APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY 6, 143-152 (1981)

Energy-Transducing Membrane

II. Roles of Liquid Crystal in the Photoresponse of a Chlorophyll Liquid Crystal Membrane

MASUO AIZAWA,*† MORIO HIRANO,
AND SHUICHI SUZUKI

*Research Laboratory of Resources Utilization,
Tokyo Institute of Technology, Nagatsuta, Midori-ku,
Yokohama 227, Japan*

Received February 13, 1980; Accepted December 4, 1980

Abstract

The states of chlorophyll a (Chl a) incorporated in a liquid crystal membrane were investigated by spectrophotometry in the visible and IR regions. *N*-(*p*-methoxybenzylidene)-*p*'-butylaniline (MBBA) was used as the liquid crystal. The Chl a-MBBA (1:3 in molar ratio) showed the dihydrate-Chl a aggregate peak at 743 nm under excess water conditions. IR spectroscopic evidence indicated that the Chl a-MBBA membrane was hydrated in the dihydrate stoichiometry [Chl a-3MBBA-$2H_2O$], via the C-10 ester OC···H(H)O···Mg and the C-9 keto OC···H(H)O···Mg bondings.

Index Entries: Photosynthesis, in a liquid crystal membrane; energy transducing membrane, of chlorophyll a and liquid crystal; chlorophyll a, in a liquid crystal membrane; membrane, chlorophyll a in a liquid crystal; liquid crystal membrane, chlorophyll a in.

Introduction

A chlorophyll-liquid crystal membrane coated onto a platinum plate has recently been developed as a model photosynthetic membrane for photoenergy conversion (*1-5*). Previous papers showed that an electron was consecutively

---

†Present address: Institute of Materials Science, University of Tsukuba, Sakura-mura, Ibaraki 305, Japan

0273-2289/81/0600-0143$02.00

pumped up from a direction originating on the platinum phase to an electrolyte by illuminating a Mg chlorophyll–liquid crystal membrane. It was also shown that the direction of photoinduced electron transport depended on the metal species coordinated to the porphyrin of chlorophyll. It was also found that the incorporation of liquid crystal considerably enhanced the photo-induced electron transport. Mg chlorophyll, for instance, exhibited the most prominent photo-response in combination with *N*-(*p*-methoxybenzylidene)-*p*′-butylaniline (MBBA) at a molar ratio of 1:3. Photoelectrolysis of water was demonstrated with a photoelectrochemical system consisting of chlorophyll–liquid crystal membrane electrodes. However, the role of the liquid crystal has not been clarified. The present investigation was undertaken to elucidate the states of chlorophyll in the liquid crystal membrane.

The study of the chlorophyll a (Chl a)–H_2O interaction is of current interest because of the probable role of Chl a–H_2O aggregates in the photochemical splitting of water during in vitro solar energy conversion (*6–8*). The stoichiometry of Chl a–H_2O aggregates have been extensively investigated by Katz, Fong, and others (*9–13*). Owing to their photospectroscopic characteristics, considerable attention has been paid to the in vitro 700-nm absorbing and the 743-nm (in the 740–745 nm region) absorbing hydrated Chl a (*14, 15*).

Many other physicochemical methods including IR spectroscopy (*12*), X-ray diffraction (*16, 17*), and X-ray photoelectron spectroscopy (XPS or ESCA) (*14, 18*) have been applied to the characterization of the Chl a aggregates. In the present paper, the possible aggregation of Chl a in a liquid crystal membrane was studied.

Experimental

Materials

Chl a was extracted and isolated from fresh spinach leaves according to the conventional method (*19*). Fresh spinach leaves were crushed in liquid N_2 and extracted with cooled acetone for 3 h. After filtration with gauze, petroleum ether was added to the extract. Water was added if necessary. The acetone-soluble carotenoids and proteins were isolated from the chlorophyll mixtures. After repeated isolation of the carotenoids and proteins, the petroleum ether solution of chlorophyll was applied to a sucrose column. Elution was made with petroleum ether, petroleum ether–ethyl ether (90:10 v/v%), and then petroleum ether–isopropanol (99.5:0.5 v/v%) to isolate chlorophyll a. The preparation was assayed by thin layer chromatography [benzene–*t*-butanol (90:10 v/v%)]. The contents of Chl a was calculated using the following equation (*20*).

$$\textit{Chl a}\ [\mathrm{mg/mL}] = 10.1\ \textit{absorbance}_{662} - 1.01\ \textit{absorbance}_{644}$$

N-(*p*-Methoxybenzylidene)-*p*′-butylaniline (MBBA) and 4′-heptyl-4-cyanobiphenyl (HCB) were obtained from Fuji Film Co., Ltd. (Tokyo).

Cholesteryl oleate (COL) was purchased from Tokyo Kasei Co., Ltd. (Tokyo). The memsomorphic ranges of MBBA, HCB, and COL were respectively 20–47°, 28–42°, and 17–51°C.

Preparation of the Chlorophyll-Liquid Crystal Membrane

After evaporation of the Chl a stock solution, the Chl a was dissolved in a mixed solvent of petroleum ether–benzene (9:1). For absorption spectroscopy, 0.1 mL of the resulting solution was spread on an optically homogeneous glass plate (1×3 cm^2) and dried. In the similar manner, 0.2 mL of the solution was spread on a NaCl plate for IR spectroscopy.

Spectral Measurements

Absorption spectroscopy was made using a Shimadzu Model UV 200 spectrophotometer. The Chl a-coated glass plate was fixed in a glass cuvette filled either with 0.1 *M* phosphate buffer of pH 7.0 or with air at 35 ± 0.1°C. A Hitachi Model 295 IR spectrometer was used for IR spectroscopy. IR spectra were obtained using a Chl a-coated NaCl cell with reference to a NaCl cell at 35 ± 0.5°C.

Results

Chl a Hydrates Involved in the Chl a–Liquid Crystal Membrane

In solvents such as pyridine, tetrahydrofuran, acetone, and the like, Chl a is monomeric, with solvent molecules in the axial position(s). Figure 1 shows an absorption spectrum of Chl a dissolved in acetone–H_2O (80:20 v/v%). Chl a absorbed at 664 nm, which could be assigned to monomeric Chl a. Figure 1 also includes the spectrum of Chl a membrane on a glass plate and a Chl a–MBBA membrane (1:3 in molar ratio) on a glass plate in contact with air. Chl a in membrane form showed a slightly different absorption spectrum. The membrane contained 1.4×10^{-8} mol Chl a/cm^2. The peak at 664 nm shifted to 674 nm with an appreciable broadening owing to the presence of dimeric and oligomeric Chl a.

The incorporation of MBBA in an acetone–H_2O solution containing Chl a resulted in a slight red shift. Further red shift resulted from the membrane form of Chl a–MBBA as shown in Fig. 1. The membrane contained 1.4×10^{-8} mol Chl a and 4.2×10^{8} mol MBBA/cm^2. The membrane absorbed at 683 nm.

Figure 2 shows the effects of water on the absorption spectrum of the Chl a–MBBA membrane. The membrane was in contact with a phosphate buffer of pH 7.0. The spectrum was determined at intervals. It should be noted that the peak at 683 nm diminished, and that a new peak at 743 nm appeared. The time course of the change is presented in Fig. 3.

It appears likely that the long-wavelength forms of chlorophyll so often reported in the literature are chlorophyll–water adducts. Monomeric Chl a has been reported to absorb at 665 nm, dimeric chlorophyll species absorb at 665

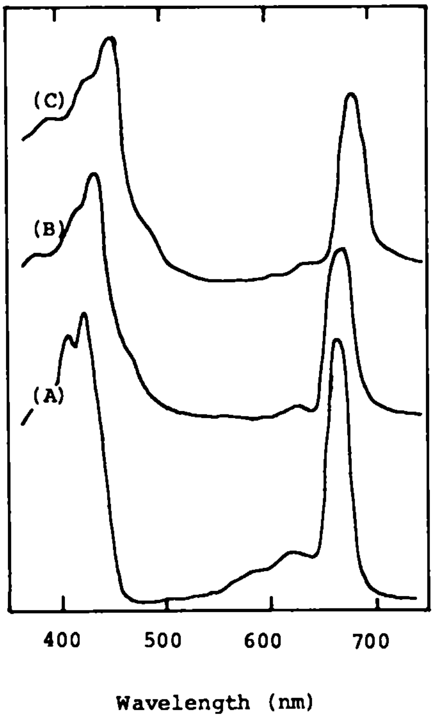

FIG. 1. Absorption spectra: (A) Chl a dissolved in a mixed solvent of acetone–H_2O (80:20 v/v); (B) Chl a membrane on a glass plate; (C) Chl a-MBBA (1:3 molar ratio) membrane on a glass plate.

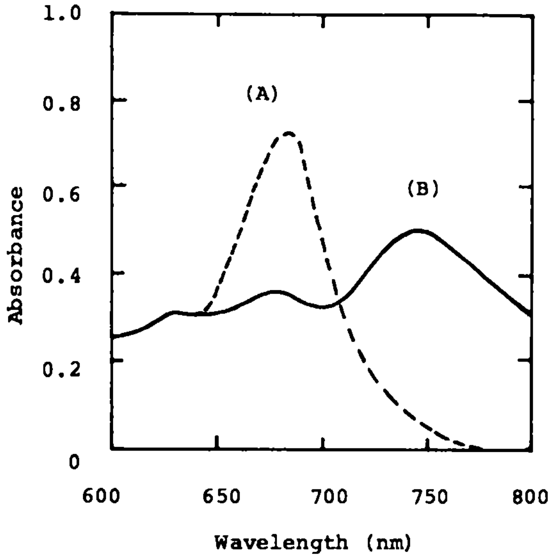

FIG. 2. Absorption spectra of a Chl a-MBBA (1:3 molar ratio) membrane contacted with water: (A) before and (B) after contacting with water.

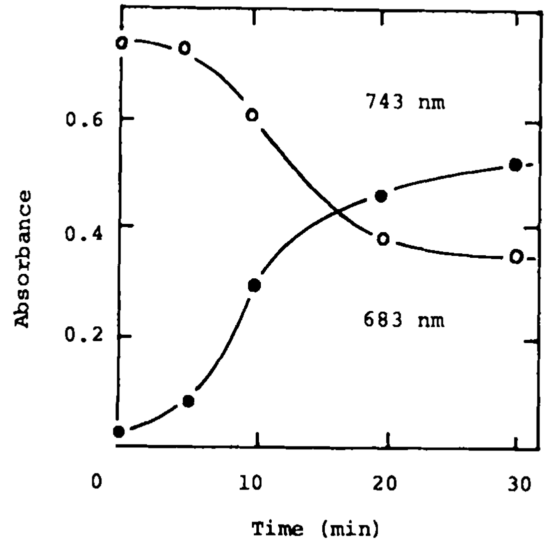

FIG. 3. Increase of the 743-nm absorbing Chl a aggregate. Absorbances at 743 nm (-●-) and 683 nm (—O—) were measured for the Chl a-MBBA (1:3 molar ratio) membrane under the excess water conditions.

nm with a shoulder at 673 nm, and oligomeric Chl a absorbs at 673nm. In contrast Chl a-H_2O adducts in hydrocarbon solvents absorb in the red at 740 nm.

This knowledge of the spectra of Chl a-water adducts leads us to the conclusion that the peak at 743 nm shown in Fig. 2 can be attributed to the formation of such Chl a-water adducts.

Intermolecular Interactions of Chlorophylls

The IR spectrum in the carbonyl region for Chl a has been assigned elsewhere (*11*). Chl a in CCl_4 solution shows ester absorptions at 1735 cm^{-1}, free keto carbonyl at 1695 cm^{-1}, and coordinated keto OC··· Mg at 1652 cm^{-1}.

Figure 4 shows the IR spectra of Chl a, Chl a-MBBA, and MBBA. Each sample was spread on an NaCl cell for IR spectroscopy. The Chl a-MBBA was prepared at a molar ratio of 1:3. The Chl a showed peaks at 1655 cm^{-1} (broad) and 1738 cm^{-1}. It indicates that the C-9 keto carbonyl is coordinated in the same manner as OC··· Mg (another porphyrin), and that the C-10 ester carbonyl is free. In contrast the Chl a-MBBA absorbed at 1703 and 1742 cm^{-1}. The peak at 1655 cm^{-1} owing to the coordinated keto carbonyl diminished, and the new peak at 1703 cm^{-1}, which could be attributed to the free keto carbonyl, appeared. The self-aggregation of Chl a via the keto carbonyl may be dissociated by the penetration of MBBA molecules. The peak at 1742 cm^{-1} appeared with shoulders.

There is clear evidence for the disaggregation of Chl a by the penetration of MBBA among CHl a molecules. The IR spectra are shown for various molar ratios of CHl a-MBBA in Fig. 5. The C-9 keto OC··· Mg aggregation peak at 1655 cm^{-1} diminishes, and the free keto CO peak at 1703 cm^{-1} increases in intensity with added MBBA.

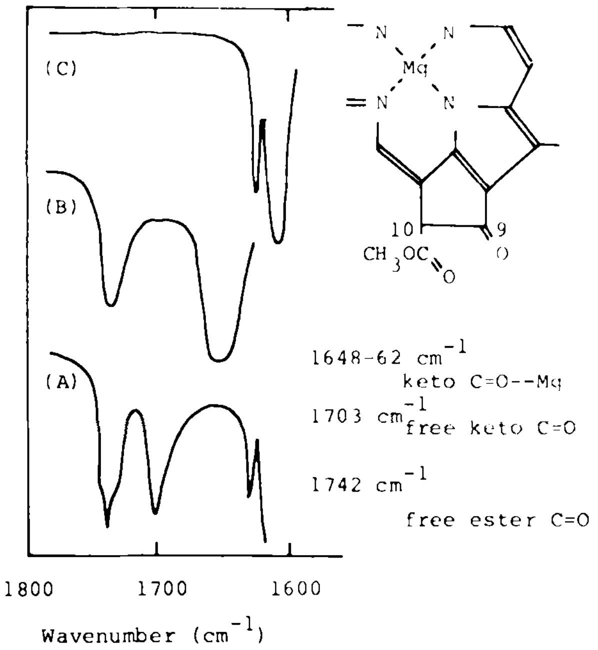

FIG. 4. IR spectra: (A) Chl a-MBBA (1:3 molar ratio) membrane: (B) Chl a membrane, and (C) MBBA membrane on a NaCl cell.

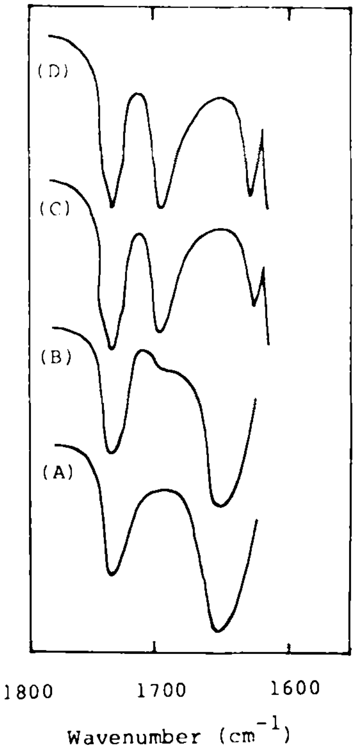

FIG. 5. The effects of MBBA on the peaks at 1655 and 1703 cm^{-1}. (A) Chl a; (B) Chl a:MBBA = 1:1; (C) Chl a:MBBA = 1:3; (D) Chl a:MBBA = 1:10.

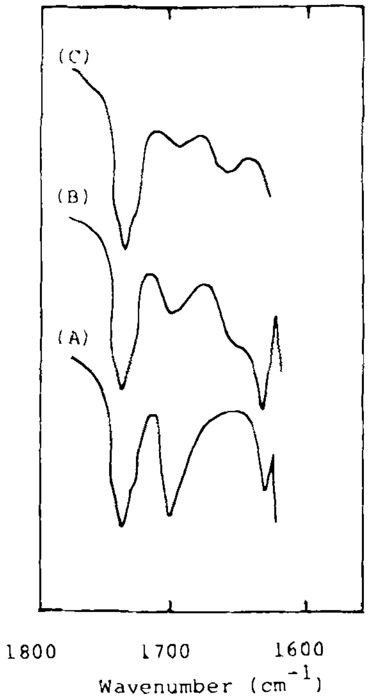

FIG. 6. IR spectra of various Chl a-liquid crystal membranes: (A) Chl a-MBBA membrane; (B) Chl a-HCB membrane; and (C) Chl a-COL membrane.

Effects of Various Liquid Crystals on the Spectroscopic Properties of Chl a

In the previous paper (*2*), it was shown that the Chl a-MBBA membrane responded most intensively to visible light, compared with the Chl-COL and Chl-HCB membranes, where COL and HCB were cholesteryl oleate and 4'-heptyl-4-cyanobiphenyl, respectively. Each membrane contained 1.4×10^{-8} mol Chl a and 4.2×10^{-8} mol liquid crystal/cm^2. The IR spectra are shown in Fig. 6. It should be noted that all the membranes gave the free keto CO peak at 1703 cm^{-1}, which resulted from the cleavage of the intermolecular interaction via keto OC···Mg bonding. The relative intensity at 1703 cm^{-1} was in the order of

$$\text{Chl a-MBBA} > \text{Chl a-HCB} > \text{Chl a-COL}$$

This order agrees well with that of the photoresponse as reported in the previous paper (*2*).

Discussion

Visible and IR spectra of the Chl a-MBBA membrane offer evidence that MBBA prevents Chl a from self-aggregation, and that Chl a is easily hydrated in the presence of MBBA.

A liquid crystal in the nematic phase can be oriented by either mechanical effects or by thin film deposition. In such a medium, dye molecules can also be oriented, especially when they are similar to the host molecules. Journeaux and Viovy showed that chlorophyll derivatives could be easily oriented in such an anisotropic medium (*21*). On the other hand, because of hydrophobicity, MBBA may have strong affinity to the phytyl chain of Chl a. It should be pointed out here that the molecular length of MBBA is approximately one-third of the phytyl chain length, and that the Chl a–MBBA membrane showed the most prominent photoresponse at the molar ratio of Chl a:MBBA = 1:3. These facts suggest that one possible scheme is that a Chl a molecule associates with three MBBA molecules along the phytyl chain. If the MBBA molecules exist in the nematic liquid crystal phase in the temperature range 20–47°C, these molecules may assume an ordered alignment with some specific inclination along the electric field.

It should be stressed that the C-9 keto carbonyl and C-10 ester carbonyl of the Chl a–MBBA membrane are free unless water is present. Under excess water conditions, water may be unusually accessible to either the C-10 ester carbonyl or the C-9 keto carbonyl. This accounts for the easy hydration of the Chl a–MBBA membrane. The hydrated Chl a may be identical to the one known as the 743-nm absorbing Chl a aggregate. However, the stoichiometry of the 743-nm absorbing Chl a aggregate has remained unsettled for a long time. The aggregate has been known as the dihydrate polymer (Chl a–$2H_2O)_n$. In the late 1960s, it was assigned a monohydrate stoichiometry by Katz, (Chl a–$H_2O)_n$ (*12, 22*). In 1975, the experimental basis for the (Chl a–H_2O) assignment was reexamined, and the dihydrate stoichiometry of the 743-nm absorbing Chl a aggregate was confirmed (*13, 23*). Fong and Koester have assumed that the structure of the 743-nm absorbing Chl a aggregate is in accord with the X-ray diffraction-determined structure of the ethyl chlorophyllide dihydrate (*16, 17*). Based on these historical investigations, we concluded that the dihydrate stoichiometry, (Chl a–$2H_2O)_n$, was the most probable in the Chl a–MBBA membrane under excess water conditions.

Traditionally, the C-10 keto carbonyl has been thought to play the dominant role in Chl a aggregation. However, under excess water conditions, the C-10 ester carbonyl group becomes engaged in cluster interactions with the H_2O molecules, leaving the C-9 keto carbonyl available for exo-bonding interactions of the type proposed by Boxer and Closs (*24*), Fong et al. (*25, 26*), and Katz et al. (*27, 28*). Clark has also confirmed this interpretation (*29, 30*). It was then postulated that the Chl a–MMBA membrane was hydrated in the dihydrate stoichiometry (Chl a–3MBBA–$2H_2O$) via the C-10 ester OC···H(H)O···Mg and the C-9 keto OC···H(H)O···Mg bondings. The postulated structure is schematically illustrated in Fig. 7.

References

1. Aizawa, M., Suzuki, N., Takahashi, F., and Suzuki, S. (1977), *J. Solid-Phase Biochem.* **2**, 111.

2. Aizawa, M., Suzuki, N., Hirano, M., and Suzuki, S. (1978), *Electrochim. Acta* **23**, 1061.

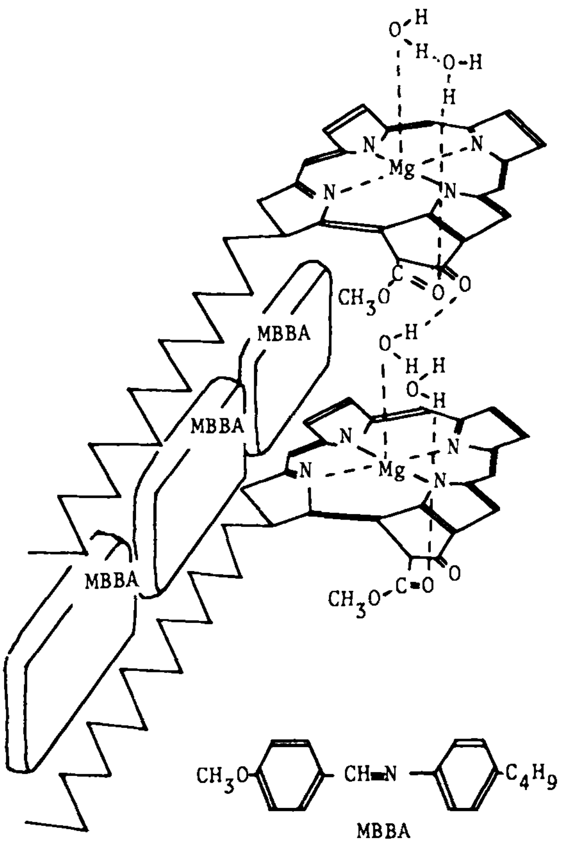

FIG. 7. Schematic representation of the postulated structure of the Chl a-MBBA membrane under the excess water conditions.

3. Aizawa, M., Hirano, M., and Suzuki, S. (1978), *Electrochim. Acta* **23,** 1185.
4. Aizawa, M., Hirano, M., and Suzuki, S. (1978). *Electrochim. Acta* **24,** 89.
5. Aizawa, M., Hirano, M., and Suzuki, S. (1978). *J. Membrane Sci.* **4,** 251.
6. Fong, F. K., and Galloway, L. (1978). *J. Amer. Chem. Soc.* **100,** 3594.
7. Fong, F. K., and Winograd, N. (1976), *J. Amer. Chem. Soc.* **98,** 2287.
8. Fetterman, L. M., Galloway, L., Winograd, N., and Fong, F. K. (1977), *J. Amer. Chem. Sco.* **99,** 653.
9. Rabinowitch, E. (1938), *Nature,* **141,** 39.
10. Jacobs, E. E., Vatter, A. E., and Holt, A. S. (1954), *Arch. Biochem. Biophys.* **53,** 228.
11. Katz, J. J., Dougherty, R. C., and Boucher, L. J. (1966), *The Chlorophylls* (Vernon, L. P., and Seely, G. R., eds.) Academic Press, New York, pp. 185-221.
12. Ballschmitter, K., and Katz, J. J. (1969), *J. Am. Chem. Soc.* **91,** 2661.
13. Fong, F. K., and Koester, V.J. (1976), *Biochim. Biophys. Acta* **423, 52.**
14. Brace, J. G., Fong, F. K., Karweik, D. H., Koester, V. J., Shepard, A., and Winograd, N. (1978) *J. Amer. Chem. Soc.* **100,** 5203.
15. Shipman, L. L., and Katz, J. J. (1977), *J. Phys. Chem.* **81,** 577.
16. Strouse, C. E. (1974), *Proc. Natl. Acad. Sci., US* **71,** 325.
17. Chow, H. C., Serlin, R., and Strouse, C. E. (1975) *J. Amer. Chem. Soc.,* **97,** 7230.
18. Winograd, N., Shepard, A., Karweik, D. H., Koester, V. J., and Fong, F. K. (1976), *J. Amer. Chem. Soc.* **98,** 2369.

19. Strain, H. H. and Svec, W. A., (1966), *The Chlorophylls* (Vernon, L. P., and Seely, G. R., eds.) Academic Press, New York, p. 21.
20. Smith, J. H. C., and Benitez, A. (1969), *Modern Methods of Plant Analysis,* Vol. 1 (Paech, K., and Tracey, M. V., eds.), *Springer-Verlag, Berlin, p. 142.*
21. Journeaux, R., and Viovy, R. (1978), *Photochem. Photobiol.* **28,** 243.
22. Cotton, F. A., and Wilkinson, G. (1972), *Advanced Inorganic Chemistry,* 3rd ed., Wiley-Interscience, New York, N.Y., p. 218.
23. Fong, F. K., and Koester, V. J. (1975), *J. Am. Chem. Soc.* **97,** 6888.
24. Boxer, S. G., and Closs, G. L. (1976), *J. Amer. Chem. Soc.* **98,** 5405.
25. Fong, F. K., Koester, V. J., and Polles, J. S. (1976), *J. Amer. Chem. Soc.* **98,** 6406.
26. Fong, F. K., Koester, V. J. and Galloway, L (1977), *J. Amer. Chem. Soc.* **99,** 2372.
27. Shipman, L. L., Cotton, T. M., Norris, J. R., and Katz, J. J. (1976), *Proc. Natl. Acad. Sci. USA* **73,** 1971.
28. Wasielowski, M. R., Studier, M. H., and Katz, J. J. (1976), *Proc. Natl. Acad. Sci. USA* **73,** 4282.
29. Clarke, R. H., and Hobart, D. R. (1977), *FEBS Lett.* **82,** 155.
30. Kooyman, R. P. H., Shaafsma, T. J., and Kleibeuker, J. F. (1977), *Photochem. Photobiol.* **26,** 235.