THE EFFECTS OF 1-PROPANOL ON BEHAVIOUR OF HUMAN SERUM ALBUMIN IN ALKALINE SOLUTION

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Dedicated to the memory of Professor Jiří Dvořák.

The effects of 1-propanol and to a certain extent of ethanol on human serum albumin were studied over the pH range 7 – 13.3 and alcohol concentrations up to 20 vol.%. In some cases behaviour of the native preparation was compared with albumin cleared of weaker bound fatty acids. The data obtained by UV-spectrophotometry were discussed from the point of view of individual types of chromophores as well as in a broader context of the secondary structure. The results can be summarized as follows: (a) Partial removal of bound fatty acids has an influence on the dissociation of tyrosines. (b) The effect of alcohols on this dissociation is rather complex, the permitivity of the solvent being only a part of it. (c) At high alkaline pH a series of peaks in the fourth-derivative absorption spectra appear in the region 305 – 320 nm. These peaks were shown to correspond to buried dissociated tyrosines. (d) In the presence of 1-propanol a small conformational transition of albumin molecule is observed at pH below 9.

Human serum albumin (HSA) belongs to the most thoroughly studied blood proteins not only for relative availability, but because of its physiological importance and physicochemical properties as well. The first property studied in detail was an extraordinary ability of HSA to bind reversibly different ligands[1-4]. One group of them, fatty acids, turned out to be an important factor determining the stability of this protein (for discussion of fatty acids binding see Kragh-Hansen[5], for general questions of ligand binding Steinhard and Reynolds[6]).

The relation between pH and protein binding of HSA was investigated by Tanford[7], who found that approx. 40 carboxylic groups buried in the native conformation are exposed to the solvent when pH is lowered to 4 – 4.5. In alkaline media three types of variously accesible tyrosines were found[8] and identified later on the basis of primary structure of HSA elucidated independently by Meloun with coworkers[9] and Brown[10].

Beginning from the pioneer studies on the conformation of both human and bovine serum albumin based on hydrodynamic[11,12], potentiometric[13] and optical studies[14], three dimensional structure of this protein was proposed[15]. HSA contains 585 amino acids forming one chain with 17 disulfide bridges. Characteristic structural features of

this protein are three repeating domains of very similar conformation. Recently a low resolution (6 Å) crystallographic survey of HSA was performed[16,17]. Molecule of HSA is a flexible body which is able to undergo pH-dependent steric transitions assuming in this way one of five isomeric forms: N (native), F (fast), E (expanded), B (basic) and A (aged). These forms, except for B and A, differ in their α-helix content, the highest being in N form (≈ 55 %, ref.[15]).

The aim of this work was to study simultaneous effects of a small aliphatic alcohol and pH on the structure and stability of HSA. The effect of alcohol is rather complex; in the first approximation it can be considered a result of the alcohol binding to the protein and of the change in the structure of the solvent. At the same time attempt was undertaken to obtain additional information about the role of fatty acids in stabilization of HSA.

EXPERIMENTAL

Materials

Human serum albumin (medical purposes quality) was a product of IMUNA (Šarišské Michaľany), its electrophoretic purity exceeded 97%. As confirmed by polyacrylamide gel electrophoresis the content of dimer was negligibly small. Prior to experiments the protein was desalted on Sephadex G-25 (Pharmacia, Uppsala) column. HSA solutions were prepared by dilution of the stock solution in redistilled water, the concentration of which was determined gravimetrically (samples were dried in vacuo above P_2O_5 at 105 °C). Molecular weight of HSA was assumed to be 66 500 (ref.[15]).

In some experiments the defatted preparation was used (DFA HSA) prepared according to Lee and McMenamy[18]. In this case desalting procedure did not precede this treatment and removal of fatty acids and desalting was carried out in a single step. Concentration of salts in eluant was determined by conductivity. For an estimation of fatty acid (FA) content the modification of the method[19] for the determination of FA in the whole plasma was worked out[20].

Reagents, all of analytical grade purity, were products of Lachema (Brno), except for N-acetyl-L-tyrosine ethylester (NATyrE) produced by Sigma. Volumetric solutions of KOH and NaOH were carbonate free. Throughout this paper the concentration of alcohol will be expressed as a volume fraction (φ) in per cents. As confirmed by gas chromatography alcohols used for the experiments did not contain any impurities.

Measurements

Titrations of HSA were performed in two ways:

A) Potentiometric titration. In this case 2 – 3 wt.% HSA solution was titrated in a continuous way using volumetric solution of 0.2 M KOH, resp. 0.2 M HCl. The values of pH were measured with a pHM 4c pH-meter (Radiometer, Copenhagen) equipped with the glass electrode G-202B and calomel electrode K 401 from the same manufacturer. Solid KCl was added to reach the ionic strengh 0.1. Temperature was kept constant at 25 ± 0.1 °C. The average number of bound/dissociated protons, $\bar{h}$, was calculated using usual equation[21]:

$$\bar{h} = c / g\,[1 - \text{antilog}\,(\text{pH}_1 - \text{pH}_2)].$$

In this equation c is the concentration of KOH (mol dm^{-3}) which in the same volumes of HSA solution resp. blank leads to pH_1 (HSA) or pH_2 (blank). The concentration g of the protein is expressed in g dm^{-3}.

B) Spectrophotometric titrations were carried out with a SP 8800 spectrophotometer (Pye Unicam) using the difference technique. For each point of the titration curve fresh solution was prepared by mixing the HSA stock solution with appropriate amount of Britton–Robinson's buffer and alcohols. For pH's higher than 11.5 volumetric solutions of NaOH instead of the buffer were used. As a reference the same solution of pH 7.1 was used; starting concentration of HSA in all samples was 0.2 wt.%. In these experiments final pH of the solutions was measured with an OP-281/1 pH-meter with combined electrode OP-0808P (Radelkis, Budapest), or a pHM 64 pH-meter (Radiometer, Copenhagen) with combined electrodes GK-2401C or GK-2402B. The relation between difference absorbancy (ΔA) and the number of tyrosyl groups of the type i (N_i) with the dissociation constant pK_i is expressed by the equation[22]

$$\Delta A = c\, l \sum_{i=1}^{M} N_i \Delta\varepsilon_i \,.\, 10^{-pK_i}/(10^{-pH} + 10^{-pK_i}),$$

where c is protein concentration, l is optical path, M is the number of different types of tyrosines and $\Delta\varepsilon_i$ is the differential molar absorption coefficient of the dissociated group of the type i. The pK_i values were determined from this equation using the least-square non-linear method on the assumption that there are no differences within an i-th group. Values of $\Delta\varepsilon_i$ were calculated from absorbancies of NATyrE solutions volume changes of which upon addition of alcohols was taken in consideration. The excess partial molal volumes were taken from Franks[23]. Calculations were performed for the values obtained at the wavelength 244 nm.

Determination of pH. In mixed solvents pH-meter readings should be corrected for the solvent effects on the activity coefficient of H_3O^+ ions as well as for its direct influence on electrodes. In this work for each pH-meter and each set of electrodes correction was made as described previously[24].

Fourth-derivative spectrophotometry (FD spectrophotometry). In order to enhance small differences in ultraviolet absorption of HSA an attempt was made to use the fourth-derivative of the absorption curve. The method described by Padros and collaborators[25] was employed. These measurements were made with a SP 8800 spectrophotometer (Pye Unicam), for the wavelenghts 238 – 330 nm. Optical path was 1 cm, registration speed 10 nm s^{-1}.

CD spectra were registered on a Jasco ORD/UV-5 spectropolarimeter with CD equipment. Concentration of HSA varied between 0.1 wt.% (for the range 250 – 210 nm) and 0.2 wt.% (300 – 250 nm). For the estimation of percentual α-helix content following equation[26] was used:

$$\alpha\,[\%] = \frac{-[\theta]_{222} + 3\,100}{35\,000}\,100,$$

where $[\theta]_{222}$ is the molar residual elipticity calculated according to Freifelder[27].

RESULTS

Removal of Fatty Acids

It turned out that the method of Lee and McMenamy[18] does not lead to the complete removal of all FA bound to HSA. The analysis of the original native HSA revealed the average number of 2.85 mol of FA bound per one mole of HSA. After treatment 1.5 mol of FA were still bound. This technique should thus be characterized rather as a

partial removal of FA, obviously of these not firmly bound. The remaining FA molecules are presumably attached to the two high affinity sites[5] in the first binding region of HSA situated in the subdomain III A–B. This treatment of HSA made it nevertheless possible to judge about stabilizing effect of weaker bound fatty acids.

Titration Behaviour of HSA

The alkaline part of HSA titration curve is shown in Fig. 1 for the native protein in water solution at ionic strength 0.1 and in 20% propanol. As expected the alkaline part of the titration curve in alcohol is slightly rotated in the direction of the lower pH and the difference between both curves increases markedly with pH. This difference expressed as $\Delta\bar{h}$ can be plotted against pH (inserted in Fig. 1). The resulting differential curve suggests that dissociation in propanol occurs in steps, when additional groups dissociate compared to the same process in water. After initial almost linear increase the first step is observed between pH's ≈ 9.3 and 10.2 followed by the next to pH ≈ 11. Very sharp increase in the difference titration curve seems to exhibit tendency to reach a limiting value of $\Delta\bar{h}$, which cannot be determined due to irreversibility of HSA titration at such an extreme pH. The linear part of the $\Delta\bar{h}$ vs pH curve represents additional dissociation of 1.5 groups in the presence of propanol, the first and second step further 2 and 4 groups, respectively. In the third step 15 groups dissociate up to pH 12.3.

In the spectrophotometric titration solely dissociation of accessible tyrosines (in the native or seminative state) was considered. Because of pK of fully masked groups (close to 13, ref.[28]) it can be supposed that they do not dissociate to a significant extent below pH 12. For calculation of pK's certain assumption must be made concerning

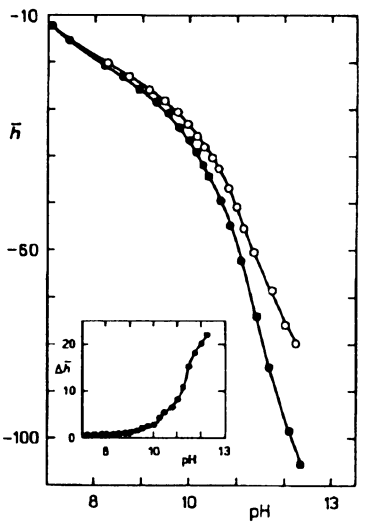

FIG. 1
The titration curves of HSA in aqueous solution (○) and in 1-propanol (20%: ●). The curve is drawn by hand; for the sake of clarity only a part of the experimental points is shown. Inserted: the difference between both curves ($\Delta\bar{h}$) as a function of pH ($\bar{h}$ is the number of dissociated protons per mole of HSA)

tyrosyl groups in general. As found by Morávek and coworkers[8] there are three kinds of tyrosyl groups in HSA: one fully exposed residue (pK_1), nine partially exposed (pK_2) and eight fully buried. In the present paper it was assumed that $\Delta\varepsilon_i$ does not differ for fully and partially exposed tyrosyl groups[22]. The values of protein absorbancy were then compared with data obtained with the model compound (NATyrE). Based on the distribution of tyrosines among two classes their pK values were optimized in order to reach the best fit with the experimental titration curve. The results are given in Table I. In order to separate the effect of permitivity change caused by propanol from other possible effects (e.g. steric) this experiment was repeated with the addition of ethanol in such concentration that the resulting permitivity (69) was the same as the permitivity of the propanol solution used (69.5). Two important conclusions can be drawn from the data given in Table I: 1. pH changes do not correlate with the permitivity of the solution; 2. even partial removal of FA is reflected by dissociation behaviour of tyrosines.

CD Spectra of HSA

Opposite to the previous observation CD spectra of HSA and DFA HSA did not differ significantly what can be considered a proof that differences shown in the titration curves are not due to the changes of the secondary structure. CD spectra of both preparations of HSA showed a small negative maximum at 262 nm corresponding to disulfide bridges. This part of the spectrum, between 250 and 300 nm, did not change neither with pH nor with the addition of alcohol. The conformation of S–S bonds is thus not altered up to pH 12.

In the region 250 – 210 nm negative maximum corresponding to peptide bond shifted with increasing alcohol concentration from 217.5 nm (0% propanol) to 220 nm (15% propanol). This effect is due to the decrease in the permitivity of the solvent as well as to the increase in α-helix content when further parts of peptide bond are transformed into less polar environment. The effect of added propanol on α-helix content is shown in Fig. 2 as a function of alcohol concentration. At moderately alkaline pH the curves are identical their characteristic feature being increase in α-helix content after the first addition of alcohol. It is in agreement with generally accepted conception of the stabilizing effect of small aliphatic alcohols on proteins[20–31]. The content of α-helix decreases gradually with increasing pH, but even at low concentration propanol supports formation of α-helix even at pH 12.7, when HSA molecule is significantly destabilized. The α-helix content at the constant propanol concentration decreases with pH stepwise (Fig. 3). The first, small decrease, is observed at pH 10 – 10.5, the second, very abrupt one, occurs in the pH region 11.8 – 12.8.

FD Spectrophotometry

An attempt was undertaken to use the FD spectrophotometry for elucidation of behaviour of chromophores in HSA. This method was applied in the same way as described by its authors[32,33] and the FD spectrum of native HSA is shown in Fig. 4. As crucial parameters of these spectra the amplitude a and the ratio $R = a_1 / a_2$ are considered, first of them reflecting the state of phenylalanines, while the second one reacts in a more complicated manner on tyrosines and tryptophanes simultaneously. FD spectra were measured as a function of alcohol concentration as well as of pH. Their general feature at high pH's was very strong decrease of a accompanied by the blue shift of λ_2 by up to 6 nm. At the same time distinct peaks appeared in the region 305 – 320 nm (Fig. 5).

Behaviour of tyrosine residues could be estimated qualitatively only because of rather high error in determination of R. For both HSA preparations the parameter R increased with pH its value being lower for HSA up to pH 10. Above pH 11 the picture was opposite with R higher for DFA HSA.

DISCUSSION

The influence of small aliphatic alcohols on proteins is of complicated nature. On general approach at least following two processes should be taken in consideration:

a) The sole presence of alcohol leads to pronounced changes in the structure of water described only qualitatively so far. These changes are reflected for example by sharp

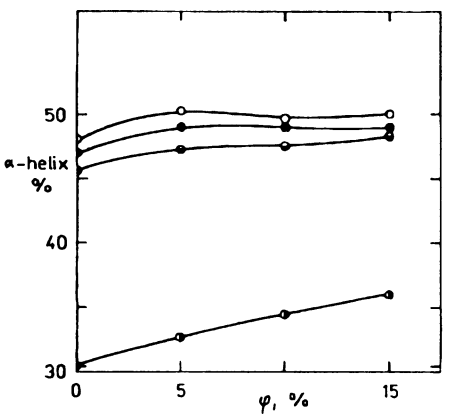

FIG. 2
The α-helix content of HSA as a function of 1-propanol concentrations (volume fraction of alcohol φ, %): pH: ○ 7.7, ● 9.9, ◒ 10.5, ◑ 12.7

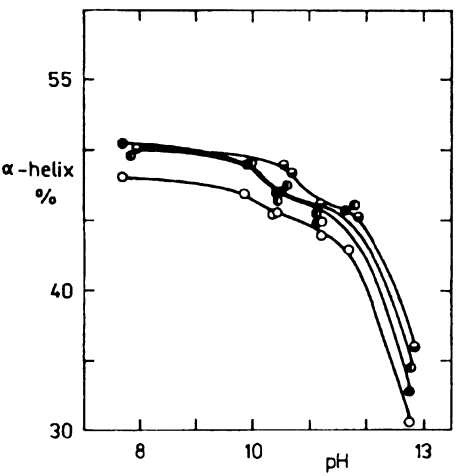

FIG. 3
The α-helix content of HSA as a function of pH. Concentrations of 1-propanol (volume fraction, %): ○ 0, ● 5, ◐ 10, ◒ 15

decrease in the excess enthalpy of mixing (for data see Westmeier[34]) with the minimal value for 1-propanol at molar fraction 0.1. The concentration of alcohols chosen for our experiments fall within this region. At higher alcohol concentrations water is supposed[35] to return to more normal behaviour. Another macroscopic quantity important for behaviour of proteins is the permitivity of the solvent (the data used in this paper are by Åkerlöf[36]).

Both these effects are acting simultaneously, but while the structural changes of water could have exerted influence on the protein molecule which is difficult to predict, decrease in permitivity in alcoholic solutions enhances the electrostatic interactions.

b) The second process occurring in mixed solvents of this kind is the binding of alcohols to protein molecules. No high specifity can be expected, because small aliphatic alcohols can be bound by hydrogen bonds as well as by hydrophobic interactions. With increasing hydrocarbon chain the second type of binding should be expected to prevail. As regards the binding of small alcohols to proteins no reliable data are available hitherto.

Considering all these facts our results will be discussed from two standpoints: 1. on the level of individual groups in the protein molecule; 2. on the level of the secondary structure and of the conformational changes of the protein molecule. Both approaches have certain points in common.

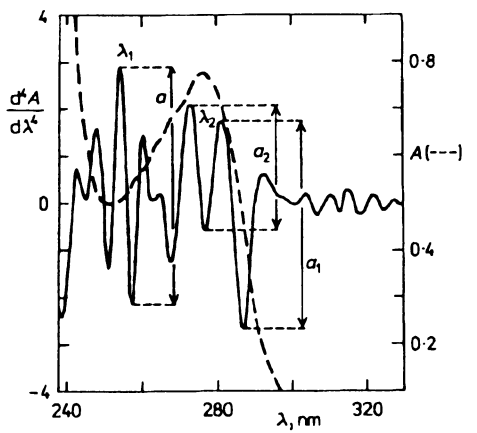

FIG. 4

The fourth-derivative spectrum of HSA. The amplitude a reflects the state of phenylalanines, the ratio a_1/a_2 reflects behaviour of tyrosines; dashed line shows the absorption spectrum; arbitrary units

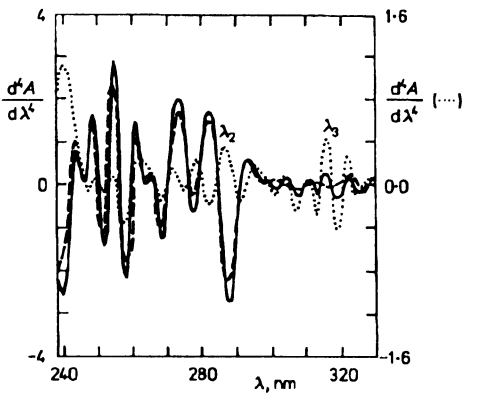

FIG. 5

The fourth derivative spectrum of HSA: (pH: 7.1 (———), 10.0 (– – – –), 13.3 (· · · · ·), arbitrary units

Changes of Behaviour of Individual Groups

The alkaline part of the titration curve of HSA shows (Fig. 1) that the presence of 1-propanol results in easier ionization of titratable groups, what is in the first approximation explained as the effect of lower permitivity of the solution. Our results with the dissociation of tyrosines revealed more complex picture.

The first question was the influence of fatty acids on the dissociation of these groups. The differences between pK's of tyrosines in native and DFA HSA (Table I) demonstrate that even removal of weaker bound FA exerts a destabilizing effect on this protein, particularly on the second class of tyrosines partially buried. Investigation of the dissociation of tyrosines was performed with two alcohols, ethanol and 1-propanol, keeping the permitivity the same for both solutions. Under such conditions the electrostatic effects should be the same and thus any difference in ionization behaviour of tyrosines should obviously be of another, non-electrostatic origin.

As evident from the Table I, the addition of alcohols resulting in higher α-helix content has marked effect on the dissociation of tyrosines. The effect of FA removal on pK_2's is almost compensated by alcohols. The dissociation of the first tyrosine is significantly hindered in the presence of ethanol.

Any discussion about ionization behaviour of tyrosines is complicated by the fact that they are located in environments of very differing hydrophobicity. If hydrophobic profile of HSA is calculated on binary basis as a sliding window of 9 amino acids[37] it is apparent that the majority of tyrosines is located in regions of higher hydrophobicity

TABLE I
The dissociation constants of tyrosines in the native (HSA) and partially defatted (DFA HSA) albumin, and the differences ΔpK = pK(HSA) – pK(DFA HSA)

pK	Albumin	Solvent		
		water	water/ethanol[a]	water/1-propanol[b]
pK_1	HSA	9.68	9.71	9.64
	DFA HSA	9.58	9.99	9.69
ΔpK_1		+0.10	–0.28	–0.05
pK_2	HSA	11.88	12.03	11.77
	DFA HSA	11.46	11.94	11.63
ΔpK_2		+0.42	+0.09	+0.14

[a] φ = 20%; [b] φ = 15%.

but there is no significant correspondence between their accesibility and hydrophobicity (Table II).

If pK_1 is the value for the only accesible tyrosine (411), then the difference in pK's (Table I) means that removal of FA, although not complete, did touch their binding region[5] in the domain III of HSA molecule. Removed FA are then substituted by molecules of alcohols, but ethanol because of its smaller size can get in a closser contact with the structures of the protein molecule. The interaction of ethanol based on hydrogen bonds and hydrophobic interactions allows firmer binding than it is the case of 1-propanol with prevailing hydrophobic interactions. Stronger binding of ethanol could have hindered the dissociation of Tyr 411 as evidenced by ΔpK_1 in the presence of this alcohol compared with the corresponding value for 1-propanol.

Simultaneously with this kind of interaction electrostatic forces are enhanced due to lowering of permitivity in the solutions of alcohols. This effect should be reflected by pK values as well. In the alkaline region tyrosines are an exception since their dissociation yields a negatively charged form. Consequently, on decrease of permitivity their pK should increase. The electrostatic contribution to this change can be calculated[38] as the activity coefficient γ_e. For phenolic hydroxyl groups dissociating as an acid their pK^* in a mixed solvent can be expressed as

$$pK^* = pK_W + \log \gamma_e ,$$

where pK_W is the value in aqueous solution. When phenolic –OH is approximated as a sphere of the diameter $2 \cdot 10^{-8}$ cm, and for the relative permitivity 70, the value of $\log \gamma_e$ is +0.10. This value can be compared with experimental data expressed as the

TABLE II

The distribution of tyrosines according to the hydrophobic profile[37] of HSA molecule (sliding window of 9 amino acids: pe partially exposed, b buried group according to Meloun et al.[9]: the only fully accessible is Tyr 411 in the window 5)

	The value of the sliding window for Tyr as the central amino acid							
	4		5		6		7	
	pe	b	pe	b	pe	b	pe	b
Residue No.	341	353	30	84	161		150	332
	401		334	138	148			370
	497			140	452			
				263				
				319				
			411					

difference $\Delta pK = pK^* - pK_W = \log \gamma_e$ (Table III). It is apparent at once that the electrostatic contribution is not the sole effect of alcohols on the dissociation of tyrosine. These data support the assumption of stronger binding of ethanol to DFA HSA. The effect of 1-propanol on this preparation of albumin is in better agreement with the above calculation what can be explained by the less direct influence of this alcohol, mediated by the changes in the structure of the solvent, what results in the decreased permitivity.

In the native HSA the picture is quite different. While for ethanol ΔpK values correspond more to electrostatic contribution as the prevailing effect when the protein molecule is fully occupied by FA, 1-propanol must exert additional influence on the conformation of HSA, which is not detectable by circular dichroism. These effects, nature of whose should be studied, make the dissociation of tyrosines easier.

The FD spectra evaluated in the region 270 – 320 nm support the conclusion drawn from spectrophotometric titration. The parameter R increases with strenght of hydrogen bond and with heterogeneity of chromophores[33]. Both these factors are present here. Heterogeneity of tyrosines based on their hydrophobicity was discussed already (Table II); the state of hydrogen bonds is revealed by the maximum of FD spectrum located in the vicinity of 280 nm. In the presence of 1-propanol this maximum is blue shifted from 282 nm (pH 7 – 10) to 276 nm (pH 13.2). This shift is an evidence of the formation of hydrogen bonds with tyrosines as the proton acceptors[33]. This observation suggests that with increasing pH the heterogeneity of tyrosines increases due to structural changes of HSA molecule when more groups are exposed to the solvent. Simultaneously, with dissociation of partially buried tyrosines hydrogen bonds are formed between them and alcohols, dissociated tyrosines being here proton acceptors. In DFA HSA more space is left to alcohol molecules to get closer to the protein. This higher possibility of hydrogen bond formation is reflected by the parameter R which is higher for DFA HSA at pH above 11.

TABLE III
The electrostatic contribution to pK's of tyrosines in the presence of ethanol and 1-propanol, $\Delta pK \equiv \log \gamma_e$

ΔpK	Ethanol [a]	1-Propanol [b]
ΔpK_1 [c]	+0.03	+0.04
ΔpK_2 [c]	+0.15	−0.11
ΔpK_1 [d]	+0.41	+0.11
ΔpK_2 [d]	+0.48	+0.17

[a] φ = 20%; [b] φ = 15%; [c] HSA; [d] DFA HSA.

At pH 13 the value of λ_1 and R are the same as those found earlier[32] for the model compound (NATyrE) in dimethylformamide which suggests that in these conditions only non-dissociated buried groups absorb. At higher pH's a series of peaks appear in the region 305 – 320 nm (Fig. 5), which was not described by the authors[32] of the FD spectroscopy. The number of peaks and their location corresponds to a relatively narrow Gaussian absorption band with λ_0 = 316.5 nm. This band appears at pH 11.2 and its intensity increases with pH. All these facts support the evidence that it is the absorption band of dissociated buried tyrosines. The position of the maximum λ_3 is in excelent agreement with this value for dissociated phenol in dimethylformamide[39]. It further supports the hypothesis[39] that buried dissociated tyrosines absorb in a different manner compared with accesible chromophores.

The Secondary Structure and Overall Shape of HSA Molecule

As expected, α-helix content increases in the presence of 1-propanol (Fig. 3). The marked step at pH ≈ 10 observed in the aqueous solution corresponds to B → A transition, which is accompanied by deeper structural change concerning particularly the α-helix content. The effect of 1-propanol at constant pH is not as pronounced (Fig. 2), but evidently the largest change takes place upon the first addition of alcohol. Certain interrelation can be observed between the number of additionally dissociated protons $\Delta\bar{h}$ (insert in Fig. 1) and the shape of α-helix vs pH curve (Fig. 3). A steep increase in $\Delta\bar{h}$ is observed from pH 10 when α-helix content starts to decrease rapidly. From pK_2 we can estimate that tyrosines dissociate at higher pH's so that this portion of $\Delta\bar{h}$ vs pH curve reflects the dissociation of lysines. Their pK should be lowered due to the lower permitivity of the solution. Like the other amino acids in HSA lysines are heterogeneous with respect to their hydrophobicity. One of the effects of small aliphatic alcohols is enhancement of these differences. Then groups which formed rather homogeneous set in the native protein and aqueous solution are divided into certain subsets in the presence of alcohols, these subsets differing for example in their dissociation.

Assuming that no charged ligands are bound to HSA in the alkaline region[1,2] data from Fig. 3 can be plotted as a function of the net charge Z represented by the number of dissociated protons (Fig. 6). In the absence of 1-propanol decrease in α-helix content is almost linear beginning from Z = –25 (corresponds to pH ≈ 9) so that the conformational change is predominantly governed by electrostatic repulsion arising from increasing negative charge. Stabilizing effect of alcohol is manifested by the shift of this almost linear part of the curve by approximatly 20 elementary charges to more negative values.

The FD spectra studied in the region of phenylalanine absorption yielded the dependence of the parameter a on pH (Fig. 7). Decrease in this parameter is a sign of a transfer of phenylalanines into a more polar environment. Marked decrease in a observed in aqueous medium at pH slightly below 10 corresponds to B → A transition. In this

process α-helix content is reduced what leads to exposure of phenylalanines to aqueous medium. In the presence of 1-propanol the sharp decrease in α-helix content is shifted by almost one unit to higher pH (15% alcohol) what is in accord with the stabilization

TABLE IV
The distribution of phenylalanines according to the hydrophobic profile[37] of HSA molecule (sliding window of 9 amino acids)

	The value of sliding window for Phe as the central amino acid						
	3	4	5	6	7	8	9
Residue No.	102	36	11	149	326	27	330
	134	49	19	156	551		
	568	127	70	157			
		206	228	165			
		223	374	211			
		309	395	377			
		502	403	554			
		509	488				
			507				

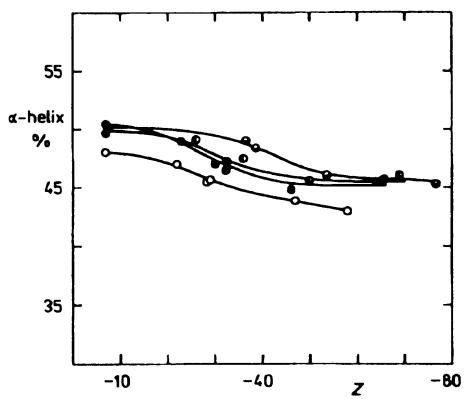

FIG. 6
The α-helix content as a function of the net charge Z of HSA (Z is the number of the elementary charges per one molecule of protein). Concentration of 1-propanol (volume fraction, %): ○ 0, ● 5, ◐ 10, ◒ 15

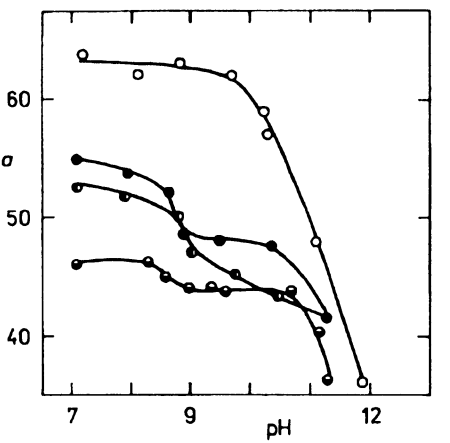

FIG. 7
The parameter *a* of FD spectrophotometry as a function of pH. Concentration of 1-propanol (volume fraction, %): ○ 0, ● 5, ◐ 10, ◒ 15

of proteins by alcohols. But at pH 8.5 another step appears in the presence of 1-propanol. In this region no significant decrease in α-helix content is observed (Fig. 3). This pH corresponds to $Z \approx -22$ and the curve shown in Fig. 4 is slightly sigmoidal here, what can be a sign of an extra conformational transition in HSA molecule under the influence of 1-propanol.

The nature of this transition cannot be interpreted on the basis of the data available at present and will be studied separately. Phenylalanines are as heterogeneous as tyrosines but their distribution in the hydrophobic profile is markedly shifted to higher hydrophobicities (Table IV). Then even a minor change in the conformation which allows for higher contact with the solvent represents a transition from less polar environment into more polar one.

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