Prevention of Photo-oxidation of Metal-free Phthalocyanine by Incorporation into Dioctadecyldimethylammonium Bromide (DODAB) Vesicles

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The normal photo-oxidation of tetra-t-butyl metal-free phthalocyanine was prevented by its solubilization in dioctadecyldimethylammonium bromide vesicles but was evident in dihexadecylphosphate vesicles where, using the spin-trapping technique, it was revealed that ·OH radicals were generated.

Various membrane-like surfactant organized assemblies have been under extensive investigation mainly because of their potential ability to mimic the functions of biological membranes. One of the functions of molecular organization in natural membranes is providing a suitable microenvironment for photosynthesis, one of the most complex photochemical processes known in nature.[1]

Surfactant vesicles, defined as smectic mesophases of sur-

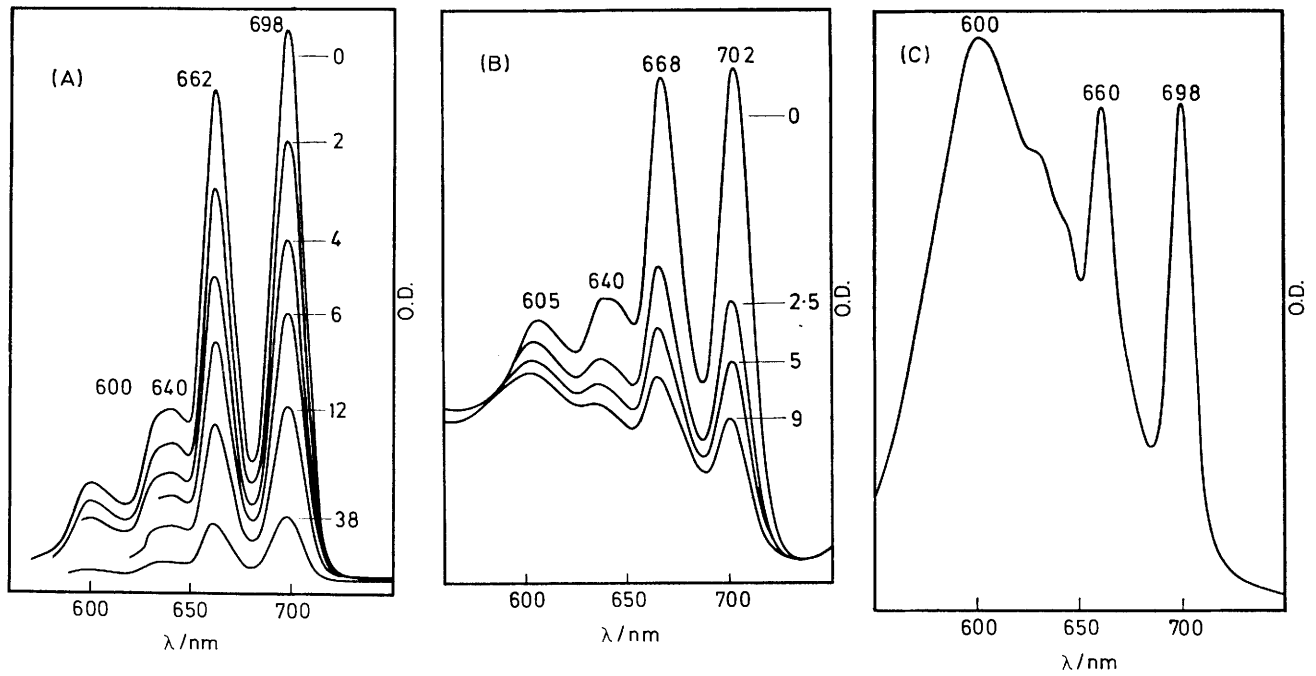

Figure 1. The absorption spectra of $Bu^t_4PcH_2$ in: (A) CH_2Cl_2, (B) DHP, and (C) DODAB vesicles. Numbers represent the time of illumination in minutes.

factant bilayers containing entrapped water, are the surfactant aggregates most closely resembling natural membranes.[2] In such systems, energy[3] and electron transfers[4,5] as well as photoionization[6] have been successfully accomplished.

In this paper, we report another unique feature of surfactant vesicles. It was discovered that there is a significant protective action of positively charged vesicles against the photo-oxidation of a dye solubilized within the bilayers. No such protective mechanism was observed in negatively charged vesicles. This is the first report of such behaviour in surfactant vesicles. The system investigated consisted of positively charged dioctadecyldimethylammonium bromide (DODAB) and anionic dihexadecylphosphate (DHP) vesicles which were formed by the ultrasonic irradiation of the surfactant† and $Bu^t_4PcH_2$ (PcH_2 = phthalocyanine) in water.

The resulting solutions were blue and their absorption spectra together with that of $Bu^t_4PcH_2$ in CH_2Cl_2 are presented in Figure 1. Two bands located at *ca.* 660 and 700 nm correspond to $Q_x(0\text{–}0)$ and $Q_y(0\text{–}0)$ transitions of the monomeric metal-free phthalocyanine.[7] A blue-shifted, very broad absorption band (λ_{max} 600 nm) clearly evident only in DODAB vesicles (Figure 1C) is consistent with the absorption of aggregated $Bu^t_4PcH_2$.[8] Irradiation of both methylene chloride solution of $Bu^t_4PcH_2$ and DHP vesicles containing solubilized phthalocyanine (A 300 W Quartzline projector lamp with a Corning CS-2-61 filter was the illumination source) resulted in a gradual bleaching of the blue colour of the solutions reflected in the time-dependent reduction of the absorption bands (Figure 1A, B). The rate of the photobleaching of

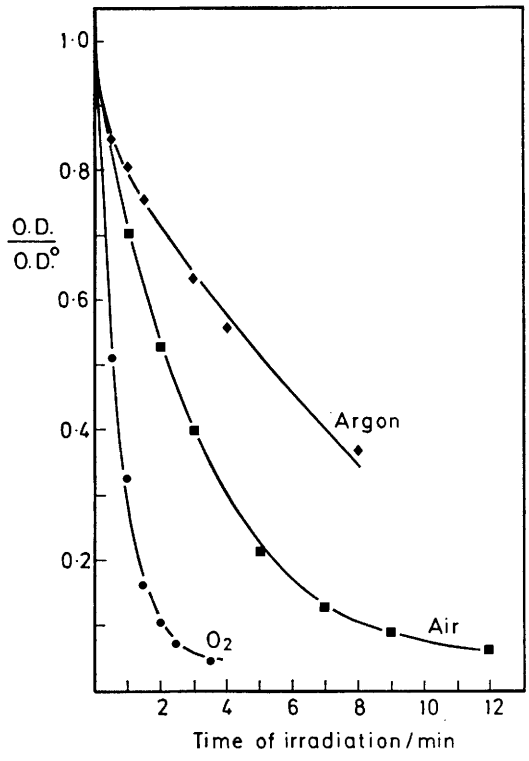

Figure 2. Destructive photo-oxidation of $Bu^t_4PcH_2$ solubilized in DHP vesicles under argon, air, and oxygen atmospheres monitored at 700 nm. O.d.° is the initial optical density which was in all cases quite similar.

$Bu^t_4PcH_2$ in DHP vesicles was found to be dependent on the concentration of oxygen (DHP solutions were purged with oxygen or argon prior to irradiation; *cf.* the normalized decays of the 700 nm band in Figure 2). This indicates that the process responsible for the decay of the dye is photo-oxidation.

† For a standard preparation of vesicles, 10 mg of surfactant (corresponding to 7.9×10^{-3} M and 9.2×10^{-3} M concentration of DODAB and DHP, respectively) were sonicated in 2 ml of water purified by passage through columns containing carbon black and ion-exchange resin. The ultrasonication was carried out for 25 min at 50 °C for DODAB and at 75 °C for DHP using a Sonicator 350 (Heat System Ultrasonic) with a microprobe at an output of 70 W. In all experiments, the stoicheiometric concentration of $Bu^t_4PcH_2$ was 1.25×10^{-5} M.

The fact that photo-oxidation still occurs with an argon-purged sample must be due to the incomplete removal of all the dissolved O_2 upon purging. The amount of solubilized phthalocyanine is *ca.* 10^{-6} M which, when compared to the normal $[O_2]$ of 2×10^{-4} in water reveals that photo-oxidation would still occur even if 99% of the O_2 was removed by purging. Interestingly, no such photobleaching of phthalocyanine solubilized in DODAB vesicles was observed under the identical experimental conditions. This result was not affected by the presence or absence of oxygen in the sample.

The mechanism of photo-oxidation of chlorophyll *in vivo* and in simulated *in vitro* environments is still unclear. $^1O_2$ has been implicated by some authors[9] whereas Harbour and Bolton[10] suggest that an hydroxyl radical, produced upon the oxidation of $OH^-(H_2O)$ may be a destructive intermediate. Since phthalocyanines are strikingly similar to porphyrins, we have attempted to follow this photo-oxidation using the technique of spin-trapping.[11]

Addition of the spin-trap, 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO)[12] to the solution of DHP vesicles (1×10^{-2} M) containing $Bu^t_4PcH_2$ did not result in an e.s.r. signal in the dark. However, when this sample was illuminated in an e.s.r. spectrometer cavity (150 W Xe-lamp with Corning CS-2-61 filter) the typical four-line spectrum of the •OH adduct of DMPO was observed[13] ($a^N = a_\beta^H = 14.9$ G). The origin of the •OH radicals in this case may result from the oxidation of $OH^-(H_2O)$ by the excited $Bu^t_4PcH_2$ molecule.

Similar experiments with DODAB vesicles containing $Bu^t_4PcH_2$, gave e.s.r. signals at least 30 times smaller than for the DHP case. The results in DHP are reminiscent of the observation of •OH radicals upon photo-oxidation of chlorophyll solubilized in Triton X-100 micelles.[10] This suggests that photo-oxidation of phthalocyanine in DHP vesicles may be similar to that of chlorophyll.

It is well known that chlorophyll and phthalocyanines can produce $^1O_2$. The role that $^1O_2$ plays in this photo-oxidation is unclear. It has been shown that nitrone spin traps react with $^1O_2$ in aqueous media.[14] A byproduct of this reaction is the hydroxyl radical adduct but it is produced (at 1×10^{-2} M DMPO) in a quantity three orders of magnitude less than the oxygen consumed by the quenching process. Hence, the •OH adduct in very small amounts in DODAB vesicles most likely results through a $^1O_2$ mechanism.

For the DHP vesicles, it was found that photo-oxidation of $Bu^t_4PcH_2$ occurs only with the solubilized monomers. The aggregate absorption remained constant long after all the molecular dye had been bleached. In addition, photolysis of vesicles which contained only the aggregate dye (the molecular dye was eliminated by prior illumination) did not result in any observable e.s.r. signals. This is consistent with the fact that the •OH radicals are produced only from soluble dye which is undergoing photo-oxidation. It should be also pointed out that variation in the ratio of aggregated to monomeric $Bu^t_4PcH_2$ did not change the kinetics of the photobleaching in either vesicle system.

Two other spin-traps were used in this study: phenyl *N*-t-butyl nitrone (PBB) and anionic sodium sulphophenyl *N*-t-butyl nitrone (SSPBN). The latter, however, caused a co-agulation of DODAB vesicles, and in the case of DHP vesicles, no signal of the •OH adduct of SSPBN was observed. Such behaviour may be accounted for by the electrostatic attraction and repulsion between positively (DODAB) or negatively (DHP) charged vesicles and the spin trap. This observation may also serve as an indication that the spin-trapping processes occur fairly close to the surface of vesicles.

There was no e.s.r. signal of •OH adduct of PBN observed in DHP vesicles. This is not unexpected since it is evident that DMPO is far more sensitive to hydroxyl radical production.[15]

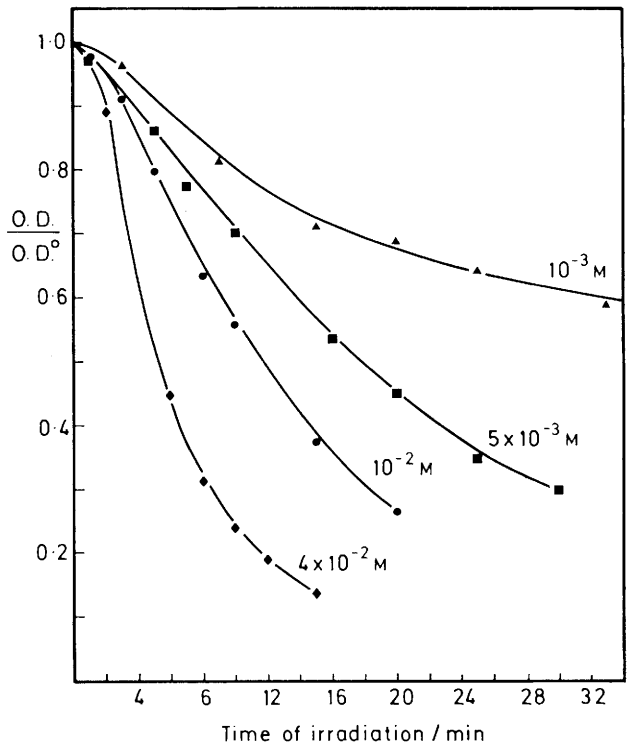

Figure 3. Photoinduced decay of 700 nm band of $Bu^t_4PcH_2$ solubilized in DODAB vesicles in the presence of different concentrations of PBN.

Interestingly, the presence of PBN in the DODAB–$Bu^t_4PcH_2$ system caused a gradual depletion of the absorption bands at 660 and 700 nm when the sample was continuously illuminated. The rate of the o.d. decay appeared to be dependent on the concentration of PBN (Figure 3) and an induction period for the decomposition of the dye was found for all concentrations of PBN. However, even at the concentration of PBN of *ca.* an order of magnitude higher than that of the surfactant (8×10^{-3} M) in the oxygen-saturated sample the rate of the dye depletion was significantly slower than that of the photo-bleaching in the DHP-oxygen saturated samples (*cf.* Figures 2 and 3). Also, there was no e.s.r. signal measurable even at the highest PBN concentration. These results suggest that the decomposition of the dye due to the presence of PBN may be different from the process responsible for the photobleaching of the dye in solvents or DHP vesicles. The photoreaction of dye with a spin trap is certainly worth noting, since nitrone spin traps are intended to be inert to the system under investigation.

Of course, there is a temptation to attribute the presented experimental results to the ionic properties of the bilayer interface. However, the role of the difference in the solubilization sites of the molecules of phthalocyanine in the vesicles' bilayers manifested by the shift in their absorption bands (see Figure 1) can not be completely discarded.

The details of the protective mechanism in DODAB vesicles is presently under investigation. It should be noted here that the chlorine analogue (DODAC) of DODAB vesicles manifested similar protective action.

In view of our results, the recently published observation about a significantly higher stability of the dimer of $C_{14}MV^+$ solubilized in DODAC vesicles toward oxygen[16] may be the manifestation of the protective mechanism described above.

The authors thank Dr. J. Duff for supplying soluble phthalocyanine and Dr. M. L. Hair for his support during this study.

Received, 11th September 1981; Com. 1087

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