A Possible Role for Plasmalogens in Protecting Animal Cells against Photosensitized Killing*

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Chinese hamster ovary (CHO) cells incorporate 12-(1'-pyrene)dodecanoic acid (P12) into membrane lipids. Exposure of P12-labeled cells to long wavelength ultraviolet light causes cell killing, presumably because excitation of the pyrene moiety (a photosensitizer) leads to the generation of reactive oxygen species. Cytotoxicity is dependent upon the concentration of P12 used to label the cells, and time of UV exposure, and the presence of oxygen during irradiation. CHO mutant cells deficient in plasmalogen biosynthesis and peroxisome assembly (Zoeller, R. A., and Raetz, C. R. H. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 5170–5174) are several orders of magnitude more sensitive to P12/UV treatment than wild-type cells, permitting direct selection of one wild-type cell in 1 × 10^6 mutant cells. A major factor responsible for the P12/UV hypersensitivity of these mutants appears to be the absence of plasmalogens. Supplementation of the mutants with 1-O-hexadecyl-sn-glycerol restores plasmalogen levels and nearly normal resistance to P12/UV treatment, whereas the biogenesis of peroxisomes is not restored. The P12/UV hypersensitivity of the plasmalogen-deficient mutants, together with the selective, P12/UV-induced decomposition of plasmalogens in wild-type cells, documented in the accompanying manuscript, suggest that the vinyl ether linkage of plasmalogens plays a direct role in protecting animal cell membranes against certain oxidative stresses.

Plasmalogens are a unique class of glycerophospholipids (1, 2), characterized by the presence of a vinyl ether substituent at the sn-1 position of the glycerol backbone (Fig. 1). These compounds are found in all mammalian cells (1, 2, 7), brain (4, 5), neutrophils (6), and macrophages (7). In typical fibroblasts, such as Chinese hamster ovary (CHO) cells (8, 9), plasmalogens make up about half of the ethanolamine-containing phospholipids (i.e., 7–10% of the total phospholipid). In the sarcolemma, about half of the choline-linked phospholipid is also recovered as the plasmalogen variant (3).

Plasmalogens were discovered 64 years ago, but no unique, biological functions have been ascribed to them (1, 2). Like other phospholipids, plasmalogens can form bilayers when dispersed in water (10). Although plasmalogens do differ from diacylglycerophospholipids in certain, subtle physical properties (10–12) and in their sensitivity to mild acid hydrolysis (10), it is not known how relevant these differences are under physiological conditions.

In order to study the biological significance of plasmalogens, we have isolated CHO mutants with a 10-fold reduced plasmalogen content (9). The primary lesion in these strains appears to be in the biogenesis of peroxisomes (9). The measurable level of N-ethylmaleimide-resistant dihydroxyacetone phosphate acyltransferase is reduced 50-fold and that of alkyl synthase is reduced 5-fold (9). These two enzymes (Fig. 2) catalyze the first steps of plasmalogen biosynthesis (1, 2, 10), and they are both localized in peroxisomes in wild-type cells (13, 14). Since subsequent enzymes of plasmalogen biosynthesis, including the alkylglycerol salvaging reactions (Fig. 2), are localized in the endoplasmic reticulum (1, 2), it is possible to restore normal plasmalogen levels without restoring peroxisomes to the mutants (see below) by supplementing the growth medium with 1-O-hexadecyl-sn-glycerol (Fig. 2). Biochemically, the plasmalogen/peroxisome-deficient CHO mutants (9) resemble diploid human fibroblasts from patients with Zellweger syndrome (16–18), but recent studies suggest that the CHO mutants do complement certain common Zellweger lines (18). The CHO system has the advantage that the cells can be cloned and can proliferate indefinitely.

We now report that plasmalogen-deficient CHO mutants are several orders of magnitude more sensitive than normal CHO cells to conditions of photodynamic stress, a treatment that results in the generation of singlet oxygen and various reactive radical species (19–21). For this purpose, we have labeled cells with a pyrene-containing fatty acid sensitizer (22), designated P12 (Fig. 1), followed by irradiation with long wavelength ultraviolet light (>300 nm). We have found that 1-O-hexadecyl-sn-glycerol supplementation of the mutant cells restores considerable resistance to this treatment, suggesting that it is the absence of plasmalogens (and not of peroxisomes) that is, at least in part, responsible for P12/UV hypersensitivity of the mutants. These findings have led us to explore the hypothesis that plasmalogens, by means of their vinyl ether linkage, can function as scavengers of reactive oxygen species, thereby possibly protecting other targets (membrane proteins, polyunsaturated fatty acids) from photodynamic damage. Chemical evidence for the remarkable lability of plasmalogens during P12/UV treatment of cells is presented in the accompanying paper (39). The results de-
Cells and Culture Conditions—The Chinese hamster ovary cell line, CHO-K1 (wild-type strain), was obtained from the American Type Culture Collection. Strains ZR-78, ZR-82, and ZR-87 were isolated as plasmalogen/peroxisome-deficient derivatives of CHO-K1, as previously described (9). All cell lines were grown at 37 °C, in a 5% CO₂, 95% air atmosphere, in Ham's F12 medium, containing 10% fetal bovine serum (Gibco), supplemented with glutamine (1 mM), penicillin G (100 units/ml), streptomycin (73.5 units/ml), and insulin (0.5 IU/ml). P12 was added to the growth medium by dilution of a concentrated stock solution (20 mM) prepared in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide to which the cells were exposed was less than 0.2% v/v, and its presence did not influence the results. When adding P12 to living cell monolayers, complete growth medium containing five times more than the desired final concentration of P12 was prepared. An appropriate amount of this material was then added to the cell cultures to achieve the P12 concentrations indicated in the experiments. 1-O-Hexadecyl-sn-glycerol was added to the growth medium in the same way as P12, except that the 1-O-hexadecyl-sn-glycerol stock solution was prepared in ethanol.

P12/UV Killing—Cells growing in plastic tissue culture dishes were incubated for the appropriate times in the presence of various concentrations of P12. Prior to irradiation, the medium containing P12 was aspirated, and the cells were washed twice with medium and then incubated in fresh medium (containing serum) but lacking P12. Cells were irradiated following the medium change by illumination for 5 min, unless otherwise indicated, with a Transiluminator (Ultraviolet Products Inc., San Gabriell, CA), placed underneath at a distance of 2 cm. A glass plate, 1 mm thick, was positioned between the tissue culture dish and the UV source to exclude ultraviolet light with wavelengths below 300 nm. The intensity at 365 nm was 1200-1400 microwatts/cm² when measured through the plastic dish with a 365 nm Blak-Ray Ultraviolet Meter (Ultraviolet Products Inc.).

Determination of Cell Viability—Two methods were used to determine the effect of P12/UV treatment on cell viability. 1) Cells were seeded at low density (100-200 cells/60-mm plate), allowed to attach for 12-24 h and treated with P12 and UV irradiation, as described above. Colonies resulting from surviving cells were counted after staining with Coomassie Blue, following outgrowth of the cells for 7-10 days. 2) Cells were seeded at a higher density (5 x 10⁴ cells/16-mm plate), allowed to attach for 12 h, and treated with P12/UV. Cell viability was evaluated 24 h later by measuring the incorporation of [3H]thymidine into acid-precipitable material during a 2-h pulse at 37 °C (25).

Conditions for Photosensitization Experiments under N₂—Phospho- phosphate-buffered saline (PBS) (26) was degassed under aspirator vacuum, and bubbled with nitrogen under sterile conditions for 45 min with fast magnetic stirring. Cells were grown in plastic tissue culture dishes and treated with increasing concentrations of P12 for 18 h. After washing with medium, the cells received 3 ml of sterile PBS (N₂-purged or normal) prior to photosensitization on top of the UV box as described above. The dishes containing N₂ purged PBS were covered with a Lucite chamber, which was purged continuously with nitrogen, 10 min before and during UV irradiation. Subsequently, the buffer was removed by aspiration and replaced with 3 ml of fresh medium. Colonies resulting from surviving cells were counted after staining with Coomassie Blue, following outgrowth of the cells for 7 days.

Incorporation and Distribution of P12 and Radioactive Fatty

![Figure 1](image1.png)

**FIG. 1.** A, structure of plasmenylethanolamine and B, 12-(1'-pyrene)dodecanoic acid (P12). The species of plasmenyl-ethanolamine shown with oleate at the sn-2 position is a common one, but arachidonate and other fatty acids may also be esterified in place of oleate.

![Figure 2](image2.png)

**FIG. 2.** Biosynthesis of plasmenylethanolamine in animal cells. PAF, platelet activating factor (2).
Acids—Cells were seeded at the concentrations indicated in the text in 20-ml glass scintillation vials (5 cm²) sterilized by heating for 18 h at 130 °C. After incubation in P12-containing medium under the various conditions indicated, the medium was removed, and cells were washed twice with 10 ml of ice-cold PBS (27), containing 2 mg/ml fatty acid-free bovine serum albumin. Next, 5.8 ml of a single phase Bligh and Dyer (28, 29) mixture, consisting of chloroform:methanol:PBS (1:2:0.8, v/v/v), was added directly to the cells. (Parallel vials containing untreated cells were used for protein determinations. These cells were washed after P12 labeling with PBS lacking bovine serum albumin, and 0.8 ml of 1 N NaOH was used to solubilize the cells prior to protein determination (30).) When larger amounts of cellular material were required for subfractionation of lipid species, cells were grown to mid-exponential in 100 mm diameter tissue culture dishes, treated with P12, and harvested as above, with the exception that the cells were scraped from the dish in 5 ml of PBS with a rubber policeman, pelleted by centrifugation, resuspended in 0.8 ml of PBS, and then added to chloroform:methanol (1:2) to form the single phase Bligh and Dyer mixture (28, 29). After addition of 0.3 mg of mouse liver phospholipids as carrier, 1 ml of PBS and 1 ml of chloroform were added to form the two-phase Bligh and Dyer system. The lower phase was collected and the upper, aqueous phase was washed once with 2 ml of fresh, pre-equilibrated lower phase. The two upper phases containing the cellular lipids were combined, dried under a stream of nitrogen and redissolved in 2 ml of chloroform:methanol (95:5). The P12 content of the lipid fraction was determined by measuring its fluorescence in a JY3 Jobin-Yvon spectrophotometer using an excitation wavelength of 344 nm and an emission wavelength of 378 nm. Absolute values were obtained by comparison of the sample values with those of P12 standard curves. Background fluorescence, obtained by extraction of cells that had not been treated with P12, was typically less than 5% of sample values. Accumulation of radioactive fatty acids by cells was monitored by liquid scintillation counting of a portion of the lipids, extracted as described above, using Patterson and Green scintillation mixture (31).

Total lipid extracts were separated into neutral and polar-lipid fractions using silica gel chromatography (32, 33). Individual phospholipid species, including plasmalogen, were separated using two-dimensional thin layer chromatography as described previously (8, 9). Radiolabeled lipid species were located by autoradiography and identified by their migration with standards (8, 9).

RESULTS

UV Sensitivity of P12-labeled Wild-type and Mutant Cells—Human leukemic cells labeled with 12-(1'-pyrene)dodecanolic acid (P12) are killed by long wavelength ultraviolet light (22). Excitation of the pyrene photosensitizer (25) under aerobic conditions presumably leads to the generation of singlet oxygen (Type II chemistry), a molecule that rapidly destroys histidine, tryptophan, methionine, and many other susceptible targets (19-21). In addition, excitation of the photosensitizer initiates radical species (Type I process) (19-21). In the case of CHO-K1 cells (Fig. 3), labeling with 2 μM (or more) P12 for 20 h sensitized the cells to a 5-min irradiation with long wavelength UV light. The extent of killing was dependent upon the concentration of P12 used to label the cells (Fig. 3), the intensity of irradiation, and the time of exposure to UV light (data not shown). Three independently isolated CHO mutants defective in plasmalogen biosynthesis and peroxisome assembly (9) were much more sensitive to P12/UV treatment than wild-type cells (Fig. 3). For instance, at 2 μM P12 there was 90–100% killing of the mutant cells, but only 20% killing of wild-type cells (Fig. 3). Labeling of mutant ZR-82 and wild-type cells with 2 μM P12 for various times (data not shown) indicated that 8 h of P12 labeling were sufficient to render the mutant cells hypersensitive to photodynamic killing.

Fig. 4 shows that both wild-type and mutant cells were more resistant to the P12/UV treatment when the system was...
purged with nitrogen in order to reduce the concentration of dissolved oxygen, as described under “Experimental Procedures.” For example, the plating efficiency of wild-type cells (Fig. 4, panel B) at 4 μM P12 and 4 min UV was reduced to 50% in aerated buffer, but it was greater than 90% in nitrogen-purged PBS. Similarly, the plating efficiency of mutant cells at 2 μM P12 and 4 min UV was 42% in normal PBS but 82% in nitrogen-purged PBS (Fig. 4, panel A). Nitrogen purging itself for 10–20 min had no toxic effect on wild-type or mutant cells. These data indicate that, besides P12 and UV light, oxygen is required to obtain full lethality, as expected for a true photodynamic effect (21).

Use of Photosensitization as a Selection Procedure—Mixing studies were performed in which P12/UV treatment was used to select wild-type cells out of a large population of mutant cells (Fig. 5). When 1 × 10⁶ mutant cells (ZR-82) were treated with 2 μM P12, followed by the usual UV irradiation, no colonies resulted (Fig. 5, panel B), although one or two colonies occasionally appeared upon repeated attempts (not shown). When 20 wild-type cells were added to 1 × 10⁶ mutant cells, P12/UV treatment consistently resulted in the appearance of 5–10 colonies (Fig. 5, panel A), equivalent to the efficiency of plating of 20 wild-type cells without any P12/UV treatment (Fig. 5, panel C). When surviving colonies obtained from such a mixing experiment (Fig. 5, panel A) were examined for the recovery of peroxisomal dihydroxyacetone phosphate acyltransferase, using colony autoradiography (9), 14 out of 16 colonies examined demonstrated wild-type levels of enzymatic activity (data not shown). Presumably, the dihydroxyacetone phosphate acyltransferase-deficient colonies represented occasional mutant cells that survived the selection procedure. Therefore, a single, P12/UV treatment would permit the selection of one wild-type cell in ~10⁴ mutant cells, and should be useful for isolation of revertants and transfectants.

Incorporation of P12 into the Lipids of Wild-type and Mutant Cells—The uptake of P12 by cells was quantitated by measuring the fluorescence of the lipids recovered from the cells with a Bligh and Dyer extraction. Labeling of the cells for 12 h at various concentrations of P12 (not shown) or at 2 μM P12 for various times (Fig. 6) revealed that the mutant cells accumulated 2–4 times more P12 than wild-type cells. Enhanced P12 incorporation was observed both in the neutral and the phospholipid fraction. Most of the P12 (83.5 and 87.6% in the wild-type and mutant, respectively) was found in the neutral lipids, which consisted primarily of triglycerides and cholesterol esters in these cells (data not shown). Unesterified P12 was also detected.

The accumulation and subcellular distribution of P12 were also examined using fluorescence microscopy (Fig. 7). Wild-type cells labeled with 2 μM P12 (Fig. 7, panel A) appeared uniformly fluorescent, while the mutant cells (Fig. 7, panel B) contained areas of intense fluorescence, presumably neutral lipid droplets.

The enhanced uptake of P12 by the mutants may account, to some extent, for their hypersensitivity to P12/UV treatment. As shown below, however, another important factor responsible for the P12/UV hypersensitivity appears to be the absence of plasmalogens. The reasons for the enhanced P12 accumulation by the mutants are unknown, but the phenomenon could be explained by the absence of the peroxisomal β-oxidation system (15), which may be essential in degrading pyrene-containing fatty acids. It is known that P12 and related analogs are not activated to the corresponding carnitine derivative, essential for transport into the mitochondria, while still β-oxidized by isolated peroxisomes (36). The fact that there was no difference between wild-type and the various mutant cells in their ability to take up [1-¹⁴C]palmitate or radiolabeled arachidonate (data not shown) provides indirect support for the view that the β-oxidation of P12 in wild-type cells occurs mainly in peroxisomes.

Bypass of the Plasmalogen Deficiency of the Mutant Cells by Alkylglycerol Supplementation—Addition of an alkylglycerol, such as 1-O-hexadecyl-sn-glycerol, to the medium resulted in the restoration of the plasmalogen pool in the mutant cells (Fig. 8), presumably because of efficient alkylglycerol salvage (Fig. 2). The extent to which the plasmalogen pools were restored could be varied over a 10-fold range and depended on the concentration of 1-O-hexadecyl-sn-glycerol in the medium (Fig. 8). In the presence of ~10 μM 1-O-hexadecyl-sn-glycerol, the plasmalogen content of the mutant cells increased by a factor of ~2–4, as compared to the absence of alkylglycerol.
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1-O-hexadecyl-sn-glycerol caused the plasmeneylethanolamine pool to be 2-fold higher than normal. No other major changes in the phospholipid composition accompanied supplementation. Interestingly, the total ethanolamine-linked phospholipid content of the cells remained unaffected by the supplementation (Fig. 8).

1-O-hexadecyl-sn-glycerol supplementation did not restore particulate catalase to these mutants (data not shown), it appeared that proper peroxisome biogenesis did not depend on the synthesis of plasmalogens. This feature facilitated the use of these mutant cells for studying plasmalogon function(s), since pleiotropic effects arising from the presence or absence of functional peroxisomes were not an issue when assessing the phenotypic consequences of 1-O-hexadecyl-sn-glycerol supplementation on the mutant cells (see below).

1-O-Hexadecyl-sn-Glycerol Supplementation Renders Mutant Cells P12/UV-resistant—When mutant cells were grown in the presence of 10–20 μM 1-O-hexadecyl-sn-glycerol to restore their plasmeneylethanolamine content, they regained resistance to P12/UV treatment (Fig. 9 and Table 1). Supplementation of wild-type cells with 1-O-hexadecyl-sn-glycerol also enhanced their P12/UV resistance slightly (Table 1). In the case of ZR-82 and other mutant cells, the restoration of P12/UV resistance was dependent upon the 1-O-hexadecyl-

**Fig. 7.** Fluorescence microscopy of P12-treated cells. Cells were grown on coverslips and treated with 2 μM P12 for 20 h. After washing with PBS, the cells were fixed and mounted as described under “Experimental Procedures.” Upper panel, wild-type cells; lower panel, ZR-82 mutant cells.

**Fig. 8.** Restoration of the plasmeneylethanolamine pool of ZR-82 mutant cells by supplementation with 1-O-hexadecyl-sn-glycerol. ZR-82 mutant cells were seeded in 60-mm diameter tissue culture dishes (5 × 10⁶ cells/dish) and allowed to attach for 2 h in 4 ml of medium. Next, 1 ml of medium containing a 5-fold excess over the desired, final 1-O-hexadecyl-sn-glycerol concentration was added. After approximately 20 h at 37 °C, 100 μl of medium containing 32P (250 μCi/ml) was added. After 3 days at 37 °C, the medium was removed, the cells were washed once with 5 ml of PBS, and then harvested in 1 ml of PBS by scraping with a rubber policeman. The cells were added to glass tubes containing 3.75 ml of chlo-roform:methanol (1:2, v/v) and 300 μg of carrier phospholipid. After 5–10 min at room temperature, 1.25 ml of chloroform and 1.25 ml of PBS were added, and the mixture was vortexed and centrifuged at 600 × g for 5 min. The lower (organic) phase was collected. The upper phase was washed once with fresh pre-equilibrated lower phase. The two lower phases were combined, the solvent was removed by evaporation under nitrogen, and the lipids were separated by two-dimensional silica gel thin layer chromatography (8, 9). The plates were sprayed with 10 mM HgCl₂ in glacial acetic acid between dimensions and allowed to dry for 20 min in a strong stream of nitrogen. This treatment cleaved the vinyl ether linkage, resulting in separation of the diacyl- and lysophosphatidylethanolamine (derived from plasmenylethanolamine) in the second dimension (8, 9). The phospholipids were localized by autoradiography and scraped directly into scintillation vials containing 1 ml of methanol. Scintillation mixture was added after 5 min, and the radioactivity was quantitated using liquid scintillation counting. PE, phosphatidylethanolamine.

**Fig. 9.** Rescue of ZR-82 mutant cells from P12/UV killing by supplementation with 1-O-hexadecyl-sn-glycerol. Cells were seeded at a density of 200 cells/60-mm plate and allowed to attach for 24 h. P12 was added at a final concentration of 2 μM for 12 h at 37 °C. The medium was changed, and the cells were irradiated for 5 min. Cells were overlaid with polyester cloth and glass beads (35) 24 h after irradiation. Polyester cloths were removed after 8 days, and the polyester-immobilized colonies, arising from the surviving cells, were stained. When the medium was supplemented with 1-O-hexadecyl-sn-glycerol (HG), it was added 24 h prior to and during the incubation with P12.
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TABLE I

Effect of 1-O-hexadecyl-sn-glycerol supplementation on P12/UV-induced cell killing

Cells were seeded in 24-well plastic dishes (16 mm diameter) at a density of 5 × 10^4 cells/well and allowed to attach overnight. Cells were then labeled at 37°C for 12 h with 2 μM P12, and washed with fresh medium, followed by UV irradiation for 5 min. 1-O-Hexadecyl-sn-glycerol-supplemented cells were grown in the presence of this material for 48 h prior to and during P12 exposure. Cells were allowed to grow for another 24 h and were subsequently labeled with [methyl-^3H]thymidine (2.5 μCi/ml) for 2 h. Finally, the amount of ^3H incorporated into acid-insoluble material was determined (25).

TABLE II

Effects of supplementation with 1-O-hexadecyl-sn-glycerol, 2-O-hexadecyl-sn-glycerol, and α-monopalmitin on P12/UV killing of mutant cells

Cells were plated out in 24-well dishes and treated essentially as described in Table I. Cells were grown in the presence of the various supplements for 24 h prior to and during P12 exposure. Each value is the average of three determinations (±S.D.).

We have discovered a phenotype associated with plasmalogen deficiency in CHO cells that suggests a special antioxidant function for plasmalogens, not previously appreciated. When mutant CHO cells lacking plasmalogens are labeled with the photosensitizer, P12, they are rendered much more susceptible to killing by long wavelength UV light than are P12-labeled, plasmalogen-containing cells. UV irradiation of the pyrene moiety of P12 under aerobic conditions generates singlet oxygen and various reactive radical species (19-21). The genetic evidence presented here makes it necessary to consider mechanisms by which plasmalogens might protect cells from reactive oxygen species.

Whatever the molecular basis for the P12/UV hypersensitivity may be, this phenotype will be very useful for further genetic studies. The possibility of selecting for wild-type (i.e. P12-resistant) revertants and transfectants, coupled with the rapid screening afforded by colony autoradiography (9), should greatly facilitate the identification of the genes involved in peroxisome and plasmalogen biogenesis. A broader analysis of P12/UV-resistant strains derived either from the mutants or from wild-type cells should also provide new insights into the mechanisms by which animal cells protect themselves against oxidative stresses.

Supplementation of the mutant cells with 1-O-hexadecyl-sn-glycerol permitted us to evaluate the relative importance of peroxisomes and plasmalogens in bringing about the P12/UV hypersensitivity. Since supplementation restores plasmalogens without restoring functional peroxisomes, it is reasonable to conclude that plasmalogen deficiency is not the cause of the failure of peroxisome biogenesis in the mutants. On the other hand, the substantial restoration of P12/UV resistance accompanying the restoration of the plasmalogen pool in 1-O-hexadecyl-sn-glycerol-supplemented mutants supports the view that plasmalogens play a role in protecting cells against P12/UV-induced damage.

The finding that α-monopalmitin also restores resistance to P12/UV treatment (Table II) without restoring the plasmalogen pool is puzzling. However, we have observed that α-monopalmitin, in contrast to 1-O-hexadecyl-sn-glycerol (Fig. 6), reduces P12 uptake by CHO cells by 40-50%. In addition, it is likely that α-monopalmitin causes the cells to accumulate triglyceride droplets, which could sequester the photosensitizer. The possibility that α-monopalmitin induces the synthesis of some other protective molecule must also be considered.

The observation that the mutant cells can utilize exogenous 1-O-hexadecyl-sn-glycerol to restore their plasmalysethanolamine pool demonstrates that the normal topography of plasmalogen biosynthesis (i.e. the localization of the first two enzymes of Fig. 2 in peroxisomes) (13-15) is not actually required for the regulation of cellular lipid composition. The reasons for the participation of peroxisomes in plasmalogen biosynthesis (13-15) in wild-type cells remain unclear. The ability of the mutant cells to utilize exogenous 1-O-hexadecyl-sn-glycerol when endogenous plasmalogen biosynthesis is deficient resembles the enhanced, specific utilization of exogenous phosphatidylcholine (37) or phosphatidylserine (38) observed in conditional mutants defective in phosphatidylcholine and phosphatidylserine biosynthesis, respectively. The mechanisms by which cells regulate the uptake of exogenous lipids, depending upon their need for them, require much further investigation. In the case of 1-O-hexadecyl-sn-glycerol supplementation it is especially striking that the total ethanolamine-linked phospholipid content remains constant (i.e. 19-20%) over a 40-fold range of plasmalysethanolamine to

| Strain        | % of untreated controls | [^3H]Thymidine incorporation into acid-insoluble material (±S.D.) |
|---------------|-------------------------|------------------------------------------------------------------|
| CHO-K1 (no supplementation) | 100% | 10.0 ± 1.0 |
| ZR-78 (no supplementation) | 50%  | 5.0 ± 0.5 |
| ZR-82 (no supplementation) | 25%  | 2.5 ± 0.3 |
| ZR-87 (no supplementation) | 12.5% | 1.25 ± 0.125 |

[^3H]Thymidine incorporation into acid-insoluble material
phosphatidylethanolamine ratios (Fig. 8). This finding indicates the existence of very precise mechanisms in CHO cells for the regulation of polar headgroup composition.

The apparent protection of cells against P12/UV-induced photodynamic damage by plasmalogens raises the question of how such protection might be achieved. In the accompanying manuscript (39), we provide evidence for the hypothesis that a direct chemical reaction between oxygen and the vinyl ether moiety of plasmalogens occurs in P12/UV-treated cells. Although the reactivity of vinyl ethers toward singlet oxygen in chemical model systems is well known (19, 20), the participation of plasmalogens in such reactions has not previously been considered. Whatever the mechanism, we demonstrate that plasmalogens are sensitive targets for reactive oxygen species in photosensitized CHO cells, and based on the phenotype of our mutants we speculate that plasmalogens might be endowed with the ability to protect other targets in membranes, such as the tryptophanyl, histidyl, or methionyl residues of proteins (19, 21). Our hypothesis does not exclude additional functions for plasmalogens, including previous theories based on physical properties (10−12).

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