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Biosynthetic machinery for C_{25},C_{25}-diether archaeal lipids from the hyperthermophilic archaeon *Aeropyrum pernix*

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ABSTRACT

Archaea that thrive in harsh environments usually produce membrane lipids with specific structures such as bipolar tetraether lipids. Only a few genera of archaea, which are hyperthermophiles or halophiles, are known to utilize diether lipids with extended, C_{25} isoprenoid hydrocarbon chains. In the present study, we identify two prenyltransferases and a prenyl reductase responsible for the biosynthesis of C_{25},C_{25}-diether lipids in the hyperthermophilic archaeon *Aeropyrum pernix*. These enzymes are more specific to C_{25} isoprenoid chains than to C_{20} chains, which are used for the biosynthesis of ordinary C_{20},C_{20}-diether archaeal lipids. The recombinant expression of these enzymes with two known archaeal enzymes allows the production of C_{25},C_{25}-diether archaeal lipids in the cells of *Escherichia coli*.

1. Introduction

Archaeal membrane lipids are easily identified by unique structures that typically involve two linear, fully saturated C_{20} isoprenoid (phytanyl) chains linked to a glycerol moiety via ether bonding [1–4]. They form a membrane that maintains low levels of ion and small-molecule leakage across a wide range of temperatures [5], which supposedly allows archaea to thrive in harsh environments such as acidic hot springs and basic salt lakes. Some archaea, mostly thermophiles but also mesophiles such as some methanogens, produce specific tetraether lipids that are believed to form by dimerization of the typical diether lipids depicted above. These dipolar (or two-headed) tetraether lipids, which have C_{40} isoprenoid (biphytanyl) chains that sometimes contain 5- or 6-membered internal ring structures, produce a strong effect to reduce leakage through the membranes containing them [6], suggesting they help archaea adapt to extreme conditions. Another type of specific lipid produced by extremophilic archaea, such as the hyperthermophilic archaea *Aeropyrum pernix* and the halophilic archaea of a few genera, includes “extended” diether lipids that have C_{25} isoprenoid chains, which are longer than the usual C_{20} chains. The C_{25},C_{25}-diether lipids isolated from *A. pernix* are known to form liposomes that are characterized by low leakage even at temperatures approaching 100 °C [7].

The biosynthetic pathways of ordinary C_{20},C_{20}-diether archaeal lipids are already known, and almost all the enzymes involved in these pathways have been identified from several examples of thermophilic or mesophilic archaea (Fig. 1) [1,3]. The precursor for the glycerol moiety of the lipids, *sn*-glycerol 1-phosphate (G1P), is formed by G1P dehydrogenase from dihydroxyacetone phosphate. Two C_{20} isoprenoid chains with double bonds are transferred from geranylgeranyl pyrophosphate (GGPP), which is biosynthesized from dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP), to G1P via the actions of two prenyltransferases: geranylgeranylglyceryl phosphate (GGGP) synthase and digeranylgeranylglyceryl phosphate (DGGGP) synthase. All double bonds in the isoprenoid chains are then reduced by geranylgeranyl reductase (GGR). We have succeeded in reconstructing this pathway in *E. coli* cells by introducing the genes of these enzymes from a mesophilic methanogenic archaeon, *Methanosarcina acetivorans* [8]. These cells produce a C_{20},C_{20}-diether lipid, diphytanylglyceryl phosphoglycerol (or archaetidylglycerol), along with its

Abbreviations: DGFGP, digeranylfarnesylglyceryl phosphate; DGGGP, digeranylgeranylglyceryl phosphate; DGFGP-glycerol, digeranylfarnesylglyceryl phosphoglycerol; DMAPP, dimethylallyl pyrophosphate; EIC, Extracted ion chromatogram; G1P, *sn*-glycerol 1-phosphate; GFGP, geranylfarnesylglyceryl phosphate; GGGP, geranylgeranylglyceryl phosphate; GFPP, geranylfarnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; GGR, geranylgeranyl reductase; IPP, isopentenyl pyrophosphate.

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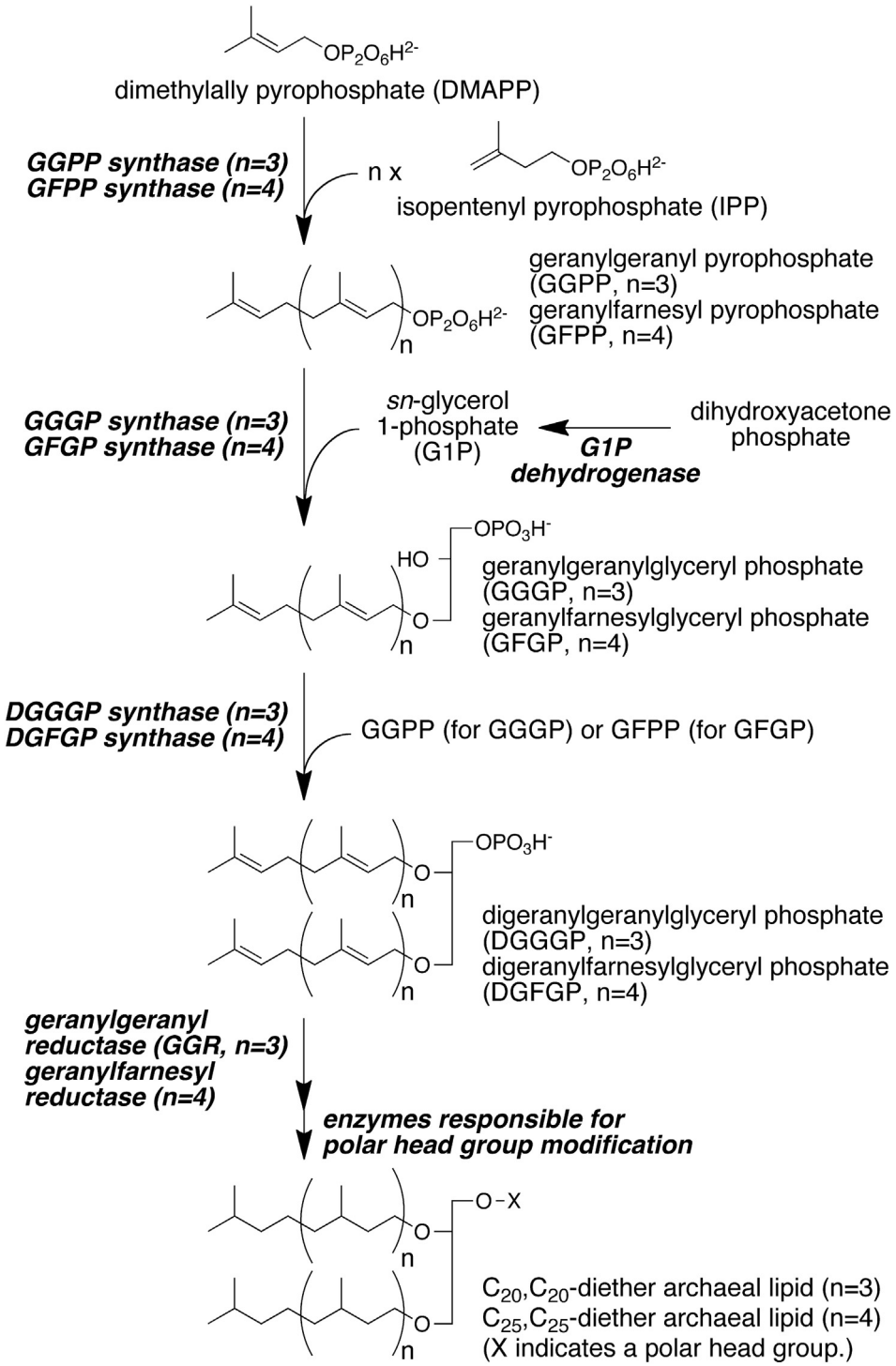

Fig. 1. The biosynthetic pathways of C_{20},C_{20}- and C_{25},C_{25}-diether archaeal lipids.

unsaturated precursors. Additionally, some archaeal enzymes that catalyze the modification of the polar head groups of membrane lipids have also been discovered. In contrast, only geranylfarnesyl pyrophosphate (GFPP, or farnesylgeranyl pyrophosphate) synthase has been identified as the enzyme responsible for the biosynthesis of extremophile-specific extended diether lipids [9,10].

In this study, we identified two new prenyltransferases and one prenyl reductase from *A. pernix*, which are responsible for the biosynthesis of C_{25},C_{25}-diether lipids (Fig. 1). The specificities of these enzymes toward biosynthetic precursors with C_{25} isoprenoid chains are in sharp contrast with those of the enzymes involved in C_{20},C_{20}-diether lipid biosynthesis, and this enabled us to synthesize a C_{25},C_{25}-diether lipid, disesterterpanylglyceryl phosphoglycerol (or C_{25},C_{25}-archaetidylglycerol), in *E. coli* cells by introducing five archaeal genes.

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