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Structural transitions in the acid-denatured ficin induced by halogenols and alkanols

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The effect of eight different alcohols including halogenols and alkanols on the structural transformation of acid-denatured ficin (ADF) at pH 2.5 was studied using far- and near-UV circular dichroism (CD) and tryptophan fluorescence. Except methanol and ethanol, other alcohols induced α-helical structure in ADF, as revealed by the increase in MRE_{222nm} values. The fluoro alcohols tested showed a higher helix-inducing potential compared to 2-chloroethanol and alkanols. Their effectiveness followed the order: 1,1,1,3,3,3-hexafluoro-2-propanol > 2,2,2-trifluoroethanol > *tert*-butanol > 2-chloroethanol > 1-propanol > 2-propanol. Near-UV CD spectra showed disruption of the tertiary structure in presence of alcohols. Tryptophan fluorescence of ADF was affected differently in presence of these alcohols, showing quenching with fluoro alcohols and enhancement with 2-chloroethanol and alkanols in the order: *tert*-butanol > 2-propanol > 1-propanol > ethanol > methanol. The obtained results suggested that the effectiveness of the alcohols correlated well with the number of fluorine atoms, bulkiness and arrangement of different alkyl groups.

Keywords: ficin, acid-denatured state, alcohol-induced state, structural transition

INTRODUCTION

The study of different conformational states of a protein has received considerable attention in the field of protein folding. These states have been produced by changing pH, temperature and concentration of chemical denaturants [1–4]. Characterization of different intermediates between native and unfolded states of a protein is an important step to understand the mechanism of protein folding [5]. The molten globule state has been shown to be a common intermediate in the folding pathway of many proteins [1, 6, 7]. Acid-denatured states of several proteins have been characterized as the molten globule state [8, 9]. On the other hand, alcohols have been found to induce α-helical structures in proteins [10–12] and these alcohol-induced states might resemble some of the intermediates in the protein folding pathway [13]. Although the exact mechanism of alcohol-induced α-helix formation in proteins remains unclear, the effect is largely correlated to the decrease in the polarity of the solvent, thus weakening the hydrophobic interactions and enhancing the formation of local hydrogen bonds to induce α-helical structures [14]. Stabilization of several acid-denatured proteins by alcohols, especially fluoro alcohols, has been well documented [15–17]. In contrast to other alcohols, both 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and 2,2,2-trifluoroethanol (TFE) have been found to possess a high helix-inducing potential in proteins [18–20]. Furthermore, HFIP has also been employed to investigate Alzheimer's amyloid peptides due to its ability to dissociate aggregates [21].

Ficin (E.C. 3.4.22.3), a proteolytic enzyme from the papain family, isolated from the latex of *Ficus* species, has a molecular mass of about 23,800 Da and possesses an essential cysteine residue at its active site [22, 23]. It is a commercial enzyme with several industrial applications such as meat tenderization, photography, chitosan depolymerisation, *etc.* [24]. Although the three-dimensional structure of ficin still remains to be investigated, a few papers have been published recently, showing the effect of pH and chemical denaturants, such as urea and guanidine hydrochloride (GdnHCl) on the conformational stability of ficin [8, 25, 26]. Members of the cysteine protease family

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i.e. papain, ficin and stem bromelain share common structural characteristics by showing resistance towards urea during destabilization in presence of GdnHCl [13, 25–27]. Such conformational stability of cysteine proteases in presence of the strong chemical denaturant urea is unusual, compared to other globular proteins [28, 29] and requires further research on their structural stability in presence of other chemical denaturants. Although effect of alcohols on both stem bromelain and papain at low pH has been reported [12, 30, 31], data on alcohol–induced structural transition in the acid-denatured state of ficin is lacking. In a previous study, acid-denatured state of ficin has been shown to accumulate between pH 3.0 and pH 2.2, based on the minimum value of the mean residue ellipticity at 222 nm (MRE_{222nm}) in the acid-induced transition [8]. Therefore, we selected the acid-denatured ficin accumulated at pH 2.5 and studied the effect of eight different alcohols including halogen alcohols (halogenols) and alkyl alcohols (alkanols) on its structural transformation using different probes such as far- and near-UV circular dichroism (CD) as well as tryptophan (Trp) fluorescence.

EXPERIMENTAL

Materials

Ficin, from fig tree (*Ficus glabrata*) latex (2 × crystallized) saline suspension, ≥1.5 units/mg protein (Lot 030M7022) as well as different alcohols, whose structures are shown in Fig. 1, *i.e.* 1,1,1,3,3,3-hexafluoro-2-propanol (Lot SHBB0681V), 2,2,2-trifluoroethanol (Lot 1366086), *tert*-butanol (Lot 58396KK), 2-propanol (Lot 77596MK) and methanol (Lot 42296LJ-199) were purchased from Sigma-Aldrich Inc., USA. 2-Chloroethanol (Lot S6221545), 1-propanol (Lot K42188897) and ethanol (Lot K42113283) were obtained from Merck Chemicals, Germany. Commercial ficin was used in all experiments without further purification. All other chemicals used were of analytical grade purity.

Protein concentration

Ficin concentration was determined spectrophotometrically on a Shimadzu double beam spectrophotometer, model UV-2450, using a specific extinction coefficient of 21.0 $(g/100\ ml)^{-1}\ cm^{-1}$ at 280 nm [22].

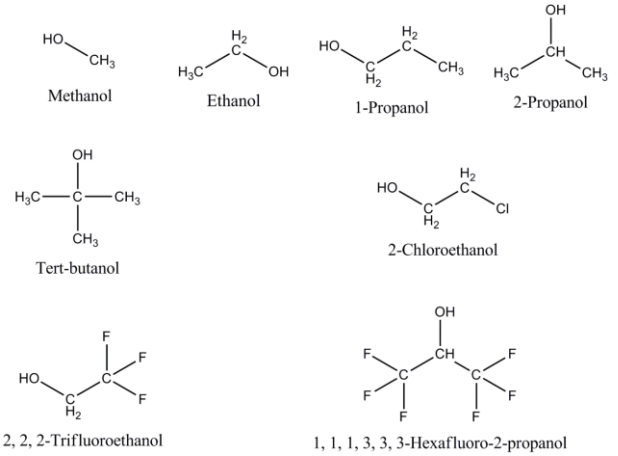

Fig.1. Structural formulae of various alcohols used in this study.

Circular dichroism spectroscopy

CD measurements were performed on a Jasco spectropolarimeter, model J-815, equipped with a thermostatically-controlled cell holder attached to a water bath, under constant nitrogen flow. The instrument was calibrated with (+)-10-camphorsulfonic acid and all measurements were made at 25°C using a scan speed of 100 nm/min and a response time of 1 sec. CD spectra in the far-UV and near-UV regions were recorded using protein concentrations and path lengths of 6.72 μM; 1 mm and 37.8 μM; 10 mm, respectively. The spectra were considered as an average of three scans. CD data were transformed into MRE values in $deg.cm^2.dmol^{-1}$ as described earlier [4]. The α-helical content was calculated from the MRE_{222nm} value following the method of Chen *et al.* [32] using the following equation:

$$\%\ Helix = (MRE_{222nm} - 2340 / 30300) \times 100$$

Fluorescence spectroscopy

Fluorescence measurements were carried out on a Jasco spectrofluorometer, model FP-6500, linked to a data recorder and equipped with a thermostatically-controlled cell holder, attached to a water bath to maintain constant temperature of 25ºC. The fluorescence spectra were recorded in the wavelength range 310–400 nm upon excitation at 295 nm using excitation and emission slits of 10 nm each and a protein concentration of 0.6 μM in a cell of 1 cm path length. Values of the fluorescence intensity obtained at different alcohol concentrations were transformed into relative fluorescence intensity by taking the value of the fluorescence intensity of acid-denatured ficin in the absence of alcohol as 100.

Preparation of acid-denatured and native forms

Acid-denatured form of ficin was prepared by dialyzing out the protein solution against 20 mM glycine-HCl buffer, pH 2.5 at 4 ºC for 72 hours and was stored at 4 ºC. Ficin solution was dialyzed against 20 mM sodium phosphate buffer, pH 7.0 under similar conditions for the preparation of the native form of the protein.

Conformational transitions

Alcohol-induced conformational transitions of ficin from acid-denatured state at pH 2.5 to alcohol-induced state were studied by taking increasing volumes (0.182–3.828 ml) of various alcohols in different tubes and adjusting the volume to 4.5 ml in each tube with 20 mM glycine-HCl buffer, pH 2.5. Then, a constant volume (0.5 ml) of a stock protein solution, prepared in the same buffer (67.2 μM and 6.0 μM for CD and fluorescence measurements, respectively) was added to each tube. The contents of each tube were mixed well and the mixture was incubated for 30 min at 25ºC before CD/fluorescence measurements. Appropriate blanks were prepared by taking similar volumes of alcohols and making the total volume to 5.0 ml with 20 mM glycine-HCl buffer, pH 2.5. Far-UV CD spectral signal was used to monitor the secondary structural changes in the protein, whereas near-UV CD spectroscopy and Trp fluorescence were employed to study the tertiary structural changes. The contribution of the blank solution (containing buffer and alcohol) in both far- and near-UV CD spectral range was directly subtracted from the CD spectra of the test solutions. Rayleigh scattering measurements were made by measuring the emission spectra of various protein samples in the wavelength range 300-400 nm upon excitation at 350 nm. Lines in the transition curves were drawn as a guide for the eyes using the curve fitting mode of SIGMAPLOT software, version 11. The concentration of alcohol required to achieve 50% helix-induced transition (mid-point concentration) or C_m value was obtained from the transition curves at 50% of the helix-transition, whereas the m (dependence of ΔG_H on alcohol concentration) values were determined in the same way as described earlier [4].

RESULTS AND DISCUSSION

Far-UV CD spectra

Figure 2 shows the far-UV CD spectra of different conformational states of ficin obtained at pH 7.0 (native state) and at pH 2.5 both in the absence (acid-denatured state) and presence of 8 M TFE (alcohol-induced state).

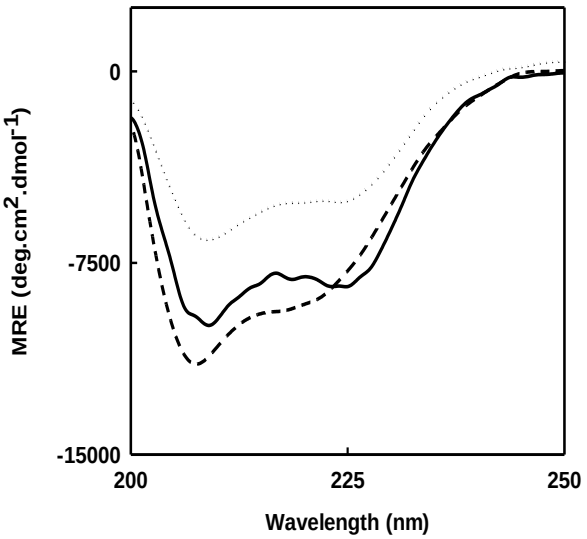

Fig. 2. Far-UV CD spectra of native ficin in 20 mM sodium phosphate buffer, pH 7.0 (──) and acid-denatured ficin in 20 mM glycine-HCl buffer, pH 2.5, both in absence (….) and presence (---) of 8 M TFE, obtained at 25ºC using a protein concentration of 6.72 μM.

As can be seen from the figure, the CD spectrum of native ficin is characterized by the presence of two minima around 208 and 222 nm, which are indicative of the presence of α-helical structure in the protein [33]. Using the MRE_{222nm} value, the percentage helical content in the native state of ficin was calculated as ~20%. This value was similar to the one reported earlier [34]. Although acid-denatured ficin at pH 2.5 retained CD spectral characteristics, it showed a significant decrease in the MRE values in the whole wavelength range of CD spectra. About 39% decrease in MRE_{222nm} was observed in the acid-denatured state of ficin by taking MRE_{222nm} of native ficin as 100%. In an earlier report [8], about 30% decrease in MRE_{222nm} was shown at pH 2.5. Such a decrease in MRE_{222nm} value suggested a significant loss (11%) in the α-helical content while it was calculated to be 9% in the acid-denatured state. Presence of 8 M TFE in the incubation mixture of acid-denatured ficin (ADF) showed a marked increase in the MRE values, which were found even higher at certain wavelengths than MRE values of the native state. A comparison of MRE_{222nm}, obtained with ADF both in the absence and presence of 8 M TFE suggested 74% increase

in MRE_{222nm} in presence of 8 M TFE, corresponding to 12% increase in the α-helical content. These results were in accordance with several earlier reports suggesting a strong helix-inducing potential of TFE [11, 15, 18, 19].

Alcohol-induced conformational transitions

Alcohols have been known to induce the formation of α-helical structure in proteins [15–18]. Alcohols with lower concentration required to produce 50% change in the structural transition are said to be more effective than others. Hence, the effectiveness of each alcohol differs from protein to protein and also varies among different alcohols [18–20]. In order to investigate the helix-inducing potential, as well as the effectiveness of various alcohols, *i.e.* fluoro alcohols (HFIP and TFE), chloro alcohol (2-chloroethanol) and both straight-chain and branched-chain alkanols (methanol, ethanol, 1-propanol, 2-propanol and *tert*-butanol), ADF was incubated at pH 2.5 with increasing concentrations of each of these alcohols for 30 min at 25ºC and induction of α-helical structure was studied using far-UV CD spectra. Figure 3 shows TFE-induced structural changes in ADF at pH 2.5 as monitored by far-UV CD spectra within the concentration range 1.0-8.0 M. Presence of TFE at lower (≤ 2.0 M), as well as higher (≥ 7.0 M) concentrations produced minimal changes in the CD spectra, whereas a marked increase in the MRE values was observed within the TFE concentration range 3.0-6.0 M.

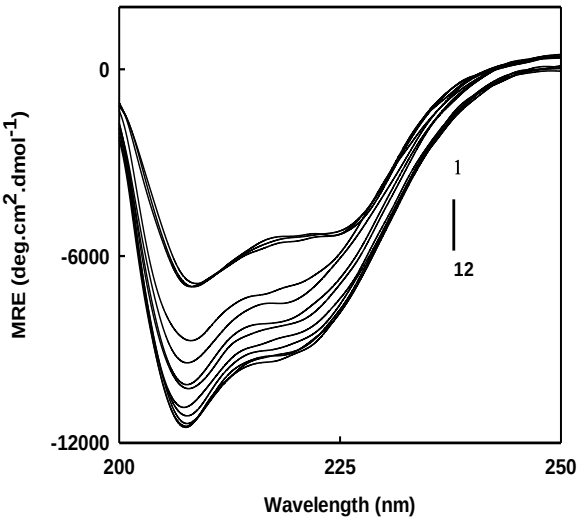

Fig. 3. Far-UV CD spectra of acid-denatured ficin in 20 mM glycine-HCl buffer, pH 2.5, obtained at 25ºC using a protein concentration of 6.72 μM in absence and presence of increasing TFE concentrations (from top to bottom): 0, 1.0, 2.0, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 7.5, 8.0 M.

In other words, a threshold concentration of TFE (~2.0 M) was required to induce significant α-helical structure in ADF, as reflected by the increase in MRE_{222nm} value which reached a maximum value at 7.0 M TFE concentration and remained unaffected thereafter (Fig. 3). Such transition was suggestive of TFE-induced refolding of acid-denatured state into a TFE-induced state, which can be clearly seen from Fig. 4 where MRE_{222nm} values of ADF obtained at different TFE concentrations are plotted against alcohol concentration.

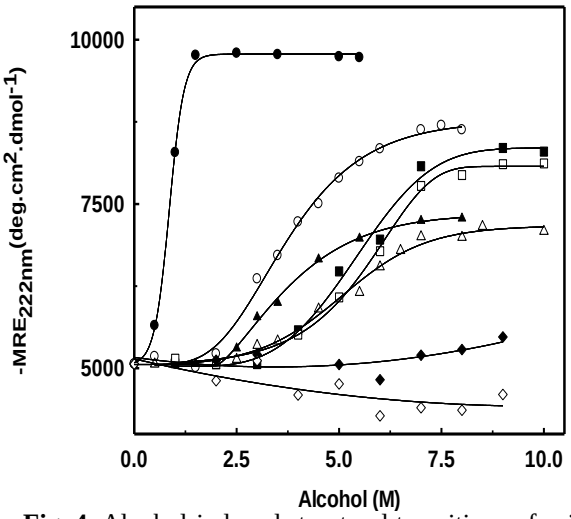

Fig. 4. Alcohol-induced structural transitions of acid-denatured ficin monitored by MRE_{222nm} at 25ºC, using a protein concentration of 6.72 μM. Transitions obtained with various alcohols are shown with different symbols: HFIP (●), TFE (○), *tert*-butanol (▲), 2-propanol (Δ), 2-chloroethanol (■), 1-propanol (□), ethanol (♦), methanol (◊).

Similar structural transitions were studied with different alcohols by monitoring the MRE_{222nm} values of ADF at increasing concentrations of these alcohols and the results are shown in Fig. 4. The alcohol concentration range selected in this study was sufficient to achieve the completion of the transition in most of the cases. Out of the eight alcohols studied, two short chain alkanols (methanol and ethanol) were found ineffective in inducing significant α-helical structure in ADF. Methanol at higher concentration was found to slightly disrupt the α-helical structure present in ADF whereas a slight increase in MRE_{222nm} value was noticed at higher ethanol concentrations. Although the remaining six alcohols were found effective in inducing the α-helical structure in ADF, differences in both the extent of the α-helical structure induced (MRE_{222nm} values) at the highest alcohol concentration and the concentration of alcohol required to achieve the maximal α- helical structure (MRE_{222nm} values) were observed.

Table 1. Characteristics of alcohol-induced transition of acid-denatured ficin obtained by MRE_{222nm} measurements.*

Alcohol	*m* (cal/mol/M)	C_m (M)	Maximum MRE_{222nm} ($deg.cm^2.mol^{-1}$)	α-Helix (%)
Alkanols				
1-Propanol	590	5.6	8117	19
2-Propanol	585	5.6	7156	16
tert-Butanol	612	3.6	7282	16
Halogenols				
2-Chloroethanol	595	5.4	8353	20
2,2,2-Trifluoroethanol	636	3.5	8624	21
1,1,1,3,3,3-Hexafluoro-2-propanol	4085	0.8	9793	25

* Values of *m*, C_m and maximum MRE_{222nm} were obtained from Figure 4 as described in the Experimental section.

Both fluoro alcohols (HFIP and TFE) showed the strongest helix-inducing potential in ADF among all alcohols used in this study. More precisely, HFIP was found to be the strongest one as it required the lowest concentration to induce the maximal α-helical structure as reflected from the highest MRE_{222nm} value (Fig. 4). Quantitative analysis of the titration curves shown in Fig. 4 was made by determining the *m* and C_m values as described in the Experimental section and these values along with the maximum MRE_{222nm} values achieved at the highest alcohol concentration and % helix content are listed in Table 1.

As can be seen from both Fig. 4 and Table 1, HFIP had the highest m value (4085 cal/mol/M), as well as the lowest C_m value (0.8 M) among all alcohols studied, whereas 2-propanol was found least effective in inducing the α-helical structure, as reflected from the lowest m value (585 cal/mol/M) and highest C_m value (5.6 M), thus requiring the highest alcohol concentration to achieve the maximum MRE_{222nm} value. Although different alcohols seem to induce different states based on the MRE_{222nm} values achieved at their highest concentration, MRE_{222nm} was still maximum with HFIP and minimum with 2-propanol (Fig. 4 and Table 1).

In general, halogenols showed higher influence in inducing the α-helical structure in ADF compared to alkanols. Several earlier studies have also shown stronger helix-inducing potential of halogenols than alkanols [11, 15, 16, 18]. Among halogenols, both HFIP and TFE were found as the most effective α-helix inducers in ADF, correlating positively with the number of fluorine (F) atoms in the alcohol, *e.g.* HFIP being more effective than TFE. This was in agreement with earlier reports, suggesting higher helix-inducing potential of HFIP due to the presence of a bulky alkyl group and abundance of F atoms [11, 15]. Although the effectiveness of halogenols has been found to increase in the group, as chlorine substituted alcohols have been shown to be more effective α-helix inducers compared to fluorine substituted alcohols [15], the presence of multiple F atoms in fluoro alcohols (HFIP and TFE) has been found to surpass the difference in effectiveness due to the presence of Cl and F atoms in halogenols [11, 15]. This is illustrated by the greater effectiveness of HFIP containing 6 F atoms in inducing α-helix than TFE containing 3 F atoms (Fig. 4 and Table 1). Among alkanols, the helix-inducing potential varied with the number of methyl groups (bulkiness) in the alcohol, being more effective with *tert*-butanol compared to 1-propanol, though branching in 2-propanol slightly decreased its potentiality. Resuming, the effectiveness of different alcohols in inducing α-helical structure in ADF was found to follow the order: HFIP > TFE > *tert*-butanol > 2-chloroethanol > 1-propanol > 2-propanol. This order was in agreement with previous reports showing similar effectiveness of various alcohols in inducing α-helix in other proteins [15, 16]. In view of the above and regardless of the mechanism, the number of the halogen atoms in halogenols and the bulkiness of the alkyl group along with their structural arrangements in the alcohol are considered to be important factors in determining the effectiveness of these alcohols in inducing the α-helical structure [11, 18, 20].

Near-UV CD spectra

In order to study the effect of alcohol on the tertiary structure of ADF, near-UV CD spectra of native ficin and ADF both in absence and presence of 8.0 M TFE were recorded in the wavelength range 250-300 nm (Fig. 5). Near-UV CD spectroscopy is a useful approach to study the tertiary structural changes in proteins by monitoring the change in the environment of aromatic amino acids [35]. As can be seen from Fig. 5, two maxima

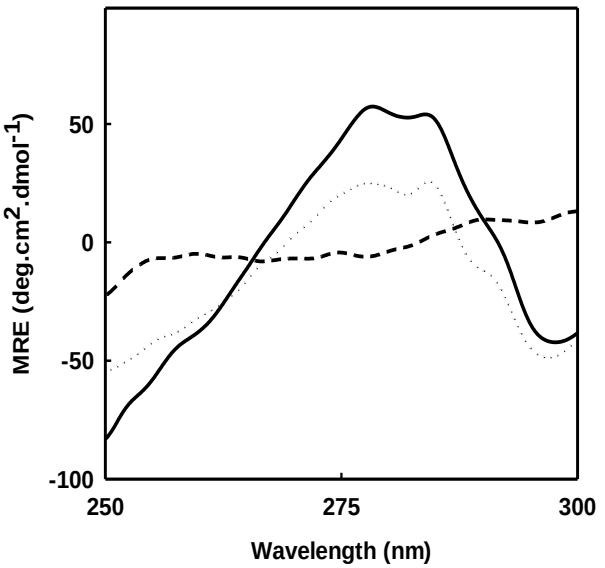

Fig. 5. Near-UV CD spectra of native ficin in 20 mM sodium phosphate buffer, pH 7.0(—) and acid-denatured ficin in 20 mM glycine-HCl buffer, pH 2.5, both in absence (…) and presence (---) of 8 M TFE, obtained at 25ºC using a protein concentration of 37.8 μM.

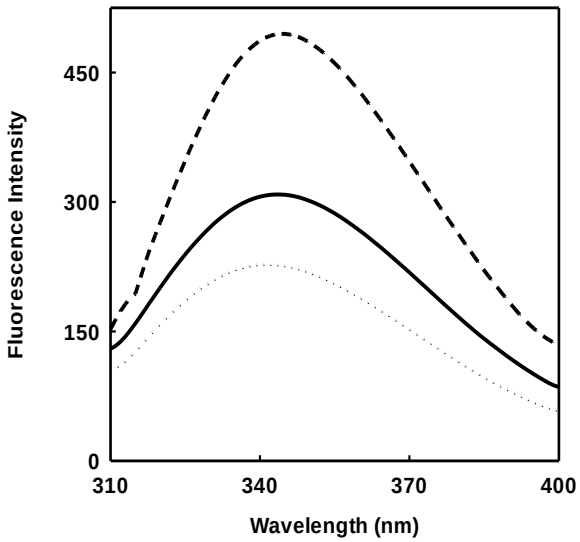

Fig. 6. Tryptophan fluorescence spectra of different conformational states of ficin obtained at 25ºC using a protein concentration of 0.6 μM upon excitation at 295 nm. Native state at pH 7.0 (—), acid-denatured state at pH 2.5 (…..) and acid-denatured state in the presence of 5.5 M tert-butanol (---).

at 277 nm and 283 nm along with a minimum at 298 nm characterized the near-UV CD spectrum of the native enzyme. These features were similar to those described in an earlier report [8]. While these signals were retained in the near-UV CD spectrum of ADF, MRE values at both maxima were significantly reduced. Presence of 8 M TFE in the ADF solution resulted in a complete loss of these signals, suggesting disruption of the tertiary structure of the protein in this case. These results agreed well with previous reports showing loss in the protein tertiary structure in the presence of alcohols at higher concentrations [36, 37].

Fluorescence spectra

To validate the results obtained from near-UV CD spectra about the alcohol-induced tertiary structural changes in ficin at pH 2.5, Trp fluorescence spectra of native ficin and ADF both in absence and presence of 5.5 M *tert*-butanol were studied (Fig. 6). As evident from the figure, the fluorescence spectrum of native ficin is characterized by the presence of an emission maximum at 344 nm, which is blue shifted by 3 nm and is accompanied by a 26% decrease in the fluorescence intensity at pH 2.5. Devaraj *et al.* [8] also reported significant decrease in the fluorescence intensity along with a blue shift in the fluorescence spectrum of ficin at acidic pH. Decrease in the fluorescence intensity at the emission maximum was indicative of the change in the microenvironment around the Trp residues from nonpolar to polar, suggesting tertiary structural changes in the protein.

These results supported our near-UV CD spectral data showing tertiary structural changes at pH 2.5. The possible reason for the small blue shift observed in the fluorescence spectrum of ficin at pH 2.5 stems from the heterogeneity of the fluorescence of multi-Trp residues [38] as ficin contains 6 Trp residues [22]. A marked increase in the fluorescence intensity along with normalization of the emission maximum (344 nm) was observed in the fluorescence spectrum of ficin at pH 2.5 in presence of 5.5 M *tert*-butanol. Such an increase in the fluorescence intensity indicated movement of Trp residues in the nonpolar environment. This seems probable, as these alcohols induced increased α-helical structure in ADF, as evident from Fig. 4. In the presence of increased helical structure in the alcohol-induced state of ficin, burial of Trp residues within and in between helical segments cannot be ruled out which might explain the increase in the fluorescence intensity. The increase in fluorescence intensity of ADF in presence of 5.5 M *tert*-butanol should not be taken as an indicator of reformation of the tertiary structure in the protein, as near-UV CD data had already confirmed disruption of the tertiary structure.

tert-Butanol-induced increase in the Trp fluorescence intensity of ficin at pH 2.5 prompted us to study the effect of various alcohols on the Trp fluorescence intensity of ADF. Titration results of ficin at pH 2.5 with increasing concentrations of different alcohols monitored by Trp fluorescence measurements at 341 nm are shown in Fig. 7.

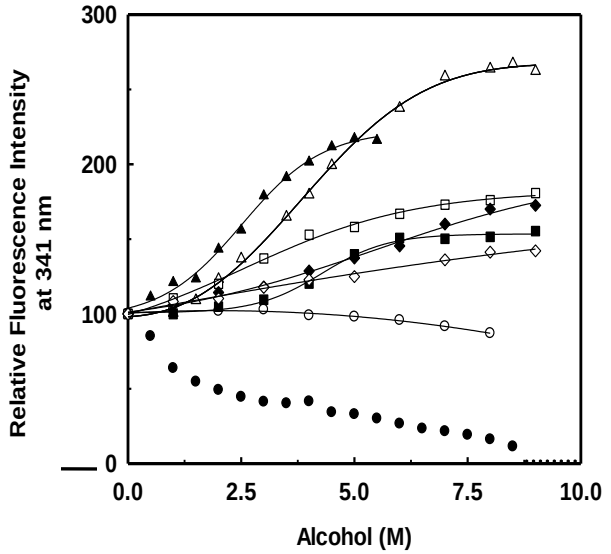

Fig. 7. Alcohol-induced structural transitions of acid-denatured ficin monitored by Trp fluorescence at 25ºC using a protein concentration of 0.6 μM upon excitation at 295 nm. Transitions obtained with various alcohols are shown with different symbols: HFIP (●), TFE (○), *tert*-butanol (▲), 2-propanol (Δ), 2-chloroethanol (■), 1-propanol (□), ethanol (♦), methanol (◊).

The possibility of aggregation in the presence of alcohol was ruled out based on the insignificant light scattering in these samples. Both fluoro alcohols (HFIP and TFE) showed anomalous behavior compared to that shown by other alcohols. For example, increasing concentrations of HFIP led to a significant quenching of Trp fluorescence in the initial range of alcohol concentrations, which sloped off at higher alcohol concentrations (Fig. 7). TFE also produced a decrease in the fluorescence intensity but it was much smaller than that obtained with HFIP and was observed only at higher alcohol concentrations. In an earlier study, specific quenching of Trp fluorescence intensity of cytochrome c was also observed in presence of HFIP [16]. Such quenching of Trp fluorescence can be ascribed to the energy transfer from Trp residues to other constituents of the protein. The decrease in the Trp fluorescence intensity of ficin at pH 2.5 in presence of lower HFIP concentrations reflected a compact denatured state, as has been shown in an earlier study with cytochrome c [16]. Contrary to it, all alkanols produced an increase in Trp fluorescence intensity with the increase in alcohol concentration. However, different alkanols showed different behavior in terms of the extent of increase in the fluorescence intensity and the concentration of alcohol required to achieve maximum fluorescence intensity for such alcohol. Of the six alkanols, both *tert*-butanol and 2-propanol were found more effective in bringing transition from acid-denatured state to an alcohol-induced state, compared to other alkanols. In general, the order of effectiveness among various alkanols was found to be: *tert*-butanol > 2-propanol > 1-propanol > ethanol > methanol. Similar order of effectiveness of various alkanols was observed in an earlier study on human serum albumin [18]. In view of these results, bulkiness of the alkyl group seems to be the main factor controlling the effectiveness of these alcohols.

As shown above, addition of both halogenols and alkanols induced α-helical structure in ADF, while with regard to disrupting the tertiary structure at higher concentrations, halogenols were more effective than alkanols. On the other hand, alkanols increased Trp fluorescence intensity while halogenols quenched it. Although many studies have shown the role of alcohols to increase the helical content of proteins, the mechanism by which alcohols induce the formation of α-helix has not been established yet [39]. A direct interaction of alcohol in micelle-like form [40] with the unfolded protein has been suggested to induce helical structure by decreasing the environmental polarity around the polypeptide chain, thus stabilizing the local hydrogen bonds [14]. The stronger ability of HFIP to form micelle-like HFIP clusters has also been proposed to explain the increase in its effectiveness in inducing helical structure. In view of these, the greater effectiveness of fluoro alcohols might be attributed to the bulkiness of the alkyl group, the presence of multiple F atoms and the formation of micelle-like clusters in an alcohol-water mixture. Although ficin showed unusual resistance towards urea denaturation [25, 26], it was found similar to other globular proteins in terms of its behavior towards alcohol-induced transition.

Both halogenols and alkanols (except ethanol and methanol) were found to induce an α-helical structure in ADF in the following order: HFIP > TFE > *tert*-butanol > 2-chloroethanol > 1-propanol > 2-propanol. However, the presence of these alcohols led to the disruption of the tertiary structure of ficin, as evident by the near UV CD spectra and Trp fluorescence. This behavior was similar to that observed with other globular proteins.

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СТРУКТУРНИТЕ ПРЕХОДИ В КИСЕЛИННО ДЕНАТУРИРАН ФИЦИН, ИНДУЦИРАНИ ОТ ХАЛОГЕНОЛИ И АЛКАНОЛИ

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(Резюме)

Изследван е ефекта на осем различни алкохоли, включително халогеноли и алканоли, върху структурната трансформация на киселинно-денатуриран фицин (ADF) при H 2.5 с помощта на далечно и близко-вълнов UV кръгов дихроизъм (CD) и флуоресценция на триптофан. Освен метанола и етанола всички останали алкохоли индуцират α-спираловидна структура на ADF, което се вижда от повишаването на MRE_{222nm}- стойностите. Флуор-заместените алкохоли показват по-висок спирало-индуциращ потенциал в сравнение с 2-хлоретанола и алканолите. Тяхана ефективност е в реда: 1,1,1,3,3,3-хексафлуоро-2-пропанол > 2,2,2-трифлуороетанол > *tert*-бутанол > 2-хлоретанол > 1-пропанол > 2- пропанол. Близките UV CD - спектри показват разкъсване на третичната структура в присъствие на алкохоли. Триптофановата флуоресценция на ADF се влияе различно в присъствие на тези алкохоли, показвайки подтискане при флуоро-алкохолите и повишение при 2-хлоретанола и алканолите в реда: *tert*-бутанол > 2-пропанол > 1-пропанол > етанол > метанол. Получените резултати показват, че ефективността на алкохолите добре се корелира с броя на флуорните атоми, размера и разположението на различните алкилови групи.