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ON THE EFFECT OF TEMPERATURE ON THE ULTRAVIOLET SPECTRA OF PROTEIN CHROMOPHORES

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The thermal perturbation difference spectra – TPDS (15–30°C) – of N-acetyl-tyrosine-ethyl ester and o-methyl-N-acetyl-tyrosine were studied in ethyl acetate and dimethyl ether with/without the addition of butanol which served as a proton donor in hydrogen bonding. In all cases the longwave shift of the absorption spectrum is shown to be a principal factor that determines the origin of TPDS and the hydrogen bonding has no effect on these spectra. These results contradict the view that the red shift of protein chromophore spectra at the elevation of temperature is a unique feature of water as a solvent. The water-inaccessible chromophores in proteins may be perturbed by temperature increase, producing red shift.

1. Introduction

One of the approaches to obtain information on the state of aromatic amino acids in proteins from the ultraviolet absorption spectra is the introduction of the controlled external factor that causes perturbation of spectra of protein chromophores and the production of difference spectra between identical preparations with/without that factor. The absorption spectra depend on temperature, so small difference in temperature may serve for this purpose. The thermal perturbation difference spectra (TPDS) [1–4] have been applied to the study of conformational state and conformational changes of a number of proteins (see [5] for a review), though the origin of these spectra has not been properly understood. For tyrosine, tryptophan and phenylalanine in water the changes of absorption spectra at the elevation of temperature to originate the TPDS are the longwave shift (0.01–0.02 nm/degree), change of intensity and broadening [6]. Can it be related with a unique feature of water as a solvent?

The majority of proteins studied show the longwave shifted maxima of TPDS [5] that were attributed to chromophores in the state of decreased polarity, partially buried in the protein interior [7]. The state of these chromophores may be correlated to that of tyrosine and tryptophan in organic solvents with higher refraction index and lower dielectric constant [7,8]. Of interest can be the study of the origin of thermal effects in absorption spectra of tyrosine chromophore in these solvents. Our task was also to clarify the problem discussed elsewhere [8], i.e. whether the hydrogen bonding of tyrosine OH group influences the thermal perturbation difference spectra. For this purpose we studied both tyrosine and OH group methylated tyrosine derivatives in ethyl acetate and diethyl ether with/without addition of butanol. We show that in these solvents, as in water, the longwave shift of the absorption spectrum at elevation of temperature is the principal factor determining the TPDS origin and that hydrogen bonding has no effect on these spectra.

2. Materials and methods

N-acetyl-tyrosine-ethyl ether (A-Tyr-EE) was a product of "Reanal", Hungary. O-methyl-N-acetyl-tyrosine (M-A-Tyr) was synthetized by Ya.P. Demchenko at the Institute of Organic Chemistry, Kiev. The procedure for TPDS determination and in-

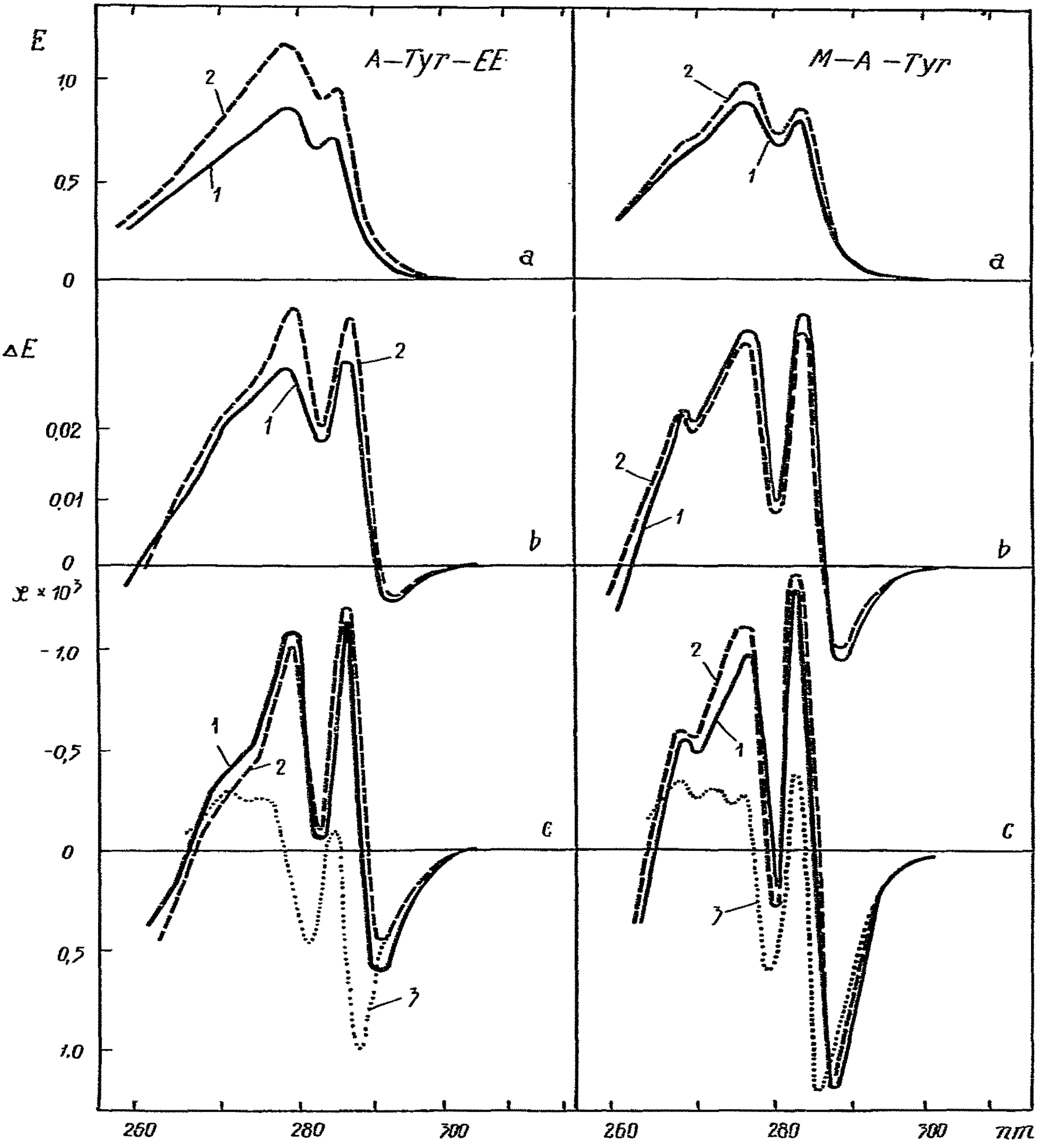

Fig. 1. The direct absorption spectra (a), experimental (b) and volume-corrected (c) thermal perturbation difference spectra of A-Tyr-EE and M-A-Tyr in ethyl acetate (1) and 9 : 1 ethyl acetate : butanol solution (2). The interval of temperature is 15–30°C. Derivative spectra (3) for A-Tyr-EE and M-A-Tyr are in ethyl acetate solution.

troduction of correction factor for thermal change of solution volume was described earlier [4,7]. The spectra are expressed in terms of the dependence on wavelength of thermal increment of extinction, κ ($\kappa = (1/E_{\max})\Delta E/\Delta t^0$), where $\Delta E/\Delta t^0$ is the change in extinction per degree and $E_{\max}$ is the extinction in the maximum of absorption spectrum. Absorption spectra were recorded on a spectrophotometer "Perkin Elmer", Mod. 402, the first derivatives were obtained using differentiation of the output signal from the spectrophotometer by means of active RC-chain [9].

3. Results and discussion

The formation of hydrogen bond in solution by OH-group of tyrosine is known to produce definite changes in absorption spectra: rise of intensity and spectral shift [10]. In ethyl acetate and diethyl ether, the solvents in which hydrogen bond is absent, this bond may be formed with the solute only if the solute is a proton donor. So, in this solvent the hydrogen bond is present for A-Tyr-EE and is absent for M-A-Tyr. On the addition of butanol the hydrogen bonds in which butanol is a proton donor are formed both with A-Tyr-EE and M-A-Tyr. The changes in the absorption spectra in ethyl acetate are seen in fig. 1. For A-Tyr-EE the 279 and 286 nm maxima are increased by 1.3 times, whereas for M-A-Tyr only a small increase in 277 and 284 nm maxima is observed.

The experimental and volume-corrected TPDS of A-Tyr-EE and M-A-Tyr in ethyl acetate are presented in fig. 1. For the solvents studied the correction factor for thermal changes of solution volume is substantial, so the corrected spectra differ significantly from the uncorrected ones. Both for A-Tyr-EE and M-A-Tyr in ethyl acetate no changes in TPDS had been noticed following the addition of butanol. The addition of butanol to diethyl ether produced the same results. These data show that the effect of temperature on the absorption spectra of tyrosyl chromophore does not depend on whether it is hydrogen-bonded or not. It may be anticipated that in the study of proteins no information on hydrogen bonding of tyrosyl residues could be obtained from TPDS.

The small magnitude of thermal effects makes the analysis of the direct spectra of absorption difficult. The information on the origin of thermal changes in these spectra may be obtained from the analysis of TPDS [6]. In case the spectral shift is the only factor, the shape of the difference spectrum corresponds to the first derivative of absorption spectrum. The derivatives of the absorption spectra of A-Tyr-EE and M-A-Tyr presented in fig. 1 show a good conformity of position of spectral maxima to TPDS. The conformity of spectra is not complete, so other factors beyond the spectral shift must be accounted for. These are: changes of intensity and width of absorption spectra. The signs and value of spectral changes may be estimated using the proposed equation [6], in which one term is proportional to the function that describes the absorption spectrum and reflects the thermal changes of intensity, and the other is proportional to the first derivative and describes the spectral shift. On the elevation of temperature the absorption spectra studied shifted to the red by 0.005–0.01 nm/degree and reduced its intensity by less than 0.1 percent/degree.

The increase of temperature reduces chromophore interaction with the solvent (the refraction index and dielectric constant being reduced), so the short-wave shift of absorption spectrum towards the position in vacuum, rather than the long-wave one could be anticipated. The shift of phenol and anisole to the red in aqueous solutions was thought to be due to melting of "isebergs" of structured water around the aromatic chromophore [11]. In the study of TPDS of tyrosine derivatives the changes in hydrogen bonding were supposed [8]. The present findings show that the long-wave shift of electronic spectra of absorption at the increase of temperature is a more universal phenomenon that is not limited to water solutions only. It may be anticipated that in the study of proteins the solvent-inaccessible chromophores, hydrogen-bonded or not, within the medium of decreased polarity may behave in the same way producing red shift, and the decreased intensity of TPDS of native proteins compared with denatured proteins and model chromophores [1,2,3,5] may reflect not only the degree of accessibility of chromophores to solvent, but also the thermal rigidity of protein matrix in the vicinity of chromophore group.

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