

AD 675746

TRANSLATION NO. 2028

DATE: 19 Oct 1967

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OCT 16 1968

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QUANTUM YIELDS OF PHOTOCHEMICAL REACTIONS OF
TYROSINE AND TRYPTOPHANE IN SOLUTIONS

Biofizika
(Biophysics)
Vol. 11, No. 4, pages 578-583, 1966

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Photochemical inactivation of enzymes under the action of ultra-violet rays occurs mainly due to energy absorbed by aromatic aminoacids, histidine, and cystine (1). Study of photochemical reactions in solutions of aromatic aminoacids and dipeptides can therefore bring us closer to understanding the mechanism of photochemical processes in proteins.

Previously it was shown that the aromatic rings of tyrosine and tryptophane can enter into both photochemical reactions: the reaction of photo-oxidation by molecular oxygen and the reaction of interaction with neighboring groups of the peptide chain (2). This study is devoted to measurement of quantum yields of these reactions at different pH values, and also to a study of the quantum yield of tryptophane photo-oxidation as a function of the wavelength of the effective radiation, and as a function of the concentration of oxygen, NaCl, glycine, and alcohol.

Methods

Domestic and imported commercial preparations of aminoacids and dipeptides were used in the study. Glycine was first purified by three-fold recrystallization from aqueous solution. In experiments studying the action of oxygen and salts on quantum yield of photo-decolorization, aqueous solutions of aminoacids at concentrations of 0.002-0.01 mg/ml were used. In studying the effect of spectral composition of irradiating light and pH of medium on photochemical reactions, solutions were prepared in 0.1 M of glycine buffer according to Serensen. Measurements were made at room temperature. The values of quantum yield of the photochemical reactions were calculated from the following formula (1):

$$\varphi = \frac{\ln(C_0/C)}{i \cdot s \cdot t},$$

where C_0 and C = concentration of aminoacid prior to irradiation and after t seconds of irradiation; i = intensity of radiation impinging on the cell containing the solution ($\text{photons} \cdot \text{cm}^{-2} \cdot \text{sec}^{-1}$); φ = quantum yield of the photochemical reaction ($\text{molecules} \cdot \text{photons}^{-1}$); s = cross section of absorption ($\text{cm}^2 \cdot \text{molecule}^{-1}$).

Calculation of s was made according to the equation (1):

$$s = 3,8 \cdot 10^{-21} \epsilon,$$

where ϵ = molar coefficient of extinction at given wavelength and pH. Values of ϵ for tyrosine and tryptophane were taken from the study (3).

Irradiation of solutions was carried out either with complete light from the SVD-120A mercury lamp, or with monochromatic radiation at the exit of the monochromator. In the latter case the light source was the mercury lamp DRSh-1000. Solutions were irradiated in quartz cells from the SF-4 spectrophotometer or in a Tunberg quartz vacuum tube. Intensity of light impinging on the cell was measured by a graduated selenium photoelement (4). In this case, corrections were made for screening effect in the solution and for reflection from quartz walls. The time of irradiation with complete light of the mercury lamp ranged from 1 to 10 minutes, and with monochromatic light — the time amounted to several hours. Photo-decolorization of aminoacids was evaluated from the luminescence of the solution. Luminescence was excited by monochromatic radiation of a mercury lamp with wavelength of 280 nanomicros. The light of luminescence passing through the second monochromator was measured with a FEU-39 photomultiplier. The photocurrent was intensified with a direct-current amplifier and was recorded on the EPP-09 self-recording potentiometer (5).

Results and Discussion

Figure 1 presents the results from measuring the quantum yield of photo-decolorization of an aqueous solution of tryptophane in air when irradiation was conducted with monochromatic ultraviolet irradiation at different wavelengths. As can be seen from this data, the quantum yield of photo-oxidation of tryptophane was the same for wavelengths of 254, 265, 280, and 289 nanomicros. In the study (6) it was shown that the tryptophane molecule contains two singlet levels corresponding to the excited state of 1L_x and 1L_a , however during the lifetime of the excited state complete redistribution of energy between these levels occurs. Therefore, the quantum yield of the photo-oxidation of tryptophane does not depend on wavelength of effective radiation, which has also been observed in actual fact. This allowed us to conduct the study of the quantum

yield of tryptophane photo-oxidation as a function of pH, oxygen, and as a function of other additives when the specimen is irradiated with the complete light of the SVD-120A mercury lamp.

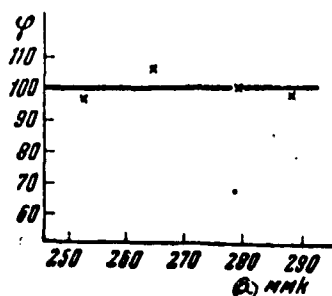


Figure 1. Quantum yield of photolysis of an aqueous solution of tryptophane (ϕ , relative units) in air as a function of wavelength of monochromatic radiation.

LEGEND: a) millimicrons

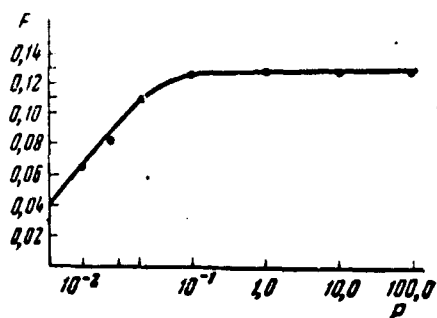


Figure 2. Rate of photolysis of tryptophane (F , relative units) as a function of air pressure over the solution (P , mm Hg).

As is commonly known, solutions of aromatic aminoacids in vacuum are fairly resistant to ultraviolet irradiation; in the presence of air oxygen however, they are rapidly subjected to photochemical degradation (2). It is obvious that for a photochemical reaction collision of an excited aminoacid molecule (or the primary unstable photo-product) with an oxygen molecule is necessary. If we know the concentration of oxygen in solution, the number of collisions of the molecules of tryptophane with oxygen per unit time can be found from the equation (10):

$$Z'_{AB} = 4\pi r D \cdot N \cdot 10^{-3} \cdot C = 7.6 \cdot 10^{21} \cdot r \cdot D \cdot C,$$

where r = total of radii of the reacting molecules; D = total of diffusion coefficients of these molecules in water; N = Avogadro's number. Adopting for the case of the collision of tryptophane with oxygen, $r \sim 4 \cdot 10^{-8}$ cm and $D \sim 3 \cdot 10^{-5}$ cm² · sec⁻¹, we find $Z'_{AB} = 9 \cdot 10^9 \cdot C$ collisions per second. From here it is easy to find the time between two collisions:

$$\tau = 1/Z'_{AB} = \frac{1.1 \cdot 10^{-10}}{C} \text{ second}$$

As we can see from Figure 2, the decrease in pressure of air over the tryptophane solution has almost no effect on the rate of the photochemical

process all the way to pressures of the order of 10^{-1} mm Hg. At these pressures, the oxygen concentration in water is approximately $1.82 \cdot 10^{-7}$ M (7), and the time between two collisions of tryptophane with oxygen is $\sim 6 \cdot 10^{-4}$ second. This evaluation is highly approximate, first of all, owing to the approximateness of the values of r and D used in the calculations, and, secondly, owing to the difficulty of precise measurement of the air pressure over the aqueous solution, when it becomes less than 1 mm Hg. Nonetheless, evidently it can be assumed that the lifetime of the photochemically active excited state of tryptophane molecules ($\sim 10^{-3}$ second) markedly exceeds the lifetime of molecules in the singlet excited state ($10^{-8} - 10^{-9}$ second). This means that photo-oxidation of tryptophane (type I reaction (2)) occurs as a result of the interaction with oxygen either of the triplet state of the aminoacid, or the primary photo-product, for example, the ion-radical (8).

It is interesting to note that glycine, ethyl alcohol, and NaCl have almost no effect on the rate of photo-oxidation of tryptophane by oxygen (cf Tables 1 and 2). Still, the concentrations of these compounds were so high (up to 2 M glycine, 25% NaCl, and 90% alcohol) that during the lifetime of the singlet excited state (taken as equal to $2 \cdot 10^{-9}$ second) aminoacid molecules can collide with glycine molecules 50 times, with NaCl molecules 15 times, and with alcohol molecules 60 times*. Why the excited indole ring of tryptophane readily reacts with the glycy moiety in glycytryptophane (type II reaction (2)) and does not react with the dissolved glycine, in spite of collision with its molecules, remains obscure. Possibly, the "cell effect" existing in the case of glycytryptophane (or in the frozen solutions of tryptophane with glycine) is essential for type II reactions.

TABLE 1
Rate of Photolysis of Tryptophane (F), and O_2 Concentration
as Functions of NaCl Concentration

NaCl, %	0.1	0.5	1	5	10	15	30	25
C, %	100	87.5	—	100	83.6	43	21.4	6.4
F, %	82	80	81	—	83	85	88.5	100

* For the calculations, r was taken as equal to 4 AU; D for glycine — $95 \cdot 10^{-4} \text{ cm}^2 \cdot \text{sec}^{-1}$ (9); for alcohol and NaCl — $2 \cdot 10^{-5} \text{ cm}^2 \cdot \text{sec}^{-1}$ (5). As in the case of oxygen, the calculation affords only estimation of the order of magnitude.

TABLE 2

Rate of Photolysis of Tryptophane (F), and O_2 Concentration
as Functions of Glycine Concentration

② Глицин [N]	0,001	0,01	0,1	0,5	1	2
C, %	100	86,4	84,8	83,3	82	82
F, %	100	99	100	101	100	100

LEGEND: a) glycine (N)

Figures 3-5 present data on the quantum yields of tryptophane, tyrosine, and glycytryptophane as functions of the pH of the medium. As can be seen from Figure 3, the form of the curve describing this function in the case of tryptophane for pH values from 3 to 10 is close to the curve of pH-dependent function of the quantum yield of tryptophane fluorescence (11). However, at $pH < 3$ and $pH > 10$, quenching of fluorescence takes place, but the corresponding decrease in quantum yield is not observed (cf Figure 3).

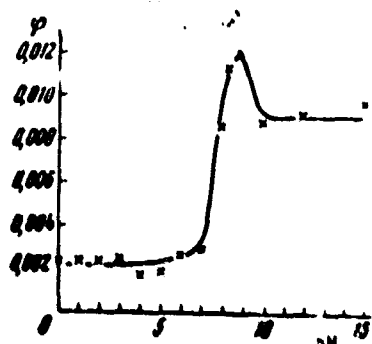


Figure 3. Absolute quantum yield of photochemical degradation of tryptophane (ϕ) in air as a function of pH of the medium.

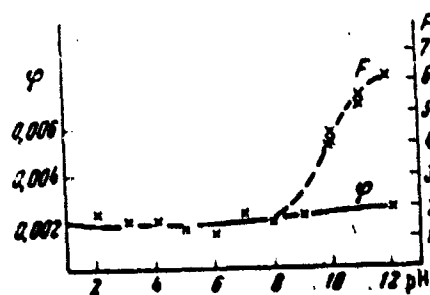


Figure 4. Rate (F, relative units) and absolute quantum yield of photolysis of tyrosine in air (ϕ) as functions of pH of medium.

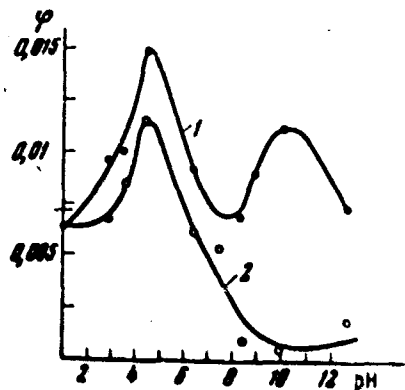
Figure 5 gives the values of the quantum yields of the photochemical degradation of glycytryptophane in vacuum (1) and also in air (2) at several pH values. As already stated, under these conditions two photochemical reactions occur in glycytryptophane: oxidation of the indole ring (type I reaction) and its interaction with the glycine moiety (type II reaction (2)). The quantum yields of these reactions can be taken as:

$$\varphi_2 = \varphi_{\text{exc}}; \quad \varphi_1 = \varphi_{\text{exc}} - \varphi_{\text{vac}}$$

LEGEND: a) vacuum; b) excitation.

where φ_{exc} and φ_{vac} = experimentally found values of quantum yields. The curve of the quantum yield of quantum yield of the photo-oxidation reaction (φ_1) for glycytryptophane is very close to the corresponding curve for tryptophane, and the absolute values are the same both in the neutral and the alkaline medium (cf Table 3). This means that in glycytryptophane the photo-oxidation reaction proceeds independently of the second reaction (interaction with glycine). As already noted earlier (2), the type II reaction (cf Table 3) is the main reaction in which glycytryptophane takes part (as also do tryptophane moieties of proteins) in a neutral medium.

Figure 5. Absolute quantum yield of photochemical degradation of glycytryptophane (φ) as a function of the pH of the medium in air (curve 1) and in vacuum (curve 2).



The quantum yield of photolysis of tyrosine is preserved approximately constant for all pH values (cf Figure 5), although at pH > 10 there occurs ionization of the phenol group, and change in the absorption spectrum and the rate of the photochemical reaction (Figure 4, curve 1). This can be explained by the fact that in the transition of tyrosine to the excited state it acquires the properties of a stronger acid ($\Delta pK = 5.4$ (12)) and therefore both in a neutral and in an alkaline medium tyrosine in the excited state is found in the form of an ion. As a result of this, in both cases the same primary photo-product is formed (13).

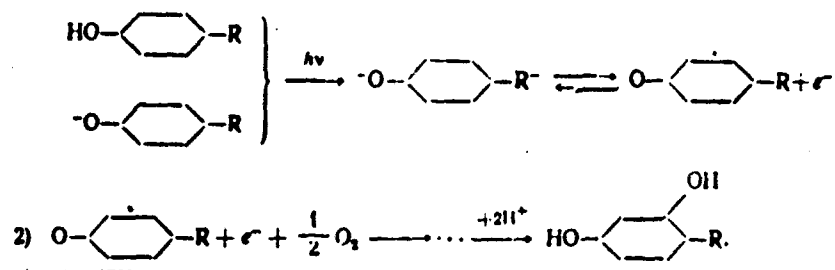
TABLE 3

Quantum Yields (φ) of Photochemical Reactions of Aromatic Aminoacids

Compound	In air (a) φ_{exc}		In vacuum (b) $\varphi_2 = \varphi_{\text{vac}}$		(c) $\varphi_1 = \varphi_{\text{exc}} - \varphi_{\text{vac}}$	
	pH 4.5	pH 10	pH 4.5	pH 10	pH 4.5	pH 10
Триптофан (e)	0.003	0.012	0.012	0.001	0.003	0.01
Глицилтриптофан (f)	0.015	0.011				
Тирозин (g)	0.003	0.003				

LEGEND: a) compound; b) in air, φ_{exc} ; c) in vacuum ($\varphi_2 = \varphi_{\text{vac}}$); d) $\varphi_{\text{exc}} - \varphi_{\text{vac}}$; e) tryptophane; f) glycytryptophane; g) tyrosine.

The reaction evidently occurs according to the following scheme:



Which stage of this chain of reactions: photo-ionization (1) or interaction with molecular oxygen (2) determines the value of the overall quantum yield, remains an open question.

Conclusions

1. An investigation has been made of absolute quantum yields of photochemical decolorization (attenuation of fluorescence of solutions upon ultraviolet irradiation) of tryptophane, glycytryptophane, and tyrosine in the presence and in a vacuum. The quantum yield of decolorization of tryptophane in air (type I reaction) did not depend on wavelength of the effective radiation and increased in the alkaline medium; the quantum yield of decolorization of glycytryptophane in vacuum (type II reaction) in an alkaline medium was reduced. In glycytryptophane reactions I and II occurred evidently independently of each other. The quantum yield of tyrosine was constant upon change in pH from 2 to 12.

2. Glycine (2 M), NaCl (1 M), and alcohol (90%; ~ 20 M) did not affect the rate of decolorization of tryptophane, although during the lifetime of the excited state (~10⁻⁸ second) the tryptophane molecule is able to collide with molecules of these compounds several dozens of times. Upon evacuation of air from the solution, a decrease in the rate of photo-oxidation of tryptophane was observed only at pressures ≤ 10⁻¹ mm Hg. This signifies that in the reaction of interaction with oxygen metastable tryptophane products take part (possibly, of the free radical type) with lifetimes of the order of 10⁻⁴ second.

T. N. Lyamina participated directly in the experiments, for which we extend heartfelt appreciation.

Received by editor
12 July 1965

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