DEPENDENCE OF THE SPECTRAL FEATURES OF PHENOLS ON SOLVENT

Spectrophotometric titration curve of tyrosine residue

M. KODÍČEK
Institute of Hematology and Blood Transfusion, 128 20 Praha, Czechoslovakia

Received 3 November 1978

1. Introduction

Wavelengths of absorption bands of aromatic compounds depend on the solvent which is used [1–3]. Attention has been focused mainly on benzene and its derivatives; for them several relations have been derived [4–6], which allow dielectric constant (or refractive index) of the solvent to be correlated with the wavelength shift of the respective aromate.

A similar detailed study of phenolic compounds, namely phenols in their dissociated form, does not exist yet. Our preliminary experiments have shown that in an alkalic medium, where their phenolic groups are dissociated, these compounds drastically change their spectral features under the influence of solvents. We assume that this finding is important namely with regard to the interpretation of spectrophotometric titration curves of proteins, the course of which depends on the spectral properties of phenolic chromophore of tyrosine residues.

2. Materials and methods

Phenol, α- and β-naphthols (Lachema, Brno) were purified by sublimation. *N*-acetyl-L-tyrosine-ethylester (Serva, Heidelberg) was used without further purification. All solvents were purified by given methods [7] and kept in the dark at 4°C.

Absorption and difference spectra were measured on Unicam SP 1750 spectrophotometer in 1 cm cells. The concentration of the samples was calculated by weighing.

In a typical experiment a solution of a sample (50 ml, $A^{1\,cm}_{max}$ 1–1.5) was prepared and the absorption spectrum was measured. Then the sodium salt of the phenolic compound was prepared by adding 0.5 ml saturated sodium isopropoxide solution in isopropyl alcohol (the stock solution was prepared by dissolving 2 g sodium in 100 ml isopropyl alcohol) and its spectrum was measured.

3. Results

Absorption spectra of phenol and sodium phenoxide with single peaks λ_1 = 269 nm (phenol) and λ_2 = 287 nm (phenoxide) are well known [8]. The spectra of α- and β-naphthols and sodium naphthoxides are depicted in fig.1, where the peaks which will be discussed later are indicated. The influence of various solvents on the spectral features of studied compounds can be analysed using table 1. It contains the wavelengths of selected peaks, their shifts produced by phenolic proton dissociation and the ratios of absorption coefficients of phenoxides and phenols. It is worth noting that with the way in which we conducted the experiment, the above-mentioned ratio is independent of concentration.

As is evident the wavelengths of the bands of the phenoxides are much more sensitive to the character of the solvent than are those of the corresponding phenols; consequently the Δλ value shows extensive fluctuations. A dramatic change occurs particularly in dimethylformamide and dimethylsulphoxide, where Δλ for phenol and β-napthol rises up to 2-fold compared to water. This shift together with the change of absorption coefficients ratio causes changes in

Table 1
Spectral characteristics of phenol, α-naphtol and β-naphtol in various solvents

	Phenol				α-Naphtol					β-Naphtol							
Solvent	λ_1	λ_2	$\lambda_2-\lambda_1$	ϵ_2/ϵ_1	λ_1	λ_2	λ_3	$\lambda_3-\lambda_1$	ϵ_3/ϵ_1	λ_1	λ_2	λ_3	λ_4	$\lambda_3-\lambda_1$	ϵ_3/ϵ_1	$\lambda_4-\lambda_2$	ϵ_4/ϵ_2
Water[a]	269	287	18	1.70	292	322	332	40	1.43	274	327	281	347	7	1.27	20	1.52
Methanol[b]	273	289	16	1.54	296	323	334	38	1.46	275	331	282	349	7	1.45	18	1.47
Octanol	273	291	18	1.56	297	324	336	39	1.49	276	333	284	351	8	1.54	18	1.50
Isopropyl alcohol	273	292	19	1.57	296	323	337	41	1.57	275	332	285	355	10	1.58	23	1.60
Triethyl-amine[c]	276	298	22	1.56	299	326	342	43	1.45	277	336	286	358	9	1.76	22	1.65
Dioxan	274	300	26	1.89	296	324	343	47	1.57	275	332	287	363	12	1.72	31	1.82
Dimethyl-formamide	275	314	39	1.88	298	324	355	57	1.52	277	335	308	386	31	2.54	51	1.81
Dimethyl-sulphoxide	275	316	41	1.94	300	325	357	57	1.77	277	335	310	390	33	2.79	55	1.89

[a] 0.05 M sodium phosphate buffer pH 7.0 and pH 10.5, respectively
[b] The dissociated forms of phenols were prepared by adding a small amount of sodium
[c] The spectra were measured in 1 mm cells

Abbreviations: λ_i, wavelength of the peak (nm); ϵ_i, absorption coefficient.
The preparation of the dissociated form of phenols is described in section 2. The numbers of the peaks of naphtols are shown in fig.1

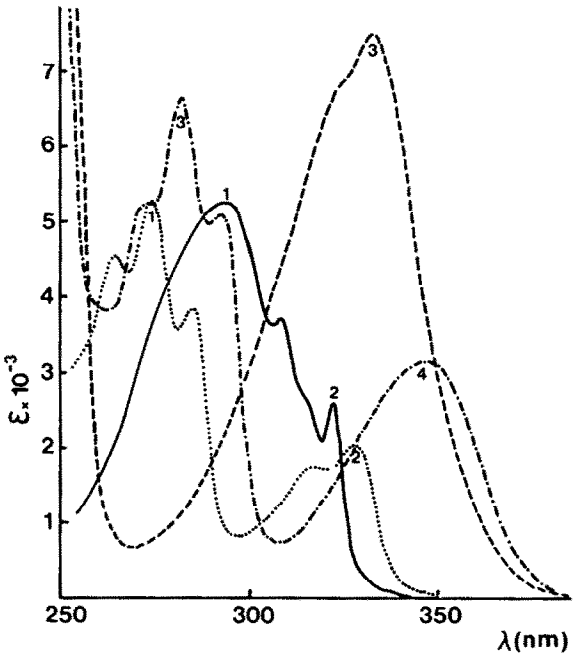

Fig.1. Absorption spectra of α-naphtol [pH 7.0 (——); pH 10.5 (- - - -)] and β-naphtol [pH 7.0 (· · · ·); pH 10.5 (—·—·)] in 0.05 M phosphate buffer. The numbers indicate the peaks in table 1.

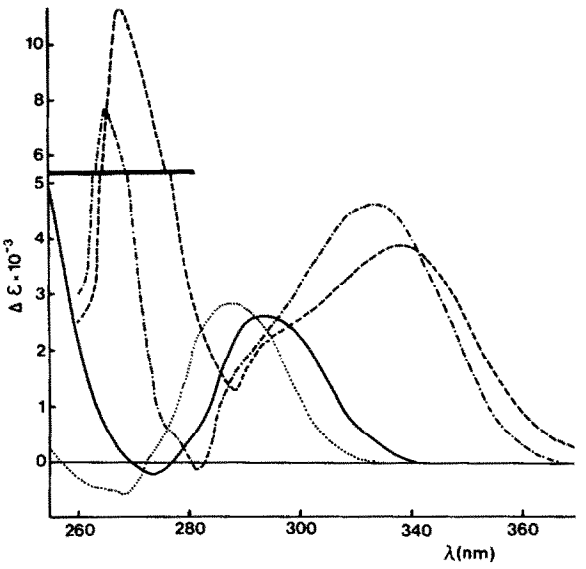

Fig.2. Difference spectra of phenol [in 0.05 M phosphate buffer (· · · ·); in dimethylformamide (—·—·)] and *N*-acetyl-tyrosine-ethylester [in 0.05 M phosphate buffer (——); in dimethylformamide (- - -)]. The spectra were registered as a difference absorbance between dissociated and associated forms of phenols.

difference spectra (fig.2) of phenol as well as *N*-acetyl-L-tyrosine-ethylester, which sets a model of the tyrosine residue in protein molecules.

4. Discussion

If we are to explain the shifts of the absorption bands of phenolic compounds we must take into account a number of factors of which the dielectric constant of the solvent and its ability for hydrogen bonding are among the most significant. Our set of solvents contained polar and nonpolar compounds as well as H^+ donors and acceptors; nevertheless, we have not yet managed to explain satisfactorily the unusually high $\Delta\lambda$ values for dimethylformamide and dimethylsulphoxide.

The fact that the shape of the difference spectrum of tyrosine residue depends on the solvent (fig.2) calls for a reconsideration of the usual method of analysis [9–11] of the spectrophotometric titration curves. This method is regarded as one of the simplest ways to study the reactivity and localisation of tyrosine side chains in proteins. In most laboratories it is not, in fact, a problem to measure the dependence of different absorbancy ΔA_{295} (or ΔA_{246}) on pH and to analyse these curves in the terms of Hasselbach-Henderson equation. This procedure is based on the assumption that the difference absorption coefficients of single tyrosyls are, in the first approximation, constant, i.e., independent from the surrounding of the chromophore.

From our result it is however evident that this assumption is not sufficiently justified. At the same time the applied solvents represent model media in which the hydrophobic tyrosine chromophores exist inside a globular protein molecule; dioxan and triethylamine are analogues of nonpolar hydrophobic amino acid side chains and the structure of dimethylformamide is reminiscent of the peptide bond. Therefore we take it for granted that the single successively dissociating tyrosines can add different contributions to the overall difference absorbancy ΔA. For this reason it is not generally possible to distinguish

reliably the contributions of single chromophores to the spectrophotometric titration curve and a discussion of the exact value of the dissociation constant, which has been determined by the above method, can be rather misleading. From this point of view the most 'dangerous' are the proteins, which contain a great number of tyrosine residues.

Acknowledgements

I wish to thank Mr Ø. Rønning (Trondheim) and Mr J. Večeř for technical assistance. I am indebted to my colleagues, Dr I. Kalousek and Dr Z. Hrkal, for helpful discussions.

References

[1] Cardinal, J. R. and Mukerjee, P. (1978) J. Phys. Chem. 82, 1614–1620.
[2] Bovey, F. A. and Yanari, S. S. (1960) Nature 186, 1042–1044.
[3] Yanari, S. and Bovey, F. A. (1960) J. Biol. Chem. 235, 2818–2826.
[4] Bayliss, N. S. (1950) J. Chem. Phys. 18, 292–296.
[5] Longuet-Higgins, H. H. and Pople, J. A. (1957) J. Chem. Phys. 27, 192–194.
[6] McRae, E. G. (1957) J. Phys. Chem. 61, 562–569.
[7] Pitra, J., Veselý, Z. and Kavka, F. (1969) Laboratorní úprava chemikálií a pomocných látek, SNTL, Praha.
[8] Wheeler, O. H. and Kaplan, L. A. eds (1956–7) Organic electronic spectral data, vol. 3, Interscience Publishers, London, New York, Sydney.
[9] Donovan, J. W. (1969) in: Physical principles and Techniques in protein chemistry (Leach, S. J. ed) p A, pp. 117–122, Academic Press, New York, London.
[10] Hermans, J. (1962) Biochemistry 1, 193–199.
[11] Kodíček, M., Hrkal, Z., Suttnar, J. and Vodrážka, Z. (1977) Biochim. Biophys. Acta 495, 260–267.