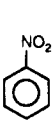
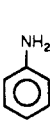
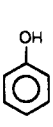
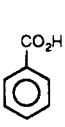
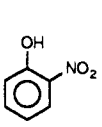
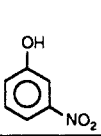
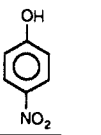
Table II. R_f Values from TLC Experiments[a]

polymer	developing solvent	R_f 						
2	MeOH	0.45	0.65	0.92	0.80	0.50	0.77	0.85
10	MeOH	0.70	0.84	0.92	0.90	0.68	0.93	0.95
silica	MeOH	0.94	0.88	0.80	0.72	0.88	0.81	0.93
2	hexane	0.45	0.30	0.19	0.53	0.45	0.0	0.0

[a] Experimental uncertainty, ±5%. Runs were carried out in triplicate.

electron micrographs (taken at 20 300 magnification) of polymers **4** and **8** are given in Figure 1. Both show porosity totally absent in polymer **10** prepared from styrene/DVB without AOT and water. Polymer **4** possesses a finer microstructure than polymer **8**, an observation consistent with BET adsorption analysis data giving surface areas for the polymer particles (150–250 mesh). Thus, as seen in Table I, polymers **4** and **8** have surface areas of 26.9 and 2.2 m^2/g, respectively. Note that the pores in the polymers are 1 order of magnitude larger than the radii of the original water pools at the monomer stage (<10 nm according to QELS data). Polymer porosity clearly reflects the propensity for pools to assemble as they become encased in polystyrene.

Although the polymerization process is complicated and not understood, three generalizations are possible from the BET data in Table I. (*a*) Polymers **2**, **4**, and **5**, made with pools of constant [AOT] but widely differing sizes (R = 14, 9.2, and 5.6, respectively), all have similar surface areas. Thus, initial pool size is, by itself, not critical to the porosity ultimately created in the polymer. (*b*) Polymers **1**, **2**, and **3**, made by using a constant pool size (R = 14) but with an increasing [AOT] and [H_2O], show a startling result; by far the largest surface area of 19.4 m^2/g was obtained with the intermediate concentration ([AOT] = 0.20 M) of the enclosed phase. A surface area of 1.3 ± 0.1 M^2/g was measured for samples made with lower ([AOT] = 0.10 M) and higher ([AOT] = 0.50 M) levels of dispersed water pools. (*c*) Polymers **6**, **7**, and **8** indicate that, at an increasing [AOT] with constant [H_2O] (and a consequent decrease in R), the area drops 10-fold from 24.0 to 2.2 m^2/g. Apparently, a small number of large microemulsion droplets are more effective than a larger number of small droplets in generating a high surface area.

TLC plates were prepared from powdered (>400 mesh) polymer **2** mixed with 10% $CaSO_4 \cdot 2H_2O$ as a binder. These plates were compared with those from polymer **10** (polystyrene made without water pools) and with commercial silica gel plates in their ability to separate the 7 compounds shown in Table II. Interestingly, the spread of R_f values is much greater for the plates based on polymer **2** than with plates made from polymer **10** or silica. Both methanol and heptane can be used with **2** to resolve the compounds although relative mobilities for the two eluting solvents are seen to be quite different. Selectivity in adsorption–elution characteristics could be extended to column chromatography. For example, the first four compounds in Table II were separated on a polymer **2** column with retention times of 6.2, 4.5, 3.7, and 4.2 min, respectively. In contrast, an identical column of polymer **10** gave retention times of 3.1, 3.0, 2.9, and 3.0 min, respectively.

Water pools in styrene/DVB were prepared by using water containing 6 mM $CuCl_2$. When the resulting polymer was dried, copper salt deposited on the walls of the pores. High-area polymer (no. **4**, 100–150 mesh) released 7% of $CuCl_2$ in 3 days and 80% in 0.5 days when extracted at 25 °C with water and methanol, respectively. Methanol must enter the pores much more efficiently than water. A similar experiment with low-area polymer (no. **1**) gave only 4% leaching in 3 days and 18% in 0.5 days with water and methanol, respectively.

We have shown that polymers prepared from reverse micellar systems can provide a variety of tailored porous surfaces. Such materials could find applications ranging from catalysis to separation science to controlled release of drugs.[10]

Acknowledgment. This work was supported by the Army Research Office. We thank Frank Horne (Georgia Pacific Corp.) for his help with the SEM studies and the Arakawa Chemical Co. (Osaka, Japan) for carrying out the BET measurements.

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Biosynthesis of Archaebacterial Membranes. Formation of Isoprene Ethers by a Prenyl Transfer Reaction

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Received September 15, 1989

Archaebacteria are a unique taxonomic group that diverged from eubacteria and eukaryotes at an early stage of evolution.[1] They inhabit environments characterized by high salt (halophiles), high temperature (thermophiles), low pH (acidophiles), or lack of oxygen (methanogens) hostile to most other life forms. Archaebacteria have several distinctive phenotypes at the molecular level, including characteristic sequences for their 5S and 16S RNAs,[2-4] unique metabolic cofactors,[5-8] and a novel architecture for the lipids in their cellular membranes.[9-12]

In contrast to the fatty acid ester motif found in other organisms, archaebacterial lipids are composed of *sn*-2,3-*O*-diphytanylglyceryl units bearing polar groups at the *sn*-C(1) position. A possible mechanism for formation of ether linkages between the glyceryl and fully saturated phytanyl moieties is a prenyl transfer reaction with an unsaturated C_{20} allylic diphosphate donor and a glyceryl acceptor with subsequent reduction of the double bonds. This hypothesis is supported by discoveries that gera-

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0002-7863/90/1512-1264$02.50/0

Table I. Evaluation of Substrates

substrates		sp act.,[a] nmol
donor	acceptor	min^{-1} mg^{-1}
1-OPP	(*S*)[^{3}H]**2**-OP	0.39
1-OPP	(*R*)[^{14}C]**2**-OP	0.01
1-OPP	[^{3}H]**2**-OH	<0.0001
1-OPP	[^{35}S]**4**-OP_S	<0.0001
(*R,R*)**5**-OPP	(*S*)[^{3}H]**2**-OP	0.07
(*RS,R,R*)**6**-OPP	(*S*)[^{3}H]**2**-OP	<0.005

[a] Determined at 37 °C by the butanol extraction assay using radiolabeled acceptor.

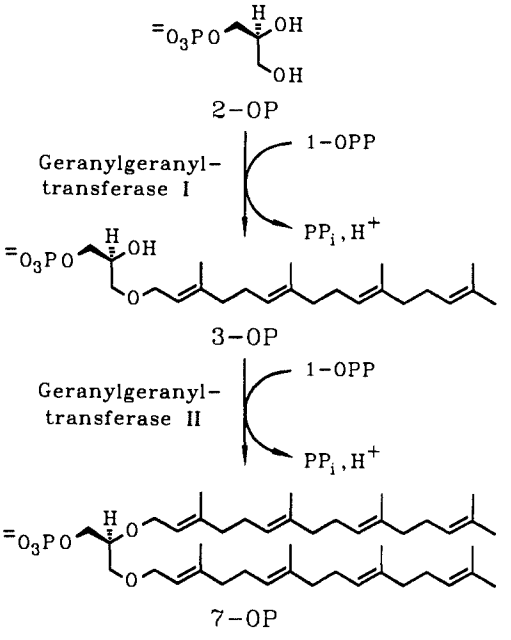
nylgeraniol[13] and glycerol[14] are efficiently incorporated into membrane lipid diethers of archaebacteria and by pulse labeling experiments.[15] We now report experiments with a cell-free extract of *Methanobacterium thermoautotrophicum* that demonstrate the ether linkages are constructed by two prenyltransferases, the first of which catalyzes condensation between geranylgeranyl diphosphate (**1**-OPP) and (*S*)-glyceryl phosphate ((*S*)**2**-OP) to generate (*S*)-3-*O*-(geranylgeranyl)glyceryl 1-phosphate ((*S*)**3**-OP).

Cell-free extracts were prepared by sonication of cells from an early stationary phase culture of *M. thermoautotrophicum*, strain *Marburg*,[16] in 60 mM *endo*-bicyclo[2.2.1]heptene-2,3-dicarboxylate, 3 mM $MgCl_2$, 5 mM KF, 1 mM dithiothreitol, pH 7.2, and centrifugation to remove cellular debris. Samples were assayed for prenyltransferase activity by incubation of 200 μM **1**-OPP and 150 μM (*S*)[1-^{3}H]**2**-OP (3 μCi/μmol) with a portion of the extract in 150 μL of buffer at 37 °C for 10 min. The reaction was terminated by addition of 100 μL of 0.5 M EDTA;[17] 1 mL of water and 3 mL of 1-butanol were added; the sample was vigorously mixed and centrifuged at 2700*g* for 25 min; a 1.0-mL portion was added to 10 mL of Optifluor (Packard); and radioactivity was determined by liquid scintillation spectrometry.

Results with **1**-OPP and (*S*)**2**-OPP and with several other compounds as prenyl donors and acceptors are given in Table I. (*S*)-[^{3}H]glycerol ((*S*)[^{3}H]**2**-OH) and [^{35}S]dihydroxyacetone thiophosphate *O*-ester ([^{35}S]**4**-OP_S) were not substrates. (*R*)[^{14}C]**2**-OP gave extractable radioactivity with **1**-OPP at approximately 3% the rate of (*S*)[^{3}H]**2**-OP. In competitive experiments, (*S*)[^{3}H]**2**-OP was strongly selected over (*R*)[^{14}C]**2**-OP or [^{35}S]**4**-OP.[18] Among the prenyl donors tested, **1**-OPP was preferred. (*R,R*)-Phytyl diphosphate ((*R,R*)**5**-OPP) was incorporated at 18% of the maximal rate for **1**-OPP, and fully saturated (3*RS*,7*R*,11*R*)-phytanyl diphosphate ((*RS,R,R*)**6**-OPP) was not a substrate.

In preparative-scale experiments, 17 mg of **1**-OPP (34 μmol) and 4 mg of **2**-OP (19 μmol) were incubated with extract from 1.5 g of cells for 5 h at 50 °C.[19] The mixture was extracted with chloroform/methanol and 1-butanol; organic solvents were removed under vacuum; the residue was resuspended in 100 mM glycine, 1 mM $MgCl_2$, 1 mM $ZnCl_2$, pH 10.4; 0.7 mg of calf intestinal mucosa alkaline phosphatase was added; and incubation was continued for 48 h at 37 °C. The mixture was extracted with ethyl ether and purified by HPLC. The major product (1.4 mg) was 3-*O*-(geranylgeranyl)glycerol (**3**-OH), and the minor product (ca. 100 μg) was 2,3-bis-*O*-(geranylgeranyl)glycerol (**7**-OH), as verified by coinjection on HPLC and comparisons of NMR and mass spectra with those of authentic samples.[20] No evidence was seen for formation of 2-*O*-(geranylgeranyl)glyceryl phosphate.[21] When the cell-free extract was centrifuged at 200000*g* for 90 min, the supernatant only catalyzed synthesis of **3**-OP from **1**-OPP and (*S*)**2**-OP, and the resuspended pellet was inactive.

Scheme I



From these experiments, we conclude that the diphytanyl lipids in archaebacteria are assembled from **1**-OPP and (*S*)**2**-OP by two prenyltransferases as illustrated in Scheme I. The first catalyzes alkylation of the primary hydroxyl of (*S*)**2**-OP and is cytosolic. The second catalyzes alkylation of the free hydroxyl in (*S*)**3**-OPP and is presumably membrane-bound. While biosynthesis of **1**-OPP follows a normal mevalonate pathway,[22,23] the route to (*S*)**2**-OP is unclear. In halophiles, glycerol kinase and glycerophosphate dehydrogenase give (*R*)**2**-OP,[24] and exogenous glycerol is incorporated into phytanyl diethers with loss of the hydrogen at C(2).[14,24] These results are consistent with biosynthesis of (*S*)**2**-OP needed for membrane by stereoselective reduction of dihydroxyacetone phosphate.[14,24] However, thermoacidophiles incorporate **2**-OH with retention of the C(2) hydrogen.[25] Thus, there does not appear to be a common route to (*S*)**2**-OP in all archaebacteria.

Acknowledgment. This work was supported by Grant GM 21328 from the National Institutes of Health.

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(17) A divalent metal is required. Activity was also seen with Mn^{2+}, Zn^{2+}, and Ca^{2+}.

(18) $^3H_{(S)2\text{-OPP}}/^{14}C_{(R)2\text{-OPP}} = 1.09$; in extractable products, $^3H/^{14}C > 10^3$. $^3H_{(S)2\text{-OPP}}/^{35}S_{4\text{-OP}_S} = 2.19$; in extractable products, $^3H/^{35}S > 10^3$.

(19) The enzyme was stable up to 67 °C. HPLC analysis of preliminary experiments with **1**-OPP and (*S*)[^{3}H]**2**-OP after a 9-h incubation show two radioactive products in a 19:1 ratio; respective retention times, 44 and 61 min on a C_{18} 10-μm Radial Pak cartridge upon elution with 75:25 MeOH/H_2O (48 min), 75:25 MeOH/H_2O → MeOH (4 min), MeOH.

(20) All new compounds gave satisfactory NMR and mass spectra. **3**-OH: ^{1}H NMR ($CDCl_3$) 1.58 (9, s, allylic methyls), 1.66 (6, s, allylic methyls), 1.9–2.1 (12, m, allylic methylenes), 3.43–3.55 (2, m, glyceryl methylene), 3.57–3.73 (2, m, glyceryl methylene), 3.8–3.9 (1, m, glyceryl methine), 4.02 (2, d, J = 6.8 Hz, H at C(1) of geranylgeranyl), 5.03–5.13 (3, m, H at C(6), C(10), C(14) of geranylgeranyl), and 5.31 ppm (1, t, J = 6.8 Hz, H at C(2) of geranylgeranyl); GCMS (EI, bis TMS ether) m/z 69, 73, 81, 93, 103, 135, 137, 147, 205, 237, 272, 302, 361, 508 (M^+). **7**-OH: ^{1}H NMR ($CDCl_3$) 1.55–1.75 (30, m, allylic methyls), 1.9–2.2 (24, m, allylic methylene), 3.43–3.55 (4, m, glyceryl methylenes), 3.68–3.76 (1, m, glyceryl methine), 4.00 (2, d, J = 6.8 Hz, H at C(1) of geranylgeranyl unit at C(3)), 4.10 (1, dd, J = 12.8 Hz, J = 6.8 Hz, H at C(1) of geranylgeranyl unit at C(2)), 4.17 (1, dd, J = 12.8 Hz, J = 6.8 Hz, H at C(1) of geranylgeranyl unit at C(2)), 5.15 (6, m, H at C(6), C(10), C(14), in geranylgeranyl), and 5.4 ppm (2, m, H at C(2) in geranylgeranyl); MS (EI, TMS ether) m/z 69, 81, 93, 103, 135, 147, 203, 272, 435, 544, 708 (M^+).

(21) Authentic samples of 2- and 3-*O*-(geranylgeranyl)glycerol were cleanly separated by HPLC on a Radial Pak cartridge.

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