THE EXCIMER FLUORESCENCE OF TRYPTOPHAN, TYROSINE AND D-GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE

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The fluorescence of tyrosine and tryptophan was analyzed at different concentrations. Tyrosine in the concentration range of 10–50 μg/ml shows a fluorescence maximum between 305 and 312 nm (depending on concentration) with an excitation maximum between 283 and 290 nm. At higher concentrations, self-quenching was demonstrated and this fluorescence becomes practically unmeasurable at 10 mg/ml. However, a new fluorescence was found on exciting a concentrated solution of tyrosine (1–10 mg/ml) between 330 and 340 nm, and measuring the fluorescence between 405 and 420 nm (fig. 1).

The excitation maximum of tryptophan, at 10–50 μg/ml, falls between 290 and 295 nm with a fluorescence maximum at 360 nm. When 1–5 mg/ml tryptophan was used, the fluorescence at 360 nm could only be measured if excited at 308–314 nm. Concomitantly, a new fluorescence band appears with activation maximum at 385 nm and fluorescence maximum at 450 nm (fig. 2).

The fluorescence of tyrosine at 310 nm is independent of pH in the range 4.0–8.7 while that at 405 nm is independent in the pH range 7.7–10.0. Below pH 7.7 enough tyrosine cannot be dissolved to measure the fluorescence at 405 nm. The fluorescence of tryptophan measured at 360 nm increases towards alkaline pH-s, while that measured at 450 nm increases with increasing acidity (fig. 3).

It is known that parallel with the concentration-quenching of monomer-fluorescence of aromatic hydrocarbons fluorescence of the activated dimer appears [1]. This has been called excimer (excited dimer) fluorescence; its maximum always lies at a wavelength higher than that of the monomer-fluorescence [2]. The appearance of excimer fluorescence as a function of

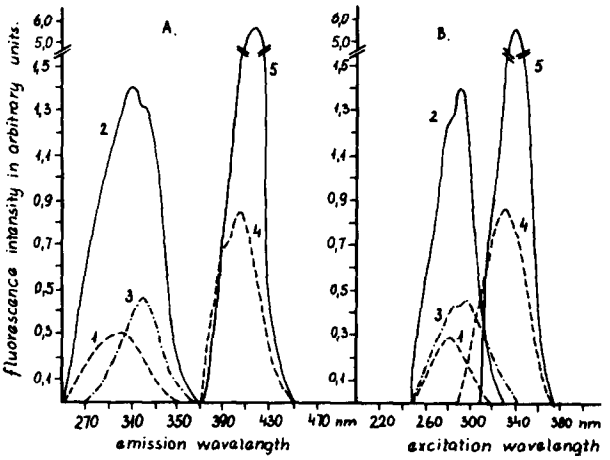

Fig. 1. Fluorescence of tyrosine solutions. L-Tyrosine (Finom Vegyszer KSz) was dissolved in 0.1 M glycine buffer, pH 7.0. An Optom PMQ II spectrophotofluorimeter was used.
A.: *Fluorescence spectra*
B.: *Excitation spectra*
A.1.: 10 μg/ml, A.2.: 50 μg/ml, A.3.: 1 mg/ml, activated at 283 nm. A.4.: 1 mg/ml, A.5.: 10 mg/ml, activated at 330 nm.
B.1.: 10 μg/ml, B.2.: 50 μg/ml, B.3.: 1 mg/ml, measured at 305 nm. B.4.: 1 mg/ml, B.5.: 10 mg/ml, measured at 410 nm.

concentration or of temperature was demonstrated with various different aromatic hydrocarbons [cf. 2, 3], acetone and other aliphatic ketones [4], nucleosides, nucleotides, and nucleic acids [5–11]. Exciplex fluorescence (fluorescence of excited complexes of indole with simple polar organic compounds) of tryptophyl residues of urea denatured proteins has also recently been published [12].

A new fluorescence band with a maximum at 460 nm was found on exciting some dehydrogenases at

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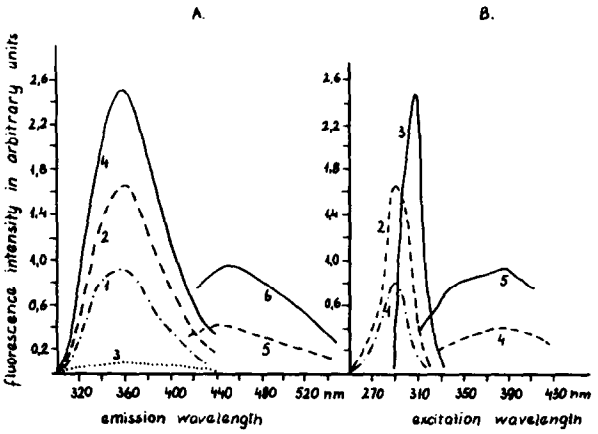

Fig. 2. Fluorescence of tryptophan solutions. L-Tryptophan (Reanal) was dissolved in 0.1 M glycine buffer, pH 7.0.
A.: *Fluorescence spectra*
B.: *Excitation spectra*
A.1.: 10 μg/ml, A.2.: 50 μg/ml, A.3.: 1 mg/ml, activated at 290 nm. A.4.: 1 mg/ml, activated at 308 nm. A.5.: 1 mg/ml, A.6.: 5 mg/ml, acivated at 390 nm.
B.1.: 10 μg/ml, B.2.: 50 μg/ml, B.3.: 1 mg/ml, measured at 360 nm. B.4.: 1 mg/ml, B.5.: 5 mg/ml, measured at 450 nm.

380 nm [13]. This fluorescence is only measurable at high protein concentrations (at least 1 mg/ml). At low protein concentrations (of the order of μg/ml) these dehydrogenases fluoresce with a maximum at 340 nm, when excited at 280 nm.

When glyceraldehyde-3-phosphate dehydrogenase was excited at 380 nm, the fluorescence intensity at 460 nm was independent of pH between 7.0 and 8.5 for the charcoal-treated enzyme (not containing firmly bound NAD, which quenches the fluorescence of the protein) and between pH 6.0 and 8.5 for enzyme containing about 3.6 moles NAD per mole*. Below these pH values (7.0 and 6.0, respectively) the intensity of the fluorescence at 460 nm increases with decrease in pH (fig. 3).

It is known that the nitroxide free-radical has a profound effect on fluorescing molecules. A tryptophyl residue of a protein may not fluoresce if a nearby site is blocked with a nitroxide free-radical [14]. Since the

* The fluorescence intensity of the protein is higher than that of tryptophan at the same concentration. This may suggest some interaction of the tryptophyl residues with other side chains in the protein, the nature of which is not yet known.

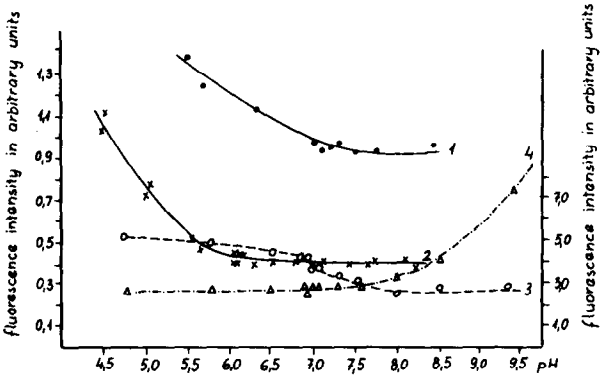

Fig. 3. pH-dependence of fluorescence. Four times recrystallized swine muscle glyceraldehyde-3-phosphate dehydrogenase prepared according to Elödi and Szörényi [19] was dissolved in 0.1 M Na-acetate: acetic acid buffer (between pH 6.0 and 4.0) or 0.1 M glycine buffer (between pH 9.0 and 6.0) in 14.5 mg/ml concentration. The protein solutions were activated at 380 nm, measured at 460 nm.
1) NAD-free enzyme, charcoal-treated [13].
2) Native enzyme containing about 3.6 moles NAD/mole protein.
3) and 4) Tryptophan (1 mg/ml) dissolved in the same buffers as the protein.
3) Activated at 385 nm, measured at 450 nm.
4) Activated at 310 nm, measured at 360 nm (right-hand ordinate).

paramagnetic quenching of fluorescence is effective only over a very short distance, it may be possible to determine the position of the side chain that binds the nitroxide free radical and of the fluorescent tryptophyl residues in the protein.

Nitric oxide free-radical was prepared by the method of Woolum et al. [15]. This radical can be bound to glyceraldehyde-3-phosphate dehydrogenase through SH-groups as indicated by the modification of ESR signal of the radical [16]. The nitric oxide radical inhibits the activity of the enzyme and the inhibition is probably competitive with the substrate [17].

The nitric oxide free-radical completely quenches the fluorescence of the enzyme at 460 nm.

Since only one tryptophyl residue of the enzyme is at or near the surface of the protein [18] this residue may be in the neighbourhood of the reactive SH-group in the active centre and one of those involved in the formation of excimers in glyceraldehyde-3-phosphate dehydrogenase.

The data presented in this paper suggest that the

fluorescence of some proteins in high concentration may be an excimer fluorescence. The pH-dependence of the intensity and the complete quenching by the nitric oxide free-radical indicate that tryptophyl residues may have some role in the appearance of excimer fluorescence.

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