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1,1,1,3,3,3-Hexafluoroisopropanol and 2,2,2-trifluoroethanol act more effectively on protein in combination than individually: Thermodynamic aspects

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ABSTRACT

2,2,2-Trifluoroethanol (TFE) and 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) are known to be secondary structure inducers of proteins. In order to determine the efficacy of TFE and HFIP in affecting the conformation of proteins when taken together, as compared to individually, we have studied the thermodynamics of unfolding of bovine serum albumin (BSA) in the presence of these alcohols along with the conformational characterization of the protein. A comparison of change in thermal transition temperature of the protein in the absence and presence of these alcohols in combination and individually shows that the (TFE+HFIP) mixture is a stronger stabilizer of BSA up to a certain molality as compared to addition of their individual effects. The thermodynamics of effects of these alcohols on dithiotheitol reduced BSA were also studied. The enthalpies of interaction of TFE with HFIP in aqueous solution were measured by using isothermal titration calorimetry. The endothermic molar enthalpy of interaction of TFE with HFIP suggests that these alcohols do not strongly associate with each other through polar interactions. This is a possible explanation for their stronger effect on protein stability and conformation in combination rather than individually. The circular dichroism and fluorescence spectroscopic results provide evidence for the enhancement of the secondary structure of the protein by TFE and HFIP along with internalization of tryptophan residues in more hydrophobic environment.

1. Introduction

Ever since Levinthal [1] proposed that protein folding cannot be a random process, there has been considerable interest in understanding the exact nature of protein folding [2,3]. Different models such as the hydrophobic collapse model [4], the framework model [5], and the funnel model [6,7] have been proposed. A common feature of these models is prediction of the presence of intermediates, although the structural features vary in different models. Different experiments over the years have proved that the hypothesis of intermediate states is indeed true [8–13], characterization of which is essential for elucidating the problem of protein folding. The biological importance of such non-native protein conformations under partial denaturing conditions and aggregated states cannot be ignored [14,15].

The effects of alcohols on proteins and peptides are useful for considering how protein-specific conformation is altered in an aqueous environment [16]. Alcohols weaken non-local hydrophobic interactions and enhance local polar interactions in proteins [17–21]. Therefore, alcohol-induced denaturation has usually resulted in stabilization of the extended helical rod where hydrophobic side chains are exposed and polar amide groups are shielded from the solvent [22].

Enhancement of secondary structural content in the presence of fluoroalcohols has been demonstrated in many proteins. Goodman and coworkers [23] have shown that 2,2,2-trifluoroethanol (TFE) coaxed certain medium length peptides to achieve helical conformation. This is called the TFE effect. Amongst several alcohols, TFE has been widely used due to its high potential for stabilizing the α-helical structure [24–27]. The high ability of TFE to induce helical conformation in secondary structure suggests the importance of the F atom in enhancing this effect. Therefore, alcohols with additional F atoms should be more effective than TFE. Hexafluoroisopropanol, with six F atoms has been observed to be a stronger α-helical inducer in proteins than TFE [28]. 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) has been widely used in generating

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these intermediates along with other applications in biologically important systems [29,30]. It has pK_a of 9.4 [31], and hence is more acidic than TFE (pK_a = 12.4) [32]. The presence of two $-CF_3$ groups alters its properties to a greater extent since it is a better H-bond donor and H-bond acceptor than TFE. Therefore HFIP should be potentially more powerful than TFE in perturbing interactions in proteins which are ionic, H-bonding, and hydrophobic in nature.

In view of the demonstrated importance of TFE and HFIP, it is of scientific interest to understand the action of these alcohols when taken in combination. The effect could be additive or otherwise. In this work, we have studied the effect of TFE, HFIP, and an equimolal mixture of (TFE+HFIP) on the conformation of bovine serum albumin (BSA) as a model protein. The thermodynamic and conformational aspects have been addressed.

2. Experimental

2.1. Materials

Bovine serum albumin, 2,2,2-trifluoethanol and dithiothreitol were purchased from Sigma Aldrich Company USA. 1,1,1,3,3,3-Hexafluoroisopropanol was purchased from Fluka Chemical Company and used without further purification. The purity of materials along with the details of their source, molecular formulae, and molecular weight are reported in Table 1. The water used for preparing the solutions was double distilled followed by deionization using a Cole-Parmer research mixed-bed ion exchange column. The stock solutions of BSA for all experiments were prepared by extensive dialysis of the protein at T = 277 K against the desired buffer with at least three changes. The reported pH is that of the dialysate, determined using a standard Control Dynamics pH meter at room temperature. The buffer used was 10.0×10^{-3} mol·kg^{-1} potassium phosphate at pH = 7.0. In the case of reduced protein, an additional 1.0×10^{-3} mol·kg^{-1} dithiothreitol (DTT) was added to the solution. For experiments with reduced BSA, precautions were taken to limit the exposure of solutions to atmospheric oxygen. In these experiments, solutions were deaerated and saturated with nitrogen gas.

2.2. UV–visible experiments

The concentration of BSA was determined using an extinction coefficient corresponding to $A_{280}^{1\%} = 0.68$ at pH = 7.0 [34]. For concentration determination and thermal denaturation experiments, a Jasco V-550 spectrophotometer was used, to which a Cole Parmer constant temperature circulator bath was attached. The concentration of BSA in the thermal denaturation experiments was kept at 15.0×10^{-6} mol·dm^{-3} and absorbance at different temperatures was measured at a fixed wavelength of λ = 295 nm in fixed temperature steps of 2 K in the pre- and post-transition regions, and 1 K in the transition region. The reference solution in these experiments was buffer when the measurements were made in buffer, or (buffer + cosolvent) when the experiments were made in the presence of the cosolvent. The absorbance versus temperature plots were analyzed by using the EXAM program of Kirchoff [35] to calculate the thermodynamic parameters transition temperature ($T_{1/2}$), molar van't Hoff enthalpy of unfolding ($\Delta_{vH}H_m$), and molar entropy of unfolding ($\Delta_{vH}S_m$) accompanying the thermal transitions. The reversibility of the scans was checked by heating the sample to just above the transition temperature, cooling immediately, and then reheating.

From the values of absorbance, the fractions of native and denatured protein at temperature T were calculated by using the following equations [36–38],

$$f_N = \frac{A(D) - A(T)}{A(N) - A(D)} \quad (1)$$

$$f_D = 1 - f_N \quad (2)$$

where f_N is the fraction of the native protein, f_D is the fraction of the denatured protein, A(N), A(D) and A(T) are, respectively, the values of absorbance of the protein in the native state, denatured state and at the temperature T.

2.3. Fluorescence experiments

The fluorescence experiments were done on a Perkin-Elmer LS-55 spectrofluorimeter at T = 298.5 K with a quartz cell of 1-cm path length. The protein concentration in all the experiments was kept 15.0×10^{-6} mol·dm^{-3}. The excitation wavelength was set at $\lambda_{excitation}$ = 295 nm to selectively excite the tryptophan residues, and the emission spectra were recorded in the wavelength range of $\lambda_{emission}$ = (300 to 400) nm at a scan rate of 100 nm·min^{-1}. The excitation and emission slit width were set at 5 nm each. The background spectrum containing the same amount of additive as in the sample was subtracted from all the plots.

2.4. Circular dichroism spectroscopy

The CD experiments were performed on a JASCO-810CD spectropolarimeter at T = 298 K. The protein concentration and path length of the cell were 5.0×10^{-6} mol·dm^{-3} and 0.2 cm, respectively for far UV-CD, and 20.0×10^{-6} mol·dm^{-3} and 1 cm respectively, for near UV-CD experiments. The spectropolarimeter was sufficiently purged with 99.9% dry nitrogen during the experiment. The spectra were collected at a scan speed of 500 nm·min^{-1} and a response time of 1 s. Each spectrum was baseline – corrected, and the final plot was taken as an average of three accumulated plots. The molar ellipticity [θ] was calculated from the observed ellipticity θ as

$$[\theta] = 100 \times \left(\frac{\theta}{c \times l}\right) \quad (3)$$

Here c is concentration of the protein in mol·dm^{-3} and l is path length of the cell in centimeters.

2.5. Isothermal titration calorimetry

The enthalpies of interaction of TFE with HFIP were determined on a VP-ITC procured from Microcal, USA. In order to avoid

Table 1
Compounds, molecular formula, molar mass (M_r) in g·mol^{-1}, source (S = Sigma Aldrich Co. USA, F = Fluka, TCI = Tokyo Chemical Industry Co, Japan), CAS number (*CN*), mass fraction moisture content (w), and their mole fraction purity (x) as reported by the vendors.

Compound	Molecular formula	M_r/g·mol^{-1}	Source	*CN*	w	x
2,2,2-Trifluoroethanol	CF_3CH_2OH	100.04	F	75-89-8	0.0015	>0.99
1,1,1,3,3,3-Hexafluoroisopropanol	$(CF_3)_2CHOH$	168.04	S	920-66-1	0.0010	>0.99
Dithiothreitol	$HSCH_2CH(OH)CH(OH)CH_2SH$	154.25	TCI	3483-12-3	0.0035	>0.96
Potassium phosphate	$KH_2P\,O_4$	136.09	S	7778-77-0	0.0022	≥0.98
BSA	Amino acid sequence [33]	66,400	S	9048-46-8		≥0.98