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ESTIMATION OF EFFECTIVE DIELECTRIC CONSTANTS OF SEVERAL AMPHIPHILIC MEDIA FOR 8-METHOXYPSORALEN

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The effective dielectric constants of SDS micelle, DMPC liposome and DNA for 8-methoxypsoralen were estimated to be 60, 32, and 70 respectively by fluorophotometry.

For these years attention has been paid to the photomedical and photobiological actions of furocoumarin compounds. Especially, the phototoxicity of 8-methoxypsoralen (8-MOP, 9-methoxy 7H-furo[3,2-g][1]benzopyran-7-one, CRN:298-81-7) has been used in PUVA[1] (Psoralens + UV-A : 320-400 nm radiation) photochemotherapy for skin disorder diseases. The photoreaction of 8-MOP in PUVA can be classified into the following three mechanisms in relation to the target molecules in biological organisms: 1) DNA has been considered as the typical target molecule in the phototoxic reaction in PUVA therapy. The main effect of 8-MOP in PUVA has been believed to be the inhibition of DNA synthesis.[2,3] The inhibition reaction involves the formation of a covalent crosslinking between DNA double strands through 8-MOP molecule at the 3, 4 and 4', 5' carbon double bond sites, and the reaction is independent of oxygen.[4] 2) Protein has been studied[5,6] as the target molecule in photoallergic reaction. Oxygen plays the main role in the photobinding reaction of 8-MOP to protein, and this reaction is completely different from the case 1) reaction. 3) Lipid is considered as a target molecule in photodamage of biomembrane,[7] since 8-MOP molecules in vivo are connected with biomembrane. Many results of 1) and 2) have been reported,[8-11] while the case 3) has been poorly studied. Details of the above mentioned photoreaction of 8-MOP have not been understood yet. One of the main reasons is that the photochemistry of 8-MOP in vitro has been investigated almost solely in water. The radiation in the UV-A region has been known very important for both the PUVA therapy and phototoxic reaction of 8-MOP in vivo,[4,12,13] however, 8-MOP in aqueous solution shows only weak absorption in the UV-A region.[14] As for the above problem suggestive results were observed. The absorption peak of 8-MOP changed according to the dielectric constant of solvent; i.e., the longest absorption peak of 8-MOP at 300 nm in water, while that in less polar solvent was observed at about 340 nm, distinctly in hexane.[14] Moreover, the 4', 5'-photoadduct of 8-MOP in various kinds of solvents showed the absorption band at 340-400 nm in less polar solvent than water.[15,16] For understanding the photochemical reaction of 8-MOP in vivo,

a very important factor is to clarify the effect of the medium wherein 8-MOP is incorporated. Our previous paper[14] reported that the fluorescence of 8-MOP was very sensitive to environment, i.e., the correlation between the fluorescence properties (λ_{max}^{em} and Φ_f) of 8-MOP and the solvent polarity was observed.

The present paper reports the effect of the dielectric properties of the environment, e.g., DNA and model biomembrane such as sodium dodecyl sulfate (SDS) and dimyristoyl lecitin (DMPC) liposome, on the photochemical behaviour of 8-MOP by fluorophotometry.

8-MOP was purchased from Tokyo Kasei, and the purity was checked by HPLC (Hitachi 635S). Calf thymus DNA (Sigma type I) was used, and the concentrations were determined spectrophotometrically at 260 nm (ε_p =6600 M^{-1} l cm^{-1}).[17] All the other reagents were commercially available ones. Water was distilled twice. The concentration of 8-MOP and SDS in the aqueous micelle solution was 4.0 x 10^{-5} M and 10^{-2} M, respectively. The 8-MOP - DMPC liposome was prepared by the following procedure:

8-MOP + DMPC (molar ratio 1 : 300 in benzene solution)
↓
Lyophilization
↓ 1 M KCl aqueous solution (or Tris buffer pH 7.5) 10ml
Ultrasonication (20 kHz, 303 K, Ar gas, 15 min)
↓
Centrifugation (1300 rpm, 15 min)
↓
Liposome suspension

The 8-MOP-DNA solution was prepared to contain 8-MOP and DNA at 1:50 or 1:100 molar ratio in a 0.015 M NaCl aqueous solution. The absorption and fluorescence spectra were measured at 298 K with a spectrophotometer, Hitachi EPS-3T or Shimazu MPS-2000, and a fluorescence spectrophotometer, Hitachi MPF-2A, respectively.

The fluorescence spectra of 8-MOP were observed in the SDS micelle, in DMPC liposome and in DNA. The aqueous 8-MOP - SDS micelle solution showed an intense fluorescence with a peak at 490 nm, which is shorter by 12 nm than the emission peak in water (λ_{max}^{em} 502 nm). The 8-MOP - DMPC liposome emitted a weak fluorescence with the maximum at 470 nm. In the DNA solution, the fluorescence peak of 8-MOP was observed at 497 nm and the intensity was decreased from that in water.

The linear relationship (open circle in Fig. 1) between the photon energy at fluorescence maximum of 8-MOP and the effective dielectric constant of solvent has been reported previously.[14] This linear relationship was utilized to estimate the effective dielectric constants of SDS micelle solution, DMPC liposome

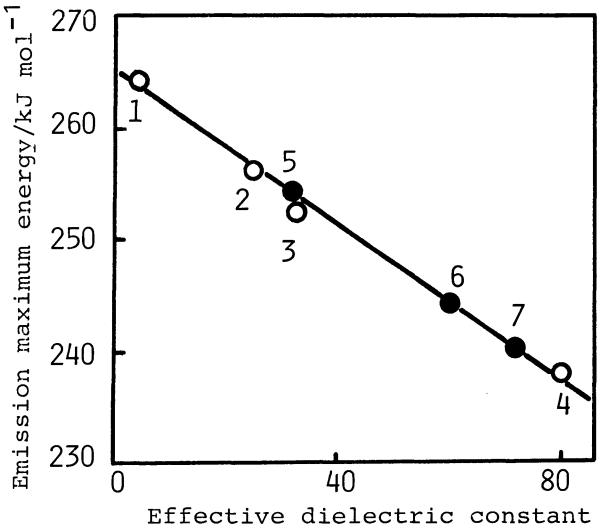

Fig. 1. Relation between fluorescence maximum energies of 8-MOP and effective dielectric constants (at 298K).

○ : 1. Chloroform 2. Ethanol
3. Methanol 4. Water
● : 5. DMPC liposome 6. SDS micelle
7. DNA (0.015 M aq. soln.)

and DNA or the incorporated 8-MOP molecules. From the observed fluorescence peak energy of 8-MOP in each medium (closed circle), the effective dielectric constant of medium was estimated by using the straight line in Fig. 1. The estimated values were 60, 32, and 70 for SDS micelle solution, DMPC liposome and DNA, respectively. These estimated dielectric constants suggest that the environment surrounding 8-MOP in vivo offers a less polar atmosphere than water. This consideration is supported by the dependency of the solubility of 8-MOP upon the solvent polarity. 8-MOP is practically insoluble in cold water, but soluble in methanol or in ethanol, freely soluble in chloroform, and turns to be slightly insoluble in dioxane or in hexane.

The number of the solubilized 8-MOP molecule per one SDS micelle was calculated 1.3 molecules at cmc, 0.0081 mol/l, on the basis of the fact that a globular SDS micelle is formed with 62 SDS molecules at higher concentration than cmc.[18] The number of the incorporated 8-MOP molecule in one DMPC liposome was estimated about 30 when one liposome is formed with 3000 - 4000 lecitin molecules. The fact that the estimated dielectric constant of SDS micelle solution and that of DMPC liposome from fluorescence of 8-MOP were smaller than the dielectric constant of water, together with the fact that 8-MOP is insoluble in water while soluble in alcohol, chloroform and hexane, suggests that the 8-MOP molecules locate rather in the micelle or the liposome than in the part of water. The location site of 8-MOP in SDS micelle is assumed to be the vicinity of the polar atmosphere of OSO_3^- group. The estimated dielectric constant for incorporated 8-MOP molecule in DMPC liposome is almost the same as in methanol, so the incorporated 8-MOP molecules seem to locate not just at the nonpolar hydrocarbon chain of DMPC, but somewhat closer to the polar group of DMPC molecule. The suggested location of 8-MOP molecules in a SDS micelle and in a DMPC liposome bilayer are illustrated in Fig. 2.

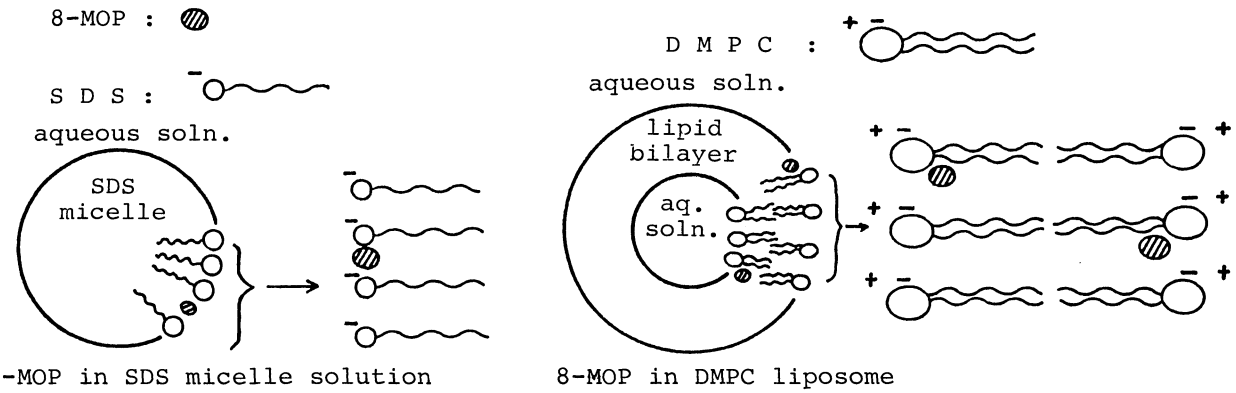

Fig. 2. Internal position of 8-MOP in SDS micelle solution and DMPC liposome.

Since 8-MOP molecule intercalates with DNA,[19] the estimated dielectric constant means the dielectric property for intercalated 8-MOP with DNA. The number of intercalated 8-MOP molecules was estimated about 6 per 100 nucleotides by measuring the dependence of 8-MOP fluorescence quenching on the DNA concentration. The observed number is consistent with the other data.[8] From the estimated dielectric constant for DNA, the interior of double stranded DNA is suggested the slightly less polar than water.

In conclusion, the effective dielectric constants of SDS micelle, DMPC liposome and DNA for 8-MOP were estimated to be 60, 32, and 70, respectively. These values suggest that the medium for 8-MOP in vivo offers less polar dielectric environment than water.

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