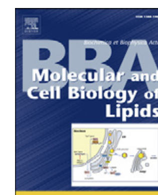
Biochimica et Biophysica Acta 1811 (2011) 607–616

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbalip



The thermoacidophilic archaeon *Sulfolobus acidocaldarius* contains an unusually short, highly reduced dolichyl phosphate

Ziqiang Guan [a], Benjamin H. Meyer [b], Sonja-Verena Albers [b], Jerry Eichler [c,*]

[a] Department of Biochemistry, Duke University Medical Center, Durham, NC 27710, USA
[b] Molecular Biology of Archaea, Max-Planck-Institute for Terrestrial Microbiology, D-35043 Marburg, Germany
[c] Department of Life Sciences, Ben Gurion University of the Negev, Beersheva 84105, Israel

ARTICLE INFO

Article history:
Received 6 May 2011
Received in revised form 16 June 2011
Accepted 17 June 2011
Available online 1 July 2011

Keywords:
Archaea
Dolichol
Electrospray ionization mass spectrometry
Polyprenol
Polyprenol reductase
Sulfolobus acidocaldarius

ABSTRACT

Polyprenoids, polymers containing varied numbers of isoprene subunits, serve numerous roles in biology. In Eukarya, dolichyl phosphate, a phosphorylated polyprenol bearing a saturated α-end isoprene subunit, serves as the glycan carrier during N-glycosylation, namely that post-translational modification whereby glycans are covalently linked to select asparagine residues of a target protein. As in Eukarya, N-glycosylation in Archaea also relies on phosphorylated dolichol. In this report, LC-ESI/MS/MS was employed to identify a novel dolichyl phosphate (DolP) in the thermoacidophilic archaeon, *Sulfolobus acidocaldarius*. The unusually short *S. acidocaldarius* DolP presents a degree of saturation not previously reported. *S. acidocaldarius* DolP contains not only the saturated α- and ω-end isoprene subunits observed in other archaeal DolPs, but also up to five saturated intra-chain isoprene subunits. The corresponding dolichol and hexose-charged DolP species were also detected. The results of the present study offer valuable information on the biogenesis and potential properties of this unique DolP. Furthermore, elucidation of the mechanism of α-isoprene unit reduction in *S. acidocaldarius* dolichol may facilitate the identification of the alternative, as yet unknown polyprenol reductase in Eukarya.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Polyprenoids correspond to a group of hydrophobic polymers that comprise up to 100 isoprene subunits linked head-to-tail, with the resulting chain presenting a hydroxy group at the α-end [for review, see 1,2]. Reduction of the double bond in the α-end isoprene subunit gives rise to the dolichols. While polyprenoids serve a variety of biological roles [1,3–5], phosphorylated polyprenols and dolichols are central components in pathways of N-glycosylation, a post-translational modification involving the covalent attachment of glycans to select asparagine residues of target proteins. Across evolution, asparagine-linked glycans are initially assembled on phosphopolyprenol carriers, namely dolichyl phosphate (DolP) in Eukarya and Archaea and polyprenol phosphate (typically undecaprenol phosphate (UndP)) in Bacteria [1,2,6].

The biosynthesis of polyprenoids begins with formation of the building blocks of isoprene-based molecules, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). IPP and DMAPP are generated by either the classic mevalonate (MVA) pathway or by the mevalonate-independent deoxyxylulose phosphate (DOXP) pathway [7–10]. The subsequent union of DMAPP and several IPP subunits produces polyprenoids. These condensation reactions are mediated by prenyltransferases, which are also responsible for determining polyprenol *cis*/*trans* stereochemistry. In this manner, all-*trans* farnesyl (C_{15}) or geranylgeranyl (C_{20}) diphosphate are elongated into *cis*/*trans* polyprenol diphosphates.

Despite over 40 years of research and the resulting wealth of information available on polyprenol biosynthesis, many details remain unclear, particularly concerning those steps involved in the conversion of polyprenol diphosphate to DolP. It is generally thought that polyprenol diphosphate undergoes two rounds of dephosphorylation to produce polyprenol. Reduction of the polyprenol α-position isoprene subunit yields dolichol, which is in turn phosphorylated to produce DolP [11]. Most of the enzymes catalyzing these reactions have, however, yet to be identified. Recently, mammalian SRD5A3 and its yeast ortholog, DFG10, were shown to be polyprenol reductases

Abbreviations: Acetate, Ac; Chloroform, $CHCl_3$; *cis*-polyprenyl diphosphate synthase, CPDS; Collision-induced dissociation, CID; Deoxyxylulose phosphate, DOXP; Dimethylallyl diphosphate, DMAPP; Dolichyl phosphate, DolP; Geranylgeranyl diphosphate, GGPP; Geranylgeranyl reductase, GGR; Isopentenyl diphosphate, IPP; Methanol, MeOH; Mevalonate, MVA; Multiple reaction monitoring, MRM; Tandem mass spectrometry, MS/MS; Undecaprenol phosphate, UndP

* Corresponding author at: Dept. of Life Sciences, Ben Gurion University of the Negev, P.O. Box 653, Beersheva 84105, Israel. Tel.: +972 8646 1343; fax: +972 8647 9175.
E-mail address: jeichler@bgu.ac.il (J. Eichler).

1388-1981/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbalip.2011.06.022

responsible for converting polyprenol into dolichol [12]. Nonetheless, some dolichol is produced in cells lacking the encoding genes, indicating that additional polyprenol reductase(s) must exist. Moreover, those phosphatases responsible for transforming polyprenol diphosphate into polyprenol remain unknown.

The search for as yet unidentified enzymes involved in DolP biogenesis may benefit from the study of Archaea. Archaea are a group of microorganisms that comprise a distinct branch of the tree of life that also includes eukaryal and bacterial limbs [13]. Although their ubiquitous distribution is now clear [14,15], Archaea remain best known as extremophiles, namely organisms able to thrive in some of the most physically challenging environments on Earth, being found at extremes of temperature, pH and salinity and other conditions seemingly inhospitable to life [16]. Possibly in response to their harsh surroundings, Archaea have often adopted unique biochemical strategies. For example, in the archaeal version of the MVA pathway, mevalonate-5-phosphate is decarboxylated to produce isopentenyl phosphate, which is in turn phosphorylated to generate IPP [17–19]. This differs from the classic version of the pathway, where mevalonate-5-phosphate is phosphorylated to yield mevalonate-5-pyrophosphate, which is subsequently decarboxylated to form IPP. Likewise, examples of archaeal DolP structurally distinct from their eukaryal counterparts have been presented. In C_{55} and C_{60} DolP of the halophilic archaeon, Haloferax volcanii, shown to participate in N-glycosylation, both the **α**- and the **ω**-position isoprene subunits are saturated [20–22]; in the eukaryal lipid, only the α-position isoprene subunit is saturated [1,2].

In the following, another unusual DolP of archaeal origin is reported. *Sulfolobus acidocaldarius*, a thermoacidophilic archaeon that grows optimally at 80 °C and pH 2 [23], contains a version of the molecule that is both shorter than eukaryal or other known archaeal DolP and which presents a degree of saturation never seen before.

2. Materials and methods

2.1. Strain and growth

S. acidocaldarius (DSM639) were grown in Brock's medium at 76 °C, pH-adjusted to 3 using sulphuric acid and supplemented with 0.1% (w/v) tryptone [23].

2.2. Lipid extraction

A *S. acidocaldarius* lipid extract was prepared according to Bligh and Dyer [24]. Briefly, 1.6 ml of PBS, 2 ml chloroform ($CHCl_3$) and 4 ml methanol (MeOH) were added to 0.5 g of lyophilized *S. acidocaldarius* cell powder in a 15 ml glass tube with a Teflon-lined cap to yield a single phase ($CHCl_3$:MeOH:PBS = 1:2:0.8, v/v) solution. Following intermittent mixing by vortex for 5 min, the mixture was sonicated in a water bath for 15 min at room temperature (25 °C). The sample was centrifuged (3000 rpm) for 5 min at room temperature and the supernatant was transferred to a fresh 15 ml glass tub with a Teflon-lined cap. Two milliliters $CHCl_3$ and 2 ml PBS were added to yield a two-phase Bligh-Dyer mixture ($CHCl_3$:MeOH:PBS = 2:2:1.8, v/v). After mixing, the sample was centrifuged (3000 rpm) for 5 min at room temperature to separate the phases. The upper phase was removed and the lower $CHCl_3$ phase was dried under a stream of nitrogen. The dried lipid extract was stored at −20 °C until further analyzed.

2.3. LC-ESI/MS

Normal phase LC-ESI/MS of the *S. acidocaldarius* lipid extract was performed using an Agilent 1200 Quaternary LC system coupled to a QSTAR XL quadrupole time-of-flight tandem mass spectrometer (Applied Biosystems, Foster City, CA). An Ascentis Si HPLC column (5 μm, 25 cm × 2.1 mm) was used. Mobile phase A consisted of chloroform/methanol/aqueous ammonium hydroxide (800:195:5, v/v/v). Mobile phase B consisted of chloroform/methanol/water/aqueous ammonium hydroxide (600:340:50:5, v/v/v/v). Mobile phase C consisted of chloroform/methanol/water/aqueous ammonium hydroxide (450:450:95:5, v/v/v/v). The elution program consisted of the following: 100% mobile phase A was held isocratically for 2 min and then linearly increased to 100% mobile phase B over 14 min and held at 100% B for 11 min. The LC gradient was then changed to 100% mobile phase C over 3 min and held at 100% C for 3 min, and finally returned to 100% A over 0.5 min and held at 100% A for 5 min. The total LC flow rate was 300 μl/min. The post-column splitter diverted ~10% of the LC flow to the ESI source of the Q-Star XL mass spectrometer, with MS settings as follows: Ion spray voltage (IS) = −4500 V, Curtain gas (CUR) = 20 psi, Ion source gas 1 (GS1) = 20 psi, De-clustering potential (DP) = −55 V, and FP = −150 V. Nitrogen was used as the collision gas for MS/MS experiments. Data acquisition and analysis were performed using the instrument's Analyst QS software.

Reverse phase LC-ESI/MS of the *S. acidocaldarius* lipid extract was performed using a Shimadzu LC system (comprising a solvent degasser, two LC-10A pumps and a SCL-10A system controller) coupled to a QSTAR XL quadrupole time-of-flight tandem mass spectrometer (as above). LC was operated at a flow rate of 200 μl/min with a linear gradient as follows: 100% of mobile phase A was held isocratically for 2 min and then linearly increased to 100% mobile phase B over 14 min and held at 100% B for 4 min. Mobile phase A consisted of methanol/acetonitrile/aqueous 1 mM ammonium acetate (60/20/20, v/v/v). Mobile phase B consisted of 100% ethanol containing 1 mM ammonium acetate. A Zorbax SB-C8 reversed-phase column (5 μm, 2.1 × 50 mm) was obtained from Agilent (Palo Alto, CA). The MS operating conditions were as above.

2.4. LC-multiple reaction monitoring

Normal phase LC coupled with multiple reaction monitoring (MRM) was performed using an Agilent 1200 Quaternary LC system interfaced to a 4000 Q-Trap hybrid triple quadrupole linear ion-trap mass spectrometer equipped with a Turbo V ion source (Applied Biosystems). The LC conditions were the same as described above. The post-column splitter diverted ~10% of the LC flow to the Turbo V ion source. MRM was performed in the negative ion mode with MS settings as follows: CUR = 20 psi; GS1 = 20 psi; ion source gas 2 (GS2) = 30 psi; IS = −4500 V; heater temperature (TEM) = 350 °C; interface heater = ON; DP = −40 V; entrance potential (EP) = −10 V; collision cell exit potential (CXP) = −5 V. The MRM pairs are as follows: 719.6/79.0 (C_{45} DolP from *S. acidocaldarius*), 917.8/79.0 (C_{60} DolP from *Hfx. volcanii*), 1392.2/79.0 (C_{95} DolP from human fibroblasts), and 845.6/79.0 (C_{55} UndP from *Escherichia coli*).

3. Results

3.1. S. acidocaldarius contains short, highly saturated DolP

To begin analysis of *S. acidocaldarius* DolP, normal phase LC-ESI/MS of a lipid extract was performed in the negative ion mode using a high resolution QSTAR XL quadrupole time-of-flight tandem mass spectrometer (Fig. 1A). The mass spectrum averaged from those acquired during the retention time of 20–21 min shows prominent monoisotopic ion peaks of *m/z* 651.559, 719.605 and 787.688, corresponding to the $[M-H]^-$ ions of C_{40}, C_{45} and C_{50} DolP containing five saturated and three, four and five unsaturated isoprene units, respectively (Fig. 1B). These measured ion masses are in agreement with the calculated values of the $[M-H]^-$ ions of C_{40}, C_{45} and C_{50} DolP, namely *m/z* 651.549, 719.611 and 787.674,

Download English Version:

https://daneshyari.com/en/article/8303235

Download Persian Version:

https://daneshyari.com/article/8303235

Daneshyari.com