Light-Induced Electron Transfer from a Lipid-Soluble Zinc(II) Porphyrin to Various Electron Acceptors in Frozen Vesicle Solutions: Effects of Cholesterol on the Solubilization Sites and Radical Yields

Marie-Pierre Lanot and Larry Kevan*

Department of Chemistry, University of Houston, Houston, Texas 77204-5641 *(Received: September 7, 1988; In Final Form: December 15, 1988)*

The photoionization of zinc tetraphenylporphyrin (ZnTPP) has been studied by electron spin resonance (ESR) at 77 K in rapidly frozen dipalmitoylphosphatidylcholine (DPPC) vesicle solutions, and in the presence of various electron acceptors like tetrachloro- and tetrabromobenzoquinone (TCBQ and TBBQ) and potassium ferricyanide ($K_3Fe(CN)_6$). The photoproduced $ZnTPP^+$ yield was found to increase, and photoinduced anion formation was also detected for added tetrahalobenzoquinones. Incorporation of cholesterol in these systems altered the $ZnTPP^+$ radical yields via two main structural changes: (1) ZnTPP–water contact increased due to intercalation of cholesterol between the surfactant headgroups, and (2) TCBQ moved deeper into the vesicle bilayer because of increased bilayer fluidity.

Introduction

Photoredox processes in vesicle solutions have attracted attention as mimicking electron transport across biological membranes and as possible means of energy storage.[1,2] Zinc(II) porphyrins and various electron acceptors have proven to be suitable to optimize such systems, for they allow a variety of spatial organization within or at the surface of the vesicle.[3-6]

The lipid-soluble zinc(II) 5,10,15,20-tetraphenylporphyrin (ZnTPP) is solubilized in the present work in DL-α-dipalmitoylphosphatidylcholine (DPPC) vesicle solutions. The quenching of the ZnTPP triplet state by electron acceptors which are differently located in frozen DPPC vesicles is investigated by electron spin resonance (ESR) at 77 K. Electron-transfer reactions within the DPPC bilayer are examined by using the uncharged lipophiles tetrabromo- and tetrachlorobenzoquinones (TBBQ and TCBQ) as electron acceptors. Hydrophilic ferricyanide ions, which do not penetrate the lipid bilayer, are used to mimic electron-transfer reactions across bilayer–water interfaces. An increase in the $ZnTPP^+$ yield is detected by ESR in these three cases. In the presence of TCBQ or TBBQ photoinduced formation of the $TCBQ^-$ or $TBBQ^-$ anions is also observed by ESR.

Another method to control the DPPC vesicle structure and thus the photoionization efficiency is to add cholesterol to the DPPC vesicle. Cholesterol can enter into phospholipid bilayers to a level of one molecule of cholesterol per molecule of phospholipid without destroying the structure of the lipid bilayer.[7] Structural changes, however, are caused at the vesicle surface and within the vesicle by addition of cholesterol. In the present study, cholesterol is added to DPPC vesicles containing ZnTPP with (1) no electron acceptor, (2) tetrachlorobenzoquinone, and (3) potassium ferricyanide. The photoionization at 77 K of ZnTPP in these three systems is also investigated by ESR. Cholesterol appears to influence the ZnTPP photoionization yield by its ability to intercalate between the headgroups of the phospholipid molecules[8] and its function of controlling the hydrocarbon chain fluidity of the surfactants within the vesicle.[7,9] It is deduced that ZnTPP increases its water contact and that TBBQ moves deeper into the vesicle bilayer when 50 mol % cholesterol is added.

Experimental Section

DPPC and cholesterol were obtained from Sigma, ZnTPP and the electron scavengers TCBQ and $K_3Fe(CN)_6$ were purchased from Aldrich. TBBQ was obtained from Alfa. All these chemicals were used without further purification.

Stock solutions of DPPC, ZnTPP, TCBQ, TBBQ, and cholesterol were prepared in chloroform. After a chloroform solution of DPPC-containing ZnTPP was evaporated under nitrogen flow, the resulting film was sonicated in an appropriate aqueous buffer solution following the method of Oettmeier et al. as follows.[10] The buffer solution contained 0.1 M sodium phosphate, 0.1 M sodium pyrophosphate, and 1.0 M EDTA in triply distilled water, adjusted to pH 7.0 with sulfuric acid. The sonication was done at 50–55 °C for 1 h with a Fisher Model 300 sonic dismembrator, operated at 30–35 W with a 4 mm o.d. microtip to form the vesicles. This produces largely unilamellar vesicles[10] and no vesicle filtration was done. The concentrations used for DPPC and ZnTPP were respectively 45 and 1.0 mM.

Lipophilic electron acceptors, TCBQ and TBBQ, were added in the desired amount to the initial ZnTPP–DPPC mixture in chloroform to obtain 15 mM concentrations of acceptor. The same procedure was used in the study of the effect of the addition of 20 and 50 mol % cholesterol to DPPC vesicles. Stock solutions of $K_3Fe(CN)_6$ were prepared in triply distilled water and small portions of it were added with a microsyringe to ZnTPP–DPPC vesicle solutions. Studies were done in the range 0–20 mM potassium ferricyanide. The vesicle solutions were sealed into 2 mm i.d. by 3 mm o.d. Suprasil quartz tubes and frozen rapidly by plunging the tubes into liquid nitrogen.

Photoirradiations were carried out with a 150-W xenon lamp, at 77 K, with a 10-cm water filter and Corning No. 5030 (320 nm $< \lambda_{irr} <$ 580 nm) or No. 3686 ($\lambda_{irr} >$ 520 nm) glass filters. Samples were irradiated for 30 min. Previous work shows that this gives a plateau yield.[20] Electron spin resonance spectra were recorded at 77 K on a Bruker ESP 300 ESR spectrometer.

Results

The average yields of photoinduced paramagnetic products are plotted with error bars showing the extremes observed. Table I

(1) Grätzel, M. In *Photochemical Conversion and Storage of Solar Energy*; Connolly, J. S., Ed.; Academic Press: New York, 1981; Chapter 5.
(2) Balzani, V.; Scandola, F. In *Photochemical Conversion and Storage of Solar Energy*; Connolly, J. S., Ed.; Academic Press: New York, 1981; Chapter 4.
(3) Fendler, J. H. *Acc. Chem. Res.* **1980**, *13*, 7-13.
(4) Mauzerall, D. In *The Porphyrins*; Dolphin, D., Ed.; Academic Press: New York, 1978; Vol. IV, Chapter 2.
(5) Pileni, M. P. *Chem. Phys. Lett.* **1980**, *71*, 317-321.
(6) Chandrashekar, T. K.; Van Willigen, H. *Chem. Phys. Lett.* **1986**, *106*, 237-241.
(7) Chapman, D. In *Membrane Fluidity in Biology*; Aloia, R. C., Ed.; Academic Press: New York, 1983; Vol. 2, p 15.
(8) Yeagle, P. L.; Hutton, W. C.; Huang, C.; Martin, R. B. *Biochemistry* **1977**, *16*, 4344-4349.
(9) Schreier-Mucillo, S.; Mersh, D.; Dugas, H.; Schneider, H.; Smith, I. C. R. *Chem. Phys. Lipids* **1973**, *10*, 11.
(10) Oettmeier, W.; Norris, J. R.; Katz, J. J. *Z. Naturforsch.* **1976**, *31b*, 163.

0022-3654/89/2093-5280$01.50/0

TABLE I: Properties of Electron Acceptors[13,18]

electron acceptor	abbrev	opt absorpn data λ_{max}, nm	opt absorpn data ϵ, M^{-1} cm^{-1}	solvent	E_0, mV	g^a
1,2,4,5-tetrachloro-*p*-benzoquinone	TCBQ	360	2.7×10^2	methanol	742	2.057
		280	1.9×10^3			
1,2,4,5-tetrabromo-*p*-benzoquinone	TBBQ	305	1.2×10^4	ether	746	2.088
potassium ferricyanide	$K_3Fe(CN)_6$	419	1.1×10^3	H_2O	410	
		302	1.9×10^3			
		269	1.4×10^3			

[a] ESR parameter of radical anion.

TABLE II: Relative Changes in Photoionization Yields of $ZnTPP^+$ upon Cholesterol Addition

	λ_{irr},[b] nm	cholesterol[a] 20 mol %	cholesterol[a] 50 mol %
1 mM ZnTPP	320–580	0 ± 5%	+89 ± 5%
	>520		+40 ± 5%
1 mM ZnTPP + 2.5 mM TCBQ	320–580	−36 ± 8%	−47 ± 3%
1 mM ZnTPP + 10 mM TCBQ	320–580	0 (1 expt)	0 (1 expt)
1 mM ZnTPP + 15 mM $K_3Fe(CN)_6$	320–580	−22 ± 2%	−74 ± 6%
	>520		+50 ● 15%

[a] The percent difference is calculated in each case with respect to the yield found with 0% cholesterol. [b] Irradiation light.

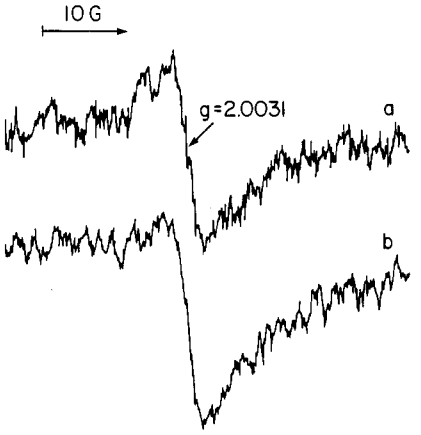

Figure 1. ESR spectra at 77 K after irradiation of (a) $ZnTPP^+$ and (b) $ZnTPP^+$ when 50 mol % cholesterol is present in DPPC vesicle solutions with [ZnTPP] = 1 mM.

shows wavelength (λ_{max}) and molar extinction coefficients (ϵ) of the intense optical absorption bands, oxidation–reduction potentials (E_0) of the electron acceptors used, and the ESR parameters of their reduction products. Table II summarizes the relative changes in photoionization yields of $ZnTPP^+$ upon cholesterol addition.

a. No Electron Acceptor. Photoirradiation at 77 K of DPPC vesicle solutions containing ZnTPP produces an ESR spectrum with a singlet at $g = 2.0031$ and with $\Delta H_{pp} = 5.5 \pm 0.1$ G, which is characteristic of the $ZnTPP^+$ radical π-cation.[11] Upon addition of cholesterol, the $ZnTPP^+$ ESR line becomes asymmetric after irradiation in the 320–580 nm wavelength range and shows by double integration a ~90% increase with 50 mol % cholesterol in DPPC vesicle solution (Figure 1). Cholesterol with an absorption band between 214 and 253 nm[12] does not absorb in the 320–580 nm wavelength region. Addition of cholesterol to the ZnTPP–DPPC vesicle solution does not induce any blank signal before irradiation. Also cholesterol alone in DPPC vesicle solution does not produce any detectable radical upon irradiation at 77 K. After irradiation with $\lambda > 520$ nm the $ZnTPP^+$ ESR signal

(11) Fajer, J.; Davis, M. S. In *The Porphyrins*; Dolphin, D., Ed.; Academic Press: New York, 1978; Vol. IV; Chapter 4.
(12) Gibbons, G. F.; Mitropoulos, K. A.; Myant, N. B. *Biochemistry of Cholesterol*; Elsevier Biomedical Press: Amsterdam, 1982; Chapter 3.

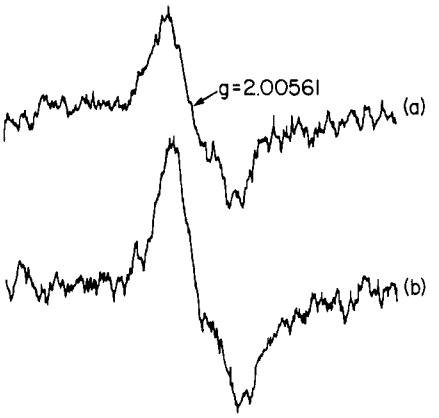

Figure 2. ESR spectra at 77 K *before* irradiation of DPPC vesicle samples containing (a) 2.5 mM TCBQ and (b) 1 mM ZnTPP and 2.5 mM TCBQ.

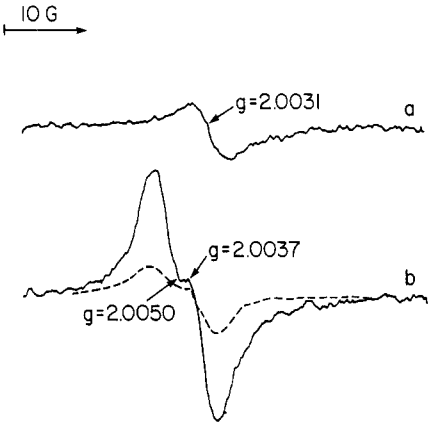

Figure 3. ESR spectra at 77 K of DPPC vesicle solution with (a) 1 mM ZnTPP, and (b) 1 mM ZnTPP and 2.5 mM TCBQ, before (---) and after (—) irradiation. All spectra are recorded on the same vertical scale. Note that no dark signal is seen in (a).

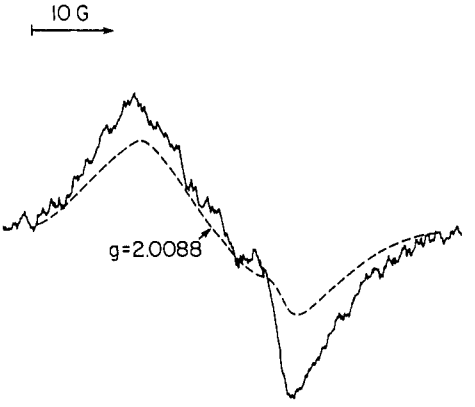

Figure 4. ESR spectra at 77 K of DPPC vesicle solutions containing 1 mM ZnTPP and 10 mM TBBQ, before (---) and after (—) irradiation. All spectra are recorded on the same vertical scale.

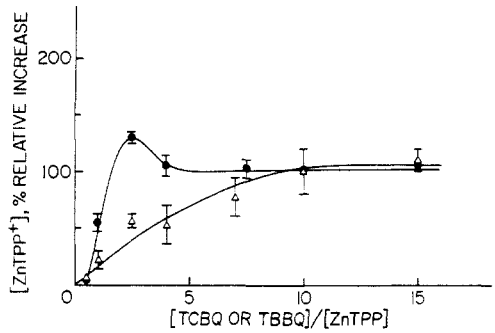

Figure 5. Relative increase in ZnTPP photoionization yield at 77 K in DPPC vesicle solution as a function of increasing [electron acceptor]/[ZnTPP] ratio, with [ZnTPP] = 1 mM. TCBQ (●) and TBBQ (△) are used as electron acceptors.

remains symmetric and shows only a ~40% increase with 50 mol % cholesterol.

b. Tetrahalobenzoquinones as Electron Acceptors. In DPPC vesicle solution with TCBQ or TBBQ as an electron acceptor an ESR signal is observed before irradiation at 77 K, with or without ZnTPP in the system (Figures 2–4). This dark signal increases in intensity with increasing concentration of tetrahalobenzoquinones. This signal, characterized by $g = 2.0055$ or $g = 2.0088$, corresponds to the $TCBQ^-$ or $TBBQ^-$ anion radicals,[13] respectively. These anions must be produced by chemical reaction during the vesicle preparation, since no signal is observed for TCBQ or TBBQ in CH_2Cl_2 solution at 77 K before irradiation. This chemical reduction appears to be partly due to chemical reaction with ZnTPP since the intensity of the signal is between 50 and 100% greater when the porphyrin is present (Figure 2). TCBQ, with a weak absorption band at $\lambda_{max} = 360$ nm ($\epsilon = 1.0 \times 10^2$ M^{-1} cm^{-1}),[14] is likely to absorb some of the light used ($320 \text{ nm} < \lambda_{irr} < 580$ nm). Indeed, after irradiation at 77 K there is production of some $TCBQ^-$ radical in CH_2Cl_2 and an increase of the blank signal for TCBQ alone in DPPC. However, when a filter with $\lambda_{irr} > 520$ nm is used, there is no production of $TCBQ^-$ radical. The tetrabromobenzoquinone, having an absorption band at 320 nm,[14] does not produce any signal upon irradiation with blue light in CH_2Cl_2 solution at 77 K.

After irradiation at 77 K in the presence of ZnTPP and TBBQ or TCBQ, an asymmetric ESR singlet is observed. This signal is the superposition of the benzoquinone anion reduction product at $g = 2.0057$ for $TCBQ^-$ and $g = 2.0031$ for $Chla^+$. The addition of the two peaks results in the cancellation of the central part of the signal between $g = 2.0037$ and $g = 2.0050$; for example, see the case of TCBQ (Figure 3). By double integration of the high-field part of that signal and after subtraction of the dark signal, an increase is observed in the $ZnTPP^+$ yield with a maximum of ~100% at a [TCBQ]/[ZnTPP] ratio equal to 2.5 and a [TBBQ]/[ZnTPP] ratio equal to 10 (Figure 5).

When cholesterol is added to DPPC vesicle solutions containing ZnTPP and 2.5 mM TCBQ no change in the line shape of the signal obtained previously is observed. However, the photoionization yield of ZnTPP decreases by ~47% for 50 mol % of cholesterol. When the TCBQ concentration is increased to 10 mM, no change in yield is seen by the addition of 50 mol % cholesterol.

c. $K_3Fe(CN)_6$ as Electron Acceptor. In DPPC vesicle solution with $K_3Fe(CN)_6$ as an electron acceptor an ESR signal of $ZnTPP^+$ is observed before irradiation at 77 K. Although $K_3Fe(CN)_6$ has an absorption band at 419 nm, only a very weak signal is produced by irradiation at 77 K of ferricyanide alone in frozen vesicle solution or frozen bulk solutions. After photoirradiation of a DPPC vesicle solution at 77 K containing 1 mM ZnTPP and

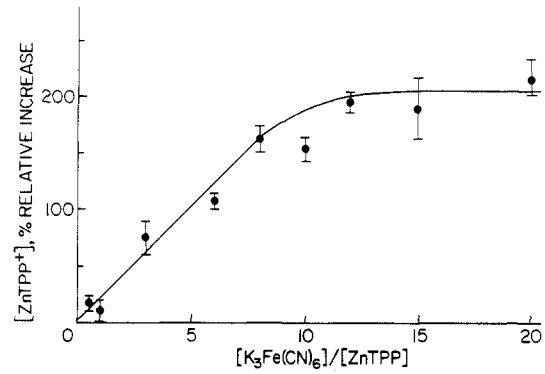

Figure 6. Relative increase in ZnTPP photoionization yield at 77 K in DPPC vesicle solution as a function of increasing $K_3Fe(CN)_6$/ZnTPP ratio, with [ZnTPP] = 1 mM.

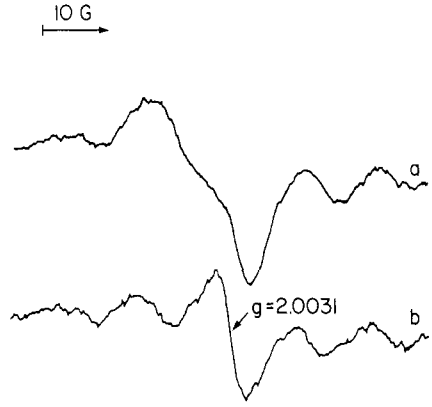

Figure 7. ESR spectra at 77 K of (a) 15 mM $K_3Fe(CN)_6$ + 50 mol % cholesterol in DPPC vesicles, and (b) 1 mM ZnTPP + 15 mM $K_3Fe(CN)_6$ + 50 mol % cholesterol in DPPC vesicles. Note that the resolution in (a) is not as good as in (b).

variable concentrations of $K_3Fe(CN)_6$, the $ZnTPP^+$ yield, measured from the height of the signal and after correction for the blank signal, increases to 200% greater than the one obtained with no ferricyanide (Figure 6). The optimum $[K_3Fe(CN)_6]/[ZnTPP]$ ratio appears to be about 15 as shown in Figure 6.

When 50 mol % cholesterol is added to a DPPC vesicle solution containing ZnTPP and 15 mM $K_3Fe(CN)_6$, the signal height decreases by about 75% after irradiation at $\lambda = 320$–580 nm. Moreover, a quintet ESR signal with a ~15 G splitting overlaps with the $ZnTPP^+$ signal (Figure 7b). This quintet is also observed with about the same intensity after irradiation at the same wavelength of a DPPC vesicle solution containing 50 mol % cholesterol and 15 mM $K_3Fe(CN)_6$ only (Figure 7a). After irradiation at $\lambda > 520$ nm no such quintet is observed with or without ZnTPP and a 50% increase in the $ZnTPP^+$ signal intensity is seen upon addition of 50 mol % cholesterol.

d. ESR Power Saturation Measurements. ESR power saturation measurements for $ZnTPP^+$ in frozen vesicle solutions with and without potassium ferricyanide were carried out at 77 K by measuring the peak height of $ZnTPP^+$ as a function of incident microwave power. The saturation curves are compared to the ones obtained with $Chla^+$ under the same conditions (Figure 8). The characteristic relaxation times of $ZnTPP^+$ are found to be much shorter than those of $Chla^+$. Addition of potassium ferricyanide to ZnTPP–DPPC samples does not affect the shape of the power saturation curve, whereas the presence of $K_3Fe(CN)_6$ in chlorophyll samples increases the power necessary to saturate $Chla^+$.

Discussion

a. Radical Formation in the Absence of Cholesterol. According to these results, $ZnTPP^+$ formation is enhanced by the presence of either hydrophobic or hydrophilic electron acceptors. $ZnTPP^+$ is formed via the ZnTPP triplet state, by intersystem crossing from photoexcited ZnTPP*. Tollin et al.[15–18] studied

(13) Blois, Jr., M. S.; Brown, H. W.; Maling, J. E. In *Free Radicals in Biological Systems*; Blois, Jr., M. S., Ed.; Academic Press: New York, 1961; p 117.

(14) Sober, M. A.; Harte, R. A. In *Handbook of Biochemistry*; Chemical Rubber Co.: Cleveland, OH, 1968.

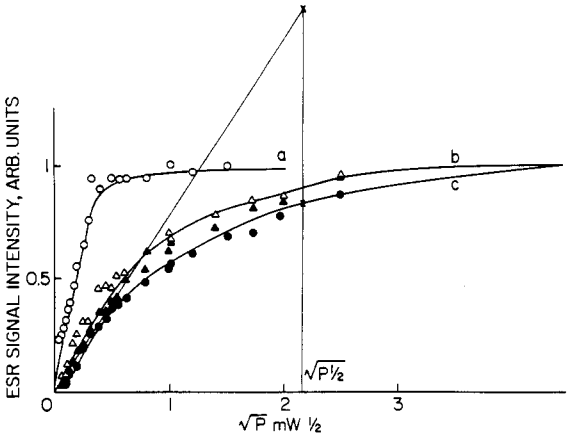

Figure 8. ESR power saturation curves at 77 K of DPPC vesicle solutions containing (a) $Chla^+$ (○), (b) $ZnTPP^+$ (△) and $ZnTPP^+$ and $K_3Fe(CN)_6$ (▲), and (c) $Chla^+$ and $K_3Fe(CN)_6$ (●). Added amounts of Chla or ZnTPP and $K_3Fe(CN)_6$ were 1 and 15 mM, respectively. The vertical line marked $(P_{1/2})^{1/2}$ denotes the 50% saturation value for curve c; see text.

photoreactions of chlorophyll (Chla) in vesicles containing *p*-benzoquinone by laser flash photolysis. From decreases in the triplet decay rate in the presence of quinone (Q) it was shown that the quinone quenches the triplet. This also resulted in the production of long-lived $Chla^+$ and Q^- species. A formation mechanism was proposed which involved a $(Chla^+\cdots Q^-)$ caged radical pair and which may also be proposed for the photoionization of ZnTPP.

The tetrahalobenzoquinones have comparable hydrophobic characters to ZnTPP and can solubilize close to the porphyrin particularly at high [electron acceptors]/[ZnTPP] ratios.[5] These electron acceptors enhance the $ZnTPP^+$ yield as shown in Figure 5. The maximum seen for TCBQ is not understood.

The water-soluble potassium ferricyanide is found to quench ZnTPP triplet by electron transfer more efficiently than the tetrahalobenzoquinones. Ferricyanide ions are symmetric and have a high charge density. They do not penetrate the lipid bilayer and are solubilized in the external hydration layer of the vesicle, possibly close to the $N(CH_3)_3$ group of the DPPC molecules as concluded from investigations of vesicle structures by NMR.[19] It is thus concluded that the porphyrin is located close to the vesicle interface to allow such efficient quenching by $K_3Fe(CN)_6$. This confirms our previous conclusion based on spectral absorption data about the location of ZnTPP in DPPC vesicles.[20]

The difference between the rates of magnetic relaxation of $ZnTPP^+$ and $Chla^+$ is not understood. They are both thought to be in similar microenvironments in the vesicle. As a result of the short relaxation time of $ZnTPP^+$, one cannot see an effect of ferricyanide ions on the saturation curve. This contrasts with the observations of $Chla^+$.[21]

b. Radical Formation in the Presence of Cholesterol. When no electron scavenger is present in ZnTPP–DPPC solutions there is formation of other radicals upon cholesterol addition and after irradiation at λ = 320–580 nm since the apparent $ZnTPP^+$ signal becomes asymmetric. The ZnTPP triplet state may participate in the formation of free radicals from the decomposition of cholesterol. These radicals have not been characterized, but they can account, at least partly, for the increase in the apparent $ZnTPP^+$ signal intensity with 320–580-nm light.

Such radicals are not formed after irradiation at $\lambda > 520$ nm, and the 40% increase in the photoionization yield upon 50 mol % cholesterol addition must be associated with structural effects due to cholesterol addition. Intercalation of cholesterol near the headgroup region increases the separation between the phospholipid headgroups.[8] This causes the vesicle surface to become more hydrated. Consequently, molecules like ZnTPP that are located close to the polar–nonpolar vesicle interface are more exposed to water in vesicles containing cholesterol than in vesicles without it.[22,23] In addition, studies of the kinetic behavior of photoproduced cations and photoejected electrons in micelles,[24] as well as electron spin echo measurements of photoproduced cation–water interactions,[25] have proven that the photoionization of a given species in surfactant assemblies is more efficient when the hydration of the photoproduced electron is facilitated. We have not been able to make good electron spin echo measurements of the water interactions of $ZnTPP^+$ because of limited signal intensity.

Cholesterol also allows greater penetration of lipophilic molecules like *p*-benzoquinone inside the bilayer[26,27] by increasing the internal fluidity or alkyl chain straightening inside the bilayer. TCBQ is therefore likely to move further from the DPPC vesicle interface upon cholesterol addition. ZnTPP, although also lipophilic, is assumed in the above argument not to be displaced from its initial site by water molecules penetrating the headgroup region upon cholesterol addition. The $H_2OZnTPP$ complex, previously proposed from spectral absorption data,[20] possibly experiences hydrogen bonding with the water molecules which penetrate the headgroup region and bind to phosphate carbonyl and *N*-methyl groups of the phospholipids, and thus does not move deeper in the DPPC bilayer. Therefore, if ZnTPP stays at its initial location site upon cholesterol addition, the average interaction distance between TCBQ and ZnTPP will increase. This can explain the observed 47% decrease in the photoionization yield of ZnTPP/TCBQ upon cholesterol addition. When 10 mM TCBQ is used instead of 2.5 mM, no effect is seen upon cholesterol addition. In that case, the lipid bilayer is too packed with benzoquinone to increase the average interaction distance from the porphyrin even if the fluidity is increased by cholesterol.

It has been demonstrated that the egg phosphatidylcholine (egg PC) is impermeable to ferricyanide.[26] Egg PC surfactant molecules only differ from DPPC by having an average of one unsaturation unit per alkyl chain.[7] The influence of cholesterol on the vesicle surface structure is expected to be the same in DPPC and egg PC vesicles. The quintet signal overlapping with the $ZnTPP^+$ signal when irradiation at λ = 320–580 nm is used probably results from sensitized cholesterol decomposition, by energy transfer from ferricyanide ions excited at 520 nm. The partial absorption of the irradiation light by ferricyanide may result in less light absorption by ZnTPP and consequently a decreased $ZnTPP^+$ yield as seen with 320–580-nm light irradiation.

This hypothesis is supported when irradiation at $\lambda > 520$ nm is used, for no quintet signal and no apparent decrease in the photoionization yield are observed. In fact, a 50% increase in the $ZnTPP^+$ signal is observed. This is about the same increase observed with no electron acceptor upon cholesterol addition and can be explained similarly as due to increased water interactions at the DPPC vesicle surface.

Another alternative to explain the increase in the ZnTPP photoionization efficiency for both no acceptor and for $K_3Fe(CN)_6$ upon cholesterol addition is a possible redistribution of ZnTPP inside the DPPC vesicle. In egg PC vesicles, above a 30 mol % concentration, cholesterol is found to dissolve preferentially near the inner vesicle surface.[26,28,29] This results in a change in Chla

(15) Ford, W. E.; Tollin, G. *Photochem. Photobiol.* **1982**, *35*, 809.
(16) Ford, W. E.; Tollin, G. *Photochem. Photobiol.* **1982**, *36*, 647.
(17) Hurley, J. K.; Castelli, F.; Tollin, G. *Photochem. Photobiol.* **1980**, *32*, 79.
(18) Hurley, J. K.; Castelli, F.; Tollin, G. *Photochem. Photobiol.* **1981**, *34*, 623.
(19) Bergelson, L. D. In *Methods in Membrane Biology*; Korn, E. D., Ed.; Plenum Press: New York, 1978; Vol. 9, pp 275–335.
(20) Lanot, M. P.; Kevan, L. *J. Phys. Chem.* **1989**, *93*, 998.
(21) Ohta, N.; Kevan, L. *J. Chem. Phys.* **1985**, *89*, 3070–3076.
(22) Haynes, D. H.; Staerk, H. J. *Membr. Biol.* **1974**, *313*, 340.
(23) Radda, G. K. In *Methods in Membrane Biology*; Korn, E. D., Ed.; Plenum Press: New York, 1975; Vol. 4, pp 97–188.
(24) Grätzel, M.; Thomas, J. K. *J. Phys. Chem.* **1974**, *78*, 2248.
(25) Narayana, P. A.; Li, A. S. W.; Kevan, L. *J. Am. Chem. Soc.* **1981**, *103*, 3603.
(26) Ford, W. E.; Tollin, G. *Photochem. Photobiol.* **1984**, *40*, 249.
(27) Hiromitsu, I.; Kevan, L. *J. Am. Chem. Soc.* **1987**, *109*, 4501.

distribution and causes radical yield increases because these latter differ between the outer and inner surfaces of the vesicles. If such redistribution also occurs in DPPC vesicles, $ZnTPP^+$ radical formation may be affected, and this may result in the increase in the ZnTPP photoionization yield both for no acceptor and for $K_3Fe(CN)_6$. In this latter case, more ZnTPP located in the outer surface can be quenched by ferricyanide ions contributing in an increase in the photoionization yield.

Finally, addition of cholesterol seems to differently affect the solubilization site of Chla in DPPC vesicles.[27] Hiromitsu et al.[27] give evidence from microwave power saturation experiments that Chla moves deeper into the bilayer when cholesterol is added because of the increase in the internal fluidity of the bilayer. The difference between ZnTPP and Chla can be interpreted by the fact that Chla with its phytol chain has less hydrophilicity than the H_2OZnTPP complex and possesses a greater mobility within the DPPC bilayer structure. This suggests a similar interpretation as found in studies of conjugated polyene in phosphatidylcholine vesicles,[23] namely that the type of binding of the solubilized probe to the bilayer headgroup region controls the overall probe mobility.

Acknowledgment. This research was supported by the Division of Chemical Sciences, Office of Basic Energy Sciences, Office of Energy Research, U.S. Department of Energy.

Registry No. ZnTPP, 14074-80-7; $ZnTPP^+$, 39732-73-5; TCBQ, 118-75-2; TBBQ, 488-48-2; DPPC, 2644-64-6; $TCBQ^-$, 17217-66-2; $TBBQ^-$, 50431-99-7; $K_3Fe(CN)_6$, 13746-66-2; cholesterol, 57-88-5.

(28) de Kruiff, B.; Cullis, P. R.; Radda, G. K. *Biochim. Biophys. Acta* **1976**, *436*, 729.
(29) Huang, C. H.; Sipe, J. P.; Chow, S. T.; Martin, R. B. *Proc. Natl. Acad. Sci. USA* **1974**, *72*, 359-362.

Correlation of Chain Dynamics and Counterion Relaxation in Semidilute Polyelectrolyte Solutions

C. J. M. van Rijn, A. J. Maat, J. de Bleijser, and J. C. Leyte*

Gorlaeus Laboratories, Department of Physical and Macromolecular Chemistry, University of Leiden, P.O. Box 9502, Leiden, The Netherlands *(Received: June 29, 1988; In Final Form: October 12, 1988)*

Reorientation of polyion segments is found to be correlated with the transverse nuclear magnetic relaxation of counterions in the semidilute regime. ^{23}Na counterion and ^2H polymer relaxation rates of selectively deuterated sodium polyacrylate have been studied as a function of the polymer charge density, the salt concentration, and the degree of polymerization. The experimental material indicates a correlation between the Na counterion and the ^2H main-chain transverse relaxation rates. Moreover, correlation times associated with the observed fast transverse relaxation appear to be in the same range (10^{-8}-10^{-6} s) for counterions and polymer nuclei. The influence of the reorientation of polyion segments as well as axial diffusion of the counterions relative to these segments on the relaxation rates of the counterions is estimated theoretically. It is concluded that the transverse relaxation rate of the counterions in the semidilute regime is mainly determined by the persistence length of the polyion.

I. Introduction

It is well-known that the quadrupolar relaxation rates of alkali ions in dilute aqueous solutions of simple salts are mainly determined by fluctuations in the orientation and position of the water molecules in the first hydration shell of the ion.[1-7]

In aqueous polyelectrolyte solutions,[8] these relaxation rates are enhanced, which indicates that additional processes contribute to the relaxation. In concentrated solutions up to 1 *m* these rates are within the extreme narrowing limit. However, a few years ago, relatively high transverse relaxation rates outside the extreme narrowing limit of the alkali counterions were found in the semidilute regime.[9-11]

Several interactions may contribute to the relaxation rate, e.g., enhancement of the solvent contribution by the polymer, site binding of the counterion to a charged group of the polyion,[12-14] and coupling between the screened electrostatic field gradient of the polyion and the quadrupole moment of the nucleus.[8]

Depending on a particular visualization of the motion of a counterion in the field of the polyion, a number of relaxation theories have been put forward. One of the earliest theories originates from van der Klink et al.[8] and focuses on the averaging of the strong radial field of the polyions by tangential diffusion of the counterions around one polyion. This theory is in accordance with the ^{23}Na relaxation rates within the extreme narrowing limit in poly(acrylic acid) (PAA) at moderately high polymer concentrations. However, relaxation rates outside the extreme narrowing limit as were found in the semidilute regime may have a different origin. Theoretical considerations at that time[9,10] indicated that the averaging of a residual coupling by axial diffusion along the polyion might cause the enhancement of the transverse relaxation.

The theory of van der Klink et al.[8] is elaborated under the assumption of two restraints; i.e., a counterion is allocated to just one polyion and the reorientation of the polyion has a negligible effect on the counterionic relaxation behavior. Halle et al.[15] studied the validity of the first restraint. In their theory they reached the conclusion that the escape of the counterion from one polyion to another one is the determining factor for the transverse counterion relaxation rate in the semidilute regime. As will be shown, the phenomena reported in the present paper cannot be explained within this framework.

(1) Hertz, H. G.; Holz, M.; Keller, G.; Versmold, H.; Yoon, C. *Ber. Bunsen-Ges. Phys. Chem.* **1974**, *78*, 493.
(2) Hertz, H. G. *Ber. Bunsen-Ges. Phys. Chem.* **1963**, *67*, 311.
(3) Hertz, H. G.; Zeidler, M. D. *Ber. Bunsen-Ges. Phys. Chem.* **1963**, *67*, 774.
(4) Hertz, H. G.; Holz, M.; Klute, R.; Stalidis, G.; Versmold, H. *Ber. Bunsen-Ges. Phys. Chem.* **1974**, *78*, 24.
(5) Hertz, H. G. *Ber. Bunsen-Ges. Phys. Chem.* **1973**, *77*, 531.
(6) Hertz, H. G. *Ber. Bunsen-Ges. Phys. Chem.* **1973**, *77*, 688.
(7) Engström, S.; Jönsson, B. *J. Chem. Phys.* **1984**, *80*, 5481.
(8) van der Klink, J. J.; Zuiderweg, L. H.; Leyte, J. C. *J. Chem. Phys.* **1974**, *60*, 2391.
(9) Levij, M.; de Bleijser, J.; Leyte, J. C. *Chem. Phys. Lett.* **1981**, *83*, 183.
(10) Levij, M.; de Bleijser, J.; Leyte, J. C. *Chem. Phys. Lett.* **1982**, *87*, 34.
(11) Levij, M.; de Bleijser, J.; Leyte, J. C. *Bull. Magn. Reson.* **1980**, *2*, 388.
(12) Russel, W. B. *J. Polym. Sci., Polym. Phys. Ed.* **1982**, *20*, 1233.
(13) Boyd, G. E.; Wilson, D. P. *Macromolecules* **1982**, *15*, 78.
(14) Delville, A.; Detellier, C.; Laszlo, P. *J. Magn. Reson.* **1979**, *34*, 301.
(15) Halle, B.; Wennerström, H.; Piculell, L. *J. Phys. Chem.* **1984**, *88*, 2482.

0022-3654/89/2093-5284$01.50/0