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Metabolism of Unusual Membrane Phospholipids in the Marine Sponge *Microciona prolifera*†

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ABSTRACT: Sponges are unique in regard to membrane phospholipid composition. Features virtually without parallel in other organisms are the predominance of the C_{26}–C_{30} polyenoic acids (demospongic acids) in the phosphatidylethanolamines (PE) and the attachment of identical acyl groups to the glycerol moiety. The biosynthesis and disposition of these unusual phospholipids were followed in the marine sponge *Microciona prolifera* where PE($\Delta^{5,9}$-26:2,$\Delta^{5,9}$-26:2) is a major molecular species. Incorporation experiments with radiolabeled fatty acids, bases, and intact phospholipids revealed the de novo biosynthesis of the two major phosphatides, phosphatidylethanolamines (PE) and phosphatidylcholines (PC), via the cytidine pathway as in higher animals, with ethanolamine selectively incorporated into PE($\Delta^{5,9}$-26:2,$\Delta^{5,9}$-26:2). Methylation of PE and random acyl chain migration across different phospholipid classes were marginal, but the exchange of PC for PE, apparently mediated by the action of phospholipase, was indicated after uptake of the unnatural PC(Δ^{9}-26:1,Δ^{9}-26:1). The present study demonstrates in the most primitive multicellular animals a phospholipid metabolic pattern similar to that in higher organisms, with unique acyl and phosphoethanolamine transferases apparently involved in the biosynthesis of the (demospongic) di-C_{26}-acyl-PE molecular species.

Phospholipids are molecules of crucial importance in the regulation of cell membrane properties and in biological signal transmission (Hirata & Axelrod, 1980). Although the major pathways of phospholipid biosynthesis are well established in both prokaryotes and eukaryotes (Vance & Vance, 1985; Hawthorne & Ansell, 1982; Mudd, 1980), extensive efforts are still being directed to the study of phospholipid class interconversion, for instance the methylation of PE to PC.[1]

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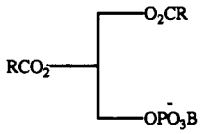
Investigations have been carried out on a variety of cells and organisms, including *Escherichia coli* (Jackson et al., 1986), yeast (Carman, 1989), a slime mold (Alemany et al., 1980), crustaceans (Chapelle, 1986), starfish spermatozoa (Tezon et al., 1986), mammalian erythrocytes (Hirata & Axelrod, 1978a,b), leukocytes (Hirata et al., 1979; Pike et al., 1979), and hepatocytes (Ridgway et al., 1989; Sundler & Akesson, 1975). The general perception is that phospholipid methylation and turnover, next to de novo biosynthesis, are highly regulated processes vital to the adjustment of membrane fluidity, biosignaling, and chemotaxis.

The most intriguing objects for the study of phospholipid metabolism, however, are the marine sponges. These oldest of multicellular animals are noted for their peculiar membrane phospholipids that virtually have no counterparts in other organisms. Over 50 different acyl components, with exceptional chain length (C_{26}–C_{30}) and unique unsaturation or substituent patterns, have been isolated from various sponge species (Lam et al., 1989). These demospongic acids (characteristic of *Demosponges*) exist in identical pairs in PE, which is notably in high abundance (around 30%). The conventional C_{12}–C_{22} fatty acids, in contrast, are more concentrated in PC (Dasgupta et al., 1986, 1987; Weissmann et al., 1988). Apparently such phospholipid arrangements confer special properties to the sponge cell membrane (Ayanoglu et al., 1988; Lawson et al., 1988) for survival in the aquatic habitat: our model membrane studies (Li et al., 1988) showed that the "symmetric" demospongic phospholipids do not interact with cholesterol or marine sterols.

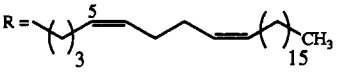
Our recent efforts to elucidate the biosynthetic pathways of demospongic acids have revealed equally striking phenomena, namely, chain elongation and desaturation patterns exclusive to marine and freshwater sponges (Hahn et al., 1988, 1989). To continue the series of investigations in comparative phospholipid biochemistry, we have attempted to examine, in the marine sponge *Microciona prolifera*, the metabolic events contributing to the unusual composition of phospholipids.

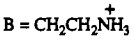
The fatty acid distribution among various phospholipid classes in *Microciona* has been reported by different researchers (Dasgupta et al., 1986; Weissmann et al., 1988; Morales & Litchfield, 1976). Despite some variations, the major head groups have been identified to be PC (35%) and PE (30%), with PS in smaller quantity (10–15%). The predominant acyl moieties of PE are the well-known $\Delta^{5,9}$-26:2 and $\Delta^{5,9,19}$-26:3 acids[2] (up to 40‰ and 25%, respectively), forming the "symmetric" molecular species PE($\Delta^{5,9}$-26:2,$\Delta^{5,9}$-26:2) (I) and PE($\Delta^{5,9,19}$-26:3,$\Delta^{5,9,19}$-26:3) (II). While these acids also exist substantially in PS (15–20%), their presence is scanty in PC (1% in most specimens), where palmitic, linoleic, and cervonic (22:6) acids predominate instead. The last acid, in particular, constitutes another major "symmetric" molecular species PC(22:6,22:6) (III) in the sponge.

Such unequal distribution of demospongic acids between PE and PC is remarkable in view of the similar abundance of these two major phosphatides in *Microciona*. It may be the result of de novo biosynthesis or other metabolic events such as base (or phosphobase) exchange and reacylation reactions (Hawthorne & Ansell, 1982; Lands & Crawford, 1976). However, the observation cannot be explained simply in terms of the affinity of PE for polyenoic acids as in other eukaryotes (Vance & Vance, 1985), since in this sponge

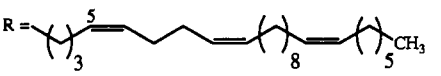


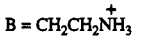
I PE($\Delta^{5,9}$-26:2, $\Delta^{5,9}$-26:2)



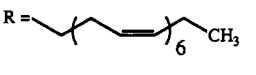


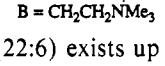
II PE($\Delta^{5,9,19}$-26:3, $\Delta^{5,9,19}$-26:3)





III PC(22:6, 22:6)





cervonic acid (22:6) exists up to 50% in PC but only 1% in PE (Dasgupta et al., 1986; Weissmann et al., 1988).

Little is known to date about phospholipid metabolism in marine sponges, although the recent demonstration of the phosphoinositide cycle in these organisms (including *Microciona*) (Weissmann et al., 1988; Muller et al., 1987; Dunham et al., 1983), where the process coordinates cell aggregation, suggests phospholipid interconversion to be an active process. In order to elucidate the phospholipid metabolic pattern in these organisms, especially biosynthesis and disposition of the unusual di-C_{26}-acyl molecular species, incorporation experiments were performed using four categories of radiolabeled precursors: (1) the [1-^{14}C]Δ^9-26:1 acid (natural abundance 1% in PE, negligible amount in PC), which is the immediate biosynthetic precursor (Hahn et al., 1988; Morales & Litchfield, 1977) of the $\Delta^{5,9}$-26:2 acid; (2) free bases and a methyl donor, [1,2-^{14}C]- and [*methyl*-^{3}H]choline, [1,2-^{14}C]ethanolamine, and [*methyl*-^{3}H]methionine; (3) CDP–[1,2-^{14}C]-ethanolamine and CDP–[*methyl*-^{14}C]choline; (4) intact phospholipids labeled at either the acyl moieties or the glycerol backbone, [3-^{3}*H*]-*sn*-PC(Δ^9-26:1,Δ^9-26:1); [$\Delta^{9,10}$-^{3}H]PC-(Δ^9-26:1,Δ^9-26:1), and [$\Delta^{9,10}$-^{3}H]PE(Δ^9-26:1,Δ^9-26:1). The PE molecular species is an assumed metabolic intermediate leading to the major PE($\Delta^{5,9}$-26:2,$\Delta^{5,9}$-26:2); the labeled di-(Δ^9-26:1)-PC species was used as an unnatural probe.

On the technical side of this study, of special significance are our successful synthesis of demospongic phospholipids tritium labeled in the glycerol backbone and the use of special solvent systems for reverse-phase high-performance liquid chromatography (HPLC) to purify the unusual molecular species. These methodologies were essential for examining the lability of phosphatides carrying the C_{26} acids.

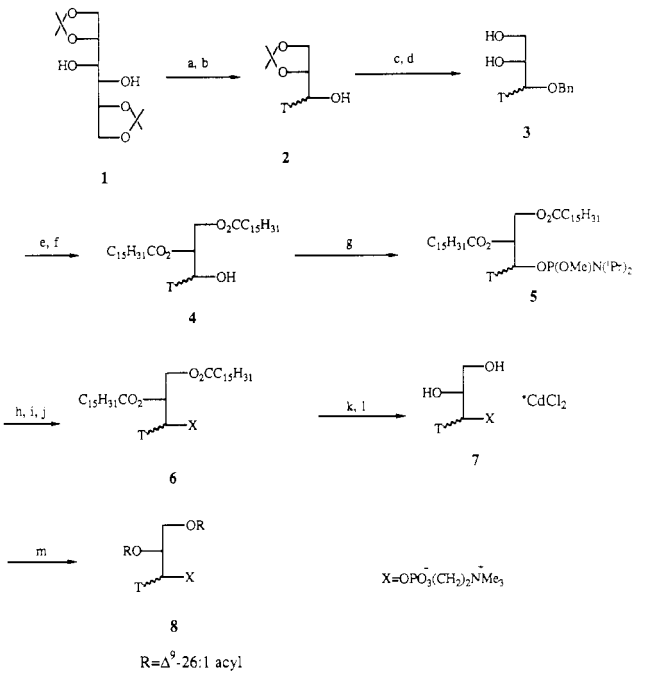
Experimental Procedures

Chemicals. [1,2-^{14}C]Choline bromide (50 mCi/mmol), [1,2-^{14}C]ethanolamine hydrochloride (100 mCi/mmol), CDP–[1,2-^{14}C]ethanolamine (51 mCi/mmol), potassium [1-^{14}C]cyanide (58 mCi/mmol), and tritiated sodium borohydride (200 mCi/mmol) were purchased from ICN Radiochemicals. [*methyl*-^{3}H]Choline chloride (78 Ci/mmol) and CDP–

[1] Abbreviations: PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; DPPC, dipalmitoylphosphatidylcholine; CDP, cytidine 5′-diphosphate.

[2] All olefinic acids described in this paper contain *Z* double bonds.

Scheme I: Synthesis of [3-^{3}H]PC (Δ^9-26:1,Δ^9-26:1)[a]



[a] (a) $Pb(OAc)_4$, EtOAc; (b) $NaBT_4$, EtOAc; (c) BnCl, $Bu_3NBn^+Cl^-$; (d) H^+; (e) $C_{15}H_{31}COCl$; (f) H_2/Pd–C; (g) $MeOPN(^iPr)_2Cl$, Et_3N, CH_2Cl_2; (h) $HOCH_2CH_2NMe_3^+OTs^-$, tetrazole; (i) PhMe, $tBuO_2H$; (j) Me_2NEt; (k) $Bu_4^+OH^-$, MeOH; (l) $CdCl_2$, H_2O; (m) Δ^9-26:1 acid, DMAP, DCC, $CHCl_3$.

[*methyl*-^{14}C]choline (60 mCi/mmol) were from Amersham Corp. L-[*methyl*-^{3}H]Methionine (200 Ci/mmol) was from Du Pont Chemicals. All other reagents were of reagent grade.

The [1-^{14}C]Δ^9-26:1 acid was synthesized as previously reported (Hahn et al., 1988). The synthesis of [3-^{3}H]PC(Δ^9-26:1,Δ^9-26:1) followed a modified procedure (Bruzik et al., 1986; Wohlgemuth et al., 1980) (Scheme I). Because of the problem of side reactions due to unsaturation and steric shielding of the head-group region by the monoenic acyl chains, introducing the choline head group at the last step, which is common to many synthetic strategies (Mena & Djerassi, 1985), had to be avoided by involving [^{3}H]DPPC as an intermediate, with the palmitoyl groups finally replaced by 9-hexacosenoyl. This acyl exchange was accomplished in three steps (k–m, Scheme I) with glycerophosphorylcholine (cadmium chloride complex) as the intermediate. Phospholipid precursors labeled at the acyl branches, i.e., [$\Delta^{9,10}$-^{3}H]PC(Δ^9-26:1,Δ^9-26:1) and its PE analogue, were synthesized according to reported procedures (Li et al., 1988; Mena & Djerassi, 1985) by tritiation of the Δ^9-acetylenic PC intermediate and subsequent conversion to the corresponding PE. The nonradioactive cocarriers for HPLC experiments, PC(Δ^9-26:1,Δ^9-26:1) and its PE analogue, were similarly prepared. The other cocarriers, PC($\Delta^{5,9}$-26:2,$\Delta^{5,9}$-26:2) and its PE analogue, were synthesized as described earlier (Ayanoglu et al., 1988).

Physical Characterization of the Nonradiolabeled Synthetic Phospholipids. 1,2-Bis[(9Z)-9-hexacosenoyl]phosphatidylcholine [*PC*(Δ^9-*26:1*,Δ^9-*26:1*)]: R_f (silica gel 60 F_{254} plate, $CHCl_3$/MeOH/H_2O 75:25:3) 0.7; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 6.8 Hz, 6 H, terminal CH_3), 1.26–1.37 (m, 72 H, aliphatic methylene protons), 1.58 (br m, 4 H, C-3 of acyl group), 1.98–2.03 (m, 8 H, allylic protons), 2.29 (t, J = 7.5 Hz, 4 H, C-2 of acyl group), 3.39 (s, 9 H, $N(CH_3)_3$), 3.88 (m, 2 H, CH_2N), 3.99 (br m, 2 H, CH_2OP), 4.09–4.43 (m, 2 H, CH_2OCO), 4.38 (br m, 2 H, PO_3CH_2), 5.22 (br m, 1 H, CHOCO), 5.34 (m, 4 H, olefinic protons). Overall yield (from D-mannitol): ca. 1%; 12 μCi in the radioactive product derived from $NaBT_4$ (1 mCi).

1,2-Bis[(9Z)-9-hexacosenoyl]phosphatidylethanolamine [*PE*(Δ^9-*26:1*)]: R_f (silica gel 60 F_{254} plate, $CHCl_3$/MeOH/H_2O, 75:25:3) 0.3; ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD 1:2) δ 0.78 (t, J = 6.8 Hz, 6 H, terminal CH_3), 1.16 (m, 72 H, aliphatic methylene protons), 1.51 (br m, 4 H, 3-C of acyl group), 1.92 (m, 8 H, allylic protons), 2.21 (t, J = 7.0 Hz, 4 H, C-2 of acyl group), 3.03 (m, 2 H, CH_2N), 3.92 (br m, 2 H, CH_2OP), 4.08 (m, 2 H, PO_3CH_2), 4.30 (m, 1 H, CH_2OCO), 5.12 (br m, 1 H, CHOCO), 5.25 (m, 4 H, olefinic protons). Overall yield (from free fatty acid): 8–10%.

Incorporation Experiments. M. prolifera was collected between April and October at a depth of 0.5 m in San Francisco Bay, CA. All incorporation experiments were conducted on live, whole sponge specimens (10 g each, dry weight) by incubation with the appropriate radiolabeled precursor (ca. 0.1 mg, either neat or in aqueous ethanolic solution) in aerated seawater around 10 °C for specific periods (maximum 2 days). For the long-term experiments with the ^{14}C-labeled hexacosenoate (Table I), precursor incubation lasted for 8 h and then the organism was transferred to fresh sea water, which was regularly replenished.

Assay of Phospholipid Classes, Molecular Species, and Fatty Acyl Components. The isolation of total phospholipids, preparation of fatty acid methyl esters, and subsequent purification and analysis (GC, HPLC) were performed as previously described (Dasgupta et al., 1986; Hahn et al., 1988). Radiolabeled phospholipids were separated according to head group by preparative thin-layer chromatography on silica gel 60 F_{254} plates with chloroform/methanol/water (74:25:3) as the eluent. R_f values for PE, PC, and PS were 0.7, 0.3, and 0.1, respectively. Appropriate spray reagents (molybdenum blue for total phospholipids, alcoholic ninhydrin for PE and PS, and Dragendorff for PC) were employed for head-group identification purposes. After elution, bands (5 mm) of phospholipids were scraped off from the plates and extracted with chloroform/methanol (1:1). Each of the PE and PC extracts was further purified by TLC, using a second solvent system, chloroform/methanol/acetic acid/water (25:15:4:2; R_f values for PE and PC were 0.6 and 0.3, respectively). The intact radiolabeled phospholipids, PC(Δ^9-26:1,Δ^9-26:1), PC($\Delta^{5,9}$-26:2,$\Delta^{5,9}$-26:2), and their PE analogues, were fractionated with nonradioactive cocarriers by reverse-phase HPLC with two Altex Ultrasphere ODS2 columns (25 cm × 10 mm i.d.) in series. The mobile phase was methanol/ethyl acetate/chloroform/acetonitrile (40:60:2:10 for PE and 60:40:2:10 for PC). Decomposition problems were alleviated by the substitution of acetonitrile for water in the previous solvent system (Dasgupta et al., 1986). The flow rate used was 1 mL/min. For radioactivity measurements, aliquots (1/5 to 1/10) of the ^{3}H or ^{14}C-labeled material were dissolved in 10 mL of organic counting scintillant (OCS), and the radioactivity was measured with a Beckman LS 7500 liquid scintillation system. All results were corrected for background radiation, calculated to the proportionate amount, and presented as disintegrations per minute by using standard solutions. All synthetic precursors were tested for purity by means of blank experiments.

Instrumental Measurements. ^{1}H NMR spectra were obtained in $CDCl_3$ or $CDCl_3$–CD_3OD with a Nicolet NT300 (300 MHz) or a Varian Associates XL-400 (400 MHz) spectrometer. Low-resolution mass spectra of known compounds were obtained with a Ribermag R-10-10 quadrupole mass spectrometer using the "SADR" (simultaneous acqui-

Table I: Radioactivity Distribution from Incorporation of the [1-^{14}C]Δ^9-26:1 Acid[a]

	obsd radioactivity (dpm)			
	Δ^9-26:1 (precursor)		$\Delta^{5,9}$-26:2	
time (day)	PE	PC	PE	PC
$^1/_2$	635000	5100	6400	<200
1	1.48 × 10^6	5300	13700	<200
2	1.71 × 10^6	7370	12200	<200
4	19600	2580	30300	<200
15	108000	870	9900	<200

[a] Experiment was carried out in April on intact sponge specimens (10 g each, dry weight). Precursor incubation lasted for 8 h, and then the organisms were transferred to fresh sea water, which was replenished regularly throughout the experiment. The radioactivity of the precursor was 20 μCi; 0.3–4% of the administered radioactivity was recovered from the fractions indicated.

Table II: Radioactivity Distribution from Incorporation of CDP-[1,2-^{14}C]Ethanolamine and CDP-[*methyl*-^{14}C]Choline[a]

		obsd radioactivity (dpm)		
precursor		4 h	10 h	25 h
CDP-[1,2-^{14}C]ethanolamine	PE	8000	15000	34000
	PC	<200	500	1200
CDP-[*methyl*-^{14}C]choline	PE	<200	<200	<200
	PC	6200	26300	65000

[a] Experiment was carried out in April. Radioactivity of each precursor was 10 μCi. Incubation took place in the presence of the precursor throughout the experiment. 0.2–0.3% of the administered radioactivity was recovered from the fractions indicated at maximum incorporation.

sition and data reduction) system.

Results and Discussion

Incorporation experiments were first carried out on intact sponge specimens using [1-^{14}C]-9-hexacosenoate as the precursor (Table I). Incubation times were optimized from the metabolic kinetics of *Microciona* (Hahn et al., 1988), with a maximum period of 15 days to follow the long-term disposition of the fatty acid. PE rapidly incorporated this precursor (radioactivity maximum at 2 days from the start of experiment), which throughout the 15-day period exhibited a 100–200-fold excess in PE without appreciable crossover to PC. The radioactivity distribution of the $\Delta^{5,9}$-26:2 acid, i.e., the immediate metabolite of the Δ^9-26:1 acid, showed a 100–300-fold excess in PE over PC throughout the experiment. These kinetic data underscore the affinity of PE for the di-C_{26}-acyl (demospongic) acids and suggest that the steady-state phospholipid composition in the organism is rapidly established at the initial stage of biosynthesis without subsequent remodeling.

De novo phospholipid biosynthesis was demonstrated in this sponge by the incorporation of [1,2-^{14}C,*methyl*-^{3}H]choline and [1,2-^{14}C]ethanolamine into the respective phosphatides (Figures 1 and 2). Further incorporation of CDP-[1,2-^{14}C]-ethanolamine and CDP-[*methyl*-^{14}C]choline (Table II) by direct injection into sponge tissues confirmed the operation of the cytidine pathway in these most primitive animals as in higher organisms (Vance & Vance, 1985; Weissmann et al., 1988). In the experiments with labeled free bases, over 99% of the ^{14}C and ^{3}H labels from choline stayed in PC, with PE essentially nonradioactive, indicating negligible demethylation of PC to PE (Figure 1). Upon coincubation with [1,2-^{14}C]-ethanolamine and L-[*methyl*-^{3}H]methionine, about 3% of the incorporated ^{14}C and a corresponding amount of ^{3}H labels were detected at various incubation times in PC (Figure 2). Apparently N-methylation of PE had occurred, although in the

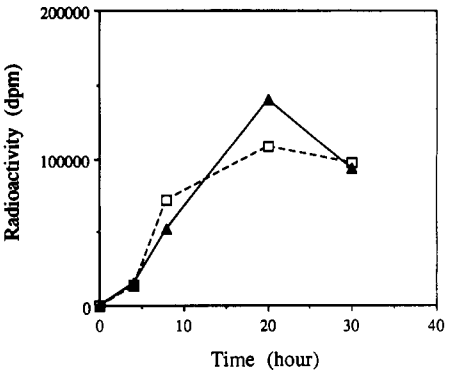


FIGURE 1: Time course of incorporation of [1,2-^{14}C]- and [*methyl*-^{3}H]choline: precursor incubation was carried out in October. The radioactivity of each precursor was 25 μCi. A maximum of 0.3% each of the ^{3}H and ^{14}C radioactivity administered was recovered from the PC fraction. Incorporation of both precursors into PC was obvious, but demethylation to PE was not observed. Radioactivity (^{3}H and ^{14}C) in the molecular species PC($\Delta^{5,9}$-26:2,$\Delta^{5,9}$-26:2) was less than 500 dpm (<0.2% of the total recovered in PC) at all incubation times: (□) ^{3}H in PC; (▲) ^{14}C in PC.

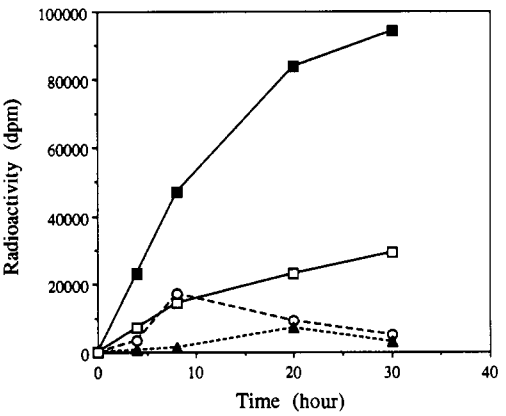


FIGURE 2: Time course of incorporation of [1,2-^{14}C]ethanolamine and L-[*methyl*-^{3}H]methionine: precursor incubation was carried out in October. The radioactivity of each precursor was 25 μCi. A maximum total 0.2% of the radioactivity administered was recovered from the PE and PC fractions. Incorporation of ethanolamine into PE was obvious: methylation of PE to PC was observed to a limited extent: (□) ^{14}C in PE; (□) ^{14}C in the molecular species PE($\Delta^{5,9}$-26:2, $\Delta^{5,9}$-26:2); (○) ^{3}H in PC; (▲) ^{14}C in PC.

absence of external stimuli the extent appears to be limited. It is likely that PE methylation in the organism serves some special functions such as membrane fluidization or signal response (Hirata & Axelrod, 1978, 1980), but judging from the present results it is not a predominant process that perturbs the static phospholipid composition.

A remarkable observation from the above base incorporation experiments was the selective incorporation of ethanolamine into the molecular species PE($\Delta^{5,9}$-26:2,$\Delta^{5,9}$-26:2), the latter accounting for about 30% of the total ^{14}C labels recovered in PE (Figure 2). In contrast, less than 0.2% of the incorporated choline was detected in the fraction PC($\Delta^{5,9}$-26:2,$\Delta^{5,9}$-26:2) (Figure 1), which existed in extremely low natural abundance, with the rest of the radioactivity residing in the shorter chain PC molecular species. Regarding the formation of "symmetric" demospongic phospholipids, a possible mechanism is the direct acylation of glycerol 3-phosphate by the C_{26}-acyl-CoA, catalyzed by an acyltransferase specific for the individual demospongic acid (in contrast to the esterification of two different acyl groups in conventional phospholipids). Subsequent introduction of the head group would involve a phosphoethanolamine transferase specific for the di-C_{26}-acylglycerol (Scheme II). An analogous pathway may ac-

Scheme II: Pathways of Demospongic Phospholipid Metabolism in *M. prolifera*

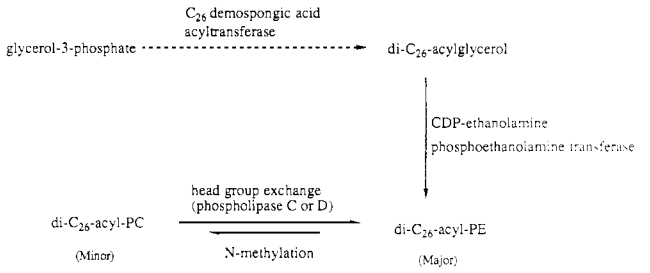


Table III: Radioactivity Distribution from Incorporation of [3-^{3}H]-*sn*-PC(Δ^9-26:1,Δ^9-26:1)[a]

molecular species	natural abundance (% of molecular species in PC or PE)	obsd radioactivity (dpm) 6 h	1 day
PC(Δ^9-26:1,Δ^9-26:1) (precursor)	<0.1	193000	128000
PE(Δ^9-26:1,Δ^9-26:1)	0.5	9600	67000
PC($\Delta^{5,9}$-26:2,$\Delta^{5,9}$-26:2)	<0.1	<500	<500
PE($\Delta^{5,9}$-26:2,$\Delta^{5,9}$-26:2)	40	<500	2300

[a] Precursor incubation was carried out on intact sponge specimens for 6 h and for 1 day in May. Precursor radioactivity was 6 μCi. 1–2% of the administered radioactivity was recovered from the fractions indicated.

count for the formation of PC(22:6, 22:6). From the affinity of choline and ethanolamine respectively for the short-chain and di-C_{26}-acyl (demospongic) phospholipids, there most likely exist in the marine sponge transferases (acyl, phosphoethanolamine, and phosphocholine transferases) that are different in specificity from those in higher eukaryotes (considering that phosphoethanolamine transferase normally is selective for hexaenoic diacylglycerols) (Lands & Crawford, 1976). The above proposition is supported by the recent evidence in *Microciona* (Weissmann et al., 1988) of the rapid de novo incorporation of the 20:4 and 22:6 acids into PC and the involvement of the corresponding diacylglycerols in cell signaling.

Some other reactions have been proposed (Vance & Vance, 1985) to account for the formation of symmetric phospholipid species (e.g., DPPC) and the introduction of polyenoic acids into phosphatides in higher organisms. These include the deacylation–reacylation cycle for forming unsaturated PC, and the condensation of lyso-PC to form symmetric PC species. Analogous pathways for PE are less known, however. Still another complex process of tailoring phospholipid composition involves the phospholipase-catalyzed base (or phosphobase) exchange reactions. In order to examine such exchange and reacylation processes in the marine sponge, incorporation experiments were performed with another group of synthetic radiolabeled phospholipids whose synthesis is described below.

[3-^{3}H]-*sn*-PC(Δ^9-26:1, Δ^9-26:1), an unnatural molecular species (**8** in Scheme I), was employed to gauge head-group lability and to follow the disposition of the biosynthetic end product of the acyl moiety (the $\Delta^{5,9}$-26:2 acid). Despite its minimal natural abundance, this di-C_{26}-acyl-PC was nonselectively absorbed, and the resulting radioactivity distribution revealed the conversion of PC to PE in progress (Table III). The substantial amount of ^{3}H labels in PE(Δ^9-26:1,Δ^9-26:1) after 6 h and 1 day was obviously due to head-group exchange, as direct demethylation of PC to PE had been shown to be insignificant (see Figure 1). A limited amount of radiolabeled PE($\Delta^{5,9}$-26:2,$\Delta^{5,9}$-26:2) was also detected after 1 day but not its PC analogue. It appears that such head-group turnover

Table IV: Radioactivity Distribution from Incorporation of [$\Delta^{9,10}$-^{3}H]PC(Δ^9-26:1,Δ^9-26:1)[a]

molecular species	obsd radioactivity (dpm) 4 h	10 h	20 h	2 days
PC(Δ^9-26:1,Δ^9-26:1) (precursor)	39400	73800	238000	156000
PE(Δ^9-26:1,Δ^9-26:1)	2900	10800	39100	44100
PC($\Delta^{5,9}$-26:2,$\Delta^{5,9}$-26:2)	400	1100	<500	<500
PE($\Delta^{5,9}$-26:2,$\Delta^{5,9}$-26:2)	<200	3200	10200	8000

[a] Precursor incubation was carried out in August on intact sponge specimens (10 g each, dry weight). Precursor radioactivity was 10 μCi. 1% of the administered radioactivity was recovered from the fractions indicated at maximum incorporation.

Table V: Radioactivity Distribution from Incorporation of [$\Delta^{9,10}$-^{3}H]PE(Δ^9-26:1,Δ^9-26:1)[a]

molecular species	obsd radioactivity (dpm) 4 h	10 h	20 h	2 days
PC(Δ^9-26:1,Δ^9-26:1)	500	1300	1100	2000
PE(Δ^9-26:1,Δ^9-26:1) (precursor)	93800	153000	186000	215000
PC($\Delta^{5,9}$-26:2,$\Delta^{5,9}$-26:2)	1300	1500	900	1100
PE($\Delta^{5,9}$-26:2,$\Delta^{5,9}$-26:2)	2100	3700	12500	17600

[a] Precursor incubation was carried out in August on intact sponge specimens (10 g each, dry weight). Precursor radioactivity was 10 μCi. 1% of the administered radioactivity was recovered from the fractions indicated at maximum incorporation.

represents a corrective mechanism for associating the C_{26}-acyl groups selectively with PE.

Incorporation experiments with phospholipids labeled at the acyl chains, i.e., [$\Delta^{9,10}$-^{3}H]PC(Δ^9-26:1,Δ^9-26:1) and its PE analogue, confirmed the operation of an exchange process of PC for PE (Table IV) as noted earlier, whereas the reverse process was sluggish: very low radioactivity was detected in PC(Δ^9-26:1,Δ^9-26:1) after the incorporation of the ^{3}H-labeled PE precursor (Table V). This apparently unilateral conversion argues against random reacylation between phospholipid classes. The dienoic $\Delta^{5,9}$-26:2 acid, which may be formed by desaturation after deacylation or at the intact phospholipid stage, displays a similar affinity for PE as the Δ^9-monoenic acid. Rationalizing the conversion of the demospongic PCs to the corresponding PEs by assuming the involvement of a PC-selective phospholipase C is not convincing in light of a recent report on lipid metabolism in *Microciona* (Weissmann et al., 1988). However, the existence of a non-PI-specific phospholipase C is not improbable, and the diacylglycerols produced may be esterified again by action of the specific phosphocholine or phosphoethanolamine transferases assumed earlier. Although this model remains to be validated, it accounts well for the corrective turnover observed after intake of the (demospongic) di-C_{26}-acyl-PC. The possibility of base exchange (transphosphatidylation) by the action of phospholipase D, however, is uncertain. Exchange of choline for ethanolamine, if indeed occurring in the sponge, has to be a highly selective process as is evidenced by the predominance of PC(22:6, 22:6) but the virtual absence of its PE analogue. What can be said at present is only that *either* phosphocholine *or* choline is labile when associated with the C_{26} acids and easily replaced by (phospho)ethanolamine.

In summary, this study demonstrates that the two major phosphatides in *M. prolifera*, PC and PE, are biosynthesized de novo via the cytidine pathway as in higher organisms. PE methylation to PC occurs to a slight extent, whereas the exchange of PC for PE, probably mediated by the action of phospholipase (C or D), does operate after intake of the extemely minor PC(Δ^9-26:1,Δ^9-26:1) species (Scheme II). The reverse exchange, however, is minimal after incorporation of PE(Δ^9-26:1,Δ^9-26:1), indicating the preferred association of

unique demospongic fatty acids with amino phospholipids. The selective incorporation of ethanolamine and choline into the respective demospongic and short-chain phospholipids points toward the operation of a de novo biosynthetic pathway involving unique acyl and phosphoethanolamine transferases, which are specific respectively for demospongic acids and their diacylglycerols and which have not been observed in higher organisms.

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Registry No. PC (Δ^9-26:1,Δ^9-26:1), 130728-33-5; PE (Δ^9-26:1,Δ^9-26:1), 130728-34-6; PC ($\Delta^{5,9}$-26:2,$\Delta^{5,9}$-26:2), 99824-55-2; PE ($\Delta^{5,9}$-26:2,$\Delta^{5,9}$-26:2), 99824-60-9; C_{26} demospongic acid transferase, 9054-54-0; CDP-ethanolamine phosphoethanolamine transferase, 9026-19-1.

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