THE EFFECT OF SMALL ALIPHATIC ALCOHOLS ON THE DISSOCIATION OF TYROSINE

Vladimir KARPENKO[a], Jana HORALKOVA[a] and Milan KODICEK[b]

[a] *Department of Physical and Macromolecular Chemistry, Charles University, 128 40 Prague 2, Czech Republic; e-mail: karpenko@prfdec.natur.cuni.cz*

[b] *Department of Biochemistry and Microbiology, Prague Institute of Chemical Technology, 166 28 Prague 6, Czech Republic; e-mail: kodicek@staff.vscht.cz*

Received March 8, 1996
Accepted June 8, 1996

Dedicated to Professor Jaroslav Podlaha on the occasion of his 60th birthday.

Dissociation of tyrosine was studied in aqueous solutions of methanol, ethanol, 1-propanol, and 2-propanol of various concentrations, when the permittivity of all solutions had the same value. The effect of electrostatic interactions was, consequently, equal and thus the other interactions became more pronounced. On the basis of p*K* values of tyrosine –OH groups these alcohols can be divided into two groups, the first one including methanol and ethanol, the second one both propanols. The observed differences can be explained only qualitatively as a result of interaction of alcohols with tyrosine and with the surrounding water.

Key words: Tyrosine dissociation; Alcohols effect; Isopermittivity conditions.

The method of solvent perturbation UV spectroscopy is widely used for the investigation of protein structure and stability. The profound influence of small aliphatic alcohols on the protein structure has been repeatedly stated[1–3]. Simultaneously, dissociation of simple organic compounds in mixed alcoholic solvents has been studied[4]. Attention has also been paid to spectral features of all important UV-chromophores[5], particularly tyrosines, which are the only species with a dissociable side chain. In alkaline media we have to consider not only the effect on the whole protein molecule, but also direct influence of the solvent on the dissociation of tyrosines. This influence was studied from the standpoint of characteristics of tyrosine spectrophotometric titration curves with phenols as model compounds[6].

In our previous studies[3,7,8] tyrosine dissociation was found to be one of the crucial factors acting on the stability of human serum orosomucoid (acid α_1-glycoprotein) in the alkaline pH region in the systems water–small aliphatic alcohol. The lack of data concerning the dissociation of tyrosine itself in mixed alcoholic solvents inspired us to present this work. These data can be used in explanation of the effects observed with other proteins under similar conditions.

EXPERIMENTAL

Materials

N-Acetyl-L-tyrosine ethyl ester (NAcTyr; Serva, Heidelberg) of analytical grade purity served as a model compound. The content of aliphatic alcohols (analytical grade purity, products of Lachema, Brno) in the solutions is given as a volume fraction ϕ (%). The experiments were carried out at following alcohol contents ϕ: methanol (MeOH) 55%, ethanol (EtOH) 44%, 1-propanol (PrOH) 36%, and 2-propanol (iPrOH) 36%. The relative permittivity of all these solution had the same value $\varepsilon_r = 57$ at 25 °C (ref.[9]). Twice distilled water was used in all experiments. Freshly prepared carbonate-free volumetric solutions of KOH were kept under nitrogen atmosphere.

Methods

Spectrophotometric titration. These experiments were carried out with a spectrophotometer Specord M 40 (Zeiss, Jena) at 244 nm in tightly closed 1 cm quartz cells under nitrogen atmosphere. The temperature was maintained at 25 ± 0.5 °C. Difference spectroscopy was performed in both continuous and discontinuous modes. In the discontinuous one tandem cells were used[5] and a new solution was prepared for each point of the curve; in the continuous mode single cell technique was applied. The measured sample in a typical continuous experiment was a $1 \cdot 10^{-4}$ M solution of NAcTyr in an appropriate mixed solvent; the ionic strength of the solutions was maintained at 0.2 adding solid KCl. The reference sample contained the $1 \cdot 10^{-4}$ M aqueous solution of NAcTyr in 0.2 M phosphate buffer pH 7.0. Volumetric solution of KOH was added to the first cell and the same amount of water to the reference cell. This approach was based on the experimentally proved assumption that no absorption of UV-light by alcohols or phosphate buffer interfere at the wavelength used. Correction for volume changes was done in all experiments.

Data analysis. Each titration curve was constructed from approximately 60 experimental points and the p*K* was calculated using computer program Origin for sigmoidal curves. No difference was detected between the results of continuous and discontinuous method.

Determination of pH. pH was measured with a pH-meter PHM 93 (Radiometer, Copenhagen) equipped with the combined microelectrode pHC 4400 of the same manufacturer. pH-Meter readings in mixed solvents should be corrected for the effects of the solvent on both the activity coefficient of H_3O^+ ions, and the electrodes[10]. In this correction, described in detail elsewhere[7], a single term is combined of two activity coefficients of H_3O^+ ions, one representing the influence of the mixed solvents, and the other the salt effect. The liquid junction potential of the calomel electrode is disregarded in this approach[11]. On this assumption, the dependence of the pH-meter readings on the actual concentration of OH^- ions was determined in all studied solvents. The results obtained for methanolic and ethanolic systems were in a good agreement with the literature data[10]; analogous data for the propanols are not available. Then, the pH-meter readings were corrected using this experimentally determined dependence. These new values correspond thus to pH expected in an aqueous solution.

The calibration of pH-meter readings confirmed our expectation that there is no difference between pH values in aqueous and methanolic solutions (up to $\phi \approx 60\%$); with other alcohols differences were found (Fig. 1). Here pH_{exp} are the actual readings of the pH-meter, pH_{theor} are the values expected in an aqueous solution as calculated for the same amount of KOH added. For all alcohols with the exception of MeOH, correction of pH had to be made over the whole pH range examined.

RESULTS

Using corrected pH values, spectrophotometric titration of NAcTyr was performed with the alcohols tested and also in the aqueous solution as the reference (Fig. 2). The p*K* of NAcTyr in the aqueous solution was close to the value 10.07 given by Conway[12]. In mixed solvents, the p*K* values of NAcTyr differed significantly from aqueous medium (Table I). The values of the differential molar absorbtivities ΔA_{244} given in the same Table were higher when the alcohols were present than in the aqueous solution; the value for iPrOH was slightly lower.

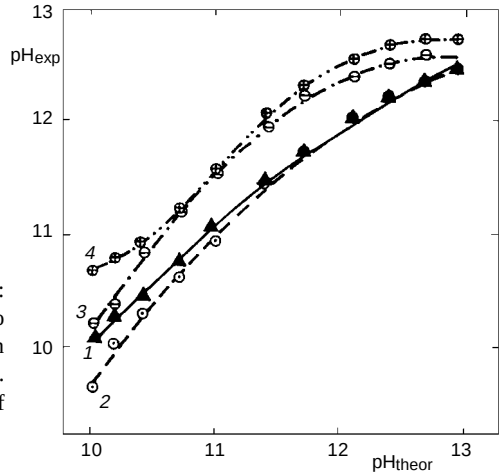

FIG. 1
Calibration of pH values in different solvents: the pH-meter readings (pH_{exp}) are related to the values expected in an aqueous solution (pH_{theor}) for the same addition of OH^- ions. Curves: *1* H_2O (the same was in presence of MeOH), *2* EtOH, *3* PrOH, *4* iPrOH

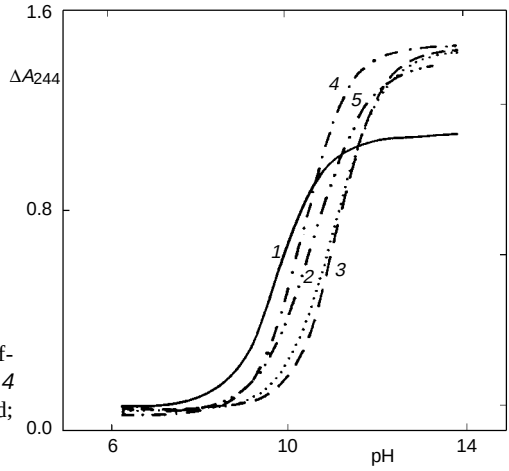

FIG. 2
Spectrophotometric titration of NAcTyr in different solvents: *1* H_2O, *2* MeOH, *3* EtOH, *4* PrOH, *5* iPrOH (experimental points omitted; approximately 60 for each curve)

DISCUSSION

The influence of small aliphatic alcohols on the aqueous solutions of amino acids and proteins is a complex process that results from the simultaneous action of three basic effects:

1) The changes of water structure that are not yet fully characterized. The dependence of the excess enthalpy of mixing (ΔH^E) on the content of alcohol[13] (the numerical data by Westmeier[14]) manifests these changes indirectly. These curves have minimum for all of the four smallest alcohols. At lower concentrations, below this minimum, alcohols are supposed to interact destructively with low-density domains of water[15]; the analogous effect was described for higher temperatures. At higher concentrations formation of microheterogeneities prevails when clusters of alcohol molecules are formed, particularly in the aqueous solutions of PrOH and iPrOH.

2) Lowering the relative permittivity enhances the electrostatic interactions in water–alcohol systems. In our work the differences in electrostatic interactions due to the permittivity have been eliminated and therefore other kinds of interactions have become apparent. Simultaneously, these concentrations of alcohols coincided with the minima on the curves ΔH^E vs alcohol content (see above).

3) The alcohols interact with dissolved substance; hydrogen bonds formed between amino acid molecules and alcohols should be expected. Alcohols compete in this respect with water molecules. On the other hand, with growing aliphatic chain, the hydrophobic interactions of alcohols with corresponding regions of the other solute become more prominent as the second type of interactions. Neither of these effects was quantified until now.

Dissociation constants in an aqueous solution (pK_w) and in the mixed solvent (pK^*) are related according to the equation[16]

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

TABLE I
The values of p*K*, of the differential molar absorbtivity ΔA_{244} ($mol^{-1}\ dm^3\ cm^{-1}$), and of the differences ΔpK for NAcTyr in alcoholic solutions

Solution	p*K*	$\Delta A_{244} \cdot 10^{-4}$	$\Delta pK \equiv \log \gamma_e$
H_2O	9.9	1.11	
H_2O–MeOH	11.0	1.45	+1.1
H_2O–EtOH	11.1	1.43	+1.2
H_2O–PrOH	10.4	1.49	+0.5
H_2O–iPrOH	10.6	1.36	+0.7

where the electrostatic contribution to the difference between them is expressed as the activity coefficient γ_e. This coefficient is a function of the dimensions of a dissociable group, of the temperature, and of the relative permittivity of the surrounding medium[16]. As apparent from the Table I, γ_e value can be higher than 1 in alcoholic solutions.

If there were solely electrostatic effects in action, then, the differences $\Delta pK \equiv \log \gamma_e = pK^* - pK_w$ should be the same in all alcohol solutions examined. However, the p*K* values given in the Table I show clearly that it was not so. The origin of this difference cannot be thus explained on the electrostatic basis and should be sought in other interactions of NAcTyr with the solvent, particularly with alcohols. When phenolic –OH group is approximated[17] as a sphere of the diameter $2 \cdot 10^{-8}$ cm, then, for the relative permittivity 57, the activity coefficient is $\log \gamma_e = +0.33 \approx +0.3$. Confronted with the last column of the Table I this result shows significant differences between this value and ΔpK's determined experimentally.

These values are a sum of more effects as mentioned above. At present attention should be turned to the fact that alcohols form two groups: MeOH, EtOH and PrOH, iPrOH. Differences between both groups can be attributed to hydrophobicity; qualitatively similar effects of EtOH and PrOH were found in experiments with human serum albumin[17].

This work was supported by grants No. 203/93/2467 and No. 203/93/0315 (both the Grant Agency of the Czech Republic).

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