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ORIGINAL PAPER

The Stabilizing Effects of Polyols and Sugars on Porcine Pancreatic Lipase

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Abstract Porcine pancreatic lipase (PPL) has been used as a biocatalyst for many years and is one of the most widely used enzymes for biotechnological applications; however, it is a rather complex mixture with various active enzymes. The present study has been undertaken to determine the effects of polyols and sugars (cosolvents) on the thermal stability of PPL preparation. The thermal stability of PPL exposed to 60°C for 10 min was enhanced in the presence of cosolvents in terms of both residual specific activity and conformational stability. Thermal denaturation, changes in circular dichroism, fluorescence spectra, apparent kinetic parameters, activity, and preferential interaction parameter of PPL preparation are discussed in terms of contributions to the mechanism of thermal stability and the activity enhancement. Partial specific volume measurement of PPL in the presence of cosolvents is presented for the first time. The preferential interaction parameter (ξ_3) was negative in all cosolvents used, and maximum hydration was observed in the presence of trehalose, where the preferential interaction parameter was −0.076 g/g. The observed increase in the thermal stability of PPL preparation in the presence of cosolvents is due to the preferential hydration of the enzyme.

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Introduction

The stability of enzymes and proteins in vitro remains a critical issue in biotechnology. Understanding the intricate balance of various factors responsible for the stability of proteins and enzymes in solution is not only an academic challenge but also has enormous implications for the pharmaceutical and biotechnology industries. Operational stability is of paramount importance for any bioprocess. The effects of cosolvents on the stability of proteins is underinvestigated. Several methods are employed to increase the stability of proteins in operational conditions, including chemical modification, use of stabilizing additives, derivatization, modification with carbohydrates, amino acid substitution, mutagenesis, and genetic engineering of enzymes [1]. We investigated stabilization of enzymes with cosolvent additives such as xylitol, sorbitol (polyols), and trehalose (sugars).

Stabilizing additives such as polyols and sugars (cosolvents) added to aqueous solution of biomolecules are known to affect protein stability and biochemical equilibria. Additives do not covalently modify the enzyme and can be useful in industrial applications [2]. Stabilizing additives that have been employed for this purpose include sugars, polyols, salts, and amino acids. A few general schemes for the mechanisms of action of these cosolvents have been proposed [3]. Sugars and polyols, except glycerol, lead to an increase in the thermal stability of proteins, and the increase in the surface tension of solvent water in their presence is considered to be a contributory force for

preferential hydration and protein stability [4]. However, the surface tension mechanism does not seem to be applicable in some cases, because an increase in the surface tension of aqueous solutions after addition of solutes has been accompanied by a decrease in the stability of proteins, and vice versa [5].

Timasheff et al. [3, 6] have proposed that cosolvents exert stabilizing effects by inducing preferential hydration of proteins; i.e., the additive tends to be excluded from the vicinity of the protein molecule [3]. Different proteins are known to interact with cosolvent molecules in diverse ways and these interactions depend on the physicochemical properties of the proteins. However, extensive cosolvent dependence studies on the thermal stability of a number of enzymes have suggested that the stabilizing effect should also depend on the nature of the cosolvent used [7, 8]. In order to resolve the anomalies observed in the stability studies, there is a need to look for correlations of the stabilization effect with several other enzymes as well.

Porcine pancreatic lipase preparation (PPL, EC 3.1.1.3) is the cheapest and one of the most widely used lipases for biotransformation of nonnatural substrates; 16% of all lipase reactions are performed with PPL preparation [9]. They constitute the most important group of biocatalysts used in various sectors, such as the pharmaceutical, bioconversion of surplus fats and oils into higher-value products for food industrial uses, surfactant, oleochemistry, and detergency industries [10]. The major component of the preparation is PPL, a glycoprotein composed of 450 amino acids with a calculated molecular weight of 50,000 Da, with six disulfide bridges, and two free cysteines [11, 12]. The structure has two domains. The larger N-terminal domain (residues 1–336) has an α/β (alpha/beta) structure, containing the catalytic triad Ser153, Asp177, His264. The C-terminal domain (residues 337–450) assumes a β-sheet, and contains the binding site for colipase [12].

There are several reports on stabilization of PPL by different methods such as immobilization ranging from adsorption, entrapment, covalent bonding, and cross-linking [13]. However, to date, there has been no systematic study on the role of cosolvent additives on structure stability, preferential interaction parameters, and thermal stability of PPL preparation. A mature understanding of PPL in the presence of cosolvents, because of their advantage versus chemical catalysts, would allow scientists to use them predictably on more sophisticated chemical structures.

We studied the effects of cosolvents on the thermal stability of PPL preparation. Preferential interaction parameters were calculated to elucidate the mechanism of stabilization by partial specific volume measurements. This is supported by activity measurements, fluorescence spectroscopy, far-ultraviolet (far-UV) circular dichroic (CD) studies, and thermal denaturation measurements.

Experimental Procedure

Materials

Lipase type VI-S from porcine pancreas (PPL preparation), xylitol, sorbitol, trehalose, gum arabica, sodium taurocholate dihydrate, tributyrin, calcium chloride dehydrate, and Tris hydroxymethyl aminomethane were procured from Sigma Chemical Co. (St. Louis, MO, USA). Triton X-100 was obtained from Beckman Inc. (USA). Spectra/Por dialysis membranes were from Spectrum Laboratories Inc. (CA, USA). Sodium hydroxide, hydrochloric acid, and sodium chloride were purchased from Merck India Ltd. (Mumbai, India). All other chemicals used in this study were of ACS analytical reagent grade. Quartz triple-distilled water was used throughout in all experiments. The pH standards used for calibrating the pH meter were from Sigma Chemical Co.

Methods

Determination of Protein Concentration

The protein concentration was detected by Bradford method [14] with bovine serum albumin as a standard. The enzyme was checked for its homogeneity on sodium

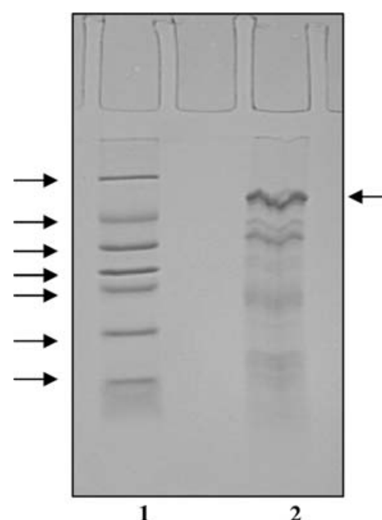

Fig. 1 SDS-PAGE pattern of porcine pancreatic lipase preparation. *Lane 1* standard proteins-BSA (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20 kDa), α-lactalbumin (14.2 kDa). *Lane 2* lipase type VI-S from porcine pancreas (sigma) (50 kDa)

dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1).

Lipase Activity Measurements

Porcine pancreatic lipase activity was determined based on titrimetric method in Mettler Toledo DL-12 titrator. The assay was carried out using tributyrin as substrate and activity was measured at 37°C [15] after preincubating the enzyme at higher temperature (60°C) for 10 min with and without cosolvents. The concentration of enzyme and cosolvents were 8.1×10^{-7} M and 0–40%. Solution containing the above concentrations were prepared and subjected to higher temperature (60°C) for 10 min. After heat treatment at higher temperature, enzyme solutions along with cosolvent were incubated for 1 h with 4 mL solution of emulsified tributyrin (110 mM) in Tris–HCl buffer (10 mM pH 7.5) containing NaCl (100 mM) and $CaCl_2$ (5 mM). The reaction mixture consisting of 4 mL substrate, 10 μL enzymes, and different concentrations of cosolvent. The reaction mixture was incubated in a Queue orbital shaker at 37°C at 100 rpm. Enzymatic reactions were terminated by the addition of 4 mL distilled alcohol. The liberated acid was titrated against 0.05 N alkali to an end-point of pH 9.5. The blank had all the above reaction mixture components except enzyme. All studies were carried out in triplicate. One unit of lipase activity corresponds to release of 1 μmol fatty acid per minute [15]. Each result represents the mean ± standard deviation (SD) of three independent experiments.

Kinetic Assays in Presence of Cosolvent

In order to see the effect of a cosolvent on the kinetic parameters ($K_{m(app)}$ and $k_{cat(app)}$) of PPL preparation, the substrate and the enzyme were preincubated in a given concentration of cosolvent. For kinetic studies, 8.1×10^{-7} M enzyme was incubated with different concentrations of cosolvent in the assay medium. The Michaelis–Menten constant ($K_{m(app)}$) and catalytic constant ($k_{cat(app)}$) of the enzyme were determined by employing the Lineweaver–Burk plot obtained from the initial velocity studies using tributyrin as substrate (ranging from 10 to 120 mM), with cosolvent for measuring the reaction rates at different conditions. The blank contained all the reaction mixture components except enzyme. Each kinetic parameter represents the mean ± SD of three independent experiments.

Fluorescence Spectroscopy Studies

Fluorescence measurements of PPL preparation under different conditions were made at 37°C using Shimadzu RF-5000 (Shimadzu, Japan) recording spectrofluorimeter. Protein concentration of 2×10^{-6} M concentration was taken in a cuvette and the spectra were recorded between 300 and 400 nm after exciting at 281 nm. Excitation and emission slit width were kept at 5 nm. Either relative fluorescence intensity or the change in the wavelength of maximum emission were plotted against cosolvent concentrations. All fluorescence measurements were recorded 10 s after excitation. The results are the average of three experiments.

Circular Dichroic Spectroscopy

The secondary structural analysis of PPL preparation in presence and absence of cosolvent was carried out with a spectropolarimeter (Jasco 810 C, Jasco, Tokyo, Japan). Far-ultraviolet circular dichroic studies were performed from 195 to 260 nm with the slits programmed to give a 1-nm bandwidth at 25°C. Dry nitrogen gas was purged into the instrument before and during use. To measure the CD spectrum of PPL preparation with and without cosolvent, sample were scanned with a protein concentration of 5×10^{-6} M in a 1-mm cell in the wavelength range of 195–260 nm with 0.1-nm increments. The blank spectra without enzyme were subtracted from the sample spectra. Mean residue ellipticity values were calculated using a value of 115 for mean residue weight. The molar ellipticity values were obtained at 1-nm intervals by using the equation:

$$(\theta)_{\mathrm{MRW}} = \frac{(\theta)\mathrm{obs} \times \mathrm{MRW}}{10 \times d \times C}, \quad (1)$$

where (θ)obs is observed ellipticity, d is the path length in cm, C is a protein concentration in g/ml, and MRW is mean residue weight of the protein. The secondary structural analysis was done with the help of instrument software [16].

Thermal Denaturation Studies

The effects of concentration of cosolvents on the thermal denaturation profile of PPL preparation were studied using Varian Carry 100 Bio UV–visible spectrophotometer. The setup consisted of an electronically controlled thermal cuvette holder with increment in temperature up to 0.1°C/min. The change in absorbance of about 1 mL protein having an absorbance of 0.4–0.5 in each case was monitored at 287 nm as a function of temperature in the range 30–90°C with 1°C increment with appropriate blanks. A protein concentration of 7.8×10^{-6} M was used for all the experiments. Respective buffers were used in the reference cell. Spectral data were stored and analyzed using instrument software. Apparent thermal denaturation temperatures

($T_{m(app)}$) were calculated either by first-derivative plot of absorbance or van't Hoff plot [17]. The fraction of protein in the unfolded state (F_u) is given by:

$$F_u = \frac{Y_F - Y}{Y_F - Y_U} \quad (2)$$

where Y_F is the absorbance of protein solution in the native state, Y_U is the absorbance of protein solution in unfolded state, and Y is the absorbance of the protein solution at different temperature. The apparent thermal denaturation temperature ($T_{m(app)}$) is defined as the temperature at which the value of F_u is 0.5. The results are the average of three experiments.

Partial Specific Volume Measurements

The partial specific volumes of PPL preparation were measured using an Anton Paar DMA 5000 density meter at 20.00 ± 0.003°C according to the standard procedure [18]. The densities of the solvents and of the protein solutions were measured, and the apparent partial specific volume ϕ, was calculated using the equation:

$$\phi = 1/\rho_0(1 - (\rho - \rho_0)/\mathrm{C}), \quad (3)$$

where ρ is the density of the solution in g/ml, ρ_0 is that of the solvent in g/ml, C is the protein concentration in g/ml, and ϕ is the apparent partial specific volume [19, 20]. The value of ϕ was plotted as a function of protein concentrations. The value was extrapolated to infinite dilution to get the partial specific volume of the protein $\bar{\mathrm{v}}$. In preferential interaction measurements, two types of apparent specific volumes were measured. The first, ϕ_2^0, was measured under the conditions at which the molal concentration, m_3, of diffusible component 3 was kept identical to the solvent and solutions. The second, $\phi_2'^0$, was measured under the conditions at which it was the chemical potential of component 3, i.e., μ_3, which was kept constant between solution and reference solvent and which can be attained to a close approximation by dialyzing the protein solution against the solvent. The data were analyzed using three-component systems; components 1, 2, and 3 were water, protein, and cosolvent, respectively. The preferential interaction parameter for a three-component system was calculated using the standard equation:

$$\xi_3 = (\partial g_3/\partial g_2)_{T,\mu 1,\mu 3} = 1/\rho_3(\phi_2^0 - \phi_2'^0)/(1 - \upsilon_3\rho_3), \quad (4)$$

where g_i is the concentration of component i in grams per gram of water, μ is its chemical potential, T is the thermodynamic temperature, ρ_3 is the density of the third component (cosolvent), ϕ_2^0 and $\phi_2'^0$ is the partial specific volume of protein at isomolal and isopotential conditions, and υ_3 is the partial specific volume of component 3 [19, 20].

Results and Discussion

Lipase Activity Measurements

Activity measurements of PPL preparation at 60°C for 10 min in the presence of cosolvents showed that all the cosolvents protected the residual specific activity of PPL preparation. Trehalose was the best stabilizer of PPL residual specific activity, followed by sorbitol and xylitol, respectively. The residual specific activity of the control without heat treatment taken was taking as 100%. Under conditions in which PPL retained only 3% activity, presence of trehalose caused retention of 78% of PPL activity (Fig. 2). Other cosolvents provided stabilizing effects, with 66% and 60% protection of activity in 40% sorbitol and xylitol, respectively. Thermodynamic measurements coupled with biological activity at higher temperature might be a valuable tool for screening additives in enzyme formulations for protection against various degradation mechanisms causing protein conformational destabilization associated with loss of (or decline in) biological activity. The thermostability of PPL preparation was a time- and dose-dependent phenomenon.

The protection of enzymes at higher temperature was due to the ability of the cosolvents to replace water molecules in the medium [7, 21]. This property has also been correlated with the ability of the cosolvents to increase the viscosity of the medium. The mechanism of activity protection was further supported by chaperonins-like action of

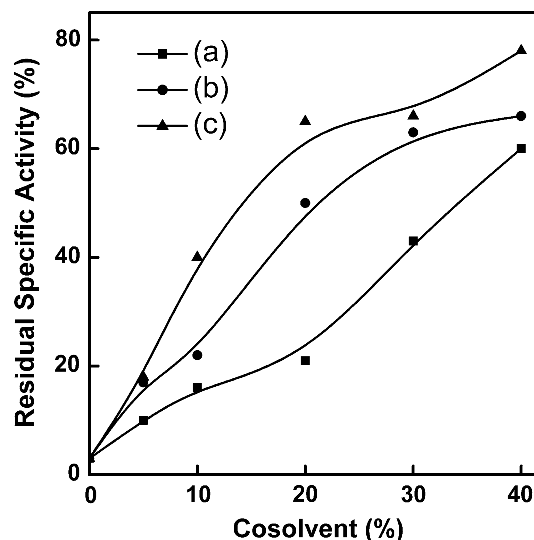

Fig. 2 Thermal inactivation profile of hydrolytic activity of porcine pancreatic lipase preparation in presence of different concentrations of (*a*) xylitol, (*b*) sorbitol, and (*c*) trehalose. The reaction mixture was exposed to 60°C for 10 min with and without cosolvent. Hydrolysis was initiated by addition of 110 mM emulsified tributyrin as a substrate. Each result represents the mean ± SD of three independent experiments

cosolvents on protein [22, 23]. Chaperonins behave as a molecular thermometer, which can inhibit the release of aggregation-prone proteins during heat shock and restore protein folding and release after heat shock. The above result clearly indicates that cosolvents protected PPL activity against thermal inactivation. The protection effects were directly related to length of cosolvent carbon chains and number of hydroxyl groups.

Kinetics of Thermal Stability

The functional activity parameters ($K_{m(app)}$ and $k_{cat(app)}$) of PPL preparation in absence and presence of cosolvents at several concentrations were determined using Lineweaver–Burk or double-reciprocal plots (Table 1). Native PPL preparation had an apparent K_m of 5.4 mM, and k_{cat} of 194×10^3 min^{-1}. After exposure to 60°C for 10 min the control PPL had an apparent K_m of 18.5 mM and k_{cat} of 2.5×10^3 min^{-1}. In the presence of cosolvents at higher temperature the apparent K_m dropped to 6.5, 7.9, and 9.1 M in presence of 40% trehalose, sorbitol, and xylitol respectively, with corresponding increases in apparent k_{cat} values to 152, 128, and 116×10^3 min^{-1}, respectively. The decrease in apparent K_m and increase in the catalytic constant showed that the activity retention of PPL was enhanced by presence of cosolvents. Enzyme substrate interactions involve conformational shifts from one microstate to another with altered surface area, and this process was apparently affected by the stabilizing solutes. A stabilizing solute may limit the mobility of enzyme domains, leading to lower apparent K_m values with increase in catalytic constant k_{cat} in presence of cosolvents [24].

Intrinsic Fluorescence Spectra

In order to understand the mechanism of stabilization, changes in the fluorescence emission spectra of PPL preparation in presence of cosolvents were determined. The intrinsic fluorescence spectra of PPL at higher temperature was an excellent parameter to monitor the polarity of the environment of tryptophan. Tryptophan emission is sensitive to the microenvironment and a red-shift is observed with an increase in the polarity. Fluorescence spectra were monitored after PPL was exposed to 60°C for 10 min in presence and absence of cosolvents (Fig. 3A, B, C). Incubation at 60°C for 10 min caused a shift in the emission maximum from 343 nm (for the unheated enzyme) to 350 nm, with a decrease in fluorescence intensity. This red-shift reflects increased polarity in the microenvironment of the emitting molecule. A blue-shift in the emission maximum, accompanied by an increase in fluorescence intensity, took place when cosolvents were added. The increase in the intensity was gradual over the entire range of concentration used (Fig. 3A, B and C). The wavelength of maximum emission shifted from a control value of 350 nm to a value of 343 nm in presence of various concentrations cosolvent.

Fluorescence can indicated changes in the polarity of the microenvironment of tryptophan but must be interpreted with caution. Incubating proteins at higher temperatures usually leads to a red-shift in the emission maximum of tryptophan due to increased polarity of the microenvironment of the emitting molecule. Melo et al. [25] observed this temperature effect with *Chromobacterium viscosum* lipase. We observed a blue-shift in the presence of all cosolvents, together with an increase in fluorescence intensity, indicating an increase in the polarity of the microenvironment around tryptophan. Cosolvents are known to alter the structure of water in protein solution [3].

Table 1 Apparent kinetic parameters of porcine pancreatic lipase preparation in absence and presence of different concentrations of cosolvent after exposure to 60°C for 10 min

Cosolvent conc. % (w/v)	K_{cat} (10^3 min^{-1})	K_m (mM)	K_{cat}/K_m
Control[a]	194 ± 8	5.4 ± 0.8	35925
Control[b]	2.5 ± 0.4	18.5 ± 2	135
40% Xylitol	116 ± 6	9.1 ± 1.0	12747
40% Sorbitol	128 ± 7	7.9 ± 0.9	16243
40% Trehalose	152 ± 9	6.5 ± 0.6	23384

Values are mean ± SD of three independent experiments

[a] Native porcine pancreatic lipase preparation in 10 mM Tris–HCl buffer, pH 7.5

[b] Heat-treated porcine pancreatic lipase preparation in 10 mM Tris–HCl buffer, pH 7.5

Cosolvent-Induced Secondary Structural Changes

Far-UV CD spectra have been shown to be an ideal technique to monitor temperature-dependent conformational changes in PPL preparation (Fig. 4). The enzyme exhibited one minimum at 208 nm. The addition of cosolvents had a major effect on the secondary structural characteristic of the PPL preparation. At higher temperature in the absence of cosolvent, the secondary structure content decreased from 23% in the unheated enzyme to 3%, indicating almost complete unfolding of the PPL preparation. The presence of cosolvent limited the decrease in α-helical content by 10%, 15%, and 11% in 30% trehalose, 30% sorbitol, and 30% xylitol concentration, respectively, with slight changes in β-structure. Secondary structure retention at higher temperature in presence of cosolvents is seen to depend upon the ability of cosolvents to preserve the native structure of the protein molecule under denaturing conditions, reminiscent of

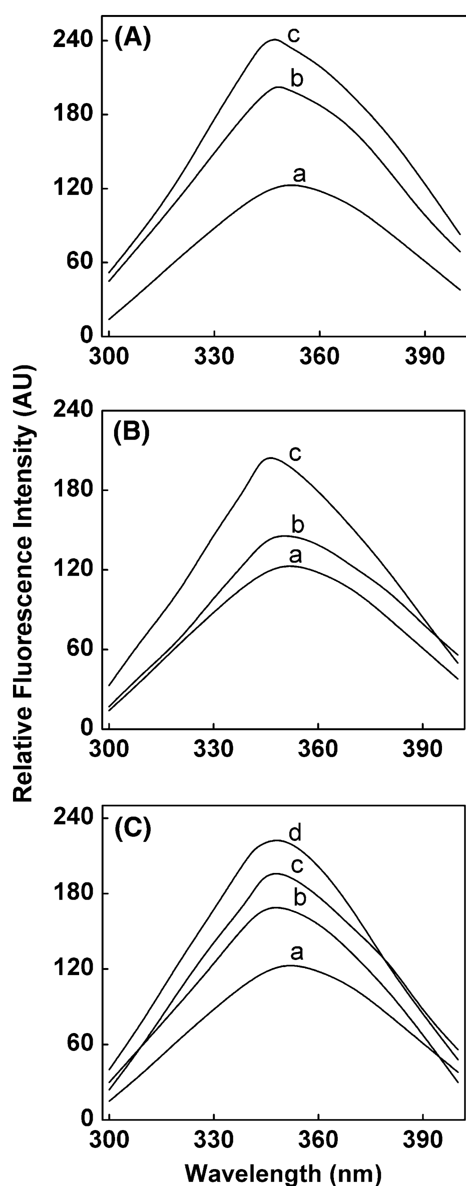

Fig. 3 Fluorescence emission spectra of porcine pancreatic lipase preparation: **A** in the absence (*a*) and presence of 10% (*b*) and 20% (*c*) xylitol. **B** In the absence (*a*) and presence of 20% (*b*) and 30% (*c*) sorbitol. **C** In the absence (*a*) and presence of 10% (*b*), 20% (*c*), and 30% (*d*) trehalose in 10 mM Tris–HCl buffer, pH 7.5 after exposure to 60°C for 10 min at 283 nm excitation and emission in the range of 300–400 nm

chaperonins. Far-UV CD protein spectra primarily reflect the spatial arrangements of amide groups: the ability of cosolvents to stabilize proteins against denaturing stresses originates from the unfavorable interaction of the osmolytes with the peptide backbones [26]. Because, the peptide backbones are highly exposed to cosolvents in the denatured state, the osmophobic effect preferentially raises the free energy of the denatured state, shifting the equilibrium in favor of the native state.

Thermal Denaturation Profile of Porcine Pancreatic Lipase Preparation in Cosolvent

A concentration-dependent shift in the apparent thermal denaturation temperature of PPL preparations as a function of cosolvent concentration took place (Fig. 5A, B and C). The apparent thermal denaturation temperature (T_m) increased in a concentration-dependent manner from the control value of 48°C in presence of cosolvents. The structuring of water by cosolvents appears to be a dominant factor that governing PPL preparation stability. It is proposed that enhancement of surface hydrophilicity led to an increase in the essential energy for exposing hydrophobic groups to water in the unfolding process. In other words, the tendency to protect the native structure increased. In the presence of nonpolar residues near a protein, cosolvents are known to increase the thermal stability by a combination of interaction such free energy changes, solvophobic, and surface tension effects [4, 26]. The increase in the apparent T_m of PPL preparation may be attributed to the nature of the cosolvent and the different effects they exert, such as preferential exclusion, solvophobic interaction between the peptide backbone and osmolytes, and surface tension effect [27, 28].

Preferential Interaction Studies in Presence of Cosolvent

The interaction of PPL preparation with cosolvent was investigated. Isomolal and isopotential partial specific volume measurements allowed calculation of preferential interaction parameters (ξ_3) (Fig. 6). The preferential interaction parameter increased with the increase in

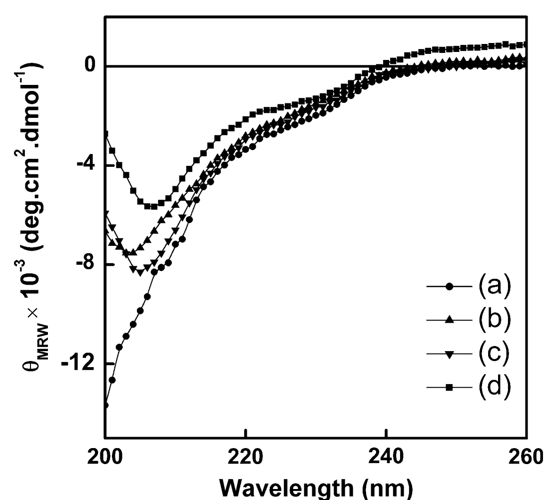

Fig. 4 Far-UV CD spectra of porcine pancreatic lipase preparation in 10 mM Tris–HCl buffer, pH 7.5 after exposure to 60°C for 10 min. **a** Control, **b** in presence of 30% xylitol, **c** 30% sorbitol, and **d** 30% trehalose. The data obtained represent the average of four runs

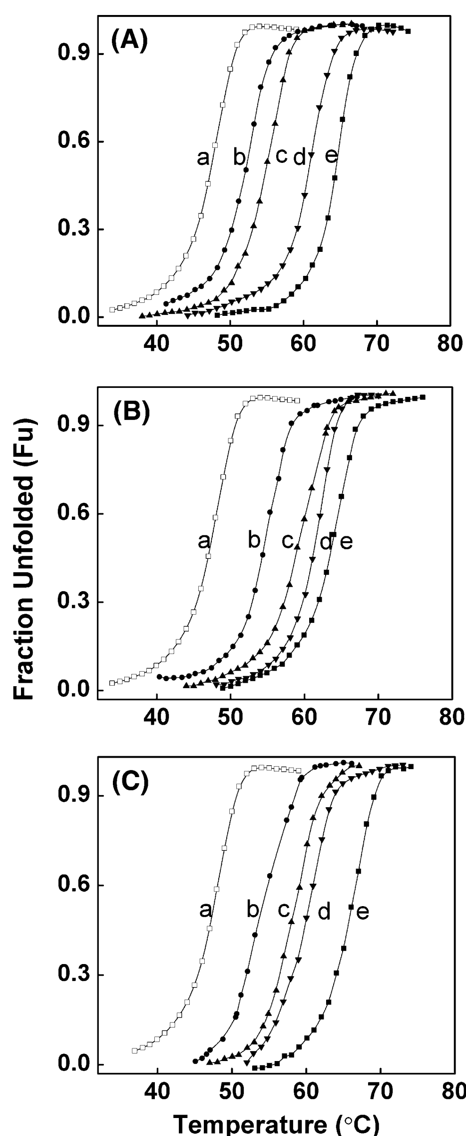

Fig. 5 Thermal denaturation profile of porcine pancreatic lipase preparation. **A** In the absence (*a*) and presence of 10% (*b*), 20% (*c*), 30% (*d*), and 40% (*e*) xylitol. **B** In the absence (*a*) and presence of 10% (*b*), 20% (*c*), 30% (*d*), and 40% (*e*) sorbitol. **C** In the absence (*a*) and presence of 10% (*b*), 20% (*c*), 30% (*d*), and 40% (*e*) trehalose in 0.01 M Tris–HCl buffer, pH 7.5. The absorption spectra were recorded as a function of temperature at 287 nm

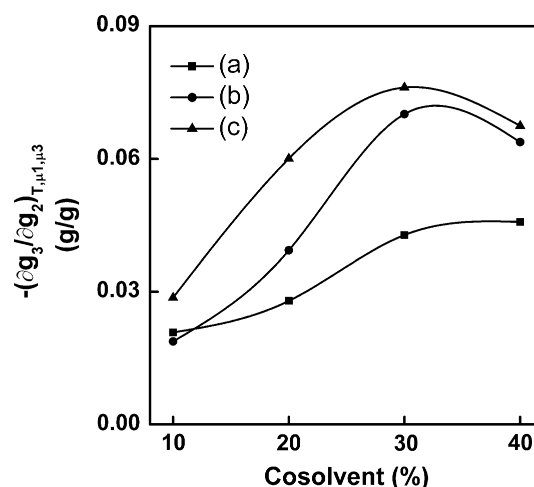

Fig. 6 Effect of cosolvents on preferential interaction parameter (ξ_3) of porcine pancreatic lipase preparation in presence of (*a*) sorbitol, (*b*) xylitol, and (*c*) trehalose in the concentration range of 0–40% w/v. The values were calculated from the isomolal and isopotential partial specific volume measurements

concentration of each cosolvent. Partial specific volume, solvent composition, preferential interaction parameter, and related interaction parameters of PPL preparation in different cosolvent are summarized in Table 2. In all the cases, $(\partial g_3/\partial g_2)_{T,\mu1,\mu3}$ was found to be negative, indicating a deficiency of these cosolvent in the immediate domain of the protein, i.e., preferential hydration. The maximum hydration was observed with 30% trehalose, and the lowest hydration took place in 10% xylitol. The extent of negative interaction depends on the cosolvents and the concentration. As shown in Table 2 the corresponding preferential hydration parameter, $(\partial g_1/\partial g_2)_{T,\mu1,\mu3}$ is almost independent of cosolvent and concentrations, showing the highest value of 0.267 g/g in 10% trehalose, and the lowest value of 0.084 g/g in 40% sorbitol.

The partial specific volume measurements showed that the preferential interaction parameter dominated the protein stability of the PPL preparation. The effects of cosolvents on protein stability can be explained by preferential exclusion from the protein surface. Protein surface area increases in denaturing conditions. Any state of the protein that has an increased surface area should be thermodynamically less favorable than states that are more compact. A critical assumption for this conclusion is that the degree of preferential exclusion varies directly with protein surface area and is not altered by changes in the chemical properties of the exposed surface. Furthermore, it is primarily the unfavorable interactions of the peptide backbone with cosolvents that give rise to the increased protein chemical potential in solutions [22]. A number of studies by Timasheff and Xie [6] on different cosolvent system such as xylitol, sorbitol, and trehalose have explained the phenomenon of preferential hydration. Preferential hydration is a thermodynamic phenomenon that reflects the inability of cosolvents to interact with protein molecule; thus, it leads to an exclusion of these cosolvent components from the protein domain. These data clearly suggest that, although all the cosolvents used in this study tend to stabilize protein, the mechanism by which individual cosolvents bring about the stability may be different depending upon the nature and concentration of the cosolvent used. It is clear that there is a considerable increase in the thermal stability of PPL preparation in

Table 2 Preferential interaction parameters of porcine pancreatic lipase preparation as a function of cosolvent concentrations

Cosolvent	Conc. % (w/v)	ϕ_2^0 (ml/g)	$\phi_2'^0$ (ml/g)	g_3 (g/g)	m_3 (mol of solvent/ 1,000 g H_2O)	$(\partial g_3/\partial g_2)_{T,\mu1,\mu3}$ (g/g)	$(\partial g_1/\partial g_2)_{T,\mu1,\mu3}$ (g/g)	$(\partial m_3/\partial m_2)_{T,\mu1,\mu3}$ (mol/mol)
Xylitol	0	0.728 ± 0.001	0.729 ± 0.001					
	10	0.728 ± 0.001	0.734 ± 0.001	0.107	0.70	−0.019 ± 0.003	0.174 ± 0.02	−6.15 ± 1.0
	20	0.727 ± 0.001	0.739 ± 0.002	0.231	1.52	−0.039 ± 0.005	0.170 ± 0.02	−12.9 ± 1.5
	30	0.725 ± 0.001	0.745 ± 0.002	0.377	2.47	−0.070 ± 0.01	0.186 ± 0.02	−23.0 ± 3.1
	40	0.728 ± 0.001	0.745 ± 0.002	0.549	3.61	−0.064 ± 0.01	0.116 ± 0.01	−20.9 ± 2.5
Sorbitol	10	0.730 ± 0.001	0.737 ± 0.001	0.107	0.59	−0.021 ± 0.002	0.194 ± 0.02	−5.70 ± 0.8
	20	0.731 ± 0.001	0.740 ± 0.001	0.230	1.26	−0.028 ± 0.003	0.121 ± 0.01	−7.65 ± 0.9
	30	0.732 ± 0.001	0.745 ± 0.002	0.374	2.05	−0.043 ± 0.004	0.114 ± 0.01	−11.7 ± 1.2
	40	0.733 ± 0.001	0.746 ± 0.002	0.544	2.99	−0.046 ± 0.004	0.084 ± 0.01	−12.5 ± 1.4
Trehalose	10	0.729 ± 0.001	0.739 ± 0.001	0.107	0.31	−0.028 ± 0.004	0.267 ± 0.02	−4.18 ± 0.2
	20	0.726 ± 0.001	0.746 ± 0.002	0.229	0.67	−0.060 ± 0.006	0.261 ± 0.02	−8.17 ± 1.6
	30	0.729 ± 0.001	0.753 ± 0.002	0.372	1.08	−0.076 ± 0.007	0.204 ± 0.01	−11.1 ± 1.9
	40	0.730 ± 0.001	0.750 ± 0.002	0.541	1.58	−0.067 ± 0.006	0.124 ± 0.01	−9.83 ± 1.8

presence of these cosolvents because of preferential hydration, and cosolvents stabilize proteins by shifting the denaturation equilibrium toward the native state. Thus the structuring of water in presence of cosolvents appears to be the dominant factor that governs such a stabilization process. However, we stress there is no unique molecular model for the influence of a cosolvent on protein stability.

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