0.2 and 0.6. Two and one half milliliters of this diluted *Micrococcus luteus* suspension was taken into a spectrophotometer cell and 0.1 mL of an appropriately diluted lysozyme sample/blank was added to it. The rate of decrease of absorbance at A450 was monitored by UV spectrophotometer during a total incubation period of 5 minutes at 25°C. Slope ($\Delta A_{450\ nm}$/min) of the linear portion of the curve between $A_{450\ nm}$ and time was used to calculate the biological activity of lysozyme in enzyme unit (EU).[12] A decrease of 0.001 $A_{450\ nm}$/min was defined as 1 EU. Biological activity of lysozyme in terms of EU/mL was determined by using following formula:

$$\text{EU of lysozyme/mL sample} = \frac{(\Delta A_{450nm}/\text{min Test} - \Delta A_{450nm}/\text{min Blank})(df)}{(0.001)(0.1)} \quad (1)$$

where *df* is the dilution factor; 0.001 is the change in absorbance at A as per the unit definition; and 0.1 is the volume (in mL) of the sample/standard used.

Amount of lysozyme in samples was determined by bicinchoninic acid (BCA) method[13] and was used for the calculation of specific enzyme activity (EU/mg) of lysozyme by using the following formula:

$$\text{EU/mg of lysozyme} = \frac{EU/mL\ sample}{mg\ of\ lysozyme/mL\ sample} \quad (2)$$

Data Analysis

Statistical comparisons were made using Student *t* test and analysis of variance (ANOVA). The level of significance was $P < .05$.

RESULTS

Figure 1 shows the DSC thermograms of lysozyme in the absence (control) and presence of various concentrations of NaCl. A decrease in the peak height of transition was observed with increasing concentrations of NaCl. **Table 1** shows the quantitative values of Tm and ΔH of lysozyme solutions containing various concentrations of NaCl. A decrease in the Tm and ΔH values of lysozyme were observed with increasing concentration of NaCl, which were significantly lower ($P < .05$)

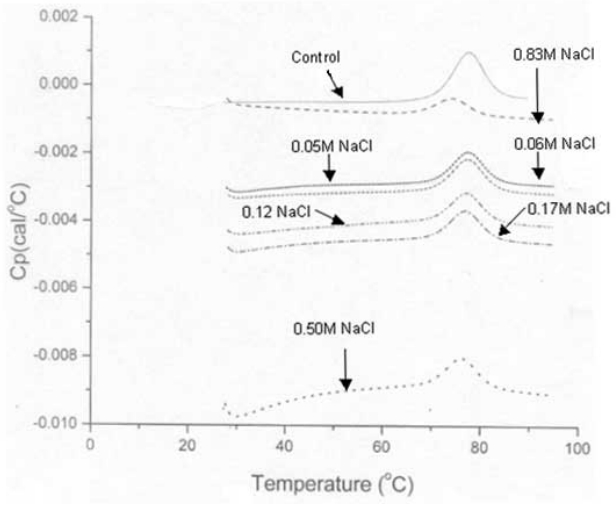

Figure 1. DSC thermograms showing the effect of NaCl on conformational stability of lysozyme.

Table 1. Effect of Sodium Chloride on Midpoint Transition Temperature, Calorimetric Enthalpy, and Specific Enzyme Activity of Lysozyme*

Sodium Chloride	Transition Temperature, Tm (^oC)	Calorimetric Enthalpy, ΔH (Cal/mol) × 10^4	Specific Enzyme Activity (EU/mg) × 10^3
Control	77.1 ± 0.1	9.6 ± 0.4	47.0 ± 1.5
0.05M NaCl	77.0 ± 0.1	9.0 ± 0.3	45.0 ± 1.0
0.06M NaCl	76.7 ± 0.2	8.9 ± 0.5	44.2 ± 1.0
0.12M NaCl	76.5 ± 0.2†	8.8 ± 0.5	41.0 ± 1.7†
0.17M NaCl	76.0 ± 0.6†	8.4 ± 0.1†	40.9 ± 0.9†
0.50M NaCl	75.1 ± 0.5†	7.5 ± 0.9†	37.0 ± 1.0†
0.83M NaCl	73.7 ± 0.6†	5.9 ± 0.5†	29.0 ± 1.8†

*All values are expressed as mean ± SD, n = 3. Control = 0.14mM lysozyme solution without NaCl.

†Significantly lower ($P < .05$) in comparison with control.

Table 2. Effect of Polyols on Midpoint Transition Temperature, Calorimetric Enthalpy, and Specific Enzyme Activity of Lysozyme Subjected to Aggregation/Precipitation by Sodium Chloride*

Polyols	Transition Temperature, Tm (^oC)	Calorimetric Enthalpy, ΔH (Cal/mol) × 10^4	Specific Enzyme Activity, (EU/mg) × 10^3
Control	73.7 ± 0.6	5.9 ± 0.5	29.0 ± 1.9
Mannitol	75.9 ± 0.8†	7.3 ± 0.6†	36.0 ± 0.8†
Sucrose	75.3 ± 0.4†	6.8 ± 0.4†	33.0 ± 0.6†
Lactose	75.2 ± 0.2†	6.7 ± 0.3	33.0 ± 0.4†
Glycerol	74.9 ± 0.4	6.2 ± 0.8	30.0 ± 1.2
Propylene glycol	71.4 ± 0.5	5.4 ± 0.3	27.0 ± 1.2

*All values are expressed as mean ± SD, n = 3. Control = 0.14mM lysozyme containing 0.83M NaCl but no polyol.

† Significantly greater ($P < .05$) in comparison with control.

in comparison with control for samples containing 0.12M or higher NaCl. Lysozyme containing 0.83M NaCl showed maximum decrease in Tm and ΔH, hence this concentration was used in the evaluation of polyols for stabilization against aggregation. Biological activity of lysozyme preparations containing NaCl was determined by enzyme activity assay. A greater decrease in specific enzyme activity was found in samples containing a greater amount of NaCl, which were significantly lower ($P < .05$) in comparison with the control for samples containing 0.12M or higher NaCl (**Table 1**). **Table 2** shows the Tm and ΔH values of lysozyme containing NaCl with or without polyols. All of the polyols significantly ($P < .05$) increased Tm and ΔH values for lysozyme in comparison with the control, except propylene glycol, which rather decreased Tm and ΔH. Greater increase in Tm and ΔH was found with mannitol followed by sucrose, lactose, and glycerol. Also, the mannitol containing lysozyme sample showed greater specific enzyme activity than lysozyme samples containing sucrose, lactose, and glycerol. All of the polyols except propylene glycol showed significantly ($P < .05$) greater specific enzyme activity than the control.

Table 3 shows numerical values of Tm and ΔH of lysozyme samples (pH 7.0) kept at 60°C for 24 hours in the presence or absence of polyols. The control lysozyme sample did not show any transition peak. Sucrose significantly increased ($P < .05$) Tm and ΔH values in comparison with other polyols. The same trend was found for specific enzyme activity data. The control sample showed an extremely low amount of specific enzyme activity (30 ± 2 EU/mg) for lysozyme. Greater specific lysozyme activity was found in the sample containing sucrose, followed by lactose, glycerol, and propylene glycol.

Table 3. Effect of Polyols on Midpoint Transition Temperature, Calorimetric Enthalpy, and Specific Enzyme Activity of Lysozyme Subjected to Deamidation at Neutral Ph*

Polyols	Transition Temperature, Tm (^oC)	Calorimetric Enthalpy, † ΔH (Cal/mol) × 10^4	Specific Enzyme Activity, † (EU/mg) × 10^3
Control	No Transition Peak		0.03 ± 0.002
Lysozyme + mannitol	74.4 ± 0.8	8.0 ± 0.7	39.0 ± 1.9
Lysozyme + sucrose	74.5 ± 0.4	8.5 ± 0.4	42.0 ± 1.4
Lysozyme + lactose	73.9 ± 0.9	8.2 ± 0.7	40.0 ± 1.0
Lysozyme + glycerol	73.4 ± 0.6	7.7 ± 0.4	37.0 ± 1.2
Lysozyme + propylene glycol	73.3 ± 0.6	7.2 ± 0.3	35.0 ± 1.0

*All values are expressed as mean ± SD, n = 3. Control = 0.14mM lysozyme (pH 7.0) solution without any polyol.
†Presence of polyols significantly increased ($P < .05$) Tm, ΔH, and specific enzyme activity in comparison with control.

Figure 2 shows the DSC thermograms of lysozyme samples (pH 2.0) kept at 60°C for 24 hours in presence/absence of polyols. The DSC thermograms show the effect of polyols on the conformational stability of lysozyme against deamidation by direct acid hydrolysis. The values for Tm and ΔH are shown in **Table 4**. All of the polyols caused a significant ($P < .05$) increase in Tm and ΔH values in comparison with the control. A greater increase in Tm and ΔH values was found with sucrose followed by mannitol, lactose, glycerol, and propylene glycol. All the samples containing polyols showed significantly ($P < .05$) greater specific lysozyme activity in comparison with the control. Lysozyme containing sucrose showed greater specific enzyme activity followed by mannitol, lactose, glycerol, and propylene glycol.

Figure 3 shows DSC thermograms of lysozyme samples containing H_2O_2 (50% vol/vol) in absence (control)/presence of polyols. Control sample did not show any thermal transition peak. All the lysozyme samples containing polyols showed distinct transition peak. **Table 5** shows Tm and ΔH values for lysozyme containing H_2O_2 (50% vol/vol) in absence (control)/presence of polyols. Lactose caused significantly ($P < .05$) greater increase in the Tm and ΔH values than other polyols. Control sample showed extremely low specific lysozyme activity (41 ± 1 EU/mg) (**Table 5**). Lactose containing lysozyme sample showed significantly ($P < .05$) greater specific enzyme activity than other polyols.

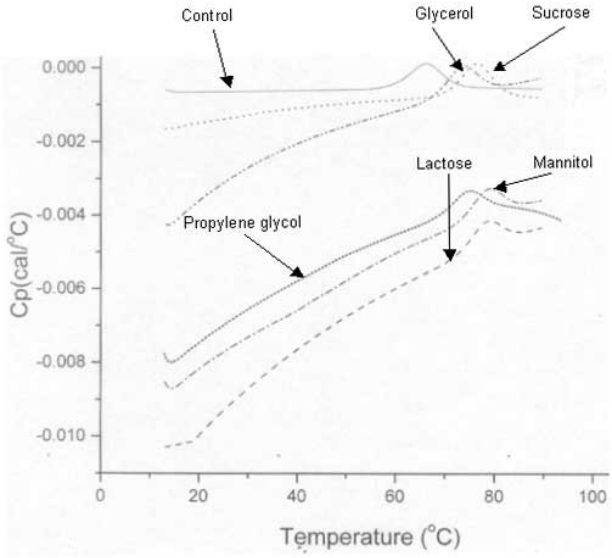

Figure 2. Effect of polyols on conformational stability of lysozyme during deamidation by direct acid hydrolysis.

DISCUSSION

DSC is ideally suited to the study of protein thermal denaturation in solution since it measures the forces stabilizing the conformational structure directly and is therefore model independent.[14] The characterization of protein unfolding, using several biophysical methods, has led to the notion that a loss in compact structure resulting in nonnative conformational change has a dramatic effect on aggregation,[15] deamidation,[16] and oxidation.[17] Biophysical studies have provided information about the relationship between protein unfolding and degree of stability.[18] Tm and ΔH have been attributed to a level of stability provided by additives under screening studies.[19]

CP buffer was used because it covers a sufficiently wide pH range and has a small enthalpy of ionization, which minimizes heat effects due to protonation

Table 4. Effect of Polyols on Midpoint Transition Temperature, Calorimetric Enthalpy, and Specific Enzyme Activity of Lysozyme Subjected to Deamidation by Direct Acid Hydrolysis*

Polyols	Transition Temperature, † Tm (^oC)	Calorimetric Enthalpy, † ΔH (Cal/mol) × 10^4	Specific Enzyme Activity, † (EU/mg) × 10^3
Control	66.0 ± 0.1	4.7 ± 0.3	23.0 ± 1.0
Lysozyme + mannitol	73.1 ± 0.2	8.1 ± 0.8	40.0 ± 1.3
Lysozyme + sucrose	73.4 ± 0.3	8.6 ± 0.4	42.0 ± 1.7
Lysozyme + lactose	72.9 ± 0.7	8.0 ± 0.9	39.0 ± 1.0
Lysozyme + glycerol	71.4 ± 0.4	7.2 ± 0.6	35.0 ± 1.1
Lysozyme + propylene glycol	71.5 ± 0.5	7.0 ± 1.0	34.0 ± 1.0

*All values are expressed as mean ± SD, n = 3.Control = 0.14mM lysozyme (pH 2.0) solution without any polyol
†Presence of polyols significantly increased (P < .05) Tm, ΔH, and specific enzyme activity in comparison with control.

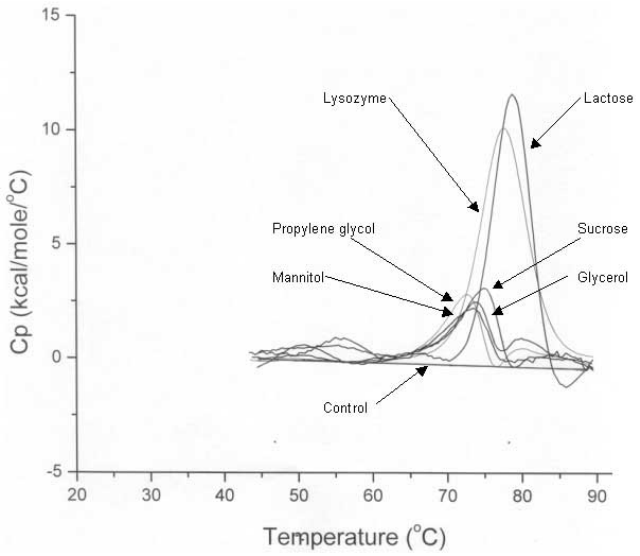

Figure 3. DSC thermograms of lysozyme treated with hydrogen peroxide in absence (control)/presence of polyols.

changes during the denaturation reaction.[20] CP buffer (pH 4.4, 62.2mM) was used because it provided maximal conformational stability to lysozyme as indicated by the determination of Tm and ΔH from DSC thermograms (data not shown).

Thermodynamic parameters have been found dependent on salt concentration in a complex way as determined by DSC. Tm and ΔH initially decrease with increasing salt concentration, and eventually that trend is reversed. The predominance of stabilizing or destabilizing interaction depends on the concentration and nature of the salt present in the solution, which determine the preferential interaction of salt with folded or unfolded state leading to aggregation/precipitation of the protein.[21] A decrease in Tm the protein.[21] A decrease in Tm and ΔH for lysozyme solution was observed with an increase in NaCl concentration (**Table 1**). NaCl (0.83M) decreased the Tm and ΔH values greater than other concentrations of NaCl; therefore it was used in screening of polyols for protection against destabilizing effect of NaCl.

Deamidation of Asn[22] and Gln amino acid residues have been favored at neutral to alkaline pH and at 40°C to 70°C.[23] The lysozyme solution was kept at pH 7.0 and 60°C for 24 hours in the absence (control)/presence of polyols. There are a total of 10 Asn residues in lysozyme, which could be potential sites for deamidation. Neighboring groups around Asn may further facilitate deamidation.[24] In lysozyme 27, 37, 44, 46, 59, and 66, Asn are directly linked with Gly, Ser, Thr, Thr, Ser, and Gly residue, respectively.[25] These amino acid residues are implicated to increase the deamidation rate under neutral or alkaline conditions. In particular, Gly has been reported to increase multifold deamidation of Asn.[26] Substitution of these amino acid residues with bulky amino acid residues generally retards the rate of deamidation reaction in a neutral or alkaline medium.[5] Moreover, 37 Asn linked with Ser in lysozyme is situated in substrate binding pocket.[25] Hence, deamidation of Asn residues might have led to severe conformational perturbation of lysozyme in the control sample resulting in nonappearance of the thermal transition peak (**Table 3**), which is also supported by minimal specific activity (30 ± 2 EU/mg). However, all the lysozyme samples containing polyols showed distinct transition peak as evidenced by their Tm and ΔH values (**Table 3**), and substantial specific enzyme activity. This finding indicates preservation of conformational integrity of lysozyme by these polyols against

Table 5. Effect of Polyols on Midpoint Transition Temperature, Calorimetric Enthalpy, and Specific Enzyme Activity of Lysozyme Subjected to Oxidation by Hydrogen Peroxide*

Polyols	Transition Temperature, † Tm (^oC)	Calorimetric Enthalpy, † $\Delta H(Cal/mol) \times 10^4$	Specific Enzyme Activity, † $(EU/mg) \times 10^3$
Control	No Transition Peak		0.041 ± 0.001
Lysozyme + H_2O_2 + mannitol	72.5 ± 0.1	1.2 ± 0.8	6.0 ± 0.1
Lysozyme + H_2O_2 + sucrose	74.3 ± 0.2	1.8 ± 0.5	9.0 ± 0.1
Lysozyme + H_2O_2 + lactose	78.4 ± 0.3	7.4 ± 0.8	36.0 ± 0.2
Lysozyme + H_2O_2 + glycerol	72.8 ± 0.2	1.4 ± 0.8	7.0 ± 0.1
Lysozyme + H_2O_2 + propylene glycol	71.5 ± 0.4	1.7 ± 0.3	8.0 ± 0.1

*All values are expressed as mean ± SD, n = 3. Control = 0.14mM lysozyme containing 50% vol/vol hydrogen peroxide but no polyol.

† Presence of polyols significantly increased ($P < .05$) Tm, ΔH, and specific enzyme activity in comparison with control.

stressing condition reported to cause deamidation at neutral pH.

Under conditions of strong acids (pH 1-2), deamidation by direct hydrolysis of the amide side chain and main chain becomes more favorable than deamidation via formation of cyclic imide at neutral pH.[27] Asp-Gly sequence found in lysozyme at positions 48, 101, and 103 is more liable to get deamidated by direct acid hydrolysis.[28] We found significant ($P < .05$) decrease in the Tm and ΔH values for lysozyme in the control sample in comparison with the freshly prepared lysozyme solution (**Table 4**). Lysozyme solution containing polyols showed significant ($P < .05$) increase in the Tm and ΔH values in comparison with the control, which indicates protection provided by the polyols against deamidation by direct acid hydrolysis. Sucrose increased the Tm and ΔH values of lysozyme to a greater extent than other polyols. It appears that the deamidation of lysozyme at neutral pH is more predominant than acidic pH. A transition peak of lysozyme was observed in the acidic control sample but not in the neutral control sample. These findings are supported by specific enzyme activity data also.

Lysozyme containing hydrogen peroxide (**Figure 3**) did not show any transition peak, which may be because of oxidation of Met (12 and 105 residues) to Met sulfoxide and/or His (15 residue) to 2-oxo-His.[29] Lysozyme mixed with polyols prior to the addition of hydrogen peroxide showed a distinct transition peak (**Figure 3**) with a significantly ($P < .05$) greater Tm and ΔH values in comparison with the control. Lactose, being a reducing sugar, is expected to nullify the oxidative efficiency of hydrogen peroxide and causes greater increase in the Tm and ΔH values than other polyols.

Polyols are reported to be used as stabilizers during formulation development and in final formulations.[30-32] The role of polyols in stabilizing lysozyme can be explained in 2 ways. The first possibility is that these additives restrict conformational changes by forming hydrogen bonds with surface groups on lysozyme.[33] Such interactions would tend to preserve the native conformations as well as protect buried groups from exposure to deamidation, oxidation, aggregation, or other adverse modifications.[24] Propylene glycol has fewer hydroxyl groups than other polyols. Therefore, it was unable to prevent NaCl from interacting unfavorably with lysozyme. The second possibility is that the polyols are preferentially excluded from the protein domain, thereby increasing the free energy of the system. Thermodynamically, preferential exclusion of polyols leads to protein stabilization, since the unfolded state of the protein becomes thermodynamically even less favorable in the presence of polyols.[34] Exclusion of polyols from the protein domain is related to the higher cohesive force of the polyols water solvent system.[35]

CONCLUSION

Thermodynamic measurements coupled with biological activity might be a valuable tool for screening additives in liquid protein formulations for protection against various degradation mechanisms causing protein conformational destabilization associated with loss of (or decline in) biological activity. No single polyol could provide maximal protection against all protein destabilization routes. Therefore, polyols should be selected on the basis of possible destabilization mechanism for a particular protein likely to be encountered during formulation or process development.

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