[Chem. Pharm. Bull. 31(11)4167—4171(1983)]

Biphasic Photolysis of Riboflavin. II.[1] Effect of Gelatin on the Photolysis

YUKIO SATO,* SACHIKO YAMASATO, and YASUO SUZUKI

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980, Japan

(Received February 14, 1983)

The photolysis of riboflavin in the presence of gelatin with a low-intensity light source was investigated. It was found that the time course of photolysis was triphasic. The first break in the time course was attributed to dissociation of dimeric riboflavin. The second break may result from a change of relative predominance between the reactive species, *i.e.*, the singlet and triplet states of excited riboflavin. The changes of thermodynamic parameters are discussed from the viewpoint of riboflavin stability. The data suggest that gelatin promotes the photolysis of riboflavin *via* energy transfer from protein, though it has a stabilizing effect in the initial stage of irradiation.

Keywords——riboflavin; gelatin; photolysis; thermodynamic parameter; fluorescence quenching; photosensitizer; energy transfer

We reported previously that the biphasic photolysis of riboflavin shows an induction period or initial rate of photolysis and then reaches a maximum value with a low-intensity light source.[1] A similar biphasic photolysis of riboflavin was also reported by Owen and O'Boyle.[2] They observed that the addition of gelatin to an aqueous solution of riboflavin reduced the rate of aerobic photolysis. They explained the effect of gelatin as being due to a gradual transition from a singlet state to a triplet one. The present investigation was undertaken to examine in detail the photolysis of riboflavin in the presence of gelatin with a low-intensity light source.

Experimental

Materials——Riboflavin was a gift from Toa Eiyo Chemical Co., Ltd., and was used without further purification. Gelatin was purchased from Wako Pure Chemical Industries, Ltd. Aqueous solutions of gelatin were used after filtration. All other chemicals used were of reagent grade. Deionized redistilled water was used throughout this study.

Methods——The apparatus and procedure were the same as in the method described previously.[1] All of the data are means of two or three determinations.

Results

The Effect of Gelatin on the Photolysis of Riboflavin

When a solution of riboflavin was irradiated with a low-intensity light in the presence of gelatin, the kinetic patterns were rather complex (Fig. 1). It should be noted that the time course of photolysis was triphasic. The rate in each phase follows a zero-order kinetic pattern rather than a pseudo first-order process. The extrapolations of the linear second and third phases at various gelatin concentrations each intersected at specific positions. The occurrence of the second break in the photolysis process is related to the addition of gelatin. It is of interest that the more rapid second phase was followed by the slower third phase. This is not

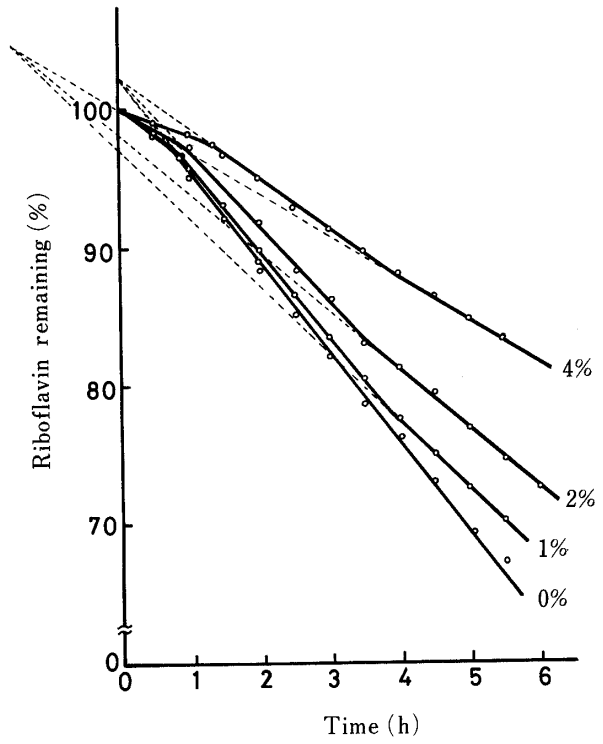

Fig. 1. Effect of Gelatin on the Photolysis of Riboflavin in 0.05 M Phosphate Buffer (pH 7.0) at 25 °C
[Riboflavin] = 5×10^{-5} M.

TABLE I. Thermodynamic Parameters for the Photolysis of Riboflavin

	E (kcal/mol)	ΔS^* (cal/mol degree)	ΔF^* (kcal/mol)
Riboflavin			
1st phase	10.3	−21.5	16.7
2nd phase	10.0	−21.5	16.4
Riboflavin–gelatin (2%)			
1st phase	12.6	−14.3	16.9
2nd phase	8.4	−26.5	16.3
3rd phase	7.9	−29.3	16.6

consistent with the result of the study of Owen and O'Boyle showing that the biphasic photolysis of riboflavin consisted only of an induction period followed by the maximum rate phase, despite the presence of gelatin.[2] The duration of each phase depended on the temperature. On elevation of the temperature, the third phase disappeared because of the rapidity of photolysis. From the temperature dependency of the degradative reaction, one can estimate the thermodynamic parameters for each phase. Apparent energies of activation, E, were graphically estimated from an Arrhenius plot. The values of activation entropy change, ΔS^*, and free energy change, ΔF^*, in each phase are given in Table I. In the presence of gelatin, the activation energy was increased only in the first phase and was decreased in the later phases. This indicates that the effect of gelatin on the stability of riboflavin to light disappeared in the later phases. The values of ΔS^* decreased successively. The values of ΔF^* showed little change.

The Effect of Electrolytes on Riboflavin Photolysis

Figure 2 shows plots illustrating the effects of electrolytes on the photolysis of riboflavin.

The duration of the initial phase and the rate were affected by the addition of electrolytes in all systems. Potassium iodide reduced the rate of photolysis in each phase more effectively than potassium chloride. As is clear from Fig. 2(b), in the presence of gelatin a rate decrease and a reduction of the duration of the second phase were caused by the addition of electrolytes. It is clear that the difference of effects between potassium iodide and potassium chloride on the photolysis of riboflavin resulted from more effective quenching by the former than the latter. However, in the presence of gelatin, the quenching effects of these electrolytes were not very different.

Fluorescence Properties of Riboflavin during Photolysis

The rates of photolysis of aqueous solutions of riboflavin were examined spectrofluoro-

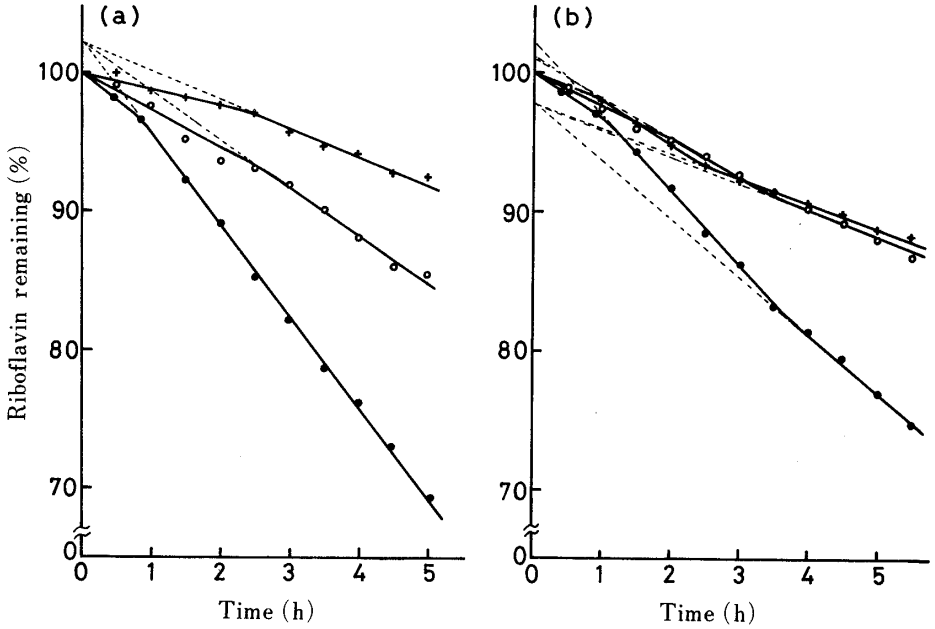

Fig. 2. Effect of Electrolytes on the Photolysis of Riboflavin in 0.05 M Phosphate Buffer (pH 7.0) at 25 °C [Riboflavin] = 5×10^{-5} M, [KCl] = 1×10^{-5} M, [KI] = 1×10^{-5} M

(a), in the absence of gelatin; (b), in the presence of 2% gelatin. (—●—), riboflavin alone; (—○—), in the presence of KCl; (—+—), in the presence of KI.

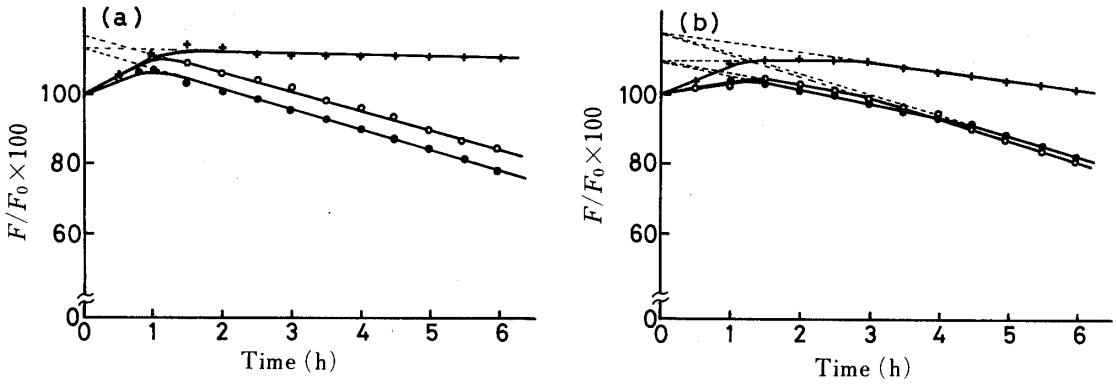

Fig. 3. Effect of Electrolytes on the Fluorescence Intensity Change of Riboflavin in 0.05 M Phosphate Buffer (pH 7.0) at 25 °C [Riboflavin] = 5×10^{-5} M, [KCl] = 1×10^{-5} M, [KI] = 1×10^{-5} M

(a), in the absence of gelatin; (b), in the presence of 2% gelatin. (—●—), riboflavin alone; (—○—), in the presence of KCl; (—+—), in the presence of KI.

metrically under various conditions. When riboflavin solution was irradiated, the fluorescence intensity first increased, and then decreased. A deviation from a linear zero-order kinetic pattern was observed when the F/F_0 value (F: fluorescence intensity) was less than about 65% of the initial value.[1,3] In general, the quantum yield of fluorescent dyes in monomeric form is higher than that of associated ones. Therefore, the initial increase in the fluorescent intensity might reflect the dissociation of associated riboflavin. In the presence of gelatin, a triphasic kinetic pattern was observed, in accordance with the data given above. Figure 3 shows the results of photolysis of riboflavin in the presence of electrolytes. Apparently, potassium iodide repressed the reactive species of excited riboflavin, without affecting the kinetic pattern. The influence of potassium iodide upon the kinetic behavior seemed to be remarkable in the second and third phases of the time course. These results indicate that excited states of riboflavin are involved in the second break in the time course.[2]

Discussion

Factors Influencing on Rate of Photolysis

From the spectrophotometric and fluorometric analyses, two consecutive phases were observed in the kinetic behavior of the photolysis of riboflavin with a low-intensity light in an aqueous solution.

Two explanations for the biphasic photolysis are possible. First, the first phase in the time course is due to the association of riboflavin molecules.[1] The ground state of riboflavin effectively determines the rate of photolysis. Second, the break in the time course results from a change of the major reactive species in the excited state of riboflavin. In this case, the kinetic behavior of the photolysis is essentially affected by the excited states. Two cases can be considered: (1) the singlet state is the major reactive species; (2) the triplet state is predominant ("singlet mechanism" and "triplet mechanism," respectively). Owen and O'Boyle[2] observed biphasic photolysis of riboflavin in the presence of a high concentration of gelatin. They suggested that as the concentration of gelatin is increased, the triplet mechanism assumes a greater importance. The maximum value of the rate is that arising from the triplet mechanism because of its activity. In the present study with gelatin, however, the maximum rate appeared in the second phase. This is in striking contrast to the result of their study. Kurtin *et al.*[4] reported a rapid initial rate for the photolysis of riboflavin in pyridine, corresponding to the change in the second and third phases in the present study. They proposed two explanations for the two consecutive lines in the rate curve. First, the slower rate curve is due to the absorbance at 450 nm contributed by a steady state concentration of intermediates such as semiquinone formed from riboflavin. Second, the break in the rate curve results from a decrease in the quantum yield due to products of the reaction. The latter explanation is consistent with the excited mechanism. The rapid second rate implies that the second break arises from a gradual transition from a reaction in which the triplet state is the important reactive species to one in which the singlet state predominates. It appears that in the case of the low-intensity light source the triplet species consumed in the early stage will not be formed abundantly in the system. Thus, the contribution of the singlet species to the rate should be predominant. On the other hand, in the case of a high-intensity light source the predominance of the triplet state is due to the continuous supply of the triplet species. The change in mechanism in the two cases may result from the difference of energies absorbed per unit time and the availability of the triplet species.

Effect of Gelatin on the Photolysis

The addition of gelatin would result in a planar configuration of riboflavin and increase the probability of radiative transition.[2] Thus, the participation of the triplet state may

become more significant with a low-intensity light source. The characteristic effect of gelatin on the photolysis can be appreciated from the thermodynamic parameters (Table I). From the values of E in each phase, it is obvious that the protective effect of gelatin is restricted to the first phase in the photolysis process of riboflavin. The data in Table I strongly suggest that the added gelatin acts a photosensitizer in the later phases. It seems reasonable to assume that such an effect arises from light absorbed by the protein of gelatin. That is, excitation energy is transferred from protein to riboflavin by a nonradiative resonance process involving the aromatic amino acid residues in the protein. Although there are several possible combinations of energy transfer modes from protein to riboflavin, the most likely explanation for the present result is energy transfer from a singlet state of protein ($E_s = 2.5$—$2.9\,\mathrm{eV}$)[5] to riboflavin triplet ($E_t = 2.05\,\mathrm{eV}$) leading to the photolysis of riboflavin.[5-8]

On the basis of the efficiency of the singlet excitation energy transfer, this reaction is energetically possible although it is a spin–forbidden process. This hypothesis of an energy transfer mechanism from protein to riboflavin seems to be consistent with a relatively small change of the ΔF^* values compared with the changes of other parameters. It should be emphasized that gelatin promotes the photolysis of riboflavin *via* energy transfer although gelatin has a stabilizing effect against light in the initial stage of irradiation. The small ΔS^* values imply that the decrease of degree of freedom is small in the later phases. This result may reflect energy transfer from protein to riboflavin in the rigid gelatin solution. The stabilizing effect of gelatin in the initial stage should be related to the increased rigidity of the solution and the induced planarity of riboflavin configuration.

References and Notes

1) This paper follows on a previous report entitled "Biphasic Photolysis of Ribiflavin with a Low-Intensity Light Source"; Y. Sato, M. Yokoo, S. Takahashi, and T. Takahashi, *Chem. Pharm. Bull.*, **30**, 1803 (1982).
2) E. D. Owen and A. A. O'Boyle, *Photochem. Photobiol.*, **14**, 683 (1971).
3) C. T. Shin, B. J. Sciarrone, and C. A. Discher, *J. Pharm. Sci.*, **59**, 297 (1970).
4) W. E. Kurtin, M. A. Latino, and P-S. Song, *Photochem. Photobiol.*, **6**, 247 (1967).
5) R. C. Nelson, *J. Chem. Phys.*, **39**, 112 (1963).
6) A. Pullman, "Modern Quantum Chemistry," Part III, ed. by O. Sinanoğlu, Academic Press, New York and London, 1965, pp. 283—312.
7) P-S. Song, *J. Phys. Chem.*, **72**, 536 (1968).
8) P-S. Song and T. A. Moore, *J. Am. Chem. Soc.*, **90**, 6507 (1968).