PHOTOSENSITIZATION OF LIPOSOMES BY PORPHYRINS†

LEONARD I. GROSSWEINER and GREESH C. GOYAL

Biophysics Laboratory, Physics Department, Illinois Institute of Technology, Chicago, IL 60616 (U.S.A.)

(Received January 5, 1984)

Summary

Lipid peroxidation was photosensitized in egg phosphatidylcholine (EPC) liposomes by hematoporphyrin (HP), hematoporphyrin derivative (HpD) and uroporphyrin I (Uro-I). Photosensitization by HP was type II via singlet oxygen ($^1O_2$) for the monomeric and dimeric states and type I for aggregated HP. Uro-I was an efficient type II $^1O_2$ photosensitizer. The HpD fraction enriched in the active biological component (HpD-A) was a type II $^1O_2$ photosensitizer at high and low concentrations. The spectral differences between HpD-A in buffer and solubilized in small EPC liposomes are attributed to a conformation change of a key dimer constituent from a folded to a planar geometry. The implications of the results for the action mechanism in photoradiation therapy of tumors with these porphyrins are discussed.

1. Introduction

Liposomes are versatile membrane models for the investigation of biological photosensitization. They can be prepared from almost any combination of lipids by a variety of methods [1]. Many of the photosensitization studies in the literature have been done with egg phosphatidylcholine (EPC), or with the closely related saturated lipid dipalmitoylphosphatidylcholine (DPPC), in which case liposomes are readily prepared by swelling the dry lipid in an aqueous phase. In certain cases cholesterol (CL) has been added to modify the membrane properties and charged fatty acids have been introduced to impart a positive or negative surface charge on the liposomes. Applications of liposomes include the following: probes for the production of active oxygen intermediates and their properties in a lipid

†Paper presented at the COSMO 84 Conference on Singlet Molecular Oxygen, Clearwater Beach, FL, U.S.A., January 4 - 7, 1984.

0047-2670/84/$3.00

matrix [2 - 10]; studies of electron transport in membranes [11 - 15]; models for membrane photophysics and photochemistry in natural processes, including photosynthesis [16 - 21], phytochrome [22] and retinal vision [23]; investigation of important biological photosensitizers including flavin pigments [24], phenothiazines [25] and porphyrins [26 - 29]; the action mechanism in clinical phototherapy procedures, especially psoralen plus UV-A [30] and photoradiation therapy (PRT) of tumors [31, 32]. (The most extensive applications of liposomes have been in connection with the delivery of drugs, which are not directly relevant to this paper (see for example refs. 33 - 35).)

In this paper recent work is summarized on photosensitization of EPC liposomes by porphyrins, emphasizing porphyrins employed for PRT, hematoporphyrin (HP), hematoporphyrin derivative (HpD) and uroporphyrin I (Uro-I). Porphyrins may be the most studied of all known photosensitizers (see for example the review of Spikes [36]). The key role of porphyrins in human porphyrias was surmized in the last century and since then there have been numerous investigations of their role in chemical and biological photosensitization, including the vast body of research on chlorophylls. Although efforts to treat cancer by the combined action of light and a photosensitizing dye have been reported since 1903 [37], the current interest in metal-free porphyrins derives from the ability of certain derivatives to localize in tumor tissue. Localization of HP in tumors was demonstrated in 1942 [38], and since 1960 much attention has been given to HpD [39 - 41], a porphyrin mixture with better localization properties than its precursor HP. The current interest in HpD as a tumor photosensitizer started in 1976 and since then the applications of PRT to human patients have grown rapidly [42]. Early workers in this field proposed that the attack of singlet oxygen ($^1O_2$) on tumor membranes is a key factor in the action mechanism [43], which motivated our applications of liposomes as membrane models. The investigation of liposome photosensitization by HpD was preceded by a study of HP [28], which has been employed subsequently as a photosensitizer in PRT [44]. Uro-I has been implicated in important human porphyrias in which there is photosensitivity [45]. There has been recent interest in Uro-I as a PRT photosensitizer of tissue with low lipid content, which led to the extension of the liposome studies to this porphyrin. The results show that liposomes can serve as useful models for the investigation of the photophysical and photochemical factors in membrane photosensitization by these clinical photosensitizing agents.

2. Experimental methods

EPC liposomes were prepared from egg yolk L-α-phosphatidylcholine (Sigma type VII-E) in chloroform. A 15 mg aliquot was evaporated to dryness at the bottom of a 10 mm test-tube with nitrogen and dispersed in 2 ml

of 0.01 M phosphate buffer (PB) (pH 7.0 or pH 7.4) by vortexing for 3 min. The liposomes obtained by swelling for 3 h at 3 °C were large multilamellar vesicles (LMVs) with a size distribution from 0.6 to 2 μm [1]. Small unilamellar vesicles (SUVs) were obtained by sonicating the LMVs for 2 h at 30 °C under nitrogen using a Heat Systems Ultrasonics model W-10 sonicator with a 2.4 mm titanium alloy tip. After sonication, the suspension was centrifuged for 2 h at 50 000g (where g is the gravitational constant) at 5 °C. The SUV size in the supernatant was estimated by gel filtration on a column 30 cm × 14 mm of Sephacryl S-1000 (Pharmacia Inc.). Elution with pH 7.0 PB at 3 ml h^{-1} gave a single band at $V_e/V_0 = 1.80$ (where V_e is the elution volume and V_0 is the void volume) corresponding to a mean diameter of about 40 nm [46]. HP dihydrochloride (Sigma Chemical Company) was incorporated prior to swelling the LMVs followed by three stages of centrifugation (15 000g for 5 min). The resulting HP concentrations in the LMVs were 0.4 wt.% for an initial 1.0 wt.% and 0.09 wt.% for an initial 0.1 wt.%. HP was incorporated in the SUVs by diffusion from the external buffer. Crude HpD was obtained from Porphyrin Products (Provo, UT) as "hematoporphyrin D" and prepared by the method of Dougherty *et al.* [43]. The crude acetylated HpD was alkaline hydrolyzed and neutralized following the procedure of Dougherty *et al.* [43]; it is this material that is designated as HpD in this paper. HpD was incorporated in the LMVs prior to swelling followed by gel filtration on Sephadex G-50 fine (Pharmacia Inc.) to remove the external dye. HpD was incorporated in the SUVs by diffusion from the external buffer, which was shown to be essentially complete by comparing the light scattering optical density of the SUVs at 335 nm (A_{335}) after elution on Sephadex G-50 with A_{398} of the incorporated HpD. The components of HpD were obtained by polyacrylamide gel filtration on Bio-Gel P-10 (Bio-Rad Laboratories) using PB as the eluant. The gel bed was 27 cm × 10 mm and the flow rate was 9.6 ml h^{-1}. Uro-I was obtained from Porphyrin Products and used as received.

The liposome suspensions were irradiated in 1 cm cuvettes with 200 W Hg–Xe lamps in Oriel Corporation model 6137 lamp housings powered by Oriel model 8500 power supplies. The incident intensity was measured with an Eppley thermopile at regular intervals. Typical values were 290 mW cm^{-2} with a Corning CS number 0-52 filter (for $\lambda > 350$ nm) and 245 mW cm^{-2} with CS number 3-70 filter (for $\lambda > 500$ nm). Approximately 210 mW cm^{-2} of the incident energy was carried by wavelengths $\lambda > 750$ nm. The temperature was controlled to 0.1 °C and the liposomes were bubbled with oxygen or nitrogen during irradiation at controlled flow rates. Membrane lysis of LMVs was monitored by the decrease in A_{750}. Lipid peroxidation was measured by the increase in A_{235}/A_{215} [47] and the production of malonyldialdehyde (MDA) based on the thiobarbituric acid method in which A_{530} was measured [48]. The liposomes were stored under nitrogen at 3 °C and used within 3 days. All experiments were repeated at least once with fresh liposome preparations. The solutions were made with doubly distilled water. The other chemicals were standard biochemical grades and used as received.

3. Results

3.1. Liposome photosensitization by hematoporphyrin

HP is a strongly photosensitizing metal-free porphyrin, which is moderately hydrophobic with a tendency to aggregate in aqueous media [49]. Lysis of LMVs was observed when the liposomes were irradiated with visible light ($\lambda > 500$ nm) in the presence of incorporated or externally added HP. Typical data in Fig. 1 for 1 μM HP show faster lysis under oxygen than under nitrogen and an incubation period that was longer under nitrogen. The rate of lysis with 15 μM HP was almost the same with oxygen and nitrogen. The changes in the HP Soret peak and extinction coefficient in going from 1 to 15 μM indicate that self-association was significant, where the dominant state at 1 μM was probably a mixture of monomers and dimers [49]. A summary of results in Table 1 shows that 10 mM azide ion completely protected against lysis by 1 μM HP and led to partial protection for 15 μM HP. Similar results obtained with 10 mM 1,4-diazabicyclo[2.2.2]-octane (DABCO), with a weaker protective effect than with azide. Table 1 summarizes experiments with incorporated HP. There was complete protection with 0.1 M azide and 0.1 M DABCO and under nitrogen for 0.1 wt.% HP and significant acceleration in D_2O buffer. However, for 1.0 wt.% HP, oxygen had little effect and the strong protection by high azide concentrations was the same with oxygen and nitrogen. The results indicate that monomeric and/or dimeric HP photosensitized lysis via a type II mechanism

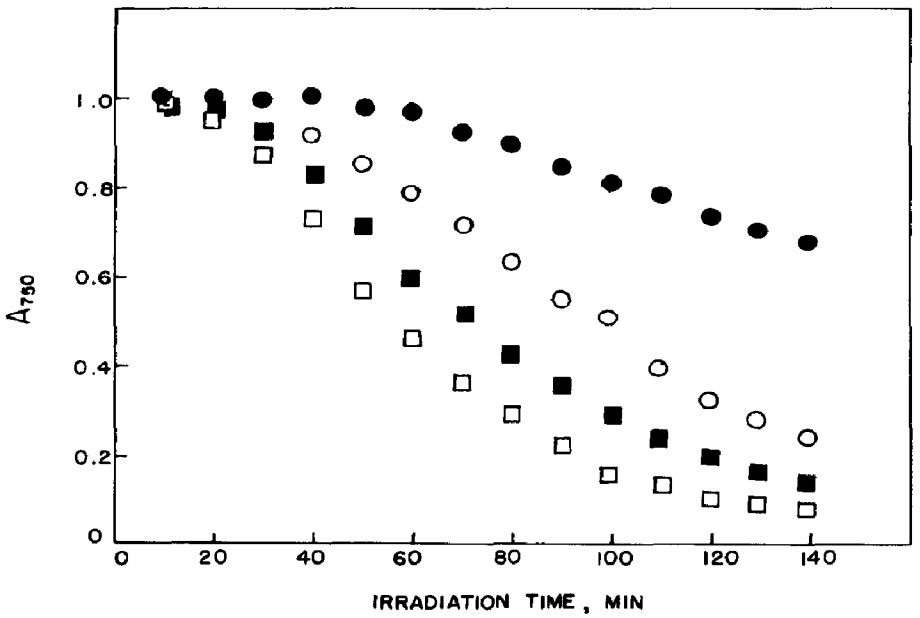

Fig. 1. Photosensitized lysis of EPC liposomes by HP (pH 7.0; 25 °C; $\lambda > 500$ nm): ●, 1 μM HP under nitrogen; ○, 1 μM HP under oxygen; □, 15 μm HP under oxygen; ■, 15 μM HP under nitrogen.

TABLE 1

Photosensitized lysis of large egg phosphatidylcholine liposomes by hematoporphyrin

HP concentration	*Additive*	$F_{20}(O_2)$ [a]	$F_{20}(N_2)$ [a]
1 μM[b]	None	(1)	1.7
1 μM	10 mM DABCO	2.9	
1 μM	10 mM azide	No lysis	
15 μM[b]	None	0.6	0.7
15 μM	10 mM DABCO	1.4	
15 μM	10 mM azide	2.0	
0.1 wt.%[c]	None	1.6	No lysis
0.1 wt.%	D_2O	1.0	
0.1 wt.%	0.1 M DABCO	No lysis	
0.1 wt.%	0.1 M azide	No lysis	
1 wt.%[c]	None	2.7	3.2
1 wt.%	1 mM azide	2.8	2.8
1 wt.%	10 mM azide	5.1	5.1
1 wt.%	100 mM azide	25.7	25.7
1 wt.%	22 wt.% CL	2.9	2.9

[a] Relative fluence for 20% lysis ($\lambda > 500$ nm; 25 °C).
[b] Added to external medium (pH 7.0).
[c] Incorporated in liposomes (pH 7.0).

involving $^1O_2$. Type I photosensitization was observed with aggregated HP. However, a concentrated HP solution may photosensitize previously undyed liposomes via the type II mechanism after monomerization by diffusion of the HP into the membrane [27, 50]. The protection by azide ion for 1 wt.% HP in the presence and in the absence of oxygen is attributed to quenching of the HP triplet state. This result and the low quenching constant for azide quenching of HP fluorescence in buffer, *e.g.* 0.14 l mol^{-1} for 22 μM HP [28], indicate that the type I pathway is mediated by the HP triplet state. The negligible effect of CL on the lytic rates (Table 1), observed also with methylene blue photosensitization of LMVs [10], contrasts with evidence that HP photosensitized CL oxidation in EPC liposomes [29] and DPPC liposomes [26]. The product in both cases was 3β-hydroxy-5α-cholest-6-ene-5-hydroperoxide, specifically identified with the reaction of $^1O_2$. The comparison indicates that the reaction of $^1O_2$ with CL in EPC liposomes is slow compared with its reaction with unsaturated fatty acid sites and that such oxidation of CL to the 5α-hydroperoxide did not affect the lytic damage to the membrane.

3.2. Liposome photosensitization by hematoporphyrin derivative

A part of this work was done with LMVs and three forms of HpD: (a) the brown powder prepared from HP by the methods of Lipson [39 - 41] and Dougherty [43] referred to as acetylated HpD; (b) the alkaline hydrolyzed derivative of acetylated HpD [43] referred to as Hpd; (c) the first major fraction of HpD separated by gel chromatography with Bio-Gel

TABLE 2

Effect of oxygen on photosensitized lysis of large egg phosphatidylcholine liposomes

Sensitizer	$S(O_2)$ [a]
0.1 wt.% HP	$\gg 10$
1 wt.% HP	≈ 1
0.1 wt.% crude HpD [b]	2.6
3 wt.% crude HpD	1.0
0.01 wt.% HpD	≈ 4
5 wt.% HpD	1.6
50 μM HpD-A [c]	2.7
1.8 wt.% HpD-A	4.3

[a] $S(O_2) = F_{20}(N_2)/F_{20}(O_2)$.
[b] Acetylated HpD.
[c] Added to external medium; otherwise incorporated in liposomes.

P-10 [31] referred to as HpD-A. The effect of oxygen on the photosensitized lysis of LMVs for these derivatives is summarized in Table 2, where $S(O_2) \equiv F_{20}(N_2)/F_{20}(O_2)$. The effect of concentration on $S(O_2)$ with acetylated HpD was similar to HP. However, HpD and HpD-A had $S(O_2) > 1$ at relatively high concentrations, indicative of a type II contribution to lytic damage. HpD is a mixture of interconvertible porphyrins in various aggregation states and therefore the results are not directly applicable to *in vivo* photosensitization by HpD after localization in tissue. The tumor localizing and photosensitizing component of HpD has been identified with a moderately hydrophobic constituent with a strongly blue-shifted Soret peak in aqueous media by means of high pressure liquid chromatography [51], reverse-phase thin layer chromatography [52] and polyacrylamide gel filtration [53]. This porphyrin comprises approximately 30 wt.% HpD and at least 60 wt.% HpD-A [54]. It has been tentatively identified as the covalent dimer, dihematoporphyrin ether (DHE), an isomer of which is bis-1-[3(1-hydroxyethyl)deuteroporphyrin-8-yl] ethyl ether [55]. The data in Table 3 show that 1.7 wt.% HpD-A photosensitized lysis of LMVs and lipid peroxidation. In addition, there was significant photobleaching of the HpD-A as measured by A_{365}. The onset of rapid lysis after the incubation period (A_{750}) was preceded by the production of MDA to a constant level (A_{530}), suggesting that lipid peroxidation preceded membrane lysis.

The diffusion of HpD-A into small EPC liposomes (SUVs) mimicked the spectral changes observed when HpD localizes in tumor tissue. The Soret band shifted from 363 ± 1 nm in buffer to 398 nm and the first fluorescence band intensity increased fourfold with a red shift from 615 to 630 nm [32, 50]. The photosensitization data for SUVs are summarized in Table 4, showing the formation of conjugated lipid hydroperoxide (A_{235}/A_{215}), MDA production (A_{530}) and photobleaching of HpD-A (A_{398}). Although HpD-A

TABLE 3

Photosensitization of large egg phosphatidylcholine liposomes by the hematoporphyrin derivative enriched in the active biological component

Irradiation time[a] (min)	A_{750}[b]	A_{530}[c]	A_{365}[d]
0	1.00	0.00	2.05
25	0.86	0.35	1.43
40	0.83	0.42	1.11
60	0.67	0.38	0.96
80	0.59	0.35	0.76

[a] 1.7 wt.% HpD-A in LMVs; pH 7.4; $\lambda > 350$ nm; oxygen; 39 °C.
[b] Light-scattering optical density (relative to unity).
[c] MDA production.
[d] Photobleaching of HpD-A.

TABLE 4

Photosensitization of small egg phosphatidylcholine liposomes by the hematoporphyrin derivative enriched in the active biological component

Irradiation time[a] (min)	A_{235}/A_{215}[b]	A_{530}[c]	A_{398}[d]
0	0.61	0.00	3.23
30	1.20	0.095	2.59
60	1.40	0.175	1.85
90	1.44	0.255	1.17

[a] 0.3 wt.% HpD-A in SUVs; pH 7.0; $\lambda > 350$ nm; oxygen; 35 °C.
[b] Conjugated hydroperoxides.
[c] MDA production.
[d] Photobleaching of HpD-A.

showed spectral shifts similar to HP after diffusion into SUVs [27, 50], the HpD-A Soret band was unchanged by hundredfold dilution and Beer's law was obeyed [56], which argues against ordinary van der Waals and hydrogen binding aggregation. An alternative explanation is that the key component of HpD-A, presumably DHE, is always monomeric, which would explain the high values of $S(O_2)$ in Table 2. The large blue shift of the Soret band and fluorescence quenching of dilute HpD-A in buffer can be explained by a *conformation change*, such as the stabilization of a folded dimer in aqueous media and a planar configuration in the SUV membranes. In concentrated HpD-A solutions ordinary aggregation probably contributes to the spectral shifts.

3.3. Liposome photosensitization by uroporphyrin I

Uro-I is reported as non-dimerizing in water [57]. However, the addition of 6.7 μM Uro-I to SUVs at 3.75 mg ml^{-1} EPC (700:1) led to a shift of the Soret peak from 398 nm in pH 7.4 buffer to 408 nm, accompanied by band narrowing and a twofold higher intensity. These spectral changes are indicative of monomerization in the SUVs. Irradiation of this system ($\lambda >$ 500 nm) induced rapid photobleaching of the Uro-I (A_{408}) and a low level of lipid peroxidation (A_{235}) (Table 5). A red shift was minimally perceptible with 50 μM Uro-I added to SUVs (100:1), which is consistent with the partition coefficient of Uro-I between buffer and EPC liposomes of 51 ± 10 [29]. Irradiation in this case ($\lambda >$ 350 nm) led to rapid lipid peroxidation accompanied by extensive photobleaching of the Uro-I (Table 5). Photobleaching of Uro-I was equally fast without liposomes under oxygen and nitrogen. The rate of photobleaching of 7 μM Uro-I in pH 7.4 buffer was reduced by 12% in the presence of 0.1 M DABCO under oxygen and nitrogen, indicative of a negligible contribution of $^1O_2$ to this process.

TABLE 5

Photosensitization of small egg phosphatidylcholine liposomes by uroporphyrin I

Irradiation time (min)	*6.7 μM Uro-I* [a]		*50 μM Uro-I* [b]	
	A_{235} [c]	A_{408} [d]	A_{235} [c]	A_{398} [d]
0	3.88	2.75	3.23	11.26
10	4.13	0.516	6.08	8.45
20	4.14	0.399	8.43	6.11
30	—	—	10.23	3.91
40	4.19	0.406	11.65	2.19
50	—	—	12.41	1.12
60	4.16	—	13.05	0.86

[a] pH 7.4; $\lambda >$ 500 nm; 25 °C; oxygen.
[b] pH 7.4; $\lambda >$ 350 nm; 25 °C; oxygen.
[c] Conjugated hydroperoxides.
[d] Photobleaching of Uro-I.

4. Discussion

The frequently employed assays for photosensitized liposome damage include lysis, marker release and lipid peroxidation. The data in Table 4 for the photosensitization of SUVs by HpD-A indicate that A_{235} assays for earlier lipid peroxidation than MDA production (A_{530}). This conclusion is consistent with measurements on autoxidation of soybean PC liposomes showing that monohydroperoxides were formed and disappeared in the early stages while the yields of trihydroperoxides increased during the later stages [58]. The limited studies on marker release show that the rate of marker release parallels the rate of membrane lysis as assayed by light scattering [1,

30]. This is an important factor for LMVs because light scattering views only the largest liposomes in a given size distribution. The data in Table 3 show that lipid peroxidation photosensitized by HpD-A attained a relatively constant level during the incubation period for rapid lysis. A similar relationship was found for toluidine blue photosensitization of EPC liposomes [1]. This sequence of events suggests that lipid peroxidation precedes massive membrane disruption. The observation that LMVs were photosensitized by 8-methoxypsoralen (8-MOP) in the external medium and in the EPC membranes but not in the internal aqueous phase, where the environment of the 8-MOP was probed by fluorescence polarization, suggests that peroxidation of the innermost membrane is not readily converted to lysis [59]. These factors may be involved in the unusual agitation effects observed with LMVs photosensitized by methylene blue, HP and HpD [10, 28], in which the rate of lysis increased with increasing gas bubbling rate. Pulse irradiation experiments showing that the extent of dark lysis after irradiation depended on the radiation dose indicate that hydrodynamic stresses promote lysis of photochemically damaged liposomes. It has not been established whether the effect of agitation is purely mechanical or whether it involves the transfer of lipid peroxides from inner to outer membrane walls.

The photoreduction of HP to a free radical under anaerobic conditions has been demonstrated by means of flash photolysis [60] and electron spin resonance [61], where the reducing agents included pyrogallol, hydroquinone, ascorbate and catechol. Flash photolysis spectra of 8 - 51 μM HP at pH 7.2 gave transient spectra identified with the triplet state (440 nm) and the reduced radical anion (630 - 720 nm) [62]. Photosensitized oxidation of tryptophan and tryptamine in aqueous and micellar solutions gave evidence for mixed type I and type II mechanisms. The former was attributed to electron transfer from the HP triplet state to the indole and the latter was attributed to $^1O_2$ [63, 64]. The optical photosensitized oxidation of tryptophan occurred at methanol:water ratios of 30:70 for 80 μM HP, where the Soret peak (391 nm) and spectral pattern of the visible bands are indicative of dimer formation [65]. The production of $^1O_2$ from 20 μM HP (pH 8.0) was shown unambiguously with the "*p*-nitrosodimethylanaline" (RNO) method [66], in which the $^1O_2$ is scavenged by an imidazole to form a transannular peroxide which, in turn, induces the bleaching of RNO. Our RNO results led to $^1O_2$ quantum yields at 546 nm of 0.19 for HP, 0.65 for Uro-I and 0.00 for HpD-A in PB (to be reported elsewhere). The quantum yield for $^1O_2$ formation from aqueous HP has been measured as 0.27 in sodium dodecylsulfate–D_2O at 532 nm [67]. However, the overall quantum yields of photoprocesses sensitized by HP are much lower, *e.g.* as follows: photo-oxidation of histidine (pH 7.0, air), 0.044 [27]; photodynamic inactivation of lysozyme (pH 7.0, air), 0.0052 [27]; photodynamic inactivation of subtilisin BPN′ (pH 7.0, oxygen), 0.0018 [68]; photosensitized oxidation of tryptophan in human serum albumin (pH 8.0, oxygen), 0.0082 [68]; photosensitized oxidation of 2,2,6,6-tetramethyl-4-piperidone hydrochloride (pH 10, air), 0.0031 [69].

The photosensitized lysis of liposomes via type I and type II mechanisms is consistent with the photochemical results with HP. The type II reaction mediated by $^1O_2$ can be attributed to the "ene" reaction with unsaturated fatty acid sites. This process leads to lipid peroxidation and eventually to membrane rupture. There is ample evidence that $^1O_2$ generated by sensitizers localized within the liposomal membrane can escape to the external aqueous phase prior to reacting, *e.g.* the protection by the water-soluble $^1O_2$ acceptors azide ion and DABCO (Table 1). The same conclusion followed from direct measurements of the $^1O_2$ lifetime for generation in D_2O and in SUVs prepared with EPC incorporating methylene blue or 2-acetonaphthone as photosensitizers [9]. A recent investigation on the lysis of 1.5 μm EPC liposomes by $^1O_2$ generated in the gas phase indicates that approximately 3000 surface interactions were required for a lytic event [70]. Taking 72 Å^2 for the surface area of an EPC molecule gives the estimate of one hit per 3000 EPC molecules for lysis. These gas phase data were obtained for conditions of very rapid gas bubbling, and the lytic efficiency of $^1O_2$ may be significantly lower with still liposome suspensions. The anoxic type I mechanism observed with high HP concentrations (Table 1) probably results from electron transfer from the HP triplet state to an unsaturated fatty acid site.

Chromatographic analyses of HpD indicate that it is a mixture of HP, the two isomers of hydroxyethylvinyldeuteroporphyrin (HVD), PP and the tumor localizing and photosensitizing component, presumably DHE [51 - 53]. The liposome studies were made with the HpD-A component, which is at least twofold enriched in DHE. Although the strongly blue-shifted Soret peak of HpD-A suggests that it is highly aggregated [53], this structure cannot explain the apparent absence of dispersion by dilution [53, 56]. An alternative structure for HpD-A in aqueous media is a folded-over DHE core hydrogen bonded to the other porphyrins, so as to form a small aggregate. The large Soret peak red shift and fluorescence enhancement when HpD-A diffused into the SUVs suggest that solubilization in the lipid leads to a planar structure. Therefore, photosensitization of liposomes by HpD-A should be similar to monomeric and/or dimeric HP for which the type II $^1O_2$ mechanism predominates. Our RNO results led to $^1O_2$ quantum yields in SUVs of 0.7 for HP and 0.8 for HpD-A (to be reported elsewhere).

The preliminary results with Uro-I indicate that it is an effective type II photosensitizer of EPC liposomes. Although this property is promoted by the hydrophilic character of the molecule, which inhibits aggregation in aqueous media, the consequent small partition coefficient limits the uptake of Uro-I by liposomes. However, the high yield of $^1O_2$ production with Uro-I makes possible photosensitization of lipids adjacent to the aqueous phase, as evidenced by the production of the 5α-hydroperoxide from CL in EPC liposomes irradiated in the presence of 20 μM Uro-I [29]. The rate of this process was twofold slower than with HP, whereas the same researchers found a twofold higher $^1O_2$ yield from Uro-I using the RNO method. Recent work has shown that Uro-I and Uro-I covalently coupled to an agarose gel

are good photosensitizers of the photo-oxidation of furfuryl alcohol and amino acids, with positive tests for the involvement of $^1O_2$ [71].

Photosensitization by Uro-I was accompanied by fast photobleaching of the photosensitizer, which was equally rapid under oxygen and nitrogen and did not require a substrate. In contrast, HP was photobleached rapidly by a process not requiring $^1O_2$ when complexed to serum albumin, but the photobleaching rate was negligible for HP in buffer without substrates [68]. Photosensitization of SUVs and LMVs by HpD-A was accompanied by photobleaching (Tables 3 and 4). This readily observed phenomenon does not appear to have been reported in the recent literature on photosensitization by HP and related porphyrins. The mechanism has not been identified, except for the ruling out of $^1O_2$ involvement.

The present results demonstrate that the diffusion of HpD-A into liposomal membranes leads to a fluorescent photosensitizing agent that acts via the type II $^1O_2$ mechanism. However, they do not provide an explanation for the better tumor localizing property of DHE compared with the other constituents in HpD, HP, HVD and PP. The suggestion that HpD-A consists of DHE in a folded conformation, probably weakly aggregated to the other impurity porphyrins, differs from the general view that the active component is highly aggregated via strong interactions [54, 55, 72]. The recent report of successful PRT with commercial HP [44] has been attributed to the presence of 15 - 20 wt.% DHE, compared with 45 - 50 wt.% in HpD [73]. The DHE content may not be the only factor, however. The competitive complexing of HP to serum proteins may promote more efficient localization of the active component in HP. Uro-I has been classified as a non-tumor-localizing porphyrin [54]. The present results and pertinent literature [29, 71] demonstrate that Uro-I is a strong type II $^1O_2$ photosensitizer in an aqueous medium, suggesting that any potential applications for PRT will involve non-lipid tissue such as bone.

Acknowledgments

This work was supported by the National Institutes of Health Grant GM 20117 and Department of Energy Contract AC02-76EV02217. This is publication DOE/EV/02217-50.

References

1 F. Szoka and D. Papahadjopoulos, *Annu. Rev. Biophys. Bioeng.*, *9* (1980) 467.
2 S. M. Anderson and N. I. Krinsky, *Photochem. Photobiol.*, *18* (1973) 403.
3 S. M. Anderson, N. I. Krinsky, M. J. Stone and D. C. Clagett, *Photochem. Photobiol.*, *20* (1974) 65.
4 B. A. Svingen, F. O. O'Neal and S. D. Aust, *Photochem. Photobiol.*, *28* (1978) 803.
5 D. J. W. Barber and J. K. Thomas, *Radiat. Res.*, *74* (1978) 51.
6 G. V. Rumyantseva, L. M. Weiner, Yu. N. Molin and V. G. Budker, *FEBS Lett.*, *108* (1979) 477.
7 M. Fragata and F. Bellemare, *Chem. Phys. Lipids*, *27* (1980) 93.

8 R. Muller-Runkel, J. Blais and L. I. Grossweiner, *Photochem. Photobiol., 33* (1981) 683.
9 M. A. J. Rodgers and A. L. Bates, *Photochem. Photobiol., 35* (1982) 473.
10 L. I. Grossweiner and J. B. Grossweiner, *Photochem. Photobiol., 35* (1982) 583.
11 D. J. W. Barber, D. A. N. Morris and J. K. Thomas, *Chem. Phys. Lett., 37* (1976) 481.
12 W. E. Ford, J. W. Otvos and M. Calvin, *Nature (London), 274* (1978) 507.
13 C. Laane, W. E. Ford, J. W. Otvos and M. Calvin, *Proc. Natl. Acad. Sci. U.S.A., 78* (1981) 2017.
14 T. Katagi, T. Yamamura, T. Saito and Y. Sasaki, *Chem. Lett.*, (1981) 1451.
15 J. S. Huebner, R. T. Arrieta and D. B. Millar, *Photochem. Photobiol., 35* (1982) 467.
16 M. Tomkiewicz and G. A. Corker, *Photochem. Photobiol., 22* (1975) 249.
17 M. Mangel, *Biochim. Biophys. Acta, 430* (1976) 459.
18 A. Mehreteab and G. Strauss, *Photochem. Photobiol., 28* (1978) 369.
19 J. K. Hurley, F. Castelli and G. Tollin, *Photochem. Photobiol., 34* (1981) 623.
20 W. E. Ford and G. Tollin, *Photochem. Photobiol., 38* (1983) 441.
21 Y. Fang and G. Tollin, *Photochem. Photobiol., 38* (1983) 429.
22 G. Georgevich and S. J. Roux, *Photochem. Photobiol., 36* (1982) 663.
23 M. Delmelle, *Photochem. Photobiol., 28* (1978) 357.
24 P. F. Heelis, B. J. Parsons, G. O. Phillips, C. Barghigiani, G. Colombetti, F. Lenci and J. F. McKellar, *Photochem. Photobiol., 30* (1979) 507.
25 E. S. Copeland, C. R. Alving and M. M. Grenan, *Photochem. Photobiol., 24* (1976) 41.
26 K. Suwa, T. Kimura and A. P. Schaap, *Photochem. Photobiol., 28* (1978) 469.
27 J. Spikes, in D. Kessel and T. J. Dougherty (eds.), *Porphyrin Photosensitization*, Plenum, New York, 1983, p. 181.
28 L. I. Grossweiner, A. S. Patel and J. B. Grossweiner, *Photochem. Photobiol., 36* (1982) 159.
29 C. Emiliani and M. Delmelle, *Photochem. Photobiol., 37* (1983) 487.
30 R. Muller-Runkel and L. I. Grossweiner, *Photochem. Photobiol., 33* (1981) 399.
31 L. I. Grossweiner and G. C. Goyal, *Photochem. Photobiol., 37* (1983) 529.
32 G. C. Goyal, A. Blum and L. I. Grossweiner, *Cancer Res., 43* (1983) 5826.
33 G. Gregoriadis, *Ann. N.Y. Acad. Sci., 308* (1978) 343.
34 G. Gregoriadis and A. C. Allison (eds.), *Liposomes in Biological Systems*, Wiley, Chichester, 1980.
35 C. G. Knight (ed.), *Liposomes: From Physical Structure to Therapeutic Applications*, Elsevier, Amsterdam, 1981.
36 J. D. Spikes, *Ann. N.Y. Acad. Sci., 244* (1975) 496.
37 H. Tappenier and A. Jesionek, *Münch. Med. Wochenschr., 1* (1903) 2042.
38 H. Auler and G. Banzer, *Z. Krebsforsch., 53* (1942) 65.
39 R. L. Lipson, *M.S. Thesis*, University of Minnesota, Minneapolis, MN, 1960.
40 R. L. Lipson, E. J. Baldes and A. M. Olsen, *J. Thorac. Cardiovasc. Surg., 42* (1961) 623.
41 R. Lipson, E. Baldes and A. Olsen, *J. Natl. Cancer Inst., 26* (1961) 1.
42 D. Kessel and T. J. Dougherty (eds.), *Porphyrin Photosensitization*, Plenum, New York, 1983.
43 T. J. Dougherty, J. E. Kaufman, A. Goldfarb, K. R. Weishaupt, D. Doyle and A. Mittleman, *Cancer Res., 38* (1978) 2628.
44 P. L. Zorat, L. Corti, L. Tomio, S. Maluta, A. Rignon, G. Mandoliti, G. Jori, E. Reddi and F. Calzavara, *Med. Biol. Environ., 11* (1983) 511.
45 W. J. Runge, *Photophysiology, 7* (1972) 149.
46 Y. Nozaki, D. D. Lasic, C. Tanford and J. A. Reynolds, *Science, 217* (1982) 366.
47 R. A. Klein, *Biochim. Biophys. Acta, 210* (1970) 486.
48 T. Asakawa and S. Matsushita, *Lipids, 15* (1980) 137.
49 S. B. Brown, M. Shillcock and P. Jones, *Biochem. J., 153* (1976) 279.
50 L. I. Grossweiner, in *Clayton Symp. on Porphyrin Localization and Treatment of Tumors*, Liss, New York, to be published.

51 J. Moan, J. B. McGhie and T. Christensen, *Photobiochem. Photobiophys., 4* (1982) 337.
52 D. Kessel, *Cancer Res., 42* (1982) 1703.
53 T. J. Dougherty, K. Wityk, D. Bellnier and T. Mang, in D. Kessel and T. J. Dougherty (eds.), *Phorphyrin Photosensitization*, Plenum, New York, 1983, p. 314.
54 D. Kessel, personal communication, 1983.
55 T. J. Dougherty, W. R. Potter and K. R. Weishaupt, in A. Andreoni and R. Cubeddu (eds.), *Porphyrins in Tumor Phototherapy*, Plenum, New York, to be published.
56 L. I. Grossweiner and G. C. Goyal, *Photochem. Photobiol., 40* (1984), in the press.
57 W. I. White, in D. Dolphin (ed.), *The Porphyrins*, Vol. V, Academic Press, New York, 1978, p. 303.
58 G.-S. Wu, R. A. Stein and J. F. Mead, *Lipids, 17* (1982) 403.
59 R. Muller-Runkel, *Ph.D. Thesis*, Illinois Institute of Technology, Chicago, IL, 1981.
60 G. Cauzzo, G. Gennari, G. Jori and J. D. Spikes, *Photochem. Photobiol., 25* (1977) 389.
61 C. C. Felix, K. Reszka and R. C. Sealy, *Photochem. Photobiol., 37* (1983) 141.
62 S. M. Murgia, A. Pasqua and A. Poletti, *Photobiochem. Photobiophys., 4* (1982) 329.
63 C. Sconfienza, A. Van de Vorst and G. Jori, *Photochem. Photobiol., 31* (1980) 351.
64 E. Rossi, A. Van de Vorst and G. Jori, *Photochem. Photobiol., 34* (1981) 447.
65 D. Kessel and E. Rossi, *Photochem. Photobiol., 35* (1982) 37.
66 I. Kraljic and S. El Mohsni, *Photochem. Photobiol., 28* (1978) 577.
67 E. Reddi, G. Jori, M. A. J. Rodgers and J. D. Spikes, *Photochem. Photobiol., 38* (1983) 639.
68 P. Richard, A. Blum and L. I. Grossweiner, *Photochem. Photobiol., 37* (1983) 287.
69 S. Cannistraro, G. Jori and A. Van de Vorst, *Photobiochem. Photobiophys., 3* (1982) 353.
70 W. C. Eisenberg, K. Taylor and L. I. Grossweiner, *Photochem. Photobiol., 40* (1984), in the press.
71 J. D. Spikes, *Int. Symp. on Porphyrins in Tumor Therapy, Milan, May 26 - 28, 1983.*
72 A. Andreoni, R. Cubeddu, S. De Silvestri, P. Laporta, G. Jori and E. Reddi, *Chem. Phys. Lett., 88* (1982) 33.
73 T. J. Dougherty, *Photochem. Photobiol., 38* (1983) 377.