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Influence of phosphate ion on the fluorescence of 3-fluorotyrosine

Short Communication

S. I. Vdovenko, M. T. Kolycheva, I. I. Gerus, and **V. P. Kukhar**

Institute of Bioorganic and Oil Chemistry, Academy of Sciences of Ukraine, Kiev, Ukraine

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Summary. 3-Fluorotyrosine fluorescence is quenched effectively by phosphate ions not only by a dynamic but also by a static mechanism owing to H-bond complex formation in ground state. 3-Fluorotyrosine pK_a values both in the ground and first excited state (8.3 and 4, respectively) are appreciably lower than those of tyrosine, thus promoting 3-fluorotyrosinate ion formation in the excited state. Additional emission owing to 3-fluorotyrosinate ion (near 350 nm) may be taken erroneously for tryptophan fluorescence.

Keywords: Amino acids – 3-Fluorotyrosine – Fluorescence – Quenching – Absorption – Phosphate

Introduction

The introduction of fluorinated aromatic amino acids into membrane proteins is regarded as extremely effective method of modification (Ghosh et al., 1985; Kuriatov et al., 1984). In particular, the substitution of native tyrosine residues by the fluorinated ones leads to little disturbances of peptide conformation (Kuriatov et al., 1984). In some cases fluorine substitution of aromatic amino acids shifts the fluorescence spectra and increases quantum yields (Chen et al., 1969; Kuriatov et al., 1984), thus allowing the use of fluorocontaining amino acids as suitable intrinsic chromophores in peptides.

The spectroscopic properties of fluorosubstituted tyrosine are the least investigated. In this work fluorescence and absorption spectra of 3-fluorotyrosine (3-F-Tyr) in aqueous solutions with various phosphate concentrations have been studied. It is known the phosphate to be important component of buffers, besides it is the specific quenching probe for analysis of the degree of exposure of tyrosine residues and peptide conformation (Shimizu et al., 1979; Ross et al., 1986).

Material and methods

Emission spectra of 3-F-Tyr were obtained with Perkin-Elmer Model 650-10 spectrofluorometer, Absorption spectra were recorded on a Specord UV-Vis Model 40 spectrophotometer. 3-F-Tyr was synthesized and purified by the method of English (1940). Solutions under investigation were prepared by mixing appropriate aliquots of the initial solutions: 1 mM 3-F-Tyr and 3 *M* K_2HPO_4. Before measurements pH of solutions were adjusted to the required value with a pH-meter by addition of concentrated HCl (10*M*) or KOH (10*M*). All solutions contained 0.1 *M* KCl and were made up in twice distilled water.

Results and discussion

3-F-Tyr fluorescence spectra are shown in Fig. 1. In the absence of phosphate the emission maximum is near 315 nm, that is a 10 nm longer wavelength shift compared with tyrosine at 305 nm (Lakowicz, 1983). Increasing phosphate concentration results in a progressive decrease in 3-F-Tyr emission at 315 nm. Concurrently, a new emission is observed at 350 nm, the emission at 315 nm being quenched practically completely at phosphate concentration 2.9 M. Similarly to tyrosine (Alev-Behmoaras et al., 1979), this new emission is a consequence of 3-F-Tyr-phosphate intraction, that is 3-fluorotyrosinate ion formation in its excited state. As it was shown earlier by Shimizu et al. (1979), Alev-Behmoaras (1964), Feitelson (1964), excited state ionization of tyrosine is facilitated by the presence of nearby proton acceptors, particularly phosphate ions. Hydrogen bonding between this acceptor and the hydroxyl moiety on 3-F-Tyr can occur in the ground state, resulting in complex poised for reaction upon excitation of 3-F-Tyr. Complex formation between 3-F-Tyr and phosphate results in red shift of absorption spectrum (about 5 nm to long waves, 2.9 M K_2HPO_4), leading to potential confusion with tryptophan fluorescence (Lakowicz,

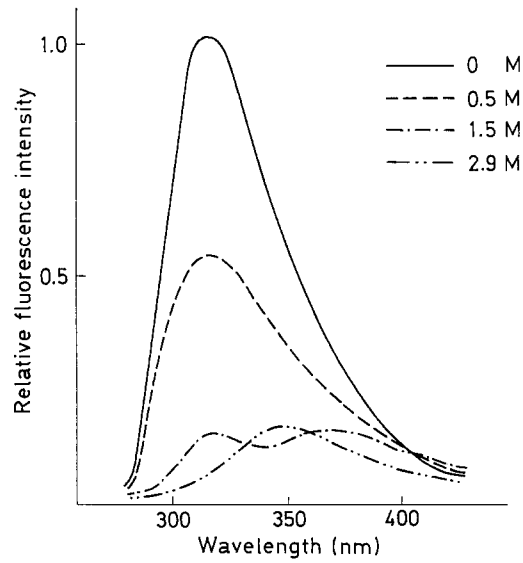

Fig. 1. Fluorescence spectra of-3-fluorotyrosine. The numbers refer to the concentration of K_2HPO_4. The pH was 7.4, t = 20°C. Excitation wavelength was 275 nm

1983). Quantitative analysis of this complexation have been carried out by the method of difference UV-spectroscopy (Moon, 1965):

$$\frac{1}{\Delta A} = \frac{1}{\Delta A_{max} K_{ass} [Q]} + \frac{1}{\Delta A_{max}}, \quad (1)$$

where ΔA – apparent optical density difference in the presence of ligand of [Q] concentration, ΔA_{max} – maximal optical density difference with completely complexed 3-F-Tyr, K_{ass} – association constant. The plot $1/\Delta A_{max}$ *versus* $1/[Q]$ is linear, so from eq. (1) $K_{ass} = 0.4 \pm 0.1\ M^{-1}$ is in good agreement with that of tyrosine ($0.5\ M^{-1}$), obtained earlier by Alev-Behmoaras (1979). Thus, 3-F-Tyr forms a complex with phosphate in the ground state, promoting the generation of 3-fluorotyrosinate ion in excited state.

The ground state pK_a of 3-F-Tyr hydroxy-group (evaluated by spectroscopic titration) is near 8.3 [in peptides, according to Kuriatov et al. (1984), this value is somewhat higher, 8.9] being lower pK_a than that of tyrosine [10, (Alev-Behmoaras et al. (1979)]. The first excited state pK_a^* of 3-F-Tyr is 4 [calculations have been made according to Förster's cycle, Sverdlova (1985)] also being lower than the pK_a^* of tyrosine 5.2–5.7, Shimizu et al. (1979). For this reason hydroxy-group deprotonation in the excited state should be (even at neutral pH) easier for 3-F-Tyr compared with tyrosine. Apparently the presence of phosphate cannot only promote this process but stabilize 3-fluorotyrosinate ion in the excited state.

Thus, quenching of 3-F-Tyr by phosphate is accompanied by a new emission peak near 350 nm due to hydroxyl moiety deprotonation. Hydrogen bonding between phosphate and 3-F-Tyr in the ground state promotes 3-fluorotyrosinate ion formation in the excited state. Selective excitation of 3-fluorotyrosinate can give emission at longer wavelengths resulting in further confusion with tryptophan fluorescence.

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Authors' addresses: S. I. Vdovenko, Institute of Bioorganic and Oil Chemistry, Academy of Sciences of Ukraine, Murmanskaja street, 1 Kiev-94, 253660, Ukraine.

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