THE PHOSPHORESCENCE OF TYROSINE OLIGOPEPTIDES

Robert F. Steiner
Laboratory of Physical Biochemistry
Naval Medical Research Institute
Bethesda, Md.

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The luminescence of the aromatic amino acids present in proteins and synthetic polypeptides has attracted increasing attention in recent years (Teale and Weber, 1957; Weber, 1961; Vladimirov and Burshtein, 1960; Nag-Chaudhuri and Augenstein, 1964; Steiner and Edelhoch, 1963). The question of energy migration between different luminescent groups is of fundamental importance to this general problem. This communication will focus attention upon energy transfer within oligomers of tyrosine. It has been found that extensive intramolecular energy transfer occurs from un-ionized to ionized tyrosine, so that the ionization of only a fraction of the tyrosines in the oligopeptide results in the appearance of the luminescence characteristic of ionized tyrosine.

The luminescent properties of tyrosine, which arise from the phenolic group, change radically upon ionization of the latter (Nag-Chaudhuri and Augenstein, 1964). At 77° K, in 50% ethylene glycol, the neutral form of tyrosine has a fluorescence maximum close to 298 mμ and a phosphorescence maximum at about 395 mμ. The corresponding maxima for the alkaline form, in which the phenolic group is ionized, are at 320 and 405 mμ, respectively. Ionization of the phenolic group increases the efficiency of intersystem crossing; the fluorescence is quenched by about 80%, while the phosphorescence is enhanced.

Since the absorption maximum of ionized tyrosine overlaps the fluorescence emission band of the un-ionized form, it is to be expected that radiationless energy transfer at the singlet level could occur by the Förster mechanism between a pair of such residues located in the same molecule (Förster, 1959). There exists in addition the possibility of energy migration at the triplet level.

Methods and materials.

Determinations of excitation and emission spectra were made using an Aminco-Bowman spectrofluorometer adapted for phosphorescence, as described in an earlier publication (Steiner, Millar, and Hoerman, 1967). Solutions were contained in quartz ESR tubes (3 mm diameter), which were reproducibly positioned within a V-shaped groove in a cuvette holder. The latter was reproducibly mounted by means of a brass clip upon a vertical bronze rod in thermal contact with a liquid nitrogen reservoir. In this manner the sample temperature was maintained close to 91° K.

All measurements were carried out in 50% ethylene glycol-0.05 M phosphate. The pH's cited are apparent values, measured with the glass electrode at 25° C, using a Radiometer pH meter. Their relation to the effective values in the glycol-water glass at liquid nitrogen temperatures remains uncertain.

Measurements of phosphorescence lifetime were made by direct observation of the decay of phosphorescence, the photomultiplier output being displayed upon the screen of a Tetronix oscilloscope. A manual shutter was used. The lifetimes were computed from the slopes of the (linear) plots of the natural logarithm of relative intensity versus time.
The difference in lifetime between ionized and un-ionized tyrosine is insufficient to produce a significant deviation from linearity over one decade when both forms are present.

The degree of ionization was obtained from measurements of ultraviolet spectra at liquid nitrogen temperatures, using an absorption cell contained in a Dewar flask equipped with quartz windows.

Tyrosine was purchased from Sigma. The following tyrosine oligopeptides were prepared by Dr. M. Wilchek of the Weizmann Institute and were obtained through the courtesy of Dr. H. Edelhoch, of the National Institutes of Health: $(Tyr)_3$, $(Tyr)_4$, and $(Tyr)_6$.

Results and discussion

At neutral pH, the luminescent spectra of $(Tyr)_3$, $(Tyr)_4$, and $(Tyr)_6$ have a general resemblance to that of un-ionized tyrosine, with similar positions of the fluorescence and phosphorescence maxima. In the pH region of tyrosine ionization, the emission spectra of these compounds change progressively into those characteristic of the ionized form. This is accompanied by a quenching of fluorescence, a fall in the fluorescence/

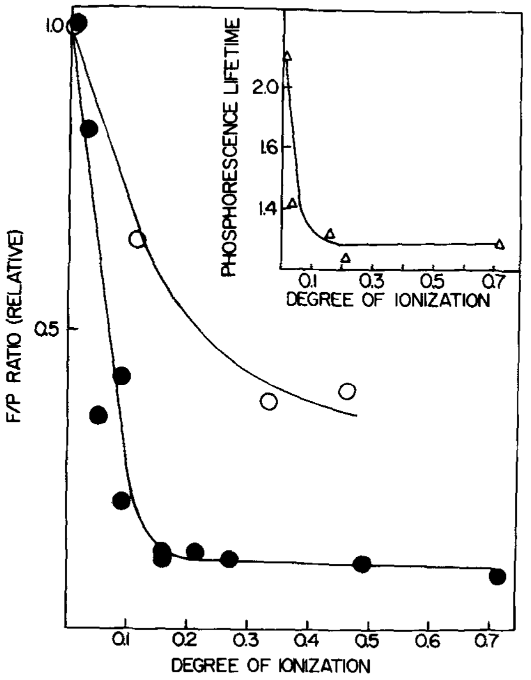

Figure 1. The relative values of the F/P ratio, excited at 263 mμ, for Tyr (○) and for $(Tyr)_6$ (●) as a function of the degree of ionization. (Insert) The phosphorescence lifetime (seconds) of $(Tyr)_6$ as a function of the degree of ionization.

phosphorescence (F/P) ratio, and a decrease in phosphorescent lifetime (Figure 1). The ratio of the limiting value of F/P approached at high degrees of ionization to that for neutral pH is about 0.10 for tyrosine and the oligopeptides.

As Figure 1 shows, the fall in F/P with increasing degree of ionization is much more rapid for $(Tyr)_6$ than for tyrosine itself. The limiting values of F/P and of phosphorescence lifetime are attained at a fractional ionization of 0.2 for $(Tyr)_6$. Thus the ionization of 1-2 groups in the hexamer of tyrosine suffices to endow it with the luminescence properties characteristic of ionized tyrosine. The oligomers $(Tyr)_3$ and $(Tyr)_4$ show profiles of F/P versus degree of ionization which are intermediate to those for tyrosine and $(Tyr)_6$.

These results indicate that, under conditions of partial ionization, the contribution of un-ionized tyrosine to the over-all luminescence is suppressed. An obvious mechanism is by intramolecular migration of excitation energy from un-ionized to ionized tyrosine.

This model may be tested by examination of the excitation spectra for phosphorescence. If emission is monitored at 400 mμ, the maxima in the excitation spectra for tyrosine and its oligomers show the expected shift to longer wave-lengths upon ionization. The position of the maximum (uncorrected) changes from 276 ± 1 mμ at pH 7 to 292 ± 1 mμ at pH 12. The contribution of un-ionized tyrosine to the excitation spectrum is negligible at wave-lengths greater than 295 mμ. Hence, for a partially ionized system, the contribution of ionized tyrosine to the excitation spectrum at any wave-length may be estimated from the height of the excitation spectrum at 300 mμ, where only the ionized form makes a contribution.

For 10^{-3} M $(Tyr)_6$ in 0.6 M KOH, where ionization should be complete, the apparent ratio of the height of the (uncorrected) excitation spectrum at 263 mμ to that at 300 mμ was 0.29. For a degree of ionization of 0.17, where F/P and the phosphorescence lifetime have their limiting values (Figure 1), the corresponding ratio for identical conditions was 0.60.

The fractional contribution of un-ionized tyrosine to excitation at 263 mμ is 0.52 (= 1-0.29/0.60). Thus un-ionized tyrosine may make a major contribution to the excitation, even under conditions where the emission spectrum corresponds to that of the ionized form.

A possible mechanism for this process is via radiationless energy transfer at the singlet level by the Förster mechanism (Förster, 1959). Since there is extensive overlap between the fluorescence band of un-ionized tyrosine and the absorption band of the ionized species, the formal requirements for radiationless exchange by this model are met (Förster, 1959; Edelhoch, Brand, and Wilchek, 1967). Since radiationless exchange would be competitive with both direct emission of fluorescence and intersystem crossing by the first excited state of un-ionized tyrosine, both the fluorescence and the phosphorescence of the latter would be quenched.

According to the above model, energy migration would occur by a single jump between an un-ionized residue and the ionized acceptor. One may also envision an alternative model in which excitation energy migrates along the chain of un-ionized tyrosines until it is accepted by an ionized site. Since the absorption and emission spectra of un-ionized tyrosine overlap to a finite extent, transfer by a Förster mechanism is feasible. Alternatively, it is possible that energy migration occurs via some form of direct interaction between a tyrosine in the excited state and an adjacent residue in the ground state.

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