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ACTION SPECTRUM FOR PHOTOINACTIVATION OF BACTERIOPHAGE LAMBDA SENSITIZED WITH 5-METHOXYPSORALEN. ROLE OF LIGHT ABSORPTION BY THE 4',5' ADDUCT

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An action spectrum for photoinactivation of phage λ sensitized with 5-methoxypsoralen was measured. The maximal action was observed at 330 nm away from the absorption peak(312 nm). This implies that the second step absorption by the 4',5' adduct is much more efficient for the inactivation than the first step absorption.

Psoralen compounds have been used as photosensitizing drugs in the photochemotherapy of psoriasis and vitiligo.[1,2] Their characteristic photoreaction with nucleic acids has been intensively studied. upon near-uv irradiation, psoralen compound can form adducts to nucleic acids by cycloaddition of the molecule at the 4',5' double bond and/or the 3,4 double bond to the 5,6 double bond of pyrimidines.[1-5] In relation to photochemotherapy of psoriasis, action spectra of 8-methoxypsoralen(8-MOP; 9-methoxy-7H-furo[3,2-g][1]benzopyran-7-one) plus near-uv treatment have been determined with respect to a variety of biological end points, i. e., inactivation of bacteriophages,[6] killing of bacterial cells,[7,8] erythemogenic action,[9] inhibition of DNA synthesis in the epidermis of mice,[10] etc. In these action spectra, maximal levels of action occurred with light in the 320-340 nm range away from the absorption peak wavelength(302 nm). This large difference in wavelength is not well elucidated. In the present study, we measured the action spectrum for photoinactivation of phage lambda sensitized with 5-methoxypsoralen(5-MOP; 4-methoxy-7H-furo[3,2-g][1]benzopyran-7-one) and attempted to explain the spectrum in terms of light absorption by the 4',5' adduct.

Psoralen derivatives used here were synthesized and kindly supplied by Hiroshi Kakishima, Cosmetics Laboratory, Kanebo Ltd. Absorption spectra were measured with a Shimadzu spectrophotometer, MPS-2000. Fluorescence spectra were measured with a Hitachi fluorescence spectrophotometer, MPF-2A. Methanol solution of 5-MOP (4×10^{-5} mol dm^{-3}) was irradiated to produce the photoproducts with near-uv light passed through glass filters(Toshiba UV-D2 and IRQ-80) at a fluence rate of 800 J m^{-2} s^{-1}. A high-pressure mercury lamp(Ushio, 500 W) was used as the light source.

Bacteriophage λ_{vir} was subjected to photoinactivation in the presence and

absence of 5-MOP. The phage titers were assayed by the agar-layer method using E. coli AB1157 as indicator bacteria. The media used were the same as described previously.[6] To the phage suspension(3 x 10^6 PFU cm^{-3}; PFU : plaque forming unit) in physiological saline, a small portion of the stock solution of 5-MOP (2.5 x 10^{-3} mol dm^{-3} in ethanol) was added (final, 5 x 10^{-5} mol dm^{-3}). After incubation of the mixture in the dark for more than 1 h, 0.5 cm^3 of the suspension was subjected to irradiation from the lateral side of a silica cuvette (10 mm path) with monochromatic light by means of a Spectro-irradiator (Japan Spectroscopic Co., Model CRM-FM) with a 5 kW Xe arc lamp and a 1200 lines mm^{-1} grating. A cut-off filter (Toshiba UV-29) was used to remove stray light at shorter wavelengths. It should be noted that the wavelength of the incident light is altered depending on the position in the cuvette. It is distributed from the value indicated as 2 nm mm^{-1} with deviations from the central position to both sides in the cuvette. The incident fluence rate was measured with a calibrated thermopile.

Figure 1 shows the survival curves of phage lambda irradiated with monochromatic light in the presence (330, 340, and 380 nm) and absence (310 and 320 nm) of 5-MOP. The surviving fraction was decreased with exponential kinetics in every

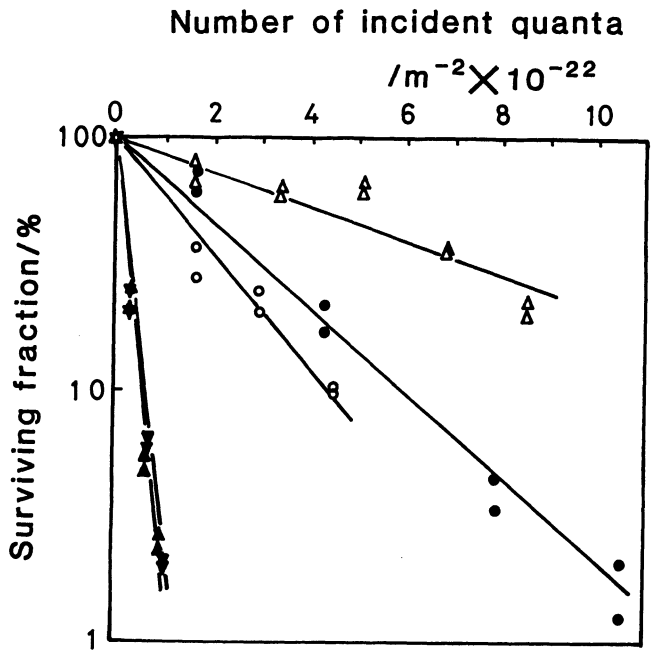

Fig. 1. Survival curves of phage lambda. ▲, ▼, ● : 330, 340, and 380 nm in the presence of 5-MOP, respectively. o, △: 310 and 320 nm in the absence of 5-MOP, respectively.

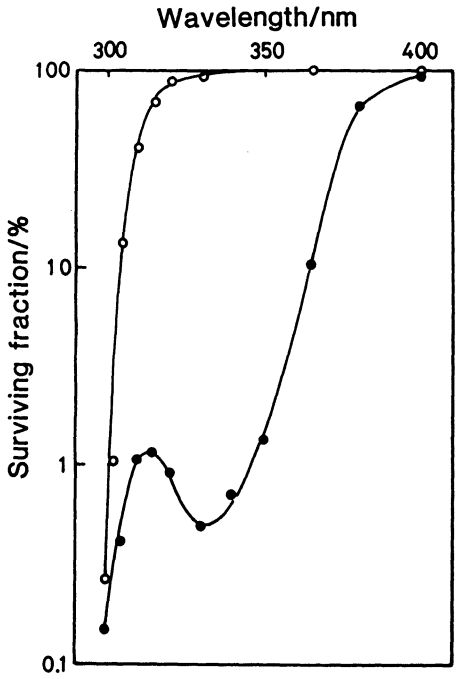

Fig. 2. Surviving fractions after monochromatic irradiation with a fixed number of quanta. ● : in the presence of 5-MOP; o : in the absence of 5-MOP.

case. Figure 2 shows the alteration of the surviving fractions after monochromatic irradiation with a fixed number of quanta (1.7 x 10^{22} m^{-2}) in the presence and absence of 5-MOP in a wavelength range from 300 to 400 nm. Near-uv light per se inactivated a significant fraction of the phage at wavelengths shorter than 320 nm.

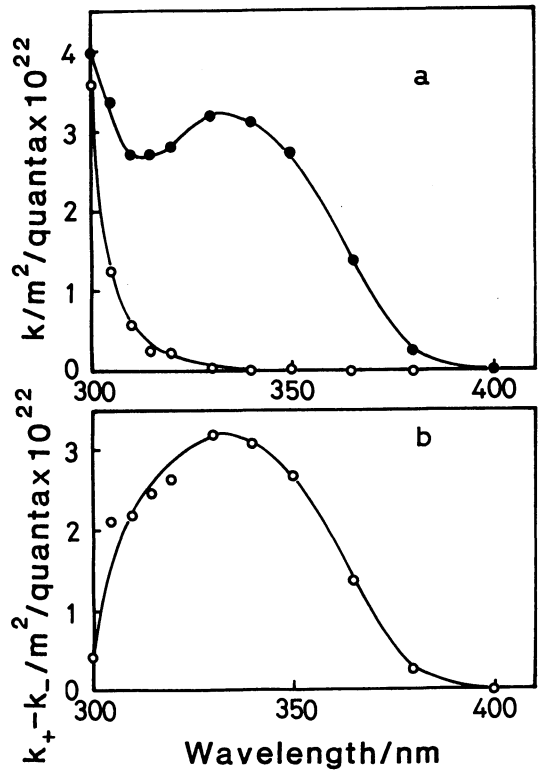

Fig. 3. Variation of the inactivation cross-section with wavelength in the presence (●) and absence (o) of 5-MOP (a) and the action spectrum for the photosensitized inactivation (b).

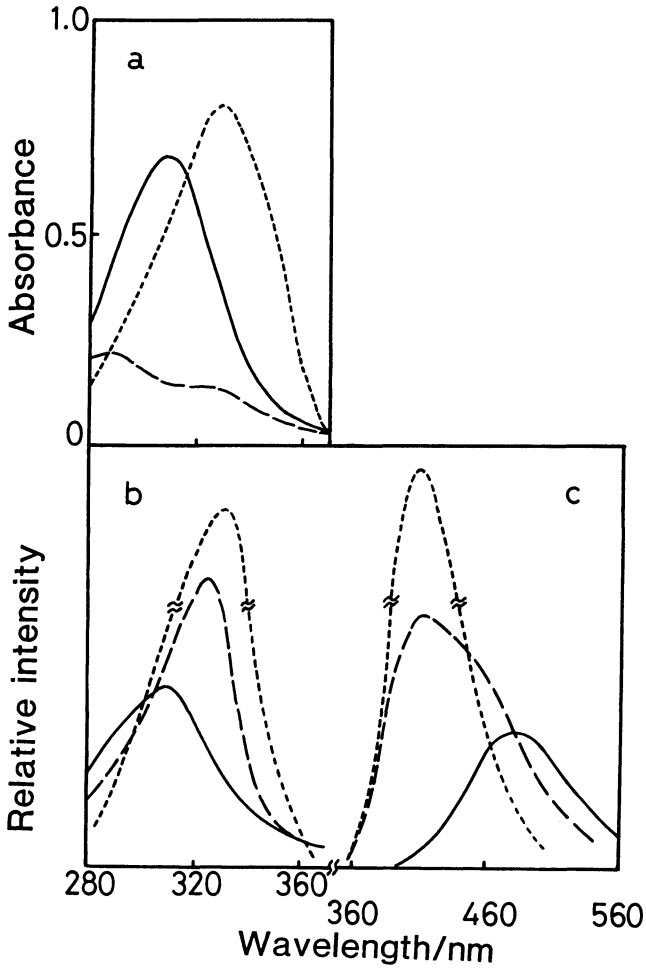

Fig. 4. Absorption (a), fluorescence excitation (b), and emission (c) spectra measured in methanol solution (4 x 10^{-5} mol dm^{-3}, saturated with Ar at 298 K). ——— : 5-MOP; — — : photoproducts produced by 90-min irradiation (in a) or the irradiated mixture (in b and c); ---- : 4', 5'-dihydro derivative. Excitation spectra : measured at each emission peak. Emission spectra : excited at each absorption peak.

When the phage was irradiated in the presence of 5-MOP, salient sensitized inactivation occurred. The sensitization efficiency was dependent on the wavelength of the incident light. Assuming that all survival curves are simple exponentials, the inactivation cross-section (k) was calculated as the reciprocal of the number of incident quanta to give a surviving fraction of 37%. Figure 3a shows the plot of the values of k_+ (in the presence of 5-MOP) and k_- (in the absence of 5-MOP) against wavelength. To obtain an action spectrum for the photosensitized inactivation, the differences between k_+ and k_- at each wavelength were plotted against wavelength (Fig. 3b). The maximal level of photosensitization was observed at 330 nm as in the case of photoinactivation of the phage sensitized with 8-MOP.[6] A marked difference between the action peak wavelength and the absorption peak wave-

length(312 nm, see Fig. 4a) was demonstrated.

The maximal photosensitization occurred in the same wavelength range as the excitation peak for fluorescence of an initial photoproduct of 5-MOP (in methanol) (Fig. 4 b). This strongly fluorescent product is a 4',5'-dihydro derivative of 5-MOP, because the wavelengths of peaks in the absorption spectrum (Fig. 4a), the fluorescence excitation spectrum (Fig. 4b) and the emission spectrum (Fig. 4c) and the retention time in HPLC of the fluorescent product[11] were consistent with those of authentic 5-methoxy-4',5'-dihydropsoralen. Since the 4', 5' bond is saturated in the furan-side monoadduct, the monoadduct is assumed to have π-electronic structure similar to those of 5-methoxy-4',5'-dihydropsoralen. The two-step formation mechanism proposed for crosslinking with psoralen or 8-MOP[4,5,12] is favorable to explanation of the action spectrum. From the present findings, we conclude that the second step absorption of quanta (ca. 330 nm) by the furan-side monoadduct of 5-MOP is much more responsible for inactivation of the biological functions of phage DNA than the first step absorption of quanta (ca. 310 nm) by 5-MOP. The present conclusion is not incompatible with the action spectra of DNA crosslinking observed in aqueous solution.[13,14]

References

1) B. R. Scott, M. A. Pathak, and G. R. Mohn, Mutation Res., 39, 29(1976).
2) E. Ben-Hur and P. -S. Song, Adv. Radiation Biol., 11, 131(1984).
3) P. -S. Song and K. J. Tapley, Jr., Photochem. Photobiol., 29, 1177(1979).
4) D. Kanne, K. Straub, J. E. Hearst and H. Rapoport, J. Am. Chem. Soc., 104, 6754 (1982).
5) J. W. Tessman, S. T. Isaacs, and J. E. Hearst, Biochemistry, 24, 1663(1985).
6) K. Suzuki, H. Fujita, F. Yanagisawa, and M. Kitakami, Photochem. Photobiol., 26, 49(1977).
7) H. Fujita and K. Suzuki, J. Bacteriol., 135, 354(1978).
8) B. A. Bridges, R. P. Mottershead, and A. Knowles, Chem.-Biol. Interactions, 27, 221(1979).
9) D. J. Cripps, N. J. Lowe, and A. B. Lerner, Br. J. Dermatol., 107, 77(1982).
10) K. H. Kaidbey, J. Invest. Dermatol., 85, 98(1985).
11) M. Sasaki, H. Kakishima, and T. Sakata, Abstract for the 25th Symposium on Photochemistry, Okayama(1984), p.309.
12) F. Dall'Acqua, S. Marciani Magno, F. Zambon, and G. Rodighiero, Photochem. Photobiol., 29, 489(1979).
13) H. Fujita, M. Sano, and K. Suzuki, Photochem. Photobiol., 29, 71(1979).
14) F. P. Gasparro, W. A. Saffran, C. R. Cantor, and R. L. Edelson, Photochem. Photobiol., 40, 215(1984).

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