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## Fluorescence and the structure of proteins

## II. Fluorescence of peptides containing tryptophan or tyrosine

The quantum efficiency of fluorescence ($Q$) of each peptide in Table I was measured by a method described in an earlier paper. The values are much lower than those for free tryptophan or tyrosine; however, this is consistent with earlier observations that peptide bonds decrease the fluorescence[1].

TABLE I

QUANTUM EFFICIENCY OF FLUORESCENCE ($Q$) OF PEPTIDES IN AQUEOUS SOLUTION AT pH 6.0 IN 0.025 M HISTIDINE BUFFER

Peptides were obtained from Mann Research Labs. and Schwarz Bioresearch, Inc. Identity and purity of the peptides were established by absorption and fluorescence spectra, Kjeldahl analyses for nitrogen, and paper chromatography of the amino acids liberated by hydrolysis in HCl or $Ba(OH)_2$.

| *Peptides of tyrosine* | $Q$ | *Peptides of tryptophan* | $Q$ |
|---|---|---|---|
| Gly–Tyr | 0.07 | Gly–Try | 0.05 |
| Tyr–Gly | 0.07 | Ala–Try | 0.06 |
| Tyr–Ala | 0.09 | Leu–Try | 0.08 |
| Leu–Tyr | 0.10 | Pro–Try | 0.05 |
| Tyr–Phe | 0.08 | Phe–Try | 0.06 |
| Tyr–Tyr | 0.08 | Try–Try | 0.09 |
| | | Try–Tyr | 0.12 |
| | | Try–Gly | 0.14 |

Values of $Q$ are similar for all peptides in Table I, and these include peptides with adjacent aromatic side-chains (Tyr–Phe, Tyr–Tyr, Phe–Try and Try–Try). Apparently the presence of a neighboring aromatic ring has little or no influence on fluorescence, either by resonance transfer of energy or by re-absorption of emitted fluorescence. In agreement with this conclusion, the phenylalanyl moiety does not contribute appreciably to the excitation of the tryptophyl or tyrosyl ring since the observed excitation spectra are identical for the pair Tyr–Phe and Tyr–Gly as well as for the pair Phe–Try and Gly–Try. Transfer of excitation energy also might occur between adjacent tryptophyl or tyrosyl residues of Try–Try and Tyr–Tyr. If so, the transfer must be efficient since the quantum efficiency of fluorescence per tryptophyl or tyrosyl residue of Try–Try and Tyr–Tyr is comparable with values for other peptides such as Try–Gly and Tyr–Gly where resonance transfer is improbable.

The most interesting peptide is Try–Tyr for which all fluorescence appears to come from the tryptophyl moiety. That is, the excitation and emission spectra correspond in every respect to those for tryptophan and the fluorescence contribution of the tyrosyl moiety is negligible. A similar phenomenon has been observed by TEALE for proteins which contain both tryptophyl and tyrosyl residues[2]. However, it is possible to demonstrate an influence of the tyrosyl group on the fluorescence of the tryptophyl moiety of the peptide Try–Tyr from the effects of pH on the fluorescence. In Fig. 1 the data for the peptide are compared with data for tryptophan and tyrosine. As discussed in the earlier paper, the fall in fluorescence in the region pH 2–3 can be ascribed to increase in the electronegativity of the carboxyl group upon association with a proton ($RCOO^- \rightarrow RCOOH$)[1]. The same effect is noted for the peptide except that the change in fluorescence is less because of the greater distance of the carboxyl group from the indole ring. In alkaline solution, an initial rise in fluorescence is noted.

A similar rise for tryptophan was ascribed to decreased electronegativity of the amino group as a proton was removed ($-NH_3^+ \rightarrow -NH_2$). The onset of this change is at a lower pH for the peptide than for tryptophan. This would be expected since the $pK_a$ for the amino group should be approx. 1.5 units lower for a di-peptide[3] than for the amino acid itself (for tryptophan, $pK_a = 9.39$). Unlike the behavior of tryptophan at still higher pH, the fluorescence of the peptide drops rapidly in the region of pH 10.3*. This fall occurs in the pH region for dissociation of the phenolic group of the peptide and a similar fall was observed for tyrosine. The fact that the fluorescence

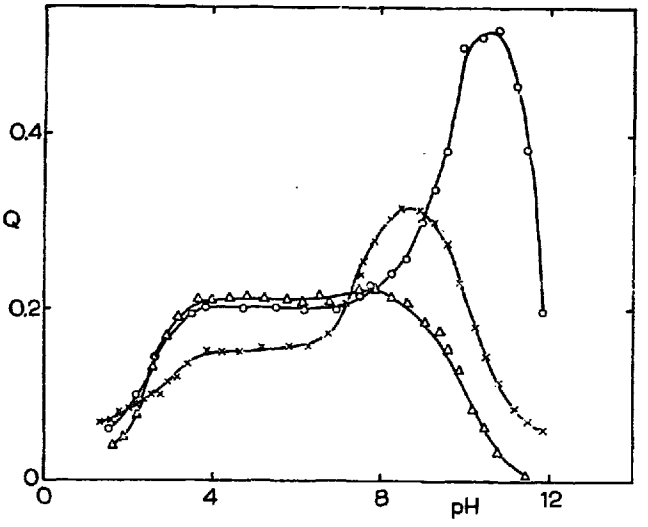


Fig. 1. Effect of pH on quantum efficiency of fluorescence of tryptophyltyrosine. △—△, tyrosine; ○—○, tryptophan; ×—×, tryptophyltyrosine.

of the peptide is sensitive to ionization of the phenolic ring even though all fluorescence arises from the indole ring indicates that some interaction must occur between the two groups. Possibly a transfer of energy from the indole ring to the ionized phenolic ring occurs with subsequent loss of energy by non-radiative processes**. A similar proposal has been advanced by STEINER AND EDELHOCH[4] to account for losses in fluorescence of proteins at high pH.

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* At pH 11 and higher, the fluorescence of tryptophan is quenched by hydroxyl ions[5].

** Ionization of the phenolic ring would lead to a rise in the molar extinction coefficient (from 1290 to 2300) and a shift of the absorption maximum from 275 mμ to 295 mμ. These changes, particularly the shift in absorption maximum, would favor transfer of energy from the indole ring to the phenolic ring.