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The mode of action of polyacetylene and thiophene photosensitizers on liposome permeability to glucose

Donald G. McRae, Etsuo Yamamoto and G H. Neil Towers *

Department of Botany, University of British Columbia, Vancouver, B C , V6T 2B1 (Canada)

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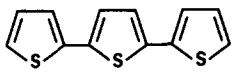
The mode of action of the two photosensitizers 1-phenylhepta-1,3,5-triyne and α-terthienyl on membrane permeability was investigated using liposomes entrapped with glucose as a model membrane system. Upon exposure to UV-A light, α-terthienyl, and to a much lesser extent phenylheptatriyne, induced leakage of glucose via a photodynamic mechanism in liposomes which had a high degree of unsaturated fatty acid side chains. Enhanced permeability to glucose in these liposomes due to the action of α-terthienyl and phenylheptatriyne involved lipid peroxidation, but neither of the two assays used to monitor lipid peroxidation (malondialdehyde and peroxide formation) was directly correlated with the increase in liposome permeability. In liposomes with highly ordered lipid where the fatty acid side chains are saturated, α-terthienyl had no effect on glucose permeability In contrast, phenylheptatriyne was very effective in increasing glucose permeability in these liposomes via a photodynamic mechanism. Addition of lysophosphatidylcholine, which perturbs the order of lipid packing, to these liposomes, completely inhibited the effect of phenylheptatriyne. Conversely, incorporation of cholesterol which increases lipid order, into egg PC liposomes, enhanced the action of phenylheptatriyne. These data suggest that under UV-A irradiation (a) α-terthienyl and phenylheptatriyne enhance permeability in liposomes with a high degree of unsaturation involving lipid peroxidation and (b) phenylheptatriyne enhances membrane permeability through some other mechanism when present in a bilayer with a highly ordered lipid environment.

Introduction

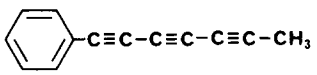
The two secondary plant metabolites phenylheptatriyne (Fig 1), a polyacetylene and the thiophene, α-terthienyl which is a polyacetylene derivative (Fig 1) [1,2] are phototoxic to a number of different organisms after exposure to UV-A (ultra violet, 320–400 nm) irradiation [1,3,4] Studies with yeast and bacteria on the requirement for oxygen indicate a strictly photodynamic mechanism (requires oxygen) for α-terthienyl, while phenylheptatriyne, can be either photodynamic or non-photodynamic depending upon the organism tested [4] Estimations of in vitro generation of singlet oxygen revealed that lower levels are produced by phenylheptatriyne, thus providing additional evidence for an alternate photochemical mechanism for phenylheptatriyne [4] Analysis of effects on nucleic acid has shown that neither of these two compounds causes the formation of interstrand cross-links in calf thymus DNA [5] nor chromosomal damage in cultured mammalian cells

* To whom correspondence should be addressed

Abbreviations egg PC, egg yolk L-3-phosphatidylcholine, DOPC, 1,2-dioleoyl-L-3-phosphatidylcholine, DPPC, 1,2-dipalmitoyl-L-3-phosphatidylcholine, DSPC, 1,2-distearoyl-L-3-phosphatidylcholine, lysoPC, lysophosphatidylcholine, stearoyl, PLePC, 1-palmitoyl-2-linoleoyl-L-3-phosphatidylcholine, SOPC, 1-stearoyl-2-oleoyl-L-3-phosphatidylcholine



α-TERTHIENYL (αT)



1-PHENYLHEPTA-1,3,5-TRIYNE (PHT)

Fig 1 Structures of α-terthienyl (αT) and 1-phenylhepta-1,3 5-triyne (PHT)

[6] when irradiated with UV-A light The lipophilic nature of these two photosensitizers suggests they partition into membrane bilayers and thus their toxicity may be explained by damage exerted primarily at the membrane level This hypothesis has been supported by experiments which demonstrate that α-terthienyl and phenylheptatriyne, when incorporated into cellular membranes, inactivate membrane-bound enzymes on irradiation with UV-A light [7,8] Further studies have revealed that when α-terthienyl and phenylheptatriyne are incorporated into erythrocyte membranes, exposure to UV-A light damages the permeability barrier resulting in K^+ leakage followed by hemolysis of red blood cells [9] The resulting increase in permeability in the erythrocyte membranes is coincident with the appearance of large lesions in the membrane surface [10]

In utilizing liposomes entrapped with glucose as a model membrane system we have attempted to examine how α-terthienyl and phenylheptatriyne present in a lipid matrix affect membrane permeability when irradiated with UV-A light This was accomplished by using phosphatidylcholine as a bilayer-forming lipid and varying the composition of the acyl side chains with respect to the degree of unsaturation In addition, the involvement of lipid peroxidation in membrane permeability was monitored and collectively the data used to provide a more comprehensive understanding of the mechanism of action of these polyacetylene and thiophene photosensitizers

Materials and Methods

Chemicals All reagents used in the glucose assay, thiobarbituric acid, phospholipids and all other lipids were purchased from Sigma Analysis of all lipids by TLC yielded only one spot with each compound GLC analyses of the methylated fatty acids prepared from each of the phospholipids confirmed the fatty acid composition Phenylheptatriyne was extracted and purified from the leaves of *Bidens alba* as described by Wat et al [5] The α-terthienyl, a gift from Dr Thor Arnason (Biology Department, University of Ottawa, Ottawa, Ontario, Canada) was further purified by eluting it twice through a column of silica gel 60 (70–230 mesh) with petroleum ether (30–60°C b p)/diethyl ether (95 5, v/v) GC-MS analysis of phenylheptatriyne and α-terthienyl indicated that both compounds were 98% pure

Preparation of liposomes All phospholipids (10 mg) used were dissolved in chloroform and dried down with nitrogen along the sides of a 12 ml test tube Residual traces of solvent were removed by drying in a desiccator connected to a vacuum pump for approx 0 5 h α-Terthienyl and phenylheptatriyne were not added initially to the phospholipids in chloroform since phenylheptatriyne is volatile under vacuum Thus, an aliquot of either α-terthienyl or phenylheptatriyne (dissolved in ethanol at 10 mg/ml and stored at −20°C) was injected into the lipid after drying This was followed immediately by addition of 1 ml of 0 3 M D-glucose and vigorously vortexing for 1 min to form large multilamellar liposomes In the case of 1,2-distearoyl-L-3-phosphatidylcholine (DSPC) and 1,2-dipalmitoyl-L-3-phosphatidylcholine (DPPC) both the dried lipid and the glucose solution were heated to 70°C and 60°C, respectively, prior to vortexing to ensure that the lipid was above the transition temperature For all other phospholipids, liposomes were formed at room temperature (24°C) and then left to equilibrate at room temperature for 0 5 h in the dark Unsequestered glucose was removed by passing the liposome suspension, at the rate of 1 drop per second, through a Sephadex G-50 (coarse) column (1 5 × 15 cm) equilibrated with 0 15 M KCl in 5 mM Tris-HCl (pH 8 0) The liposome suspension, which appeared as a milky, white suspension was collected and stored on ice

UV-A treatment of liposomes Aliquots of the liposome suspension were placed in 3 ml reaction tubes (borosilicate) These tubes were placed side

down, with a slight vertical displacement, 10 cm beneath 4 black light blue lamps (Sylvania F20T12/BLB, 320–380 nm with maximum emission at 360 nm) The reaction was carried out over a 2 h period with an energy fluence rate of 10 W/m^2 During the incubation, the temperature of the liposome suspension increased from 28°C to 31°C due to the radiant heat from the UV-A light source Reaction tubes wrapped in aluminum foil under the UV-A lights served as dark controls After 2 h, the tubes were removed, stored on ice in the dark until analysed for glucose leakage Once liposomes were placed on ice, glucose leakage was essentially halted

Assay for glucose leakage from liposomes The assay for determining glucose leakage was carried out essentially as described by Kinsky [11] A 20–50 μl aliquot of the liposome suspension was added to 1 ml of solution containing magnesium acetate (2 mM), KCl (75 mM), NaCl (75 mM), Tris-HCl buffer, pH 8 0 (25 mM), ATP (1 mM), $NADP^+$ (0 5 mM) and hexokinase (25 units) It was not necessary to add glucose-6-phoshate dehydrogenase since the hexokinase used contained sufficient levels of this enzyme as an impurity After inverting the mixture, the absorption at 340 nm was recorded using a dual beam spectrophotometer The reference cuvette contained everything except ATP, $NADP^+$ and the enzymes Total glucose was obtained by adding 20–50 μl of Triton X-100 to both cuvettes to release the remaining entrapped glucose

Malondialdehyde assay Aliquots from the liposome suspensions were assayed for malondialdehyde using the thiobarbituric acid test as described by Buege and Aust [12] The thiobarbituric acid reagent consisted of 2 4 ml thiobarbituric acid (0 35% w/v in 0 25 M HCl), 0 4 ml potassium phosphate buffer, pH 7 0 (50 mM), 0 2 ml butylated hydroxytoluene (2% w/v in ethanol) and 0 1 ml of Triton X-100 (10%) Upon adding 0 15 ml of the liposome suspension to the thiobarbituric acid reagent, the tubes were mixed and placed in boiling water for 20 min The reaction was stopped by placing the tubes in ice and the absorption at 535 nm recorded The amount of malondialdehyde present in the liposome suspension was calculated using a molar extinction coefficient of $1\,56\ \ 10^5$ for malondialdehyde

Potassium iodide assay for peroxides The extent of peroxide formation in the liposome suspension was determined essentially as described by Buege and Aust [12] A 20 μl aliquot of the liposome suspension was placed in a 5 ml screwcap test tube and flushed with nitrogen for 30 s Next, 1 ml of acetic acid/chloroform (3 2, v/v) and 50 μl of KI (6 g/5 ml), both of which had been purged of oxygen by bubbling with nitrogen, were added to the screw cap test tube and flushed with nitrogen, capped and kept in darkness for 5 min Next 3 ml of cadmium acetate (0 5 g/100 ml) was added, the vial mixed and centrifuged to separate the two layers The upper aqueous phase was withdrawn and the absorbance at 353 nm recorded The reaction was quantified using H_2O_2 as a calibration standard

Anaerobic conditions To remove oxygen, the reaction vials containing the liposome suspension were tightly fitted with a rubber septum and degassed for 5 min by inserting a needle connected to a vacuum pump In contrast to phenylheptatriyne present with dried lipid, the phenylheptatriyne present in hydrated liposomes was not lost under vacuum Oxygen-free nitrogen, obtained by passing nitrogen through a train of two glass vessels containing alkaline-pyrogallol (12 g in 200 ml of 3 5 M KOH in 10% v/v ethanol) and two glass vessels containing Tris-HCl buffer, 5 mM (pH 8 0), was passed into the reaction vial through the insertion of a needle A second needle was inserted through the septum to allow the excess nitrogen to escape After about 1 min both needles were removed from the septum and parafilm was wrapped around the septum to ensure that leakage of atmospheric oxygen did not occur during the incubation

Phospholipid assay Phospholipid content was assayed using the colorimetric method described by Stewart [13] A 20 μl aliquot of the liposome suspension was added to 2 ml of the ammonium ferrothiocyanate solution followed by addition of 2 ml chloroform The mixture was vortexed for 1 min, the lower chloroform layer removed and the absorbance at 488 nm recorded Phospholipid content was quantified using egg PC

Results

Glucose entrapped in liposomes (controls) composed solely of egg yolk L-3-phosphatidylcholine (egg PC), was released slowly over a two hour period resulting in approx 23% leakage (Fig 2) Exposure of these liposomes to UV-A light did not enhance glucose permeability compared to the liposomes maintained in darkness (Fig 2) When maintained in darkness, addition of α-terthienyl or phenylheptatriyne to egg PC in a molar ratio of 1 32 and 1 27, respectively, did not affect permeability to glucose compared to the controls (Fig 2) However, when liposomes containing α-terthienyl were exposed to UV-A light, the effect on glucose permeability was dramatic with the complete release of glucose at 1 h (Fig 2) Enhanced permeability to glucose in response to phenylheptatriyne with UV-A irradiation was much less pronounced and evident only at two h when 57% of the glucose was released (Fig 2)

When the ratio of α-terthienyl to egg PC was decreased from 1 32 to 1 210 the effect on permeability was delayed until after 0 5 h when glucose leakage became significantly higher than the controls (Fig 3) Further reduction of the ratio of α-terthienyl to lipid (1 419) still affected permeability, but the lag phase was now shifted even further until after 1 5 h (Fig 3) similar to egg PC liposomes containing phenylheptatriyne (Fig 2) There was no measurable effect on glucose permeability when the α-terthienyl to lipid ratio was reduced to 1 2097 over the 2 h incubation under UV-A light (Fig 3)

To evaluate the effect of acyl side chains on the action of α-terthienyl and phenylheptatriyne on glucose permeability, liposomes composed of phosphatidylcholine with different acyl side chains were compared (Table I and II) In liposomes composed of DSPC or DPPC, the incorporation of α-terthienyl had no effect on permeability after 1 h of incubation under UV-A light (Table I) The replacement of stearic with oleic acid at the *sn*-2 position of DSPC (1-stearoyl-2-oleoyl-L-3-phosphatidylcholine, SOPC), had no detectable effect on permeability, but when both acyl side chains in DSPC were replaced with oleic acid, as in 1,2-dioleoyl-L-3-phosphatidylcholine (DOPC), α-terthienyl exhibited a pronounced effect with 91% leakage of glucose after 1 h (Table I) Likewise, if the two points of unsaturation were on the same fatty acid, as in 1-palmitoyl-2-linoleoyl-L-3-phosphatidylcholine (PLePC), α-terthienyl completely lysed the liposomes after 1 h (Table I) Table II shows that the presence of phenylheptatriyne in DPPC and DSPC liposomes had a very significant effect on permeability with 54% and 86% of glucose being released under UV-A light, respectively When incorporated into SOPC and DOPC

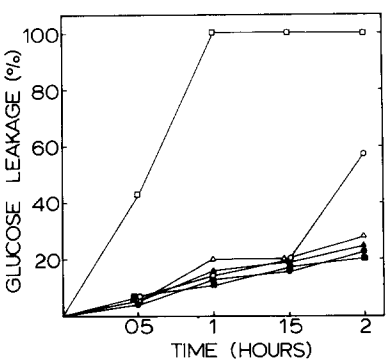


Fig 2 Effect of α-terthienyl and phenylheptatriyne on permeability to glucose with time in egg PC liposomes upon exposure to UV-A light △, Egg PC liposomes (control), ○, phenylheptatriyne present in egg PC liposomes in a molar ratio of 1 27, □, α-terthienyl present in egg PC liposomes in a molar ratio of 1 32 Open and closed symbols represent liposomes maintained under UV-A light and darkness, respectively The values represent the average of three separate experiments

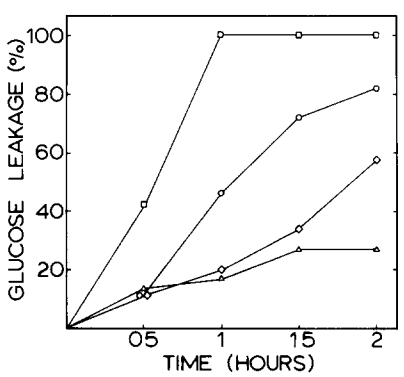


Fig 3 Effect of different concentrations of α-terthienyl on the UV-A light-induced increase to glucose permeability in egg PC liposomes with time □, α-Terthienyl present in egg PC liposomes in a molar ratio 1 32 under UV-A light, ○, ◇, △, same as above except that the α-terthienyl to lipid ratio is 1 210, 1 419, and 1 2097, respectively Values represent the average of three separate experiments

TABLE I

EFFECT OF DIFFERENT ACYL SIDE CHAINS IN PHOSPHATIDYLCHOLINE ON THE ACTION OF α-TERTHIENYL IN INDUCING GLUCOSE LEAKAGE FROM LIPOSOMES

Ratio of α-terthienyl (αT) to phospholipid was 1 32 Values represent the percentages of glucose leaked from liposomes after a 1 h incubation period Each value is the mean ± S D of three separate experiments

Liposomes	Control		αT-treatment	
	dark	UV-A	dark	UV-A
DPPC	4±1	6±1	4±1	4±2
DSPC	2±1	3±1	4±1	5±1
SOPC	16±3	17±1	13±2	15±1
DOPC	20±3	19±3	22±3	91±2
PLePC	13±2	10±2	17±3	100
Egg PC	15±3	21±5	13±3	100

TABLE II

EFFECT OF DIFFERENT ACYL SIDE CHAINS IN PHOSPHATIDYLCHOLINE ON THE ACTION OF PHENYLHEPTATRIYNE IN INDUCING GLUCOSE LEAKAGE FROM LIPOSOMES

Ratio of phenylheptatriyne (PHT) to phospholipid was 1 27 Values represent the percentages of glucose leaked from liposomes after a 2 h incubation period Each value is the mean ± S D of three separate experiments

Liposomes	Control		PHT-treatment	
	dark	UV-A	dark	UV-A
DPPC	6±1	8±1	5±2	54±9
DSPC	4±2	6±2	3±1	86±3
SOPC	19±3	25±1	23±1	44±2
DOPC	29±4	34±3	36±4	46±2
PLePC	25±1	20±5	24±1	87±1
Egg PC	25±3	27±3	23±3	57±3

liposomes, phenylheptatriyne had less of an effect on glucose leakage (Table II) However, in liposomes of PLePC, phenylheptatriyne had a dramatic effect on permeability resulting in 87% leakage of glucose after 2 h under UV-A light (Table II)

Agents which perturb or modify membrane bilayers, such as cholesterol (30 mol%), lysophosphatidylcholine, stearoyl lysoPC (10 mol%) and the free fatty acid stearic acid (10 mol%), were added to liposomes of egg PC containing α-terthienyl or phenylheptatriyne and to liposomes of DSPC containing phenylheptatriyne (Table III) The addition of lysoPC completely inhibited the action of phenylheptatriyne in DSPC liposomes under UV-A light and had no effect on the action of α-terthienyl and phenylheptatriyne in egg PC liposomes To ensure that lysoPC was being incorporated into the phospholipid bilayer of DSPC and not merely forming micelles which could then sequester the photosensitizers, the following experiment was carried out Two batches of DSPC liposomes containing phenylheptatriyne, one with and one without lysoPC, were prepared and then centrifuged to collect the liposome pellet If lysoPC micelles were being formed they would float to the top away from the liposome pellet Analysis of the liposomal pellets revealed that the amounts of phenylheptatriyene were identical in both cases and, in addition, TLC analysis indicated the presence of

TABLE III

EFFECT OF VARIOUS AGENTS WHICH PERTURB LIPID BILAYERS UPON THE MODE OF ACTION OF α-TERTHIENYL AND PHENYLHEPTATRIYNE IN ENHANCING LIPOSOME PERMEABILITY TO GLUCOSE

Ratio of α-terthienyl (αT) and phenylheptatriyne (PHT) to phospholipid was the same as in Tables I and II Values represent the percentages of glucose leaked from liposomes after either a 1 h (αT) or 2 h (PHT) incubation period Each value is the mean ± S D of three separate experiments

Treatment	PHT DSPC		PHT eggPC		αT egg PC	
	dark	UV-A	dark	UV-A	dark	UV-A
Control	3±1	86±3	23±3	57±3	13±3	100
Stearic acid (10 mol%)	2±1	81±4	22±2	58±2	15±4	100
Cholesterol (30 mol%)	1±1	78±3	20±2	90±9	18±2	79±6
LysoPC (10 mol%)	2±1	5±1	22±2	55±3	18±3	100

lysoPC in the liposomal pellet confirming that lysoPC was incorporated into the DSPC bilayer Cholesterol had no effect on phenylheptatriyne in DSPC liposomes, but when incorporated into egg PC liposomes, significantly enhanced the action of phenylheptatriyne by about 30% after 2 h In contrast, treatment of α-terthienyl-containing egg PC liposomes with cholesterol resulted in 20% less leakage after 1 h Stearic acid had no effect on any of the systems examined

HPLC analysis of ethanolic extracts from egg PC liposomes containing α-terthienyl or phenylheptatriyne showed that approximately 47% of α-terthienyl and 20% of phenylheptatriyne remained after 1 and 2 h of UV-A light treatment, respectively In addition, DSPC liposomes containing phenylheptatriyne had about 3% phenylheptatriyne left after the 2 h UV-A treatment

Involvement of lipid peroxidation in increasing glucose permeability was examined by adding various agents which quench free radicals and/or singlet oxygen (Table IV) The water soluble compounds sodium azide and ascorbic acid were both effective in reducing the amount of glucose leakage over time with the most pronounced effect in α-terthienyl-treated egg PC liposomes The two lipid soluble compounds β-carotene and α-tocopherol were also effective in reducing permeability of glucose due to the action of either phenylheptatriyne or α-terthienyl under UV-A light

Fig 4 illustrates the extent of lipid peroxidation as indicated by the levels of malondialdehyde and peroxide formation over time in egg PC liposomes containing either α-terthienyl or phenylheptatriyne Control liposomes which did not contain either α-terthienyl or phenylheptatriyne did not produce any detectable levels of either malondialdehyde or peroxide when exposed to UV-A light for 2 h Only liposomes containing α-terthienyl or phenylheptatriyne produced measurable amounts of both malondialdehyde and peroxides in UV-A light With respect to malondialdehyde, liposomes treated with α-terthienyl produced malondialdehyde which reached a maximum in 1 h and slowly declined thereafter However, for phenylheptatriyne-treated liposomes, malondialdehyde increased to levels twice those produced by the α-terthienyl-treated liposomes at the end of the 2 h incubation period

TABLE IV

EFFECT OF VARIOUS SCAVENGERS OF LIPID PEROXIDATION ON THE ACTION OF α-TERTHIENYL AND PHENYLHEPTATRIYNE ON THE PERMEABILITY OF EGG PC LIPOSOMES

Ratio of α-terthienyl (αT) and phenylheptatriyne (PHT) to egg PC was 1 32 and 1 27, respectively The concentration of sodium azide and ascorbic acid were 10 mM and β-carotene and α-tocopherol were each added at 10μg/10 mg egg PC Values represent the percentages of glucose leaked out over either a 1 h (αT) or 2 h (PHT) incubation period Each value represents the mean ± S D of three separate experiments

Treatment	αT		PHT	
	dark	UV-A	dark	UV-A
Control	13 ± 3	100	23 ± 3	57 ± 3
Sodium azide	20 ± 1	40 ± 2	22 ± 1	37 ± 3
Ascorbic acid	13 ± 1	22 ± 4	22 ± 1	28 ± 3
β-Carotene	14 ± 1	56 ± 12	22 ± 2	35 ± 7
α-Tocopherol	16 ± 1	30 ± 8	23 ± 2	28 ± 2

The peroxide formation patterns in α-terthienyl- and phenylheptatriyne-treated liposomes were somewhat similar to the patterns for malondi-

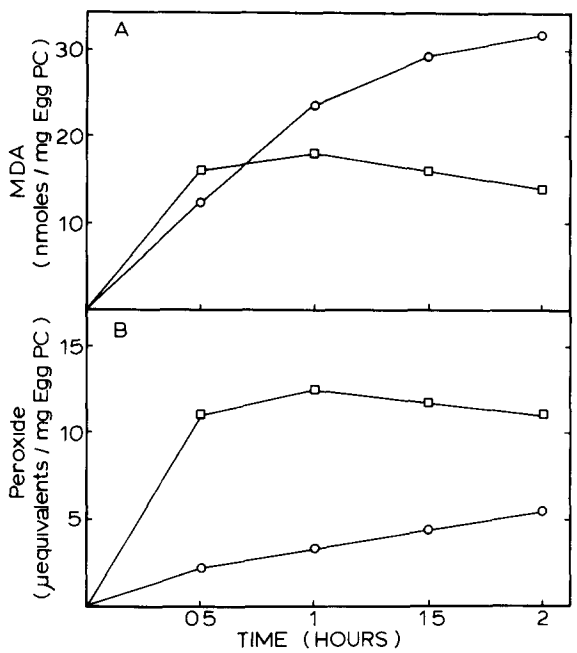


Fig 4 Effect of α-terthienyl and phenylheptatriyne on lipid peroxidation in egg PC liposomes during UV-A irradiation measured by (A) malondialdehyde and (B) peroxide formation □, ○, Liposomes containing α-terthienyl and phenylheptatriyne to lipid in a ratio of 1 32 and 1 27, respectively Values represent the average of three separate experiments

TABLE V

PHOTODYNAMIC EFFECT OF α-TERTHIENYL AND PHENYLHEPTATRIYNE ON THE PERMEABILITY OF EGG PC AND DSPC LIPOSOMES

Ratio of α-terthienyl (αT) and phenylheptatriyne (PHT) to phospholipid was the same as in Tables I and II Values represent the percentages of glucose leaked out from liposomes over either a 1 h (αT) or 2 h (PHT) incubation period Each value represents the mean ± S D of three separate experiments

Liposome	Atmospheric oxygen	Minus oxygen
Egg PC with αT	100	18 ± 2
Egg PC with PHT	57 ± 3	25 ± 2
DSPC with PHT	86 ± 3	5 ± 1

aldehyde production except that the levels of peroxide in phenylheptatriyne- treated liposomes, despite increasing continuously over time, were quantitatively lower than the α-terthienyl-treated liposomes Levels of malondialdehyde and peroxide formation measured in irradiated egg PC liposomes were caused by peroxidation of the unsaturated fatty acids and did not arise from photodecomposition products of either α-terthienyl or phenylheptatriyne This was demonstrated by irradiating DSPC liposomes containing either α-terthienyl or phenylheptatriyne In these liposomes, where the saturated fatty acids prevent lipid peroxidation, no detectable levels of either malondialdehyde or peroxide were formed

Liposomes from SOPC treated with either α-terthienyl or phenylheptatriyne under UV-A light were also monitored for the formation of either malondialdehyde or peroxide With both α-terthienyl- and phenylheptatriyne-treated liposomes, no detectable levels of malondialdehyde were present, while levels of peroxide formation in α-terthienyl-treated liposomes were approx 7 times higher than in phenylheptatriyne-treated liposomes after UV-A irradiation

The photodynamic action of either α-terthienyl or phenylheptatriyne in egg PC liposomes and phenylheptatriyne in DSPC liposomes is illustrated in Table V Clearly, the presence of oxygen was necessary for the action of both α-terthienyl and phenylheptatriyne since in the absence of oxygen, the permeability to glucose in all liposomes was reduced to within control values

Discussion

α-Terthienyl was the more effective photosensitizer in enhancing membrane permeability to glucose in egg PC liposomes (Figs 2, 3) where the fatty acid side chains have a high degree of unsaturation The effectiveness of α-terthienyl in causing glucose leakage appears to be related to the degree of unsaturation in the acyl side chains of phosphatidylcholine (Tables I and II) This was clearly demonstrated with liposomes of DSPC and DPPC (acyl side chains are saturated) where there was no effect in UV-A light Nor did the introduction of one point of unsaturation at the *sn*-2 position in SOPC have an effect on permeability to glucose over the experimental time period However, with two double bonds on the same fatty acid chain (PLePC) or with one on each side chain (DOPC), α-terthienyl dramatically enhanced membrane permeability to glucose in light By comparison, while phenylheptatriyne was able to enhance glucose leakage in liposomes with a high degree of unsaturation, such as egg PC and PLePC, the effect was much less pronounced than for α-terthienyl

Lipid peroxidation is a complex process associated with the degradation of polyunsaturated fatty acids which results in enhanced permeability to solutes in both liposomes and biological membranes [14] The presence of either α-terthienyl or phenylheptatriyne in liposomal membranes of egg PC induced lipid peroxidation when exposed to UV-A light (Figs 4A, B) The involvement of lipid peroxidation in enhancing glucose leakage in egg PC was demonstrated by utilizing various inhibitors of lipid peroxidation which all reduced permeability resulting from the action of α-terthienyl and phenylheptatriyne in UV-A light (Table IV) However, the two parameters malondialdehyde and peroxide formation are not directly correlated with increased membrane permeability to glucose This is evident from Fig 4A where malondialdehyde levels increase constantly with time in phenylheptatriyne-treated liposomes surpassing the α-terthienyl-treated malondialdehyde levels, whereas the increase in permeability to glucose for phenylheptatriyne-treated liposomes is below that for the α-terthienyl-treated liposomes even after 2 h of UV-A irradiation (Fig 2) Evidence that peroxide

content was not correlated with permeability was demonstrated with SOPC liposomes treated with α-terthienyl and phenylheptatriyne under UV-A light The data in Tables I and II indicate that glucose permeability in phenylheptatriyne-treated SOPC liposomes was enhanced 20% over the controls, while α-terthienyl-treated liposomes were unaffected Since the α-terthienyl-treated liposomes have 7 times the amount of peroxide after UV-A irradiation, peroxide content cannot be indicative of increased membrane permeability These results not only suggest that malondialdehyde and peroxide formation are not correlated with permeability, but also that α-terthienyl and phenylheptatriyne affect lipid peroxidation in egg PC liposomes in different ways

Phenylheptatriyne has a more pronounced effect on membrane permeability in liposomes of either DPPC or DSPC (Table II) which have gel to liquid-crystalline phase transition temperatures of 42°C and 52°C, respectively [15] Thus, the acyl side chains in these liposomes are in a highly ordered state at the temperature at which the assays are carried out This suggests that phenylheptatriyne, a rigid, linear molecule must be present in a highly ordered lipid environment in order to produce photo products(s) which perturb the permeability barrier of the lipid bilayer Supporting this hypothesis are the results in Table III in which lysoPC and cholesterol, agents which affect bilayer fluidity, were incorporated into egg PC and DSPC liposomes containing phenylheptatriyne LysoPC which increases lipid disorder [16] creates an environment whereby phenylheptatriyne molecules would be prevented from aligning in the proper manner required to generate the product(s) which affect permeability Thus, in DSPC liposomes addition of lysoPC reduces the effects of phenylheptatriyne on permeability In egg PC liposomes, on the other hand, the lipid is already in a fluid state, so that the addition of lysoPC has no effect on phenylheptatriyne Conversely, when the fluid lipid molecules in egg PC liposomes are induced into a more ordered state by the incorporation of cholesterol [17] the action of phenylheptatriyne on permeability is enhanced (Table III) When cholesterol is added to lipid below the transition temperature (i e DSPC), the effect is to decrease lipid order [17] However, the ineffectiveness of cholesterol in preventing the action of phenylheptatriyne in DSPC liposomes suggests that phenylheptatriyne is still present in a relatively ordered lipid environment In egg PC liposomes treated with α-terthienyl, cholesterol may react with singlet oxygen produced by the interaction of α-terthienyl and UV-A light [4,18] thereby inhibiting lipid peroxidation [14] This would explain the inhibitory effect of cholesterol on α-terthienyl in egg PC liposomes, With a few exceptions [19], lipids such as DSPC and DPPC are not found in biological membranes However, relatively high concentrations of cholesterol are found in most cellular membranes, especially in red blood cell membranes (50 mol%) [15], and thus, the presence of cholesterol in membranes could provide the necessary environment for phenylheptatriyne to cause leakage when exposed to UV-A irradiation

Lipid diacetylenes incorporated in liposomes photopolymerize below the lipid transition temperature but are inhibited above the lipid transition [20] In addition, fatty acids containing diacetylenic bonds in biological membranes also photopolymerize in response to ultraviolet light [21] Although polymerization of diacetylenes has been correlated with the reduced activity of an intrinsic membrane-bound enzyme [21], data are not available on membrane permeability Whether or not photopolymerization product(s) of phenylheptatriyne are responsible for affecting membrane permeability remains to be determined However, it is interesting to note that an ordered lipid environment is necessary for both photopolymerization of diacetylenes and the photoinduced action on permeability by the naturally occurring polyacetylene, phenylheptatriyne

Previous studies have demonstrated that in various organisms [4], the mechanism of photosensitization of phenylheptatriyne involves both photodynamic and nonphotodynamic mechanisms However, with liposomes as a model membrane system phenylheptatriyne operates only in the photodynamic mode By this mechanism, phenylheptatriyne affects membrane permeability on two different membrane types (1) membranes having a high level of unsaturation where lipid peroxidation is involved, (2) membranes in which the lipids are aligned in an ordered state The absence of any non-photodynamic effect of phenylheptatriyne on

liposome permeability suggests that in living cells where phenylheptatriyne is effective under anaerobic conditions, the mechanism of photosensitization may involve another component(s) of the membrane, possibly proteins Alteration in the conformation of membrane proteins either directly or indirectly by phenylheptatriyne may result in enhanced permeability

In accordance with previous observations, the results with α-terthienyl are consistent with a strictly photodynamic mechanism of action However, while α-terthienyl can clearly enhance membrane permeability by acting on the lipid bilayer this does not preclude the possibility that proteins, embedded in the lipid matrix of biological membranes, might also be involved in enhancing membrane permeability [18]

Further work is now in progress to establish the nature of the biophysical changes occurring in liposome membranes treated with α-terthienyl and phenylheptatriyne which result in enhanced glucose leakage Such data may provide further insights into the different mechanisms of action between the polyacetylene and thiophene photosensitizers

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