The Riboflavin-sensitized Photooxidation of Mitomycin C by Near Ultraviolet Light Irradiation

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Abstract

The riboflavin-sensitized photodestruction of mitomycin C by the irradiation with near ultraviolet light was investigated. The irradiation with near ultraviolet light abruptly decomposed mitomycin C in the presence of riboflavin, but mitomycin C slowly decomposed by irradiating mitomycin C alone. The riboflavin-sensitized photodestruction of mitomycin C was suppressed by the quenchers, such as halogen and paramagnetic metal ions. Oxygen was required for the photosensitization. These results indicate that the initial reaction species in this photosensitization of mitomycin C is riboflavin in the excited triplet state and the decay of mitomycin C is caused by the photooxidation.

Introduction

Mitomycin C (MC, Fig. 1), a potent anticancer agent, can effectively induce the development of λ-phage in lysogenic *Escherichia coli* K-12(λ) cells (*1*). It was reported that the induction of λ-phage formation was suppressed when the lysogenic cells were exposed to visible light at wavelengths between 400 and 500 nm in the presence of MC (*2, 3*). In the course of studies on the mechanism of the photosuppression, it was found that MC loses the phage-inducing and antibiotic activities on *in vitro* irradiation with visible light in the presence of riboflavin (RF, Fig. 2).
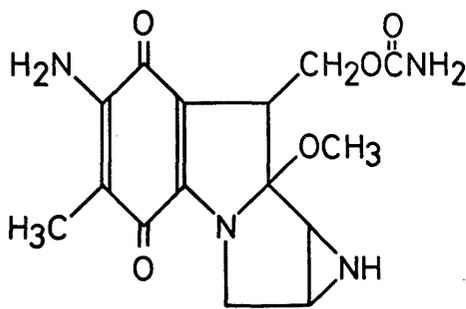
Fig. 1. Structure of mitomycin C (MC).
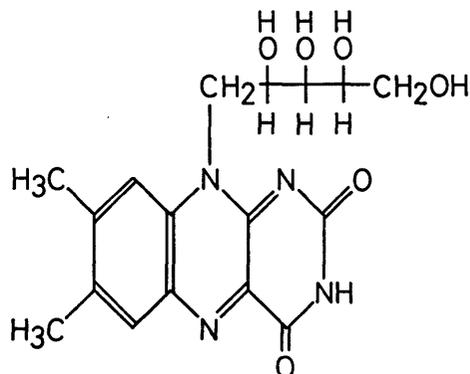
Fig. 2. Structure of riboflavin (RF).

RF is known as a favourable sensitizer for photoinactivation of various enzymes and antibiotics, and for photooxidation of indoleacetic acid and others. It has been reported that RF-sensitized photooxidation of MC involves singlet oxygen as the reactive species (4). Recently, the kinetic relation between the photoinactivation and photooxidation of MC in the presence of RF has been reported (5). MC has absorption maxima at 217 and 362 nm. The spectrum of RF is more complicated with maxima at 223, 268, 373 and 445 nm. Because the near ultraviolet(UV) light excites RF and MC at the same time, it therefore seemed of considerable interest to investigate the photodestruction of MC by the near UV light irradiation in the presence or absence of RF.

An objective of this work is to study the mechanism of the photodestruction of MC with the near UV light irradiation in the presence or absence of RF.

Materials and Methods

Materials.

MC was supplied by Kyowa Hakko Kogyo Co., Ltd. RF was obtained from Nakarai Chemicals, Ltd., and purified according to the method of Smith and Metzler (6). The other chemicals used were of G. R. grade. Distilled and deionized water was used in all experiments. The aqueous solution of MC was stored at 5°C in a refrigerator and used within a few days after preparation. The solution of RF was kept in a dark place. The aqueous solutions were not bufferized, unless indicated.

Apparatus and Procedure.

Aerobic photolysis was carried out in a open quartz cell (1 × 1 × 4 cm) at room temperature in a dark room. Light from a 100 watt high pressure mercury lamp (H type, Taika Kogyo Co., Ltd.) was passed through a Toshiba filter UV-D1A, and a radiation of about 360 nm was obtained. The intensity of the illumination was determined by means of a compensated thermopile (Kipp & Sonen, Delft, Holland) in each case. Fluorescence measurements were made on a Shimazu UM-S ultramicrofluorophotometer. The photodestruction of MC could be followed by measuring the absorbances at 362 and 445 nm by means of a Hitachi 124 spectrophotometer according to the method of Yajima and Mizunoya (5). The cross section of the photodestruction of MC was calculated from measurement of the absorbances in the initial stage of the irradiation in order to avoid the influence of photoproducts.

Results and Discussion

Riboflavin as Sensitizer.

As seen in Fig. 3, the irradiation with the near UV light abruptly decomposed MC in the presence of RF, but MC slowly decomposed by irradiating MC alone. This finding indicates that RF acts as the photosensitizer for the photodestruction of MC. It has been reported that MC is photooxidized by the irradiation of the visible light with first order kinetics in the presence of RF (4, 5). In the initial stage of the irradiation with the near UV light, the decay of MC also obeys first order kinetics with respect to the dose of the incident light as shown in Fig. 3, that is,

DOSE OF LIGHT (kJ/m^2)
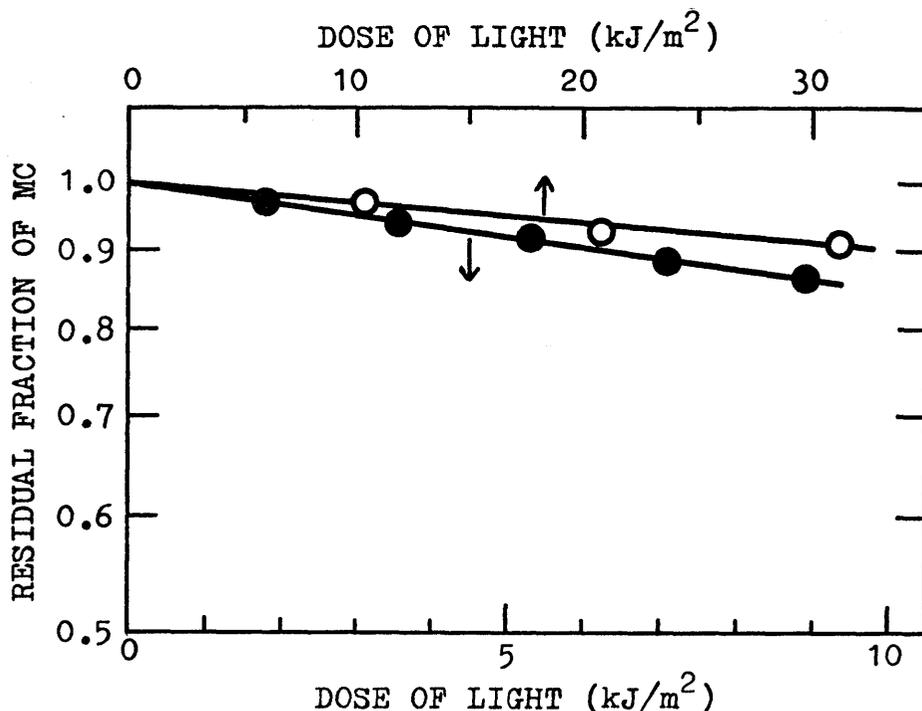
Fig. 3. Photodestruction of MC in the presence or absence of RF.   A solution containing MC (○: 6.0 μM, ●: 16.3 μM) and RF (○: none, ●: 11.4 μM) was irradiated with near UV light at 23°C.   The intensity of the incident light was 2.4 W/m^2.

$$\ln X = -\sigma D \qquad (1)$$

where X is the residual fraction of MC during the irradiation, σ is the cross section for the photodestruction, namely, the effective fraction of the incident photons and D is the dose of the incident light.   From the slope of the decay curve, σ in the presence and absence of RF were estimated to be 16.5×10^{-6} and 1.24×10^{-6} m^2/J, respectively.

To study the relationships between the cross section of the photodestruction of MC and the concentration of RF, several kinetic runs were performed at different concentrations of RF.   A plot of the cross section vs. the concentration of RF for the photodestruction of MC is given in Fig. 4.   The photodestruction of MC accelerated with an increase of the concentration of RF.   The results shown in Fig. 4 also indicate that RF sensitizes the photodestruction of MC.
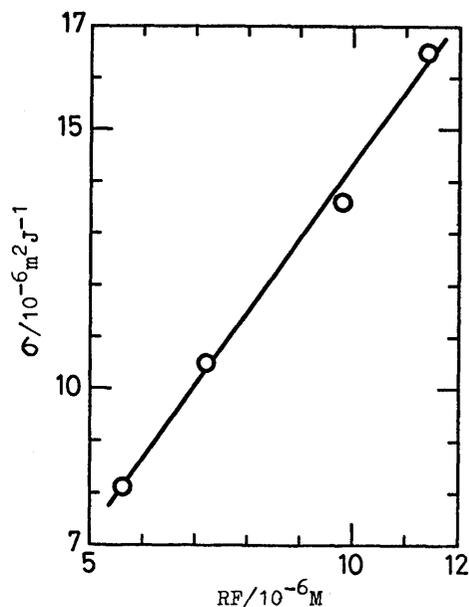
Fig. 4. Dependence of the cross section of the photodestruction of MC on the concentration of RF.   A solution containing MC (16.3 μM) and RF (5.6-11.4 μM) was irradiated with near UV light at 23°C. The intensity of the incident light was 2.4 W/m^2.

Effect of Halogen Ions.

Figure 5 shows the effect of halogen ions on the photodestruction of MC in the presence of RF. The relative cross section of the photodestruction of MC decreased with increase of atomic weight, atomic number, or ionic radius of the halogen. In other words, the heavier and the larger the halogen ion is, the more intensely the photodestruction of MC is suppressed. The addition of these halogen ions did not affect MC under dark conditions as might be expected.
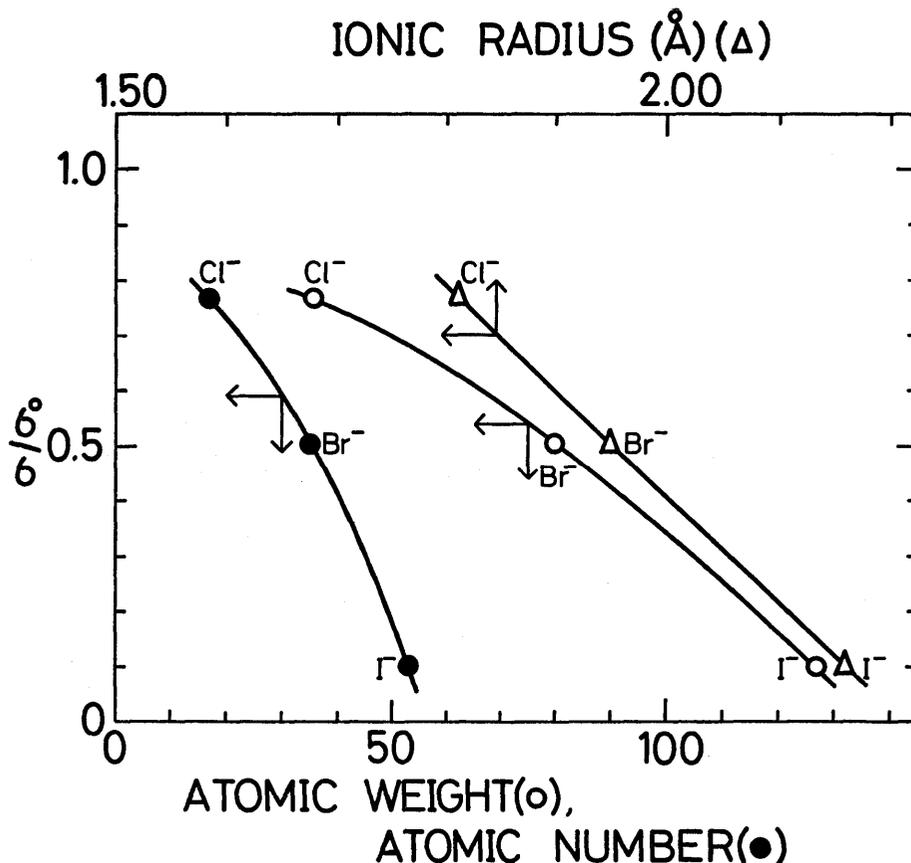
Fig. 5. Effect of halogen ions on the RF-sensitized photodestruction of MC. A solution containing MC (7.2 μM) and RF (11 μM) was irradiated with near UV light at 23°C in the presence of potassium halide (0.48 mM). The intensity of the incident light was 3.2 W/m². σ, cross section with potassium halide; σ_0, cross section without potassium halide.

If the photodestruction of MC is caused by RF molecule in the excited singlet state, the fluorescence of RF will be quenched at the same concentration of the halogen ion as that at which the photodestruction of MC is suppressed. Figure 6 shows the dependence of the cross section of photodestruction of MC and the fluorescence yields of RF on the concentration of potassium iodide. The photodestruction of MC seemed to be suppressed by 10^{-7} M or more of iodide ion. On the other hand, the fluorescence of RF was observed to be barely quenched by 10^{-3} M or more of the ion. According to Tomita

and Kim (7), the strong quenching of the phosphorescence of RF in the presence of iodide ion even below 10^{-4} M runs parallel to the inhibition of the photoinactivation of taka-amylase A.
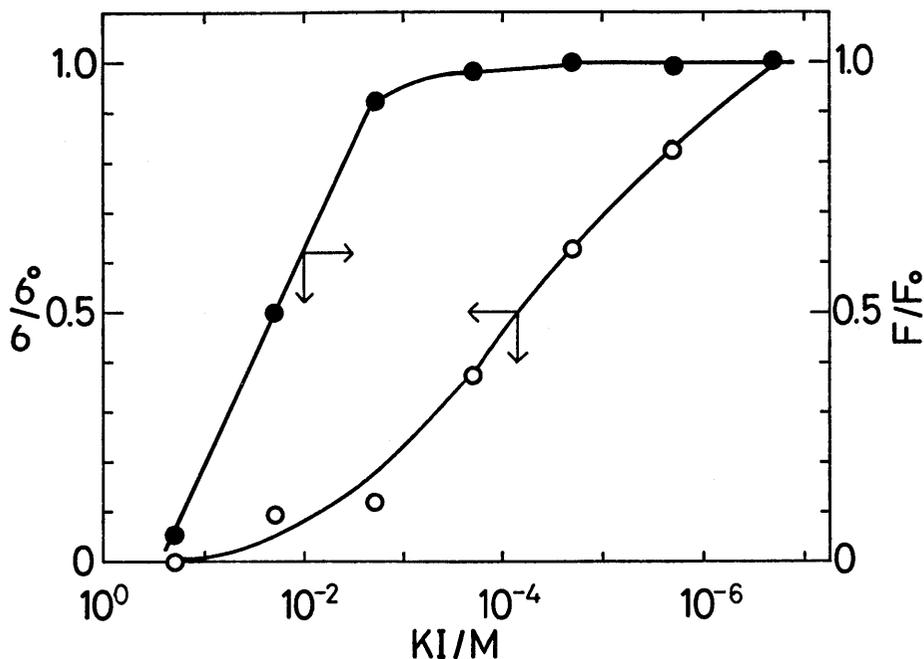
Fig. 6. Dependences of the cross section of the photodestruction of MC and the fluorescence yield of RF on the concentration of KI.   A solution containing MC (7.4 μM) and RF (9.6 μM) was irradiated with near UV light at 21°C in the presence of various concentrations of KI.   The intensity of the incident light was 1.81 W/m^2.   The fluorescence was measured at 520 nm with the exciting light at 445 nm.   The cross section, σ_0, and the fluorescence yield, F_0, were obtained without KI.

Table I shows the effect of the halogen ions on the photodestruction of MC in the absence of RF.   In this case, the relationships between the cross section of the photodestruction of MC and the halogen ions were ambiguous.   The results shown in Table I suggest that MC is not in the excited triplet state.

Effect of Paramagnetic Ions.

When a paramagnetic metal ion, such as Ni^{2+} or Co^{2+}, was added to MC solution containing RF, the photodestruction of MC was greatly suppressed in a manner similar to that when halogen ions were added, as shown in Table II.   Compared with the paramagnetic ions, the other metal ions,

TABLE I.   Effect of halogen ions on the photodestruction of MC in the absence of RF.   A solution containing MC (14.6 μM) and potassium halide (0.48 mM) was irradiated with near UV light at 23°C.   The intensity of the incident light was 3.2 W/m^2. σ, cross section with halide ion; σ_0, cross section without halide ion.

Halogen ions	σ/σ_0
None	1
Cl^-	0.58
Br^-	0.77
I^-	0.54

such as Mn^{2+}, Ca^{2+} or Ba^{2+}, did not appreciably affect on the RF-sensitized photodestruction of MC.

Figure 7 shows the dependence of the cross section of RF-sensitized photodestruction of MC and the fluorescence yields of RF on the concentration of $NiCl_2$. The photodestruction of MC seemed to be suppressed by 10^{-5} M or more of nickel ion. On the other hand, the fluorescence of RF was observed to be barely quenched by 10^{-2} M or more of the ion. The phosphorescence of RF, in contrast with the fluorescence, can be quenched on the addition of 10^{-6} M of paramagnetic ions (8).

On these evidences shown in Fig. 5-7 and Table II, it was found that the initial reactive species in the photosensitization of MC is RF in the excited triplet state. Furthermore, the quenching of the triplet RF by the halogen ions or paramagnetic metal ions

TABLE II.   Effect of metal ions on the photodestruction of MC in the presence or absence of RF.   The reaction mixtures were irradiated with near UV light at 25°C in the presence of metal ion (0.48 mM). The intensity of the incident light was 3.1 W/m^2. σ, cross section with metal ion; σ_0, cross section without metal ion.

Metal ions[a]	MC-RF[b] σ/σ_0	MC[c] σ/σ_0
None	1	1
Mn^{2+}	0.76	1.00
Co^{2+}	0.47	1.00
Ni^{2+}	0.48	1.00
Ca^{2+}	0.70	0.74
Ba^{2+}	0.83	0.62

a) Metal ions were used as chloride.
b) MC: 7.5 μM, RF: 10.4 μM.
c) MC: 17.2 μM.
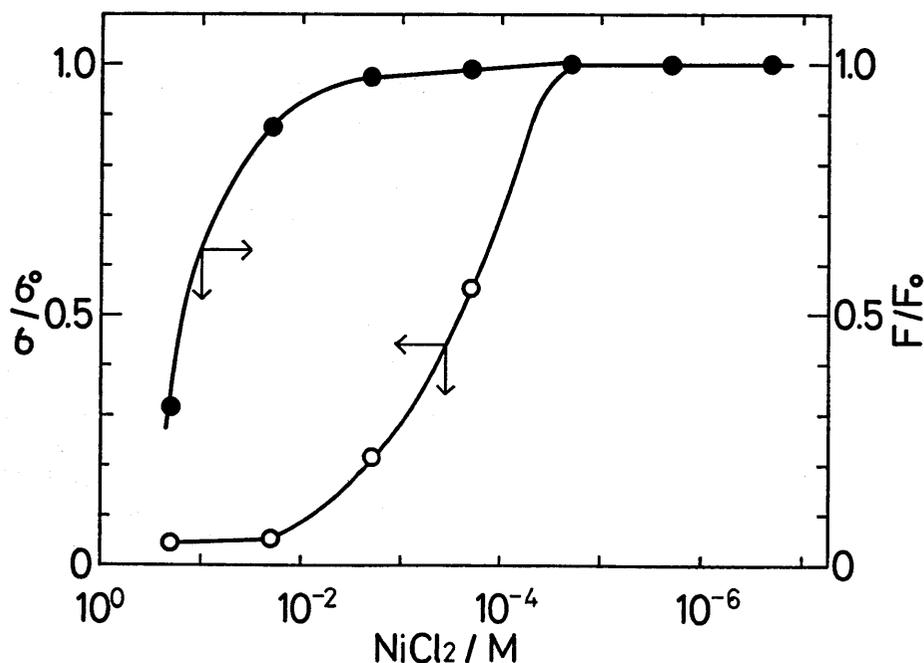
Fig. 7.   Dependences of the cross section of the RF-sensitized photodestruction of MC and the fluorescence yield of RF on the concentration of $NiCl_2$.   A solution containing MC (7.1 μM) and RF (9.8 μM) was irradiated with near UV light at 21°C in the presence of various concentrations of $NiCl_2$.   The intensity of the incident light was 3.3 W/m^2.   The fluorescence was measured at 520 nm with the exciting light at 445 nm. The cross section, σ_0, and the fluorescence yield, F_0, were obtained without $NiCl_2$.

is responsible for the suppression of the RF-sensitized photodestruction of MC.

The photodestruction of MC in the absence of RF was not entirely affected by the paramagnetic ions as shown in Table II.   This finding also suggests that MC is not in the excited triplet but in the excited singlet state.

Oxygen Requirement.

It has been known that the photoinactivation of biological materials in the presence of dye requires oxygen molecules in many cases.   To decide whether oxygen molecule participates or not in the RF-sensitized photodestruction of MC, the solution containing RF and MC was bubbled with nitrogen gas or air during irradiation with near UV light. In the solution bubbled by nitrogen gas, MC decayed slower than in aerated solution, as shown in Fig. 8.
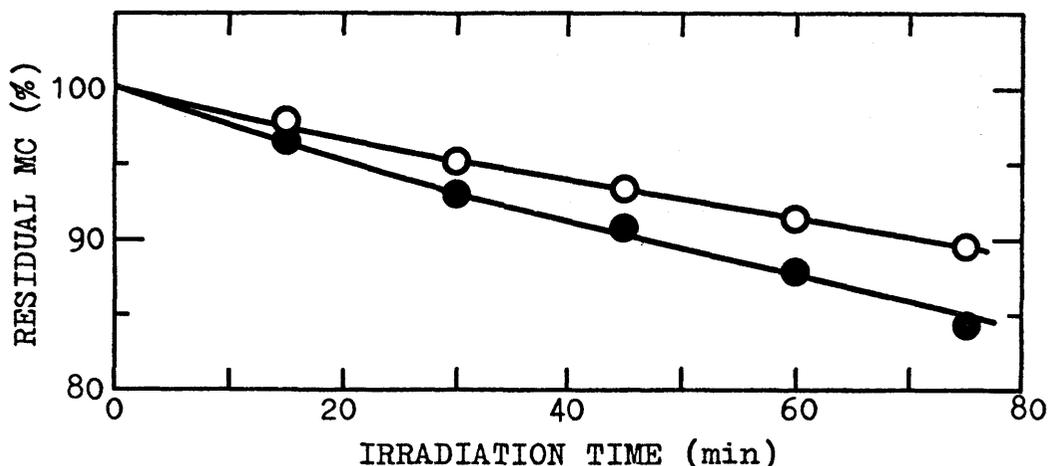
Fig. 8.   Effect of N_2 on the RF-sensitized photodestruction of MC.   A solution containing MC (9.0 μM) and RF (13.3 μM) was irradiated with near UV light at 22°C.   The intensity of the incident light was 1.5 W/m^2.   ○: N_2 bubbling, ●: aeration.

This finding indicates that oxygen is also required for the photodestruction of MC in the presence of RF.   In other words, the RF-sensitized photodestruction of MC is oxidative reaction.   A small residual decay of MC under conditions of nitrogen gas bubbling may be due to incomplete removal of the oxygen from the reaction system.

Proposal of Mechanism.

RF is known to sensitize the aerobic photooxidations of organic compounds.   Most of the dye-sensitized photooxidations, including flavin sensitizing, involve dyes in the excited triplet state as initial reactive species, although cases where the excited singlet state is involved have been documented (9, 10).   Flavin in the excited triplet state may then react directly with the substrate by hydrogen atom abstraction, or with oxygen to give either a reactive peroxide intermediate (11) or singlet oxygen (4, 12, 13).

Taking into account the results mentioned above, we now propose the following mechanism of the RF-sensitized photooxidation of MC.   (1) RF in the excited triplet

state react with oxygen molecule to give either a reactive peroxide intermediate or singlet oxygen.   (2) The reactive species which is a reactive peroxide intermediate of RF or singlet oxygen may be expected to react with MC.   (3) A part of the reactive species presumably react with MC in the excited state.

References

1. OTSUJI, N., SEKIGUCHI, M., IIJIMA, T., & TAKAGI, Y. (1959) *Nature* **184**, 1079-1080.
2. TAKENO, T., NAGATA, T., & MIZUNOYA, T., (1968) *Nature* **218**, 295-296.
3. MIZUNOYA, T. & TAKENO, T. (1971) *Photochem. Photobiol.* **13**, 369-374.
4. UEDA, K. & MIZUNOYA, T. (1979) *J. Biochem.* **86**, 857-862.
5. YAJIMA, E. & MIZUNOYA, T. (1981) *J. Biochem.* **89**, 929-936.
6. SMITH, E. C. & METZLER, D. E. (1963) *J. Am. Chem. Soc.* **85**, 3285-3288.
7. TOMITA, G. & KIM, S. S. (1965) *Nature* **207**, 975-976.
8. TOMITA, G. (1966) *Nature* **212**, 898-901.
9. BRIDGE, N. K. & PORTER, G. (1958) *Proc. Roy. Soc.* (London) **Ser. A 244**, 259-276.
10. SONG, P. -S. & METZLER, D. E. (1967) *Photochem. Photobiol.* **6**, 691-709.
11. GOLLNICK, K. & SCHENCK, G. O. (1965) in *Organic Photochemistry* p. 507, Butterworth, London.
12. KEARNS, D. R., HOLLINS, R. A., KHAN, A. U., & RADLICK, P. (1967) *J. Am. Chem. Soc.* **89**, 5456-5457.
13. SPECK, W. T., ROSENKRANZ, S., & ROSENKRANZ, H. S. (1976) *Biochim. Biophys. Acta* **435**, 39-44.