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Riboflavin-Sensitized Photooxidation of Phenothiazines in Aqueous Solution by Laser-Irradiation

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A laser-irradiation (490 nm) of the tranquilizing phenothiazines such as chlorpromazine, propericiazine, prochlorperazine, and promethazine in the presence of riboflavin gave the corresponding sulfoxides in an acidic buffer solution (pH 4.0). Fluorescence quenching and visible absorption changes of riboflavin by the binding to phenothiazines were quantitatively treated to obtain the equilibrium constants (K_c) of the complexes. The larger the K_c values, the greater the extent of photolysis was observed. Riboflavin-sensitized photooxidation was discussed on the basis of the complex formation between riboflavin and phenothiazines.

Keywords——tranquilizing phenothiazines; riboflavin; laser-irradiation; riboflavin-sensitized photooxidation; fluorescence quenching; circular dichroism; equilibrium constant of complex

Recently, a considerable attention has been paid to study the photodynamic action of light-sensitive compounds, and their consequences on biological activities.[2] The tranquilizing phenothiazines are extremely sensitive to photo-irradiation yielding mainly the corresponding sulfoxides which are less biologically active.[3] Riboflavin is known to promote the photodecomposition of other compounds *via* several mechanisms,[2-4] including energy transfer, coupled oxidation-reduction, and sensitized photooxidation. Thus, the present investigation was undertaken to examine the photo-sensitized effect of riboflavin on several phenothiazine derivatives such as chlorpromazine, propericiazine, prochlorperazine, and promethazine in air-saturated acidic buffer solution. The photo-irradiation was carried out by using an argon-laser because of its powerful and selective light source (490 nm). This kind of knowledge will provide not only a rational basis for design of formulation but also a means for improving the efficiency of the photo-sensitive drugs, from the pharmaceutical point of view.

Experimental

Materials——All the phenothiazine derivatives used were favored from Shionogi Pharmaceutical Co., Ltd. Riboflavin was commercially obtained and used without further purification. All other materials and solvents were analytical reagent grade. Deionized double-distilled water was used throughout the study.

Apparatus and Procedure——Solutions of phenothiazine derivatives (2.0×10^{-4} M) in the absence and in the presence of riboflavin (1.0×10^{-4} M) in 0.1 M phosphate buffer (pH 4.0, ionic strength 0.3) were prepared and irradiated in a 1 cm quartz cell located 20 cm from a projector equipped with 10 mW argon-laser (NEC, GLC-3000) at 36°. The intact phenothiazines in the reaction solution were analyzed by a gas chromatography (GC) described previously.[5] The absorption, fluorescence, circular dichroism (CD), and GC–mass

1) Location: *5-1, Oe-honmachi, Kumamoto 862, Japan.*
2) D.R. Sandvordeker and H.B. Kostenbauder, *J. Pharm. Sci.*, **63**, 404 (1974); F.S. Ghazy, T. Kimura, S. Muranishi, and H. Sezaki, *Life Sciences*, **21**, 1703 (1977).
3) C.L. Huang and F.L. Sands, *J. Pharm. Sci.*, **56**, 259 (1967); T. Iwaoka and M. Kondo, *Bull. Chem. Soc. Jpn.*, **47**, 980 (1974); *idem*, *ibid.*, **50**, 1 (1977).
4) P.-S. Song and T.A. Moore, *J. Am. Chem. Soc.*, **90**, 6507 (1968); D.R. Kearns, R.A. Hollins, A.U. Khan, and P. Radlick, *ibid.*, **89**, 5456 (1967).
5) K. Uekama, T. Irie, and F. Hirayama, *Chem. Lett.*, **1978**, 1109.

spectra (MS) were taken by a Shimadzu UV-200 spectrophotometer, a Hitachi MPS-3 spectrofluorometer, a Jasco J-20A spectropolarimeter, and a Shimadzu GC-MS-7000, respectively.

Results and Discussion

Figure 1 is a typical plot illustrating the effect of riboflavin on the photo-decomposition of chlorpromazine. A significant rate increase was achieved by riboflavin and the main photolytic product was ascertained to be chlorpromazine sulfoxide by GC–mass spectrometry. Any side reactions such as formations[5] of promazine and its sulfoxide were not observed in the reaction conditions. In the absence of riboflavin, however, any photolysis was not observed, since chlorpromazine has no absorption band at the wavelength subjected the laser-irradiation, as seen in Fig. 2. The other phenothiazine derivatives employed were also photodecomposed in the presence of riboflavin to give the corresponding sulfoxides, indicating a photo-sensitized action of riboflavin.

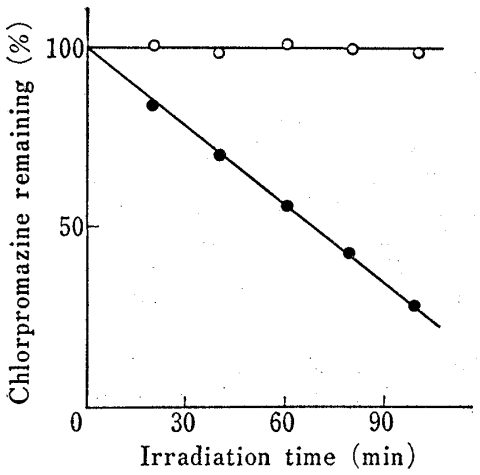

Fig. 1. Effect of Riboflavin (1.0×10^{-4} M) on the Photodecomposition of Chlorpromazine (2.0×10^{-4} M) in 0.1 M Phosphate Buffer (pH 4.0) at 36°

○: in the absence of riboflavin,
●: in the presence of riboflavin.

Figure 3 shows fluorescence quenching of riboflavin by chlorpromazine, as an example. An inner-filter effect is unlikely, because there is no spectral overlap between the absorption spectrum of chlorpromazine and the fluorescence spectrum of riboflavin (see Fig. 2). Figure 4 shows the effects of chlorpromazine on absorption and CD spectra of riboflavin in the 370—500 nm region. In the presence of chlorpromazine, the absorption peaks of riboflavin (373

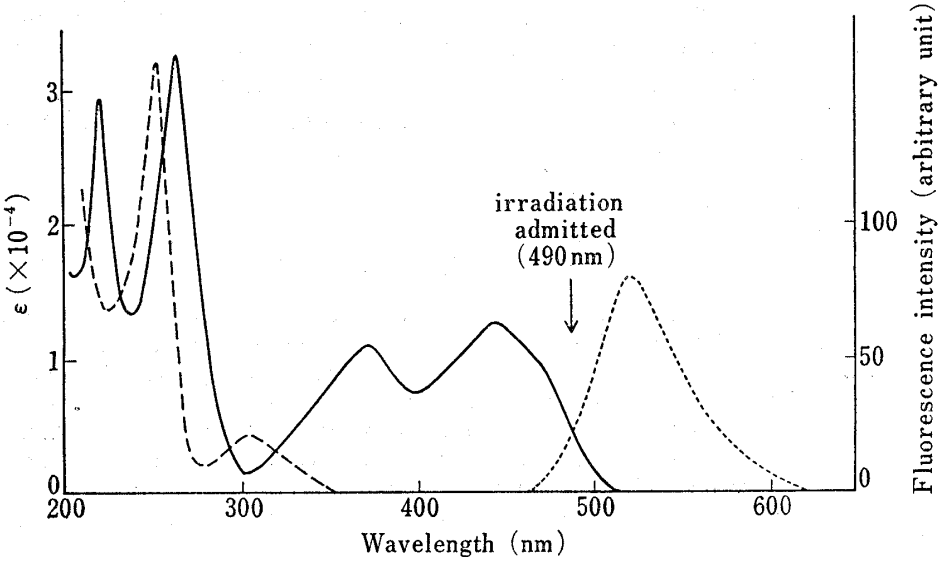

Fig. 2. Absorption Spectra of Chlorpromazine (----) and Riboflavin (——) and Fluorescence Spectrum of Riboflavin (·····) in 0.1 M Phosphate Buffer (pH 4.0) at 25°

Concentrations: chlorpromazine (5.0×10^{-5} M), riboflavin (5.0×10^{-5} M).
Excitation wavelength was 420 nm.

and 445 nm) were slightly shifted to longer wavelength with a decrease in molar absorptivity, along with a significant alteration in the CD spectrum. These spectral changes may be ascribed

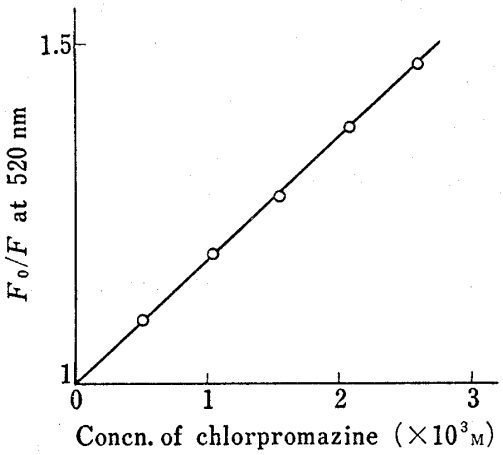

Fig. 3. Fluorescence Quenching of Riboflavin (5.0×10^{-5}M) by Chlorpromazine in 0.1M Phosphate Buffer (pH 4.0) at 25°

F_0 and F are fluorescence intensities in the absence and in the presence of chlorpromazine, respectively.

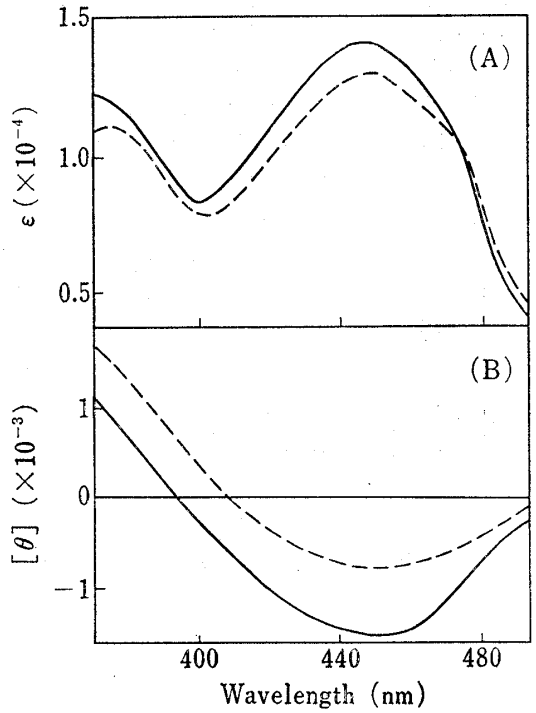

Fig. 4. The Absorption (A) and Circular Dichroism (B) Spectra of Riboflavin (5.0×10^{-5}M) in the Absence (——) and in the Presence (- - - -) of Chlorpromazine (1.0×10^{-2}M) in 0.1M Phosphate Buffer (pH 4.0) at 25°

to the intermolecular association between two planner rings (phenothiazine–isoalloxazine) as is observed in flavin-adenine dinucleotides.[6] The visible absorption changes of riboflavin were quantitatively treated by the conventional Scott's equation (1)[7] to obtain equilibrium constant (K_c),

$$\frac{a\cdot b}{d} = \frac{1}{K_c\cdot\varepsilon'} + \frac{b}{\varepsilon'} \qquad (1)$$

where a is the total concentration of riboflavin, b is the total concentration of phenothiazine derivative, ε' is the difference of the molar absorptivities for free and complexed riboflavin, and d is the change in absorbance of riboflavin by the addition of phenothiazine derivative.

Table I. The Photolytic Data and Equilibrium Constants for Tranquilizing Phenothiazine–Riboflavin Systems

Phenothiazine derivative	Decomposition[a] (%)	K_c (M^{-1})[b]	
		Fluorescence quenching	Absorption change
Chlorpromazine	43	354	352
Propericiazine	34	236	209
Prochlorperazine	26	209	194
Promethazine	22	91	80

a) The intact phenothiazines in reaction solution (after 60 min irradiation, at 36°) were analyzed by gas-chromatography.
b) Determined at 25° in 0.1 M phosphate buffer (pH 4.0), accuracy of ±5%.

6) D.W. Miles and D.W. Urry, *Biochemistry*, **7**, 2791 (1968).
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Similarly, the fluorescence quenchings of riboflavin were treated by eq. (1) to obtain the K_c values. As seen in Table I, the K_c values determined by fluorescence quenching agree very closely with those determined by absorption change, indicating a static quenching[8] of the riboflavin fluorescence by the phenothiazines. Although the experimental temperature in spectral studies was different from that in kinetic studies, it is interesting to note that the larger the K_c values, the greater the extent of photolysis was generally observed. It is well known that riboflavin is a good electron acceptor and phenothiazine derivatives are good electron donor.[9] Thus, the formation of charge-transfer complex between riboflavin and tranquilizing phenothiazine as an intermediate[10] in ground state and/or in excited state may play an important role in the present photolysis, although various mechanisms[2,4] have been proposed for riboflavin-sensitized photooxidation.

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Use of a Macroreticular Resin Containing Isothiocyanate Group as Insoluble Reagent. Removal of Primary and Secondary Amines

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A macroreticular resin containing isothiocyanate group was proposed as an insoluble reagent and applied to the removal of the primary and secondary amines. The reaction condition of this resin with primary and secondary amines were determined. Most of the aliphatic amines tested, except for primary amines having tertiary alkyl group, were retained on the resin by shaking at 50° for 2 hr. Three kinds of impurities in commercial triethylamine could be removed by using this resin.

Keywords——macroreticular isothiocyanate resin; insoluble reagent; removal of primary and secondary amines; reactivity of isothiocyanate resin with amines; removal of impurities in triethylamine

3-Nitrophthalic acid anhydride, *p*-toluene sulfonyl chloride, and phenyl isothiocyanate are known as the reagents for the removal of primary and secondary amines from the mixture of primary, secondary, and tertiary amines.[2] These reagents react with primary and secondary amines, but not with tertiary amines.

Insoluble reagents containing functional groups introduced by chemical modification of polymer support will make the separation procedure simple and easy. Although a gel type cross-linked polystyrene (1.5% divinylbenzene) containing isothiocyanate group has been

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