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Early Evolution of Membrane Lipids: How did the Lipid Divide Occur?

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Abstract The ubiquitous distribution, homology over three domains, and key role in the membrane formation of the enzymes of the CDP-alcohol phosphatidyltransferase family, as well as phylogenetic analyses of lipid synthesizing enzymes suggest that the membranes of Wächtershäuser's hypothetical pre-cells (universal common ancestor) [Mol Microbiol 47:13–22 (2003)] comprised a lipid bilayer with four types of core lipids [G-1-P-isoprenoid ether (Ai), G-3-P-fatty acyl ester (Bf), G-1-P-fatty acyl ester (Af) and G-3-P-isoprenoid ether (Bi)]. Here, a complementary hypothesis is presented to explain the difference between archaeal and bacterial lipids (lipid divide). The main driving force of lipid segregation is assumed to be glycerophosphate (GP) enantiomers, as Wächtershäuser proposed, but in the present study the hydrocarbon chains bound to each backbone are also hypothesized to affect lipid segregation. It is assumed that segregation was stimulated by different hydrocarbon chains bound to different GP backbones (Ai:Bf or Af:Bi). Because Ai and Bi are diastereomers and Af and Bf are enantiomers, Ai:Bf and Af:Bi are not equivalent. G-1-P-isoprenoid ether is provisionally assumed to segregate more easily from Bf than Bi does from Af. G-1-P-isoprenoid ether and Bf could more easily achieve the more stable homochiral membranes that are the ancestors of Archaea and Bacteria. This can explain why the extant archaeal and bacterial membrane lipids are mainly composed by Ai and Bf lipids, respectively. Because polar head groups were localized in the cytoplasmic compartment of pre-cells, they were equally carried over to Archaea and Bacteria during differentiation. Consequently, the both descendants shared the main head groups of membrane phospholipids.

Keywords Amphiphilic lipid · Archaea · Bacteria · CDP-alcohol phosphatidyltransferase · Common ancestor · Lipid divide · Membrane · Phospholipid

Introduction

All of the organisms on Earth are classified into three domains: Archaea, Bacteria, and Eucarya based on small subunit rRNA sequences (Woese et al. 1990). The classification is well supported by a number of biochemical features, the most characteristic of which are the properties of membrane lipids (including their structures, biosynthetic pathways, biosynthetic enzymes and their genes) (Kates 1978; Koga et al. 1993; Koga and Morii 2005, 2007). Therefore, several hypotheses on the differentiation of Archaea and Bacteria have been postulated based on or in relation to differences in the membrane lipids (Koga et al. 1998; Wächtershäuser 2003; Martin and Russell 2003; Pereto et al. 2004; Payandeh et al. 2007; Glansdorff et al. 2008). Almost all authors concur with the notion that the most critical distinction between archaeal and bacterial membrane lipids is the stereochemical difference in the glycerophosphate (GP) backbones of the phospholipids. The archaeal backbone is *sn*-glycerol-1-phosphate (G-1-P) and the bacterial backbone is *sn*-glycerol-3-phosphate (G-3-P), both of which are enantiomers. In general, two isoprenoid chains are bound at the *sn*-2 and 3 positions of G-1-P via ether bonds in Archaea, whereas two fatty acyl chains are bound at the *sn*-1 and 2 positions of G-3-P via ester linkages in Bacteria (this fundamental difference is designated the "lipid divide"). Although there are exceptions to

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hydrocarbon chains and ether/ester bonds, to date there are no exceptions reported for the stereochemistry of GP. Isoprenoids and fatty acids found in cells may be from membrane glycerophospholipids or, from other cellular constituents; for example, some isoprenoid compounds are involved in cell wall synthesis as a sugar-carrier lipid intermediate, and also function as respiratory chain members (ubiquinones) in Bacteria. These isoprenoid compounds are synthesized by the methylerythrytol phosphate pathway or the mevalonate pathway in Bacteria. The existence of isoprenoids or their synthesizing system does not necessarily imply the presence of isoprenoid lipid membranes in Bacteria. Although Gattinger et al. (2002) reported a significant amount of fatty acid in several archaeal species, ester-linked phospholipid fatty acid comprised less than 6% of the total phospholipid fatty acid. The exact structure of ester-linked phospholipid fatty acids in Archaea is not known. An ester phospholipid with known chemical structure composed of a GP backbone and two fatty acyl chains has never been identified in Archaea, while fatty-alcohol bonded GP phospholipids are present in Bacteria. Thus, the "lipid divide" may account for the fundamental difference in membrane lipids between Archaea and Bacteria.

The present paper discusses the differentiation of Archaea and Bacteria, and does not include Eucarya. Organisms usually comprise one enantiomer of optical isomers (for example, L-amino aids and D-sugars), while either enantiomer is present in the GP backbone of membrane lipids depending on the domain of life. How stereochemical isomers (enantiomers) with an identical plane structure and having the same role (in this case, as a backbone of membrane phospholipids) occurred separately in two fundamentally different lineages of organisms is not known, but has implications for the evolution of relevant organisms. Thus, an important biologic event may underlie this remarkable phenomenon.

Wächtershäuser (2003) proposed a hypothesis in which the enantiomeric difference in GP backbones was stressed. He designated the common ancestor cells of Archaea and Bacteria as pre-cells. The membranes of the pre-cells are composed of racemic GP (a mixture of G-1-P and G-3-P in equal amounts). Racemic GP is assumed to be carried over from the former chemical (prebiotic) evolution stage to the pre-cell stage, synthesized non-enzymatically by inorganic catalyst or GP-forming enzymes. The most recent argument of Pereto et al. (2004) is from phylogenetic analysis of G-1-P dehydrogenase and G-3-P dehydrogenase. Wächtershäser postulated that membranes made of mixed GP enantiomers (heterochiral membranes) are less stable than membranes with higher GP chirality (i.e., more homochiral membranes), and that the lipid membrane spontaneously segregated into more homochiral membrane patches. Morigaki et al. (1997) recently reported some data on the stability of homochiral versus heterochiral fatty acid vesicles. Although fatty acid bilayers differ from GP-based bilayers, their study essentially confirms one of the hypotheses (i.e., Wächtershäuser's hypothesis on different stability of hetero- versus homochiral membranes).

Pre-cells with more homochiral lipid membranes then underwent frequent collision, fusion, and division. Consequently, highly homochiral pre-cells (G-1-P-rich pre-cells and G-3-P-rich pre-cells) evolved. During this process, heterochiral pre-cells became extinct as a result of their instability. The enzymes, G-1-P dehydrogenase and G-3-P dehydrogenase, induced pre-cells to develop membranes with a pure enantiomeric GP backbone. These cells became the ancestors of Archaea and Bacteria, respectively. Because G-1-P dehydrogenase has no amino acid sequence similarity with G-3-P dehydrogenase, the stereospecificity of the enzymes could not be interchangeable (Koga et al. 1998; Daiyasu et al. 2005).

The recent phylogenetic analysis of lipid enzyme genes and recent studies on lipid biosynthesis, particularly the discoveries of the enzymes involved in the final steps of phospholipid synthesis (Morii and Koga 2003; Morii et al. 2009, 2010), resulted in new possibilities of providing evidence for the pre-cell membrane (e.g., How was it constituted?), and to answer the question of why the core lipids of Archaea and Bacteria are different while most organisms share the main polar head groups. To consider Wächtershäuser's hypothesis as a starting point for the following discussion, a hypothesis is proposed by which two kinds of core lipids (G-1-P-isoprenoid ether [Ai core] and G-3-P-fatty acid ester [Bf core]) were selected (survived) in Archaea and Bacteria. It is believed that discussing these problems will bring us closer to discovering the entity of the membrane lipids of common ancestor cells.

Characteristics of the Phospholipid Biosynthesis Pathway in Archaea and Bacteria: A Comparison

The archaeal phospholipid biosynthetic pathway and its specific characteristics have been reviewed in comparison with the bacterial counterpart (Koga and Morii 2007). After publication of the review, archaetidyl-*myo*-inositol phosphate (AIP) synthase (Morii et al. 2009) and phosphatidyl-*myo*-inositol phosphate (PIP) synthase (Morii et al. 2010) were discovered. The most notable features are briefly summarized in Fig. 1. First, the phospholipid biosynthesis pathways from Archaea and Bacteria are quite similar. The reaction order in the pathway of both domains follows the same pattern, and structural differences in the lipids are constructed intensively in the first half of the pathway (until completion of the core lipids). After formation of the core lipids by combining hydrocarbon chains with GP,

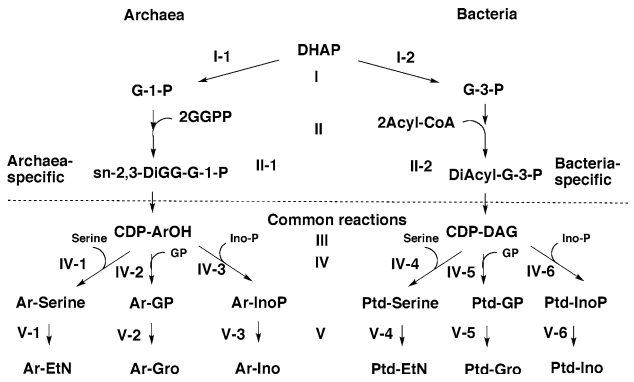

Fig. 1 Comparison of the biosynthetic pathways of major archaeal and bacterial phospholipids. *DHAP* dihydroxyacetonephos phate, *G-1-P sn*-glycerol-1-phosphate, *G-3-P sn*-glycerol-3-phosphate, *GGPP* geranylgeranylpyrophosphate, *sn-2,3-DiGG-G-1-P* 2,3-digeranylgeranyl-*sn*-glycerol-1-phosphate, *DiAcyl-G-3-P* 1,2-diacyl-*sn*-glycerol-3-phosphate, *CDP-ArOH* CDP-archaeol, *CDP-DAG* CDP-diacylglycerol, *Ar-Serine* archaetidylserine, *Ar-EtN* archaetidylethanolamine, *Ar-GP* archaetidylglycerophosphate, *Ar-InoP* archaetidylinositolphosphate, *Ar-Ino* archaetidylinositol, *Ptd-Serine* phosphatidylserine, *Ptd-EtN* phosphatidylethanoamine, *Ptd-GP* phosphatidylglycerophosphate, *Ptd-Gro* phosphatidylglycerol, *Ptd-InoP* phosphatidylinositolphosphate, *Ptd-Ino* phosphatidylinositol. Numbers *I–V* are the mode of reactions similar in Archaea and Bacteria. Numbers *I-1* to *V-6* are enzymes involved in the reactions. *I-1* G-1-P dehydrogenase, *I-2* G-3-P dehydrogenase, *II-1* DGGGP synthase, *II-2* phosphatidic acid synthase, *III* CDP-archaeol (CDP-DAG) synthase, *IV-1* Ar-Serine synthase, *IV-2* Ar-GP synthase, *IV-3* Ar-InoP synthase, *IV-4* Ptd-Serine synthase, *IV-5* Ptd-GP synthase, *IV-6* Ptd-InoP synthase, *V-1* Ar-Serine decarboxylase, *V-2* Ar-GroP phosphatase, *V-3* Ar-InoP phosphatase, *V-4* Ptd-Serine decarboxylase, *V-5* Ptd-GP phosphatase, *V*-6 Ptd-InoP phosphatase. The *upper part* of the figure shows synthesis of core lipids and the *lower half* of the figure depicts polar head group attachment reactions

amphiphilic phospholipids are synthesized by reactions to attach the polar head. The main polar head groups (L-serine, *myo*-inositol, and glycerol) are commonly found in Archaea and Bacteria, and the enzymes catalyzing these reactions in all organisms are homologous. At present, the enzymes that synthesize the major phospholipids, other than archaetidylglycerol phosphate (AGP) synthase have been characterized in vitro from at least one species from each domain. Consideration of the whole pathway highlights some remarkable characteristics of the CDP-alcohol phosphatidyltransferase family. These characteristics have not been discussed and might provide information regarding the early evolution of membrane lipids.

Characteristics of Enzymes of the CDP-Alcohol Phosphatidyltransferase Family

Physiologic Role

CDP-archaeol and CDP-diacylglycerol are central and common intermediates of the biosynthesis of various phospholipids (Hirabayashi et al. 1976; Nikawa and Yamashita 1997; Matsumoto 1997; Morii and Koga 2003, Morii et al. 2009, 2010). The polar group-specific enzymes catalyze the displacement of CMP of CDP-archaeol or CDP-diacylglycerol by a polar group to give rise to amphiphilic phospholipids.

$$\text{PS synthase}: \text{CDP-diacylglycerol} + \text{L-serine} \rightarrow \text{phosphatidylserine (PS)} + \text{CMP} \quad (1)$$

$$\text{AS synthase}: \text{CDP-archaeol} + \text{L-serine} \rightarrow \text{archaetidylserine (AS)} + \text{CMP} \quad (2)$$

$$\text{PIP synthase}: \text{CDP-diacylglycerol} + \textit{myo}\text{-inositol 1-phosphate} \rightarrow \text{PIP} + \text{CMP} \quad (3)$$

$$\text{AIP synthase}: \text{CDP-archaeol} + \textit{myo}\text{-inositol 1-phosphate} \rightarrow \text{AIP} + \text{CMP} \quad (4)$$

$$\text{PGP synthase}: \text{CDP-diacylglycerol} + \text{G-3-P} \rightarrow \text{phosphatidylglycerophosphate (PGP)} + \text{CMP} \quad (5)$$

$$\text{AGP synthase}: \text{CDP-archaeol} + \text{G-1-P} \rightarrow \text{AGP} + \text{CMP} \quad (6)$$

Amphiphilic phospholipids are synthesized for the first time at this step in this biosynthetic pathway. Because amphiphilic phospholipids are essentially required for the formation of biologic membranes, enzymes of the CDP-alcohol phosphatidyltransferase family play a key role in membrane formation. The mode of this reaction is common to the major polar groups and all the CDP-activated core lipids throughout all the domains of organisms (see the next subsection).

Ubiquitous Distribution and Homology

Only archaetidylserine (AS) synthase (reaction (2)) and AIP synthase (reaction (4)) from *Methanothermobacter thermautotrophicus* cells have been characterized in vitro as archaeal CDP-alcohol phosphatidyltransferase members. The bacterial mechanism of PI synthesis has been revised (Morii et al. 2010). Although they have not been purified, their primary structure, monomeric molecular mass, and the presence of a specific motif have been determined based on the genomic information. A BLAST search using AS synthase of *M. thermautotrophicus* as a query sequence detected a number of homologs, including AS synthase from different archaeal species and bacterial PS synthase (type II). In addition to serine phospholipid synthases, PGP synthase and PIP synthase from Bacteria and Eukarya and hypothetical proteins from various Archaea were detected with low similarity (Daiyasu et al. 2005). A database search using a *Pyrococcus abyssi* hypothetical protein homologous to *Bacillus subtilis* PGP synthase detected

PGP synthase and PI(P) synthase from a wide variety of Bacteria and Eukarya and AGP synthase and AIP synthase from Archaea (Daiyasu et al. 2005). These results suggest that the AS, AGP, AIP, PS, PGP, and PIP synthase are all homologous.

Daiyasu et al. (2005) constructed phylogenetic trees of homologous serine phospholipid synthases and inositol phospholipid synthases. The latter tree contained glycerol phospholipid synthases from various Archaea in addition to inositol phospholipid synthases. These enzymes belong to an enzyme super family, the CDP-alcohol phosphatidyltransferase family. The pfam home page (http://pfam.sanger.ac.uk/family?acc=PF01066) depicts a phylogenetic tree of thousands of the enzymes belonging to the CDP-alcohol phosphatidyltransferase family, which includes Archaea, Bacteria, and Eucarya as organisms, and phospholipid synthases with the major polar head groups. Currently, there is one known exception: that of type I PS synthase from *E. coli* and its relatives (Matsumoto 1997), which is derived from another ancestral enzyme.

The ubiquitous occurrence and homologic nature of the phospholipid synthases suggest that the ancestral enzyme(s) existed in universal common ancestor cells. Consequently, the ancestral enzyme(s) would have functioned to synthesize the amphiphilic phospholipids in universal common ancestors. Because lipid bilayer membranes comprise amphiphilic phospholipids, the universal common ancestor membrane should have lipid bilayer membranes.

Various Core Lipids in Universal Common Ancestor Membranes

Pereto et al. (2004) analyzed families of G-1-P dehydrogenase and G-3-P dehydrogenase and bacterial genes of fatty acid synthesis in Archaea. They suggested that the universal common ancestor had ancestral G-1-P dehydrogenase and G-3-P dehydrogenase, as well as those for the synthesis of fatty acids, so that it probably had a membrane with a mixture of G-1-P-fatty acids and G-3-P-fatty acids. More recently, Lombard and Moreira (2010) showed that Bacteria had not only a methylerythrytol phosphate pathway for isoprenoid biosynthesis but also had a mevalonate pathway other than horizontal gene transfer from other domains, suggesting that the universal common ancestor most likely also had a pathway for the synthesis of isoprenoid chains. Consequently, the mixture of materials of phospholipid constituents, G-1-P and G-3-P, fatty acids and isoprenoids were synthesized in the universal common ancestor. This suggests the possibility that every combination of the lipid components forms Ai, Bf, Af, and Bi with ether or ester bonds.

On the other hand, Morii and Koga (2003) investigated the core lipid specificity of two enzymes of the CDP-alcohol phosphatidyltransferase family, AS synthase of *M. thermautotrophicus* (an archaeon) and PS synthase of *B. subtilis* (a bacterium). Various chemically synthesized analogs of CDP-archaeol and CDP-diacylglycerol were used for the experiments. AS synthase and PS synthase naturally catalyzed the reactions (1) and (2), respectively. In addition, the two enzymes were active with several other unnatural substrates including Ai, Bf, Af, and Bi (Table 1). The relative activities with the unnatural CDP-activated core lipids were almost identical to or even higher than that with the natural substrate.

Such reactivity with an unnatural lipid substrate was observed not only for serine phospholipid synthesizing enzymes but also for the AIP synthase from *M. thermautotrophicus* and PIP synthase in *Mycobacterium smegmatis*. Archaetidyl-*myo*-inositol phosphate synthase catalyzes reaction (3), but CDP-diacylglycerol also served as a substrate with approximately 20% activity (Morii, personal communication). Phosphatidyl-*myo*-inositol phosphate synthase from *M. smegmatis* was active with an unnatural substrate, CDP-archaeol with a G-1-P backbone, as well as the natural substrate, CDP-diacylglycerol with a G-3-P backbone.

The phylogenetic analyses of lipid synthesizing enzymes and the wide range of reactivity of extant phospholipid synthases described above suggests that the four or more types of core lipids most likely existed in the universal common ancestors. Even if the loose core substrate specificity does not directly suggest the presence of a wide range of core lipids in the universal common ancestor, it has important significance when other evidence such as that described above, implies the presence of multiple core lipids in the universal common ancestor. If the enzyme works with only one type of core lipid, in either one of the two domains of life (Archaea and Bacteria) after divergence, then the enzyme does not work well and would not continue to survive.

Why Do Only Ai and Bf Core Lipids Exist? Involvement of Hydrocarbon Chain Interactions in the Segregation of Enantiomeric Core Lipids: A Hypothesis

There may be eight or more core lipid species composed of combinations of GPs, isoprenoid or fatty acid, and two types of linkages (ether and ester). Here, the situation is simplified by limiting a lipid molecule to containing two GP enantiomers, isoprenoid ether and fatty acyl ester lipids and the same two hydrocarbon chains. As a result, Ai (ether), Bf (ester), Af (ester), and Bi (ether) would have been present in the universal common ancestor. The fact that archaeal and bacterial homologous enzymes of the

Table 1 Reactivity of CDP-alcohol phosphatidyltransferase family enzymes to various kinds of CDP-bound core lipid substrates with an enantiomeric GP backbone, and either an isoprenoid or fatty acid

Exp. No.	CDP-alcohol substrate			Relative activity (%)			
	Core lipid	Hydrocarbon	GP backbone	ASS[2]	PSS[3]	AIPS[4]	PIPS[5]
1	Ai ether	Geranylgeranyl	G-1-P	100	80		
2	Ai ether	Phytanyl	G-1-P	86	120	100	39
3	Bi ether	Geranylgeranyl	G-3-P	149	91		
4	Bf ether	C18:1[1]	G-3-P	64	102		
5	Af ester	C18:1	G-1-P	280	143		
6	Bf ester	C18:1	G-3-P	199	100	20	100

Data from Journal of Bacteriology (2003) 1181–1189 doi:10.1128/JB.185.4.1181-1189.2003. Reproduced and amended with permission from the American Society for Microbiology, and from H. Morii (personal communication)

[1] Oleoyl

[2] Archaetidylserine synthase from *Methanothermobacter thermautotrophicus*

[3] Phosphatidylserine synthase from *Bacillus subtilis*

[4] Archaetidylinositolphosphate synthase from *Methanothermobacter thermautotrophicus*

[5] Phosphatidylinositolphosphate synthase from *Mycobacterium smegmatis*

CDP-alcohol phosphatidyltransferase family possess similar activities with unnatural substrates suggests that the three hidden activities could be retrospectively related to the ancestral enzyme present in the universal common ancestor. The wide range of reactivity of phospholipid synthases with the four types of core lipids allows us to imagine that the four types of core lipids could readily exist in the ancestor cells.

Phospholipid synthases of the CDP-alcohol phosphatidyltransferase family are ubiquitously distributed over the three domains of life and active with a wide range of CDP-core substrates. These facts suggest that the universal common ancestor membranes comprised a lipid bilayer with four types of core lipids (Ai, Bf, Af, and Bi). If this was the case, the segregation of membrane phospholipids with GP enantiomers as the backbones could cause the differentiation of G-1-P lipid with either isoprenoid or fatty acids (Ai or Af) and G-3-P lipid with either isoprenoid or fatty acids (Bi or Bf), assuming that isoprenoid lipid is ether lipid and fatty acid lipid is ester lipid. To segregate and survive as Ai and Bf lipids, not only the GP enantiomer backbones but also the hydrocarbon chains must be involved in this process. A hypothetical mechanism for the segregation of the four core lipids is presented below.

Why the G-1-P backbone combines with isoprenoid to form the archaeal core lipid (the Ai type core lipid), and why the Bf core lipid exists in Bacteria remain unanswered questions. The wide range of specificity of phospholipid synthases and retrospective inference of the phylogeny of lipid enzymes suggest that phospholipids with the four types of core lipids existed in the pre-cells and one enzyme was sufficient for phospholipid synthesis from all core lipid types.

Although Wächtershäuser assumed that lipids with each enantiomer of the GP backbone were segregated to constitute more homochiral membranes, the effect of the hydrocarbon chain interaction in the core lipids was not discussed. Here, the effect of hydrophobic hydrocarbon chain interaction on membrane segregation is speculated (Fig. 2).

Differentiation of organisms with G-1-P lipids and with G-3-P lipids does not take into account environmental selective pressure, because enantiomers are physicochemically on equal footing and therefore selective pressure would not apply.

Two types of lipids with enantiomeric GP backbones tend to segregate according to the hypothesis presented by Wächtershäuser. This is assumed to be the main driving force for membrane segregation. A complementary assumption is as follows. Segregation of two lipids would be prompted if the two lipids have different types of hydrocarbon chains, but not in the case of the two lipids with the same kind of hydrocarbon chain. Phospholipids with identical hydrocarbon chains generally tend to cluster in monophasic patches, but phase separation might occur with a mixture of different hydrocarbons. Even a slight difference in the molecular shape of hydrocarbons, e.g., DPPC and DMPC (only a two-carbon difference in fatty acids) affords phase separation under certain conditions (Mabrey and Sturtevant 1976).

At present, however, the physicochemical properties of mixed hydrocarbons of isoprenoid lipids and fatty acid lipids are not known. Isoprenoid chains and typical fatty acid chains have largely different molecular shapes; isoprenoid chains are characterized by four methyl branches on the main chain at every four carbon atoms, and most

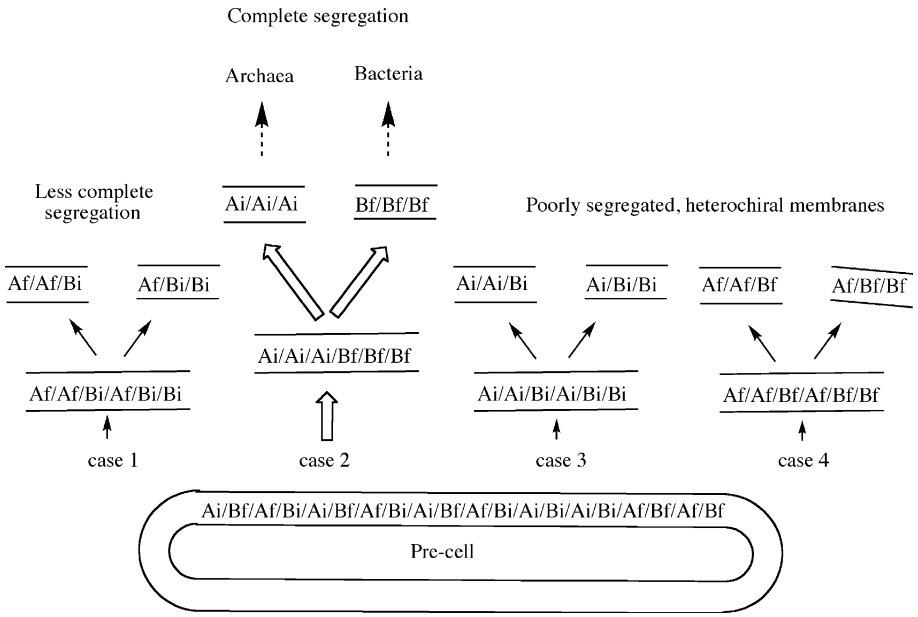

Fig. 2 A hypothetical phospholipid segregation (phase separation) model in the pre-cell membrane lipids composed of Ai, Bf, Af, and Bi cores (see text for abbreviations). According to Wächtershäuser's theory, the enantiomeric GP backbones (G-1-P (A) and G-3-P(B)) play a major role in lipid segregation, i.e., the difference in the GP backbones is the main driving force of membrane lipid segregation. In addition, interactions between hydrocarbon chains play a supplementary role in membrane lipid segregation. That is, the quite different hydrocarbon chains stimulate segregation (*case 2*, Ai:Bf), but the same kinds of hydrocarbon chains exert no additional effect on segregation (*case 3*, Ai:Bi and *4*, Af:Bf). Whereas case 1 seems to be the reverse of *case 2*, Bi and Ai are not enantiomers but diastereomers because the phytanyl group has three *R* chiral centers. *sn*-G-1-P is in (*S*)- and *sn*-G-3-P has (*R*)-configuration, respectively (see Fig. 3). Therefore, Ai:Bf and Af:Bi do not have equivalent tendencies toward segregation. Although at present, because of the lack of physicochemical data on the mixed lipids, it cannot be determined whether Ai:Bf or Af:Bi is better, it is assumed that one of the two possibilities (*case 1* or *2*) would be the most easily segregated. In *cases 3* and *4*, because the same hydrocarbons are in tendency to aggregate rather than segregate, segregation of *A* and *B* is not accelerated

isoprenoid chains of archaeal phospholipids are saturated, whereas fatty acyl chains have no or only one methyl branch and often have a *cis* double bond, at which the chain is kinked. Thus, the physicochemical properties of these chains differ and the effect of the hydrocarbon interaction on segregation is significant.

The tendency toward segregation of two types of lipids, Ai:Bf and Af:Bi, seems to be identical. Saturated isoprenoid chains (phytanyl group), however, have three chiral centers at the methyl branching points (the positions 3, 7, and 11), all of which are in an *R* configuration (Kates et al. 1967). Because the chiral center of the position 2 carbon of G-1-P is in an *S* configuration, Ai has asymmetric carbon atoms in an *S,R,R,R,R,R,R*, whereas Bi has an *R,R,R,R,R,R,R* configuration (Fig. 3). These are diastereomers. On the other hand, fatty acid generally does not have an asymmetric carbon, and the chiral center of Af and Bf are in an *S* and *R* configuration in GP, respectively.

Af:Bi and Ai:Bf are not equivalent in the stereostructure of the whole molecules. That is, Ai and Bi are diastereomers (non-enantiomeric stereo isomers with two or more chiral centers), while Af and Bf are enantiomeric (in a strict sense the two fatty acyl chains are identical). Diastereomers have different physicochemical properties as different compounds, whereas enantiomers have identical properties except for their chiral properties. Thus, Ai:Bf and Af:Bi do not have equivalent tendencies toward segregation. Although at present, because of the lack of physicochemical data on the mixed lipids, it cannot be determined whether Ai:Bf or Af:Bi is better, it is assumed that one of the two possibilities of Ai:Bf would be the most easily segregated. As a result, descendent cells with Ai lipid membranes became the ancestor of Archaea and descendent cells with Bf lipid membranes became the ancestor of Bacteria.

Cellular Localization of CDP-Alcohol Phosphatidyltransferase, Core Lipids, and Polar Head Groups

The first half of the archaeal and bacterial phospholipid biosynthetic pathway is devoted to building up core lipids. Reactions at or after the second ether or ester bond formation in these pathways proceed on the membrane. (Hemmi et al. 2004; Morii et al. 2000; Dowhan 1997; Okuyama and Wakil 1973; Wilkison and Bell 1997). That is, the construction of core lipids completed on the

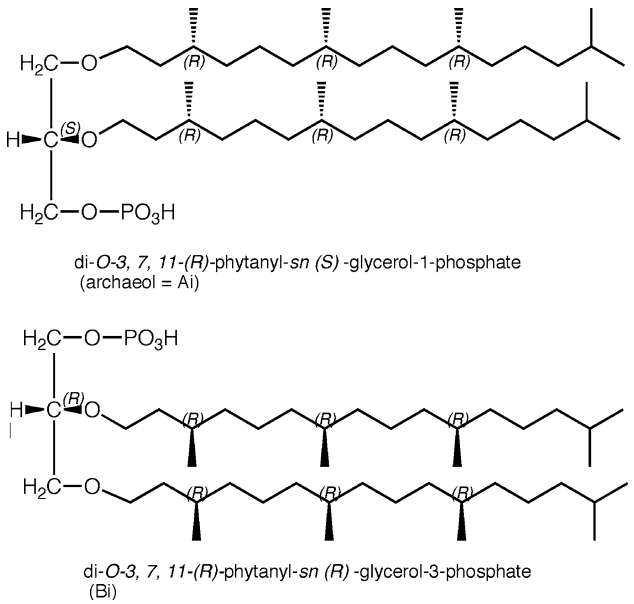

Fig. 3 Diastereomeric relationship of Ai and Bi. Ai and Bi have seven asymmetric carbons. One of them is the carbon 2 of GP (*R* and *S*) (G-1-P is (*S*) and G-3-P is (*R*)) and the other six are 3, 7, 11, 3′, 7′, 11′-carbons that are all *R*. Thus, Ai has an *S, R,R,R,R,R,R* configuration, and Bi has an *R,R,R,R,R,R,R* configuration. Therefore, Ai and Bi are diastereomers

membranes. On the other hand, polar head groups of membrane phospholipids, which are water-soluble compounds before binding to a core lipid, are synthesized from the intermediates of glycolysis or gluconeogenesis in a cellular cytoplasmic compartment. L-serine synthesis starts with phosphoenolpyruvate, GP is formed from dihydroxyacetonephosphate, and *myo*-inositol-1-phosphate is synthesized from D-glucose-6-phosphate. These intermediates and the relevant enzymes are all water-soluble and are present in the cytoplasmic fraction. After activation of a core lipid by CDP, a core lipid and a polar head group are linked together on a membrane to an amphiphilic molecule by a membrane-bound enzyme phosphatidyl-*X* synthase or archaetidyl-*X* synthase (*X* is a polar group).

The common distribution of phospholipid polar head groups may indicate that both polar head groups and their synthesizing systems were equally transferred to Archaea and Bacteria through the fission (division) of pre-cells. This, in turn, suggests that polar head groups are not a driving force in the differentiation of Archaea and Bacteria. The fact that Archaea and Bacteria possess common polar groups and enzymes suggests the presence of common ancestor cells in which polar head groups and their synthesizing systems already existed.

The cytoplasm is equally divided into both daughter cells. This is the reason for the common occurrence of polar groups in archaeal and bacterial phospholipids. CDP-alcohol phosphatidyltransferase enzymes were equally distributed in the both domains of organisms even though they were membrane-bound enzymes but they were not a driving force of membrane segregation.

Discussion

The characteristic features of the ubiquitous distribution, homology covering three domains of organisms, and reactivity to a wide range of core lipid substrates, of CDP-alcohol phosphatidyltransferase family enzymes support the hypothesis that before the differentiation of Archaea and Bacteria the membranes of pre-cells comprised a lipid bilayer with four types of core lipids (Ai, Af, Bi, and Bf). The biochemical observations and bioinformatics data support and extend Wächtershäuser's two-lipid pre-cell theory.

How the clear-cut distribution of Ai and Bf lipids occurred is one of the most pressing problems in the fields of biochemistry and evolutionary science. In this study, composition of the lipid membrane of the universal common ancestor (pre-cells) just prior to the differentiation of Archaea and Bacteria is inferred from extant biochemical knowledge. Four types of core lipids existed in the pre-cells. There is little evidence for the process of selection of two of the four types of core lipids. Therefore, the emergence of Archaea and Bacteria is based on speculation. A logical statement may clarify the cause of the lipid divide. Homochiral membranes are segregated to Archaea-like membranes and Bacteria-like membranes. If Archaea and Bacteria were differentiated by causes other than the segregation of homochiral lipid patches, Archaea and Bacteria would have an equal mix of Ai type and Bf type lipids, because lipids are in a fluid state (Singer and Nicolson 1972) and a variety of lipid species would be mixed in the membrane. This is not the case. The Ai and Bf lipids are clearly segregated. Auto(self)segregation of homochiral lipid membranes is one of the driving forces of the differentiation of Archaea and Bacteria, i.e., the "lipid divide" is caused by membrane-driven evolution. To test this hypothesis, two experimental approaches are conceivable; one is physicochemical research of liposomes of mixed phospholipids with Ai, Bf, Af, and Bi cores; the other is observation of genetically engineered bacteria, in which archaeal phospholipid synthetic genes have been inserted.

The popular viewpoint that archaeal ether lipids emerged to adapt to extreme (high temperature, high acidity, or high salt concentration) environments has one major deficiency: the *sn*-2, 3 configuration is not at all related to extremotolerance.

Archaeal lipids did not necessarily emerge to adapt to extreme environments, because archaeal polar lipids are synthesized through unstable allyl intermediates (Chen

et al. 1993; Hemmi et al. 2004; Morii et al. 2000). Although completed (saturated) ether core lipids are stable, the first and second ether intermediates, GGGP and DGGGP, are allyl ethers. Allyl ether bonds have an experimentally similar level of acid and heat stability as fatty acyl ester bonds. (Hydrocarbon chains of DGGGP and fatty acyl ester lipids are cleaved off under the same condition; Koga and Morii 2006). Further, the intermediate exists in archaeal cells in a small amount. If fatty acyl ester lipids were so fragile in extreme conditions to affect the survival of an organism, allyl ether lipids would also not be tolerant. A phosphodiester bond between a core lipid and a polar head group is shared by Archaea and Bacteria, which are hydrolyzable under identical conditions, especially for polyol head groups (glycerol and inositol). Although archaeal ether lipids appear to be tough, they also have their weak points.

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References

Chen AJ, Zhang DL, Poulter CD (1993) (*S*)-Geranylgeranylglyceryl phosphate synthase—purification and characterization of the first pathway-specific enzyme in archaebacterial membrane lipid biosynthesis. J Biol Chem 268:21701–21705

Daiyasu H, Kuma K, Yokoi T, Morii H, Koga Y, Toh H (2005) A study on archaeal enzymes involved in polar lipids synthesis by an approach linking the information about amino acid sequences, genomic contexts and lipid composition. Archaea 1:399–410

Dowhan W (1997) CDP-diacylglycerol synthase of microorganisms. Biochim Biophys Acta 1348:157–165

Gattinger A, Schloter M, Munch JC (2002) Phospholipid ether lipid and phospholipid fatty acid fingerprints in selected euryarchaeotal monocultures for taxonomic profiling. FEMS Microbiol Lett 213:133–139

Glansdorff N, Xu Y, Labedan B (2008) The last universal common ancestor: emergence, construction and genetic legacy of an elusive forerunner. Biol Direct 3:29 (http://www.biology-direct.com/content/3/1/29)

Hemmi H, Shibuya K, Takahashi Y, Nakayama T, Nishino T (2004) (S)-2,3-Di-O-geranylgeranylglyceryl phosphate synthase from the thermoacidophilic archaeon *Sulfolobus solfataricus*. Molecular cloning and characterization of a membrane-intrinsic prenyltransferase involved in the biosynthesis of archaeal ether-linked membrane lipids. J Biol Chem 279:50195–50203

Hirabayashi T, Larson TJ, Dowhan W (1976) Membrane-associated phosphatidylglycerophosphate synthetase from *Escherichia coli:* purification by substrate affinity chromatography on cytidine 5'-diphospho-1, 2-diacyl-*sn*-glycerol sepharose. Biochemistry 15:5205–5211

Kates M (1978) The phytanyl ether-linked polar lipids and isoprenoid neutral lipids of extremely halophilic bacteria. Prog Chem Fats other Lipids 15:301–342

Kates M, Joo CN, Palameta B, Shier T (1967) Absolute stereochmical configuration of phytanyl (dihydrophytyl) Groups in lipids of *Halobacterium cutirubrum*. Biochemistry 6:3329–3338

Koga Y, Morii H (2005) Recent advances in structural research on ether lipids from Archaea including its comparative and physiological aspects. Biosci Biotechnol Biochem 69:2019–2034

Koga Y, Morii H (2006) Special methods for the analysis of ether lipid structure and metabolism in archaea. Anal Biochem 348:1–14

Koga Y, Morii H (2007) Biosynthesis of ether-type polar lipids in Archaea and evolutionary considerations. Microbiol Mol Biol Rev 71:97–120

Koga Y, Nishihara M, Morii H, Akagawa-Matsushita M (1993) Ether polar lipids of methanogenic bacteria. Structures, comparative aspects, and biosyntheses. Microbiol Rev 57:164–182

Koga Y, Kyuragi T, Nishihara M, Sone N (1998) Did archaeal and bacterial cells arise independently from noncellular precursors? A hypothesis stating that the advent of membrane phospholipid with enantiomeric glycerophosphate backbones caused the separation of the two lines of descent. J Mol Evol 46:54–63

Lombard J, Moreira D (2011) Origins and early evolution of the mevalonate pathway of isoprenoid biosynthesis in the three domains of life. Mol Biol Evol 28:87–99. doi:10.1093/molbev/msq177

Mabrey S, Sturtevant JM (1976) Investigation of phase transition of lipid mixtures by high sensitivity differential scanning calorimetry. Proc Natl Acad Sci USA 73:3862–3866

Martin W, Russell MI (2003) On the origin of cells: a hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells. Philos Trans R Soc B 358:59–85

Matsumoto K (1997) Phosphatidylserine synthase from bacteria. Biochim Biophys Acta 1348:214–227

Morigaki K, Dallavalle S, Walde P, Colonna S, Luisi PL (1997) Autopoietic self-reproduction of a chiral fatty acid vesicles. J Am Chem Soc 119:292–301

Morii H, Koga Y (2003) CDP-2,3-di-*O*-geranylgeranyl-*sn*-glycerol:L-serine *O*-archaetidyltransferase (archaetidylserine synthase) in the methanogenic archaeon *Methanothermobacter thermautotrophicus*. J Bacteriol 185:1181–1189

Morii H, Nishihara M, Koga Y (2000) CTP:2,3-di-*O*-geranylgeranyl-sn-glycero-1-phosphate cytidyltransferase in the methanogenic archaeon *Methanothermobacter thermautotrophicus*. J Biol Chem 275:36568–36574

Morii H, Kiyonari S, Ishino Y, Koga Y (2009) A novel biosynthetic pathway of archaetidyl-*myo*-inositol via archaetidyl-*myo*-inositol 1-phosphate from CDP-archaeol and D-glucose 6-phosphate in the methanoarchaeon *Methanothermobacter thermautotrophicus*. J Biol Chem 284:30766–30774

Morii H, Ogawa M, Fukuda K, Taniguchi H, Koga Y (2010) A revised biosynthrtic pathway for phosphatidylinositol in mycobacteria. J Biochem 148:593–602

Nikawa J, Yamashita S (1997) Phosphatidylinositol synthase from yeast. Biochim Biophys Acta 1348:173–178

Okuyama H, Wakil SJ (1973) Positional specificities of acyl coenzyme A: glycerophosphate and acyl coenzyme A: monoacylglycerophosphate acyltransferases in *Escherichia coli*. J Biol Chem 248:5197–5205

Payandeh J, Fujihashi M, Gillon W, Pai EF (2006) The crystal structure of (*S*)-3-*O*-geranylgeranylglyceryl phosphate synthase reveals an ancient fold for an ancient enzyme. J Biol Chem 281:6070–6078

Pereto J, Lopez-Garcia P, Moreira D (2004) Ancestral lipid biosynthesis and early membrane evolution. Trends Biochem Sci 29:469–477

Singer SJ, Nicolson GL (1972) The fluid mosaic model of the structure of cell membranes. Science 175:720–731

Wächtershäuser G (2003) From pre-cells to Eukarya—a tale of two lipids. Mol Microbiol 47:13–22

Wilkison WO, Bell RM (1997) *sn*-Glycerol-phosphate acyltransferase from *Escherichia coli*. Biochim Biophys Acta 1348:3–9

Woese CR, Kandler O, Wheelis ML (1990) Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci USA 87:4576–4579