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ON THE EMISSION OF TRYPTOPHAN

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SUMMARY

Tryptophan possesses two sets of emissions, each one of which has one short-lived emission and one long-lived emission. The excitation by π–π absorption at 280 mμ gives u.v. fluorescence and blue phosphorescence under certain conditions, *e.g.* frozen in an aqueous solution containing glucose or methanol. Both emissions are sensitive to the presence of added substances: in a frozen solution without addition, the blue phosphorescence disappears, while with the decrease in the fluorescence there is a concomitant increase in the phosphorescence, resulting from energy transfer between triplets, in the presence of acetone. The excitation by a near-u.v. absorption at 350 mμ produces blue short-lived emission and green long-lived emission, which are quenched by the addition of strong acid or the formation of charge-transfer complex with dinitrophenol, etc.

INTRODUCTION

Studies of this laboratory have indicated that tryptophan plays a special role in biological energy transmission. For this reason it seems desirable to have additional details concerning the optical properties of this amino acid. Tryptophan and proteins containing tryptophan are known to show two kinds of blue emission[1-3]. One is a long-lived emission (phosphorescence) from a triplet state on excitation at about 280 mμ. This emission is accompanied by an u.v. fluorescence from an excited singlet state. The other blue emission is a short-lived one from an unknown state of the molecule when tryptophan is excited at about 350 mμ.

The further attempt presented here to elucidate the nature of the short-lived emission has revealed that tryptophan can also emit a green long-lived emission which seems to be related electronically to the blue short-lived emission, and that the couple, the blue short-lived emission and the green long-lived emission, is subject to changes different from the other couple, the u.v. fluorescence and the blue phosphorescence, in the presence of foreign substances. This report will also present evidence that nitro-compounds, such as 2,4-dinitrophenol and trinitrobenzene, which can quench the tryptophan emission, complex with tryptophan.

EXPERIMENTAL

DL-tryptophan (Eastman Organic Chemicals) was recrystallized three times from hot methanol–water (70:30).

Emission spectra at liquid-nitrogen temperatures were measured by means of the Beckman DK-1 spectrophotometer with an unsilvered Dewar flask containing liquid nitrogen in the lamp-housing compartment[2]. The exciting light was isolated from a Hanovia high-pressure xenon arc (Model 10-C-1) using a Leiss fused-quartz monochromator.

Absorption spectra were studied with the Beckman DK-1 recording spectrophotometer.

RESULTS

The results of tryptophan emission are summarised in Table I with those of indole and protein emission for comparison.

In a frozen aqueous solution, tryptophan excited by 350 mμ was found to emit a weak green long-lived emission at liquid-nitrogen temperature, as well as the blue short-lived emission at about 450 mμ (Fig. 1). Since protein emitted more strongly, the use of a narrow slit enabled two peaks to be shown at 440 mμ and at about 500 mμ (Fig. 2). This indicates that there are two different states excited by near-u.v. light, which give rise to two kinds of emission: blue short-lived emission and green long-lived emission.

The previous spectral study of a concentrated protein solution suggested the possible existence of a long-wave-length shoulder on the 280 mμ peak[2]. Investigation of the action spectrum for the short-lived blue emission of tryptophan indicated that the emission was excited in the vicinity of 360 mμ[3]. In the present study, absorption spectra in the near u.v. region were measured for both tryptophan and

TABLE I

EMISSION OF TRYPTOPHAN, INDOLE AND PROTEIN AT 77° K

Exciting light	*280 mμ*				*350 mμ*	
Emission	*Short-lived*		*Long-lived*		*Short-lived*	*Long-lived*
Tryptophan	325 mμ	345	410 435 435	~500	450	~500
Water	—	+	—	+	+	+
Glucose	++	—	++	—	+	+
Methanol	++	—	++	—	+	+
Acid	+	—	+	—	—	—
Acetone	—	—	+	+	+	+
Cysteine	—	—	—	+	+	+
Histidine	+	—	+	—	+	+
Dinitrophenol Riboflavin Folic acid	—	—	—	—	—	—
Indole	310 320		405 430 450		430	500
EPA	+		+		+	+
Acetone	—		+		+	+
Bovine serum albumin	335		410 435 455		440	~500

indole in order to detect any absorption maximum in this range. Indole did give a clear shoulder at about 360 mμ, but tryptophan did not show any such shoulder. It is probable that a low absorption maximum of tryptophan in this range may be covered by the high tail of the main absorption band around 280 mμ.

On excitation at 280 mμ in a frozen aqueous solution at 77° K the π–π u.v.

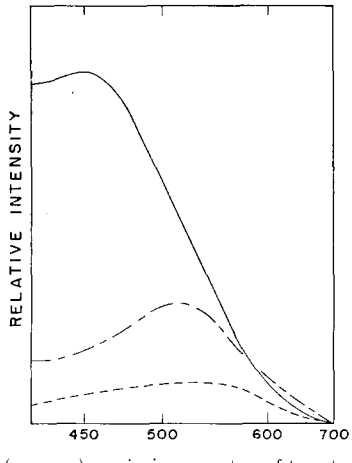

Fig. 1. (———), emission spectra of tryptophan (10^{-2} *M*) in water; (—·—), with 10^{-4} *M* dinitrophenol; (– – –), with 8 *M* HCl. Excitation: 350 mμ; T = 77° K; Slit 2 mm.

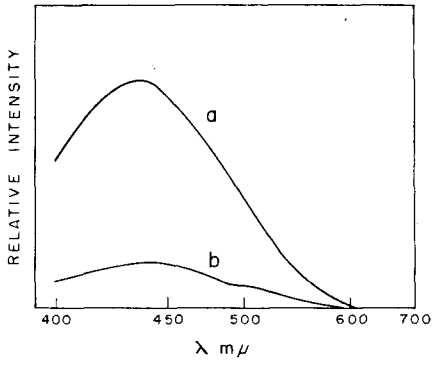

Fig. 2. Emission spectra of bovine serum albumin (10%). Excitation: 350 mμ; T = 77° K. (a) Slit 1 mm; (b) Slit 0.5 mm.

fluorescence was observed at 345 mμ with a very weak greenish long-lived emission (Fig. 3). It should be mentioned that the blue long-lived emission was not observed at this excitation[2].

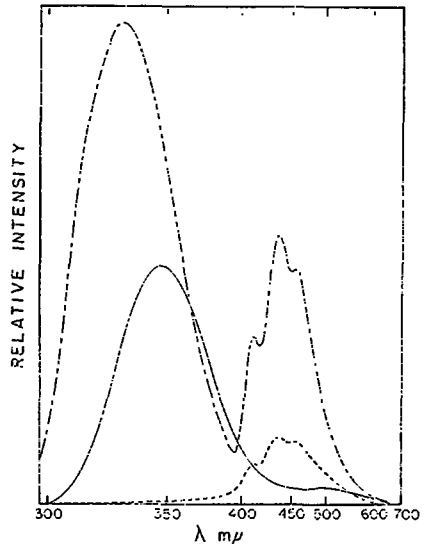

Fig. 3. (——), emission spectra of tryptophan (10^{-2} *M*) in water; (—·—), in 10% glucose; (– – –), in 80% acetone. Excitation: 280 mμ; T = 77° K; Slit 1 mm.

Glucose

When tryptophan was excited at 280 mμ in a frozen watery solution containing 0.5 ~ 10% glucose, not only the blue π–π phosphorescence[1] appeared at about 440 mμ, but also the u.v. fluorescence increased and shifted to 325 mμ compared with the fluorescence of a solution frozen without glucose (max. 345 mμ) (Fig. 3). The 325-mμ emission corresponded to the fluorescence of indole in a rigid glass medium EPA. On the other hand, in the short-lived blue emission excited at 350 mμ there was no shift in the presence of glucose, and the green long-lived emission was observed at liquid-nitrogen temperature whether glucose was present or not. The green long-lived emission had a shorter lifetime than the blue one which also had some structure. Methanol, added to water prior to freezing, had the same effect as glucose.

Acid

Tryptophan solutions were frozen immediately after the addition of 8 *M* hydrochloric acid. The u.v. fluorescence and the blue phosphorescence excited at 280 mμ remained unchanged. However, both the short-lived blue emission and the long-lived green emission excited at 350 mμ disappeared, while a very faint greenish-yellow fluorescence appeared due to a small amount of a secondary product to be described below (Fig. 1). This fluorescence increased when the solution with acid was allowed to stand at room temperature. The effect of acid on the absorption spectra in the near-u.v. region was also studied. On adding acid, the absorption initially decreased and then a new absorption gradually developed at 355 mμ in tryptophan (in concentrated hydrochloric acid) and at 337 mμ in indole (in 0.1 *M* HCl in methanol).

This secondary change of absorption and fluorescence was due to an irreversible reaction which occurs in an acidic medium.

Acetone

At the temperature of liquid nitrogen the short-lived blue emission excited by 350 mμ was not greatly changed by acetone. When tryptophan was excited at 280 mμ in the presence of acetone, however, the u.v. fluorescence at 345 mμ was considerably quenched without any shift of the maximum, and both blue and green long-lived emission were enhanced (Fig. 3). The same change was observed even in indole in a rigid glass medium (EPA) containing a small amount of acetone at 77° K (Fig. 4).

The decrease of the tryptophan fluorescence by acetone can be explained by n–π absorption (singlet-singlet transition) of acetone at 280 mμ, which is the wave-length used to excite tryptophan. The energy transfer between n–π triplet state of acetone formed from singlet excited state and triplet state of tryptophan may well result in the increased tryptophan phosphorescence. Such an energy transfer between triplet states was first shown by TERENIN AND ERMOLAEV[4] in a benzaldehyde (benzophenone)–naphthalene system.

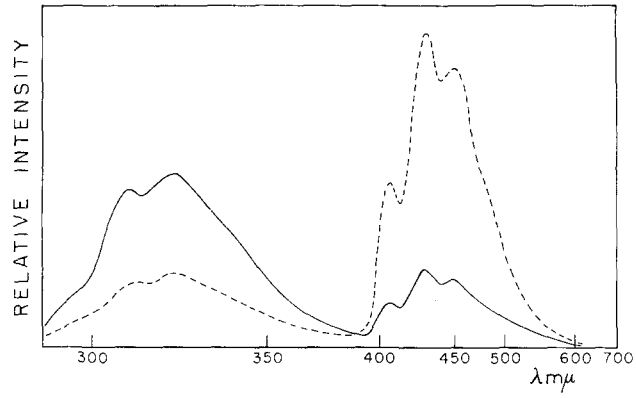

Fig. 4. (——), emission spectra of indole (10^{-2} M) in EPA; (– – –), in EPA with 2 % acetone. Excitation: 280 mμ; T = 77° K; Slit 1 mm.

Other amino acids

Of the amino acids so far studied, histidine was particularly active in its ability to enhance the blue phosphorescence in a 10^{-2} M concentration. Cysteine effectively quenched the u.v. fluorescence of tryptophan without any enhancement of blue phosphorescence.

2,4-dinitrophenol

The absorption spectra of mixed solutions of tryptophan and dinitrophenol indicated a complex formation. The absorption spectrum of dinitrophenol was found to be shifted toward a longer wave-length in the presence of tryptophan in a neutral buffer solution. Spectral changes in low concentrations of dinitrophenol in the presence of high concentrations of tryptophan indicated an association constant of about 5 l/mole. Trinitrobenzene, which is known to be a good electron-acceptor, was also found to complex with tryptophan, indole, and proteins containing tryptophan.

The change of absorption spectrum in the trinitrobenzene complex was even more marked. The measurement of difference spectra showed the absorption maximum of the trinitrobenzene-tryptophan (or indole) complex to be at about 380 mμ, while that of trinitrobenzene in itself was at 325 mμ. In recent studies[5,6], tryptophan has been found to act as an electron-donor. The formation of this type of charge-transfer complex of dinitrophenol with tryptophan in proteins may provide some insight into the mechanism of the biological action of dinitrophenol.

In the emission spectra of frozen complexes of tryptophan with nitrocompounds, all of the tryptophan emission was quenched. Instead, the complex exhibited a new weak emission at 510 mμ in the dinitrophenol-complex (Fig. 1) and at 465 mμ in the trinitrobenzene-complex at liquid-nitrogen temperatures. Dinitrophenol itself was found to emit at 557 mμ in the absence of glucose and at 510 mμ in the presence of glucose. Trinitrobenzene had the same emission at 465 mμ in a frozen solution with and without glucose. Thus, the emission of the complexes can be seen to correspond almost exactly to the luminiscence of the electron-acceptor nitro-compound. This indicates that the emission occurs from the metastable state of the electron-acceptor components. It should be made clear that the luminiscence of nitro-compounds was a delayed emission observable only at low temperature. The same phenomenon was observed in frozen complexes of tryptophan with riboflavin[5] and with folic acid[6], where the very weak emission occurred at liquid-nitrogen temperatures at 565 mμ and at 500 mμ, respectively, which corresponded to the luminiscence of riboflavin and of folic acid in a frozen solution without glucose. These emissions in the complexes did not change even in the presence of glucose, while riboflavin fluoresced at 525 mμ with a red phosphorescence, and folic acid at 425 mμ with a green phosphorescence at 485 mμ in the presence of glucose.

In these complexes the electron-acceptors have a lower excited level than tryptophan, and their lowest metastable state is lower than the excited state of the complexes[7]. This would explain the experimental results that the weak emission from the metastable state of electron-acceptors is observed in the complexes at low temperature, accompanying the quenching of tryptophan emission.

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