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Intermolecular crosslinking of fatty acyl chains in phospholipids: Use of photoactivable carbene precursors

(membranes/lipid–lipid interactions/phospholipid–protein interactions/synthetic phospholipids/photolysis)

CHHITAR M. GUPTA*, RAMACHANDRAN RADHAKRISHNAN, GERHARD E. GERBER†, WILLIAM L. OLSEN, STEVEN C. QUAY‡, AND H. GOBIND KHORANA§

Departments of Biology and Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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ABSTRACT **Phospholipids containing photolysable carbene precursors (β-trifluoro-α-diazopropionoxy and *m*-diazirinophenoxy groups) in ω-positions of *sn*-2 fatty acyl chains were prepared. Photolysis of their vesicles produced crosslinked products in 40–60% yields. Crosslinking was mostly intermolecular and occurred by carbene insertion into the C—H bonds of a second fatty acyl chain. Crosslinking products were characterized by (*i*) their gel permeation behavior, (*ii*) analysis of products formed by base-catalyzed transesterification, (*iii*) degradation with phospholipases A_2 and C, (*iv*) gas chromatography/mass spectrometry, and (*v*) use of mixtures of phospholipids carrying the carbene precursors and a phospholipid containing radioactively labeled fatty acyl groups. Nitrenes generated from the aliphatic or aromatic azido groups in phospholipids were unsatisfactory for forming crosslinks by insertion in C—H bonds.**

A central problem in membrane biochemistry is the understanding of the interactions between phospholipids and proteins. Recently, a chemical approach to the problem was described (1) which involved the use of phospholipids containing photoactivable groups as part of the fatty acyl chains. Two photoactivable groups mainly were considered: the azido group which on photolysis would generate nitrene as the intermediate, and suitably substituted alkyl diazo groups which would produce the carbene intermediates. We now report on photolytic studies of phospholipids containing these photoactivable groups. We show that carbenes photogenerated from trifluorodiazopropionyl (2) and diazirinophenoxy (3) groups give rise to extensive intermolecular crosslinks by insertion into C—H bonds. The main aim of this paper is the characterization of crosslinked products formed upon photolysis of vesicles prepared from phospholipids containing these carbene precursors. In contrast, nitrenes are not satisfactory for forming intermolecular crosslinks by insertion reactions, and these results are similar to those of Bayley and Knowles (4).

MATERIALS AND METHODS

Materials. Crude rattlesnake venom (*Crotalus adamanteus*) was used as the source of phospholipase A_2. Phospholipase C was purchased from Sigma. Phospholipids shown in Fig. 1 were synthesized according to a published procedure (5). Di-[^{14}C]-palmitoyl phosphatidylcholine ([^{14}C]Pam_2PtdCho) was synthesized from glycerophosphorylcholine by using [1-^{14}C]palmitic acid purchased from New England Nuclear.

Thin-Layer Chromatography (TLC). This was performed on EM silica gel plates. The solvents were: A, chloroform/methanol/water, 65:25:4 (vol/vol); B, ether/chloroform, 10:90 (vol/vol); and C, chloroform/methanol/acetic acid, 7:3:1 (vol/vol). Phospholipids were visualized by the molybdenum blue spray (6) and fatty acids, by the 2′,7′-dichlorofluorescein spray (7).

Phospholipid Vesicles. A chloroform solution of the phospholipid was evaporated under N_2, and 0.01 M Tris·HCl, pH 7.6/0.15 M KCl was added. After being flushed with N_2, the tube was sealed, vortex-mixed for 2 min, and then sonicated in a bath type sonicator (80 W, 80 KHz at 3.5 A) until a clear solution resulted (30–45 min). The concentration of phospholipids in the vesicles was about 3 mg/ml. Temperatures used for sonication are indicated individually.

Photolysis of Vesicles. The sample was introduced into a quartz vessel with a jacket through which circulated aqueous potassium hydrogen phthalate solution (0.5 or 2.0%, wt/vol). The vessel was placed in the center of a Rayonet photochemical reactor equipped with 16 symmetrically placed RPR 3000 Å or RPR 3500 Å lamps. The temperature of the circulating solution was controlled by a thermostat. RPR 3000 Å lamps emitted 15% of the total radiation at 254 nm; RPR 3500 Å lamps emitted no radiation below 290 nm. The radiation at 254 nm emitted by RPR 3000 Å lamps was filtered out by 0.05% phthalate solution; the 2% solution filtered out radiation essentially completely up to 315 nm. Progress of photolysis was followed either by monitoring the disappearance of the characteristic infrared frequency of azido (2100 cm^{-1}) and diazo (2140 cm^{-1}) groups or the UV absorption of diazirine and α,β-unsaturated carbonyl groups after extraction (8) of the reaction mixture aliquots.

Separation of Photolysis Products. After photolysis, the reaction mixtures were extracted (8), the organic phase was evaporated, and the residue, as a concentrated solution in chloroform/methanol 1:1 (vol/vol), was applied to a Sephadex LH-20 column (2.5 × 100 cm). Elution was performed with the same solvent at a rate of about 60 ml/hr. Fractions were monitored by their phosphorus content or radioactivity.

Digestion with Phospholipase A_2. This was performed as described by Chakrabarti and Khorana (1).

Digestion with Phospholipase C. The digestions were performed and the resulting 1,2-diacylglycerides were isolated as described by Ottolenghi (9).

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Abbreviations: TLC, thin-layer chromatography; [^{14}C]Pam_2PtdCho, 1,2-di[^{14}C]palmitoyl-*sn*-glycero(3)phosphocholine (di[^{14}C]palmitoyl phosphatidylcholine); [^{14}C]Ole_2PtdCho, 1,2-di[^{14}C]oleoyl-*sn*-glycero(3)phosphocholine (di[^{14}C]oleoyl phosphatidylcholine); GCMS, gas chromatography/mass spectrometry.

* Present address: Division of Pharmaceutics, Central Drug Research Institute, Chhattar Manzil Palace, Lucknow, India.
† Present address: Department of Biochemistry, McMaster University, Hamilton, ON, Canada.
‡ Present address: James Homer Wright Laboratory, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114.
§ To whom reprint requests should be addressed.

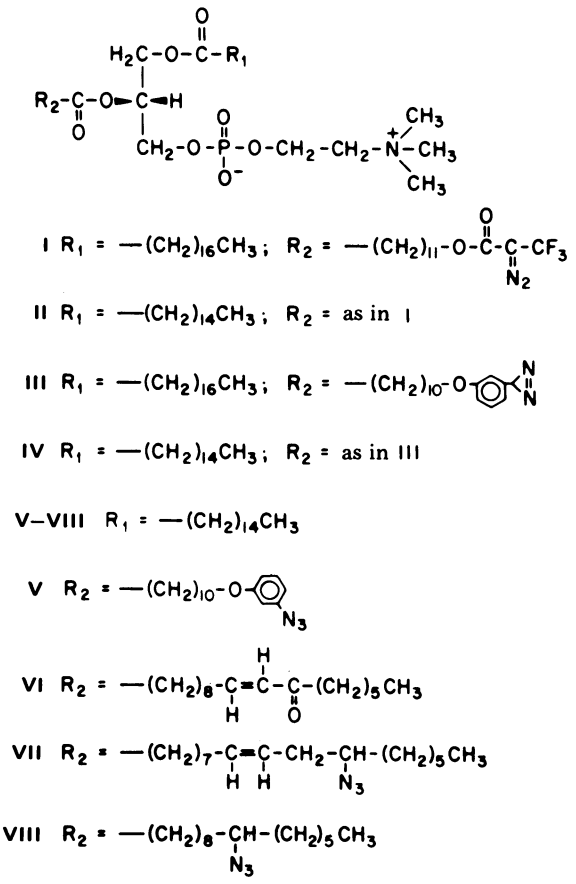

FIG. 1. Synthetic mixed diacyl phosphatidylcholines with different photoactivable groups in the fatty acyl chains.

Transesterification of Phospholipids. To a methanolic solution (1.0 ml) of the photolysis product (about 2 mg) was added 10 μl of 0.5 M sodium methoxide in methanol, and the mixture was stirred at room temperature for 4 hr. After evaporation, the residue was dissolved in chloroform (500 μl) and washed with 30 mM HCl (500 μl). The aqueous layer was removed and the organic phase containing the methyl esters was washed twice with water (500 μl). Removal of the solvent from the organic phase yielded the fatty acid esters.

RESULTS

Photolysis of 1-palmitoyl-2-ω-(trifluorodiazopropionoxy)lauroyl-*sn*-glycero(3)phosphocholine (II) vesicles and characterization of the crosslinked products

Photolysis. The phospholipid (16.5 mg; 20 μmol) in 5.0 ml of the standard buffer was sonicated for 30–40 min at 35–40°C. The formation of sealed vesicles was shown by the trapping of the expected amount of [^{14}C]glucose when the latter was included in the sonication buffer (1). Photolysis of the vesicles was performed at 28 ± 1°C using RPR 3500 Å lamps with 2% potassium hydrogen phthalate solution as the filter for 15–18 hr. (The half-life of the diazo group as measured by disappearance of the diazo group was 4 hr.) The phospholipids were then extracted (8) and chromatographed on a Sephadex LH-20 column. The elution pattern is shown in Fig. 2. Peak I contained 40 ± 5% of the total phosphorus-containing material; peak II contained the remainder.

Degradation of Crosslinked Product (Peak I, Fig. 2) with Phospholipase C. The material in peak I (5 mg) was treated with phospholipase C. The solvents were evaporated *in vacuo*

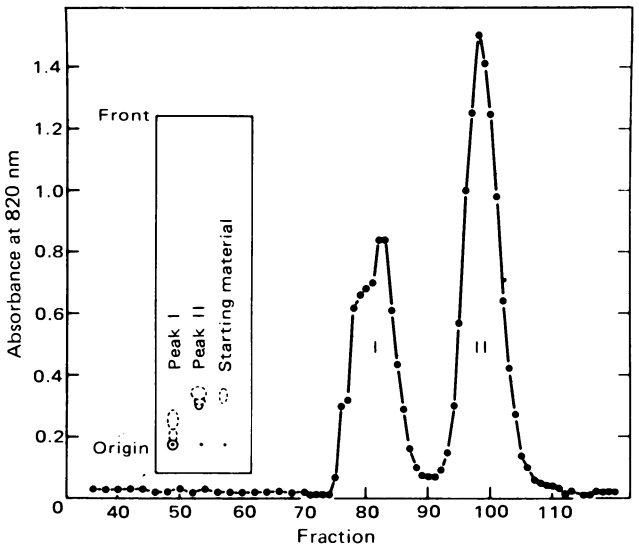

FIG. 2. Separation on a Sephadex LH-20 column (2.5 × 100 cm) of the products obtained from the photolysis of 1-palmitoyl-2-ω-(trifluorodiazopropionoxy)lauroyl phosphatidylcholine. The solvent was $CHCl_3/CH_3OH$, 1:1 (vol/vol). Rate of elution was 60 ml/hr; fractions were collected every 1.5 min. (*Inset*) Mobilities of the products on TLC.

and the residue was dissolved in 4 ml of chloroform/methanol, 1:1. After mixing with water (2 ml), the phases were separated by centrifugation. Evaporation of the organic phase yielded the diglycerides—NMR (C^2HCl_3)|δ: 5.1 (m, 1H, -*CH*-OC-$\overset{=O}{}$), 4–4.5 (d, 2H, -*CH*$_2$-*O*-C-$\overset{=O}{}$), 3.8 (d, J = 7 Hz, 2H, -*CH*$_2$-OH), 2.35 (t, 7 Hz, 4H, O-C-*CH*$_2$-*CH*$_2$-).

The aqueous phase was evaporated and the residue was resuspended in 1.0 ml of water. After removal of the precipitate formed by addition of 4.0 ml of 5% aqueous trichloroacetic acid, the supernate contained all the phosphate present in the phospholipid. Thus, all of the material present in peak I was sensitive to phospholipase C.

Transesterification of the Diglycerides. Transesterification using [^{3}H]CH_3OH followed by partitioning of the dry residue between chloroform and water gave the [^{3}H]methyl esters of the fatty acids in chloroform. Chromatography on Sephadex LH-20 gave two well-resolved peaks (a typical separation is shown below). The second peak corresponded in elution volume to methyl palmitate. Gas chromatography/mass spectrometry (GCMS) showed that it contained mainly methyl palmitate and methyl ω-hydroxylaurate. (Methyl palmitate: M^+, m/e 270; $M^+ - 29$, m/e 241; $M^+ - 43$, m/e 227; $M^+ - 31$, m/e 239. Methyl ω-hydroxylaurate: $M^+ - 18$, m/e 212; $M^+ - 31$, m/e 199; $M^+ - 32$, m/e 198; $M^+ - 30$, m/e 200.) The material in peak I corresponds to the molecular formula $C_{33}H_{59}F_3O_6$ (found, m/e 608.424; calc., m/e 608.427) which in consistent with the adduct expected from the intermolecular insertion of the carbene intermediate into the palmitoyl chain of the neighboring phospholipid molecule (Fig. 3A). Predominance of the ions derived from sequential loss of CH_3O and $CH_2CO_2CH_3$ fragments from M^+ and the formation of the products of the McLafferty rearrangement (m/e for $C_{16}H_{27}F_3O_4$, 340.198 found, 340.186 calc.; m/e for $C_{20}H_{36}F_3O_4$, 397.257 found, 397.256 calc.; m/e for $C_3H_6O_2$, 74.037 found, 74.036 calc.) were all consistent with the structure shown in Fig. 3A (10).

Characterization of Material in Peak II of Fig. 2. High-

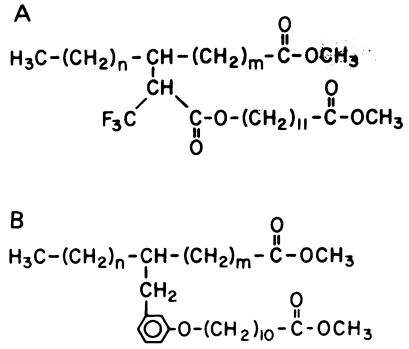

FIG. 3. General structures of the crosslinked fatty acid esters obtained by using diazo- (*A*) and diazirinophenoxy (*B*) phospholipids.

pressure liquid chromatography of material in peak II showed at least two major and two minor compounds that could not be separated in pure form. Treatment of the unseparated material with phospholipase C showed it to be completely sensitive. The diglycerides thus obtained were transesterified with [^{3}H]-CH_3OH as a label. The resulting fatty acid esters were separated on a Sephadex LH-20 column. Only 4–5% of the total fatty acid esters applied to the column corresponded to the crosslinked fatty acids. The major component corresponded to monomeric fatty acid esters and GCMS analysis indicated an approximately equal mixture of methyl palmitate (M^+, *m/e* 270) and methyl ω-hydroxylaurate ($M^+ - 18$, *m/e* 212).

Photolysis of a mixture of 1-stearoyl-2-ω-(trifluorodiazopropionoxy)lauroyl-*sn*-glycero(3)phosphocholine (I) and 1,2-di[^{14}C]palmitoyl-*sn*-glycero(3)phosphocholine ([^{14}C]Pam_2PtdCho) and characterization of the crosslinked products

Photolysis. Vesicles were prepared from an equimolar mixture of I and [^{14}C]Pam_2PtdCho, and photolysis was performed at 44 ± 1 °C, for 16 hr with RPR 3500 Å lamps and 2% aqueous potassium hydrogen phthalate filter. After extraction of the phospholipids and evaporation of the solvent, the products were separated on a Sephadex LH-20 column (Fig. 4). The behavior of the two peaks on TLC was similar, respectively, to that of the peaks in Fig. 2.

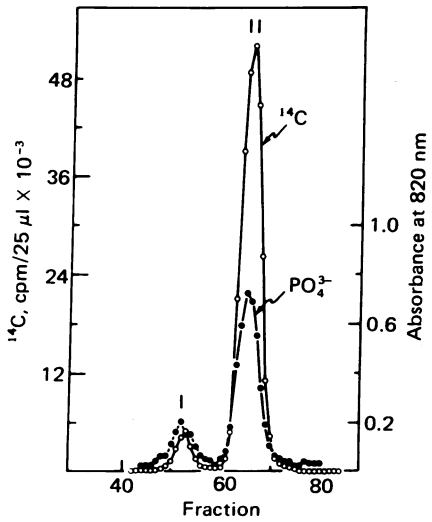

FIG. 4. Sephadex LH-20 column elution pattern of the products obtained from the photolysis of vesicles containing an equimolar mixture of 1-stearoyl-2-ω-(trifluorodiazopropionoxy)lauroyl phosphatidylcholine and [^{14}C]Pam_2PtdCho. Rate of elution was 60 ml/hr; fractions were collected every 2.0 min. Peak I accounted for 7.5–8% of the total ^{14}C.

Degradation with Phospholipase C. Treatment of a portion (4.2×10^5 cpm) of the material in peak I with phospholipase C followed by TLC of the products showed complete disappearance of the starting material. Peak II of Fig. 4 was also treated similarly. Comparison of mobilities of the diglycerides, from peaks I and II on TLC and on a Sephadex LH-20 column, showed that only peak I contained some radioactive material of molecular weight higher than that of monomeric diglyceride.

Base-Catalyzed Transesterification. The material in peak I (8.5×10^5 cpm) was treated with 5 mM sodium methoxide in methanol (1 ml) for 4 hr. The fatty acid esters formed were isolated and separated on a Sephadex LH-20 column (Fig. 5) and by TLC (data not shown). Both methods showed the presence of two radioactive compounds in approximately equal amounts. The first peak moved slower on TLC and corresponded to the dimeric fatty acid ester (Fig. 3*A*); the second peak, with higher mobility on TLC, corresponded to authentic methyl palmitate. Field desorption mass spectral analysis of the material in peak I showed it to contain crosslinked methyl palmitate (M^+ calc. for $C_{33}H_{59}O_6F_3$, *m/e* 608; found, MH^+ = *m/e* 609).

Degradation with Phospholipase A_2. The products of digestion of a part of peak I with phospholipase A_2 that were analyzed by TLC. Because the carbene formed from phospholipid I may insert into either of the two [^{14}C]palmitoyl chains in [^{14}C]Pam_2PtdCho, the radioactive products to be expected would be: (*i*) palmitic acid; (*ii*) palmitic acid crosslinked to ω-trifluoropropionoxylauric acid; (*iii*) 2-lysolecithin containing a 1-[^{14}C]palmitoyl group; and (*iv*) 2-lysolecithin containing a 1-[^{14}C]palmitoyl group crosslinked to ω-trifluoropropionoxylauric acid. The TLC pattern agreed with this expectation. Thus, there was a doublet (R_F, 0.14 and 0.16) that consisted of lysolecithins 3 and 4. Next, there was some incompletely hydrolyzed material (R_F, 0.32) and, finally, there were spots with R_F 0.87 and 0.92, which corresponded to the above crosslinked fatty acid and palmitic acid, respectively. As expected, the radioactivity in lysolecithins was equal to the sum of the radioactivity in crosslinked fatty acid and palmitic acid. From the ratio of the radioactivities present in the last two products, the extent of crosslinking of the carbene to each one of the palmitoyl chains of [^{14}C]Pam_2PtdCho was calculated to be 35% with the *sn*-2 acyl chain and 65% with the *sn*-1 acyl chain.

Quantitation of intermolecular carbene insertion: Photolysis of 1-[^{14}C]palmitoyl-2-ω-(trifluorodiazopropionoxy)lauroyl-*sn*-glycero(3)phosphocholine

After photolysis of this phospholipid, which carries a [^{14}C]-palmitoyl group at *sn*-1 and the photolabel at *sn*-2, the mixture

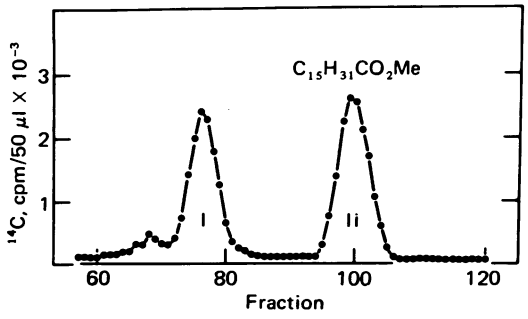

FIG. 5. Sephadex LH-20 chromatogram of the transesterification products of peak I of Fig. 4. Peak II corresponds to authentic methyl palmitate. Peak I is the crosslinked fatty acyl ester (Fig. 3*A*).

was separated and then, after transesterification, the fatty acid esters were examined on a Sephadex LH-20 column (Fig. 6). The products formed by C—H insertion (peak I) represented about 33% of the total radioactivity. Because in the above experiments the crosslinked product represented 40–45% of the total phospholipids, about two-thirds of the latter arose by the C—H insertion mechanism and the remainder by alternative mechanisms such as carbene–carbene dimerization.

Photolysis of 1-palmitoyl-2-ω-(diazirinophenoxy)-undecanoyl-*sn*-glycero(3)phosphocholine (IV) and characterization of crosslinked products

Photolysis. A suspension of the phospholipid in the standard buffer was sonicated for 20–30 min at 30–35 °C. The clear solution was photolyzed for 1 hr at 28 ± 2°C using RPR 3500 Å lamps with the 2% aqueous potassium hydrogen phthalate filter (the half-life of the diazirino group was 2 min). The products were then extracted (8) and separated on Sephadex LH-20 (Fig. 7). Peak I contained 55–60% of the total phosphorus-containing material.

Base-Catalyzed Transesterification. The material in peak I was transesterified and the fatty acid esters were separated on Sephadex LH-20. Two peaks were obtained, similar to those shown in Figs. 5 and 6. From its position, the first peak corresponded to the crosslinked fatty acid esters (Fig. 3*B*). Its analysis by high-resolution mass spectrometry showed a molecular formula of $C_{36}H_{62}O_5$ [calc., *m/e* 574.46 (Fig. 3*B*)]. Some of the more important modes of fragmentation observed were: *m/e* 544.44 (calc., *m/e* 544.42 for $C_{35}H_{59}O_4$) corresponding to the loss of a methoxyl group; *m/e* 306.22 (calc., *m/e* 306.21 for $C_{19}H_{30}O_3$) corresponding to a cleavage at the benzylic site (the benzyl cation had a high intensity in the spectrum); *m/e* 269.2 (calc., *m/e* 269.18 for $C_{17}H_{33}O_2$) corresponding to the palmitoyl chain; a series of ions of general formula $(CH_2)_nCOOMe$ (n = 1–10) were also seen in the spectra.

Photolysis of a mixture of 1-stearoyl-2-ω-diazirinophenoxy)undecanoyl-*sn*-glycero(3)phosphocholine (III) and [^{14}C]Pam$_2$PtdCho and characterization of the crosslinked products

Vesicles prepared from an equal mixture of [^{14}C]Pam$_2$PtdCho and phospholipid **III** were photolyzed for 60–90 min. Separation of the photolysis products gave a pattern essentially identical to that shown in Fig. 4. Characterization of the radioactively labeled crosslinked product was accomplished by degradations with phospholipase A_2 and phospholipase C and by transesterification as described above. After transesterification,

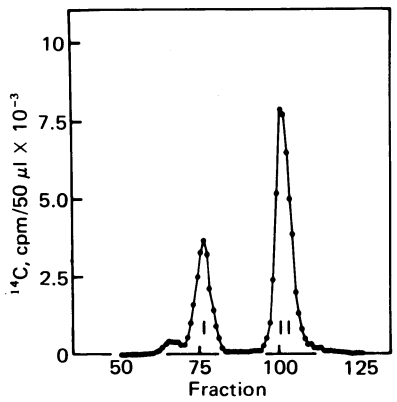

FIG. 6. Sephadex LH-20 elution pattern of the radioactive fatty acid esters obtained after transesterification of the crosslinked products derived from photolysis of 1-[^{14}C]palmitoyl-2-ω-(trifluorodiazopropionoxy)lauroyl phosphatidylcholine vesicles.

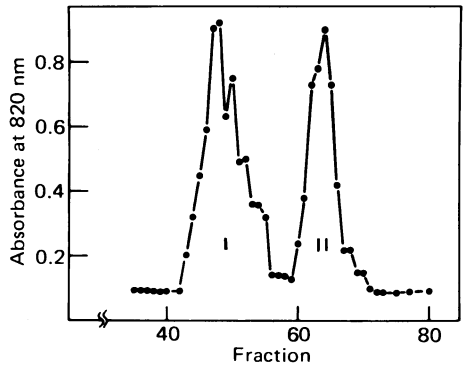

FIG. 7. Elution pattern from a Sephadex LH-20 column (2.5 × 100 cm) of the products obtained from the photolysis of 1-palmitoyl-2-ω-(diazirinophenoxy)undecanoyl phosphatidylcholine. Elution conditions as in Fig. 2.

the monomeric and crosslinked fatty acid esters were separated and analyzed by mass spectrometry. The molecular formula of the crosslinked fatty acid esters was $C_{36}H_{62}O_5$ (calc., *m/e* 574.46) with an *m/e* 574.46. Important fragments observed included *m/e* 306.22 (calc., *m/e* 306.21) for $C_{19}H_{30}O_3$ corresponding to cleavage at the benzylic site and *m/e* 269.20 (calc., *m/e* 269.18) for $C_{17}H_{32}O_2$ corresponding to the cation from the palmitoyl chain.

Photolysis of Phospholipids Containing the Azido Group. A series of photolysis experiments were performed on selected mixtures of phospholipids, with RPR 3000 Å lamps, with and without filter, at 42°C under N_2. The products after extraction were analyzed by TLC (solvent A). Radioactivity was determined after the chromatograms were cut into sections. Photolysis of 1,2-di[^{14}C]oleoyl-*sn*-glycero(3)phosphocholine ([^{14}C]Ole$_2$PtdCho) without a filter gave 2.8% of slower-traveling products; these were largely eliminated when the filter was used. When a mixture of [^{14}C]Ole$_2$PtdCho and azido lipid **VII** was photolyzed without the filter, 8–9% of slower-traveling product was formed. The use of the filter decreased this product to about 3.5%. Photolysis of a mixture of [^{14}C]Pam$_2$PtdCho and **VII** gave a barely detectable amount (1.4%) of crosslinked products, which further decreased (0.6%) when filtered light was used. The results obtained with a mixture of azido phospholipid **VIII** and [^{14}C]Ole$_2$PtdCho or a mixture of **VIII** and [^{14}C]Pam$_2$PtdCho were similar to those obtained (see above) in experiments with **VII**. The small amounts of crosslinked products formed in experiments with mixtures of phospholipids **VII** and **VIII** with [^{14}C]Ole$_2$PtdCho, even when filtered light was used, are ascribed to addition of the nitrene intermediate to the double bond in the oleoyl chain.

Irradiation of the vesicles containing 1-acyl-2-(12-azido)-stearoyl-*sn*-glycero(3)[^{32}P]phosphoethanolamine isolated from an *Escherichia coli* unsaturated fatty acid auxotroph (11, 12) did not reveal any characterizable crosslinked product. Photolysis of a mixture of 1-palmitoyl-2-(*m*-azidophenoxy)undecanoyl-*sn*-glycero(3)phosphocholine (**V**) and [^{14}C]Pam$_2$-PtdCho and workup of the products showed only about 1% of the total radioactivity eluting ahead of the monomeric phospholipid from the gel column; of this, only about one-third seemed to be a crosslinked product.

DISCUSSION

The present approach to the study of hydrophobic interactions between membrane components involves the use of photoactivable groups that are "built into" the fatty acyl chains of phospholipids. The groups used should cause minimal pertur-

bation of the hydrophobic interactions in the membrane, their photolysis should require wavelengths that are not damaging to the membrane proteins and, finally, the photo-induced intermediates should be reactive enough to insert into the C—H bonds of the saturated carbon chains.

In the present work, the trifluorodiazopropionoxy group (2) and the diazirinophenyl group (3) were incorporated into phospholipids, and photolysis of them gave extensive crosslinking reactions. Detailed analysis of the products formed on photolysis of the phospholipids alone or in the presence of an "acceptor" such as [^{14}C]Pam$_2$PtdCho or in the presence of [^{14}C]cholesterol (unpublished results) all were consistent with the above conclusion. Furthermore, quantitation of the extent of C—H insertion in the crosslinked products was performed by photolyzing 1-[^{14}C]palmitoyl-2-ω-(trifluorodiazopropionyloxy)lauroyl-*sn*-glycero(3)phosphocholine. The ^{14}C-labeled crosslinked fatty acid ester eluting in the first peak (Fig. 6) must represent the C—H insertion product. The result showed that at least two-thirds of the crosslinked products obtained in the present experiments arose from C—H insertion reactions. The remainder contained products formed by alternative intermolecular reactions.

One photoactivable group studied extensively in recent years, is the azido group, especially when linked to aromatic systems (13, 14). Various azido fatty acids have been prepared in this laboratory and incorporated into phospholipids (refs. 1, 11, 12, and Fig. 1). The present work has shown that the nitrenes generated from the aliphatic azido groups do not undergo C—H insertion reactions. Only when photolysis of azido phospholipids is performed in the presence of phospholipids containing unsaturated fatty acyl chains (e.g., Ole$_2$PtdCho) is a detectable amount of crosslinked product formed. Evidently, this results from the addition of the nitrene to the fatty acyl double bond to form an aziridine. It seems likely that the crosslinking reactions reported by Stoffel and coworkers (15), who used phospholipids containing azido oleic or mixtures of azido fatty acids with unsaturated fatty acids, involve such "addition" reactions or free radical intermediates, or both, rather than true insertion reactions. Similarly, phospholipids containing α,β-unsaturated keto groups or simply unsaturated fatty acids can yield crosslinked products when irradiated with UV light of relatively short wavelengths, which activates carbon–carbon double bonds (ref 1; present work). However, no crosslinking was observed when a mixture of phospholipid **VII** and [^{14}C]Pam$_2$PtdCho was photolysed with filtered light. Clearly, the crosslinking reactions in these cases occur only when free radical intermediates can be formed. The behavior of the latter, because of their long life and chain reactions, would be different from that of the carbene intermediates described above.

The goal of the present approach using the reactive carbene intermediates is to study the topography of hydrophobic membrane proteins. The carbene-generating groups could serve as yardsticks if a correlation could be demonstrated between the sites of intermolecular crosslinking and the length of the hydrocarbon chains carrying the photoactivable groups.

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1. Chakrabarti, P. & Khorana, H. G. (1975) *Biochemistry* **14,** 5021–5033.
2. Chowdhry, V., Vaughan, R. J. & Westheimer, F. H. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1406–1408.
3. Smith, R. A. G. & Knowles, J. R. (1975) *J. Chem. Soc. Perkin Trans. 2,* 686–694.
4. Bayley, H. & Knowles, J. R. (1978) *Biochemistry* **17,** 2414–2419.
5. Gupta, C. M., Radhakrishnan, R. & Khorana, H. G. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4315–4319.
6. Goswami, S. K. & Frey, C. F. (1971) *J. Lipid Res.* **12,** 509–510.
7. Kates, M. (1972) in *Techniques of Lipidology,* eds. Work, T. S. & Work, E. (Elsevier/North-Holland, Amsterdam), p. 436.
8. Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37,** 911–917.
9. Ottolenghi, A. C. (1969) *Methods Enzymol.* **14,** 188–197.
10. McCloskey, J. A. (1970) in *Topics in Lipid Chemistry,* ed. Gunstone, F. (Wiley Interscience, New York), p. 369–440.
11. Greenberg, G. R., Chakrabarti, P. & Khorana, H. G. (1975) *Proc. Natl. Acad. Sci. USA* **73,** 86–90.
12. Olsen, W. L., Schaechter, M. & Khorana, H. G. (1979) *J. Bacteriol.* **137,** 1443–1446.
13. Peters, K. & Richards, F. M. (1977) *Annu. Rev. Biochem.* **46,** 523–551.
14. Bercovici, T. & Gitler, C. (1978) *Biochemistry* **17,** 1484–1489.
15. Stoffel, W., Darr, W. & Salm, K. (1977) *Hoppe-Seyler's Z. Physiol. Chem.* **358,** 453–462.