Pergamon

Tetrahedron Letters 43 (2002) 451–453

TETRAHEDRON LETTERS

Biosynthesis of calditol, the cyclopentanoid containing moiety of the membrane lipids of the archaeon *Sulfolobus solfataricus*

Agata Gambacorta,[a,*] Gabriella Caracciolo,[a] Diego Trabasso,[a] Irene Izzo,[b] Aldo Spinella[b,*] and Guido Sodano[b,*]

[a]*Istituto per la Chimica di Molecole di Interesse Biologico, C.N.R., via Toiano 6, 80072 Arco Felice, NA, Italy*
[b]*Dipartimento di Chimica, Università di Salerno, 84081 Baronissi, SA, Italy*

Received 14 November 2001; accepted 16 November 2001

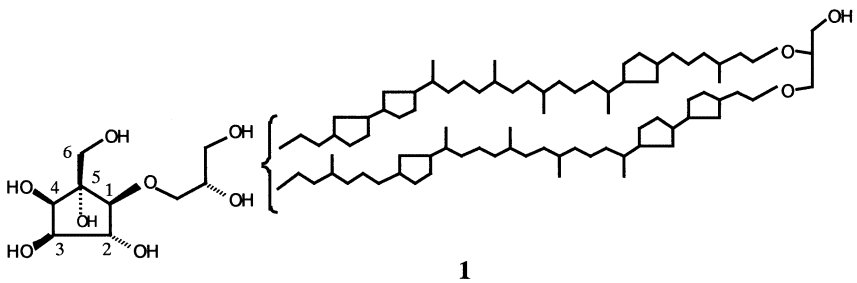
Abstract—Calditol, the cyclopentane containing moiety of the membrane lipids of the archaeon *Sulfolobus solfataricus*, could be biosynthesized from D-glucose, via an 'inositol like' pathway.

Microorganisms belonging to the Archaea domain contain membrane core lipids with unique chemical structures, characterized by a glycerol core linked to isoprenoid chains with ethereal bonding, in contrast to the ester linkages with fatty acids in bacterial and eukaryotic membrane lipids.[1,2] These lipids should preserve the membrane function under extreme conditions, such as high temperatures and strong acidity. With reference to one of the most studied species, *Sulfolobus solfataricus*, the non polar part of the mixture of polar lipids ranges, in order of increasing complexity, from the archaeols and caldarchaeols to the 'nonitol-caldarcheols'[1] (calditoglycerocaldarchaeols, CGCAs, according to Nishihara et al.[2,3]). While archaeols and caldarchaeols are widespread within the archaea and have been shown to be ubiquitous in the environment by analysis of recent sediments,[4] the CGCAs are restricted to the order *Sulfolobales* and constitute a taxonomic marker of the order.

The chemical structures of CGCAs are not fully defined. The major core lipid occurring in *S. solfataricus* could be represented by the gross structure **1**, the other CGCAs featuring a different number of cyclopentane rings in the macrocyclic part of the molecule. These lipids are in turn obtained in the laboratory by acid hydrolysis of the polar ones which are essentially glycolipids and phosphoglycolipids.[1]

The structure of the cyclitol containing moiety, called calditol, occurring in **1** and related lipids in which is linked to the rest of the molecule by ethereal bonding, has been only recently defined. The correct gross structure was first proposed by Sugai et al.,[3] while the relative and absolute configuration has been recently established to be as reported in **1** by synthesis.[5]

Several aspects of the biosynthesis of archaeal lipids have been investigated, chiefly dealing with the biosyn-



1

calditol

Keywords: microorganisms; biosynthesis; lipids.

* Corresponding authors. Tel.: +39 081 8534167; fax: +39 081 8041770 (A.G.); tel.: +39 089 965230; fax: +39 0 89 965296 (A.S.); e-mail: agambacorta@icmib.na.cnr.it; spinella@unisa.it

0040-4039/02/$ - see front matter
PII: S0040-4039(01)02187-6

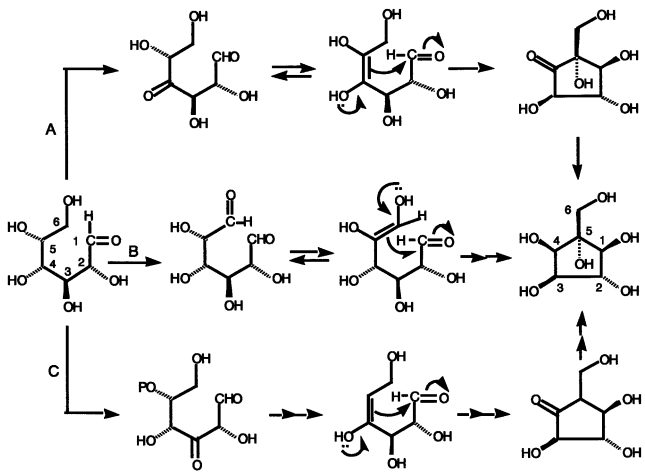
thetic origin of the terpenoidic part of the molecules and the origin of the glycerol ether bonding.[2] On the other hand, the biosynthesis of the calditol moiety was previously also investigated;[6] however, the conclusions reported in the previous study were heavily affected by the fact that at that time the proposed structure of calditol was incorrect. For these reasons we have decided to reinvestigate the biosynthesis of calditol and in particular the biosynthetic origin of the cyclopentanoid part of the molecule.[7] An additional interest resides in the fact that natural products containing carbohydrate derived cyclopentane rings are much rarer than six-membered counterparts[8] and none of them contains the cyclopentanoid substructure found in calditol.

Inspection of the structure of the cyclopentanoid part of calditol suggests that a likely biosynthetic origin would involve the formation of a 1,5 carbon bond from an aldohexose; in the case that the sugar precursor is glucose, inversion of the stereochemistry at C-4 of the precursor should occur during the bioconversion into calditol. In Scheme 1 three possible biosynthetic pathways are outlined from glucose to the cyclopentanoid part of calditol, in accord with proposed pathways for other carbohydrate-derived cyclopentane rings.[8]

Pathways A and B are defined 'inositol like' for the analogy with the extensively investigated biosynthesis of the six-membered cyclitol inositol from glucose. Both ways involve an aldol type condensation between C-1 and C-5 of glucose, the difference residing in the mode of the activation of the C-5 carbon atom. In pathway A the activation is due to the oxidation of the adjacent C-4 carbon, while in pathway B the activation is achieved by oxidation at C-6. The latter pathway was shown to be operative in the biosynthesis of the cyclopentane ring formation of allosamizoline, an aminocyclitol derivative of the chitinase inhibitor allosamidin.[9] Pathway C is analogous to the well known shikimate pathway, which is responsible for the synthesis of aromatic amino acids. Pathways A and B seem more likely for the biosynthesis of calditol since would afford directly the required carbon framework, while pathway C would involve a hydroxylation step in a later stage of the biosynthesis.

Sulfolobus solfataricus (DSM 5833) was adapted to grow on a minimum medium in which the sole carbon source was glucose, as previously reported.[10] Media containing 2% glucose (weight/volume) were utilized. In order to discriminate between the pathways A–C (Scheme 1) three experiments were planned in which fully deuterated, C-3 and C-4 deuterated glucose were administered to the culture media. The results of each experiment were monitored by ^{1}H and ^{2}H NMR on the mixture of the core lipids obtained by acid hydrolysis of the polar ones and acetylation. In other words, the mixture of the peracetates of compound **1** and companions was monitored, since it was found that the number of the cyclopentane rings in the macrocyclic part of the molecules does not affect the resonances of the cyclopentanoid part of calditol, which exhibited sharp resonances both in the proton and carbon spectra of the mixture of peracetates.

In the first experiment a mixture of fully deuterated D-glucose[11] and D-glucose (1:1 ratio) was administered as the sole carbon source to the culture medium, containing 20% of an inoculum culture of *S. solfataricus* previously grown on D-glucose. This experiment was devised in order to have a preliminary information on the loss of deuterium during the conversion to calditol. The ^{1}H NMR spectrum of the core lipid peracetates relative to the cyclopentanoid part of calditol (Fig. 1a) was virtually identical to the spectrum of the same



Scheme 1.

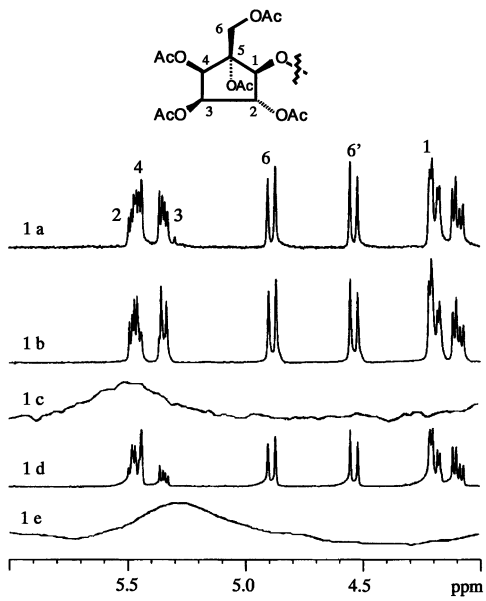


Figure 1. ^{1}H NMR and ^{2}H NMR spectra of the cyclopentanoid moiety of peracetates of calditol containing core lipids of *S. solfataricus* grown with a mixture of fully deuterated D-glucose and D-glucose (1:1 ratio) (1a), C-4 deuterated (1b, 1c) and C-3 deuterated (1d, 1e) D-glucose. The unnumbered peaks are due to the glycerol acetate unit, top right in formula **1**.

lipids obtained from a culture grown on D-glucose, giving as a first indication that no loss of deuterium occurred at the C-1, C-2, C-3, C-4 and C-6 of glucose during the conversion. The fully deuterated D-glucose was incorporated at a ca. 50% extent, as inferred by integration of the calditol's resonances against those of the six acetyl methyls, which, coming from undeuterated acetic anhydride, constitute an useful internal standard.

However, the above experiment did not indicate how the carbon framework of D-glucose is incorporated into calditol. In addition, the above result in principle does not exclude that deuterium could be lost on a carbon position and then reintroduced from another carbon position (e.g. C-5 of glucose). For these reasons two additional experiments were carried out growing *S. solfataricus* on C-3 and C-4 deuterated D-glucose, which were synthesized following previously reported procedures,[12] with minor variants that improved the yields. The C-3 deuterated glucose was obtained with 100% of deuterium at C-3 (^{1}H and ^{2}H NMR monitoring), while the C-4 deuterated one showed a 90% enrichment of deuterium at C-4. In these experiments only deuterated glucose was added to the culture medium, giving the advantage that the results can be directly monitored by ^{1}H NMR.

The proton spectrum of the acetylated calditol portion coming from the growth with C-4 deuterated glucose is reported in Fig. 1b; the spectrum is superimposed to the natural abundance spectrum due to the inoculum (ca. 20% of the isolated peracetates). It can be seen that the C-4 deuterium of D-glucose is retained at C-4 of calditol, indicating that the inversion of configuration at this carbon during the conversion from D-glucose occurs with retention of deuterium. In addition, the ^{2}H NMR spectrum (Fig. 1c), showing a single broad signal centered at the H-4 chemical shift of the calditol peracetate, indicated that deuterium was not transferred in part to other positions of calditol.

The results of the growth with C-3 deuterated glucose behave similarly (Fig. 1d). The C-3 deuterium atom of D-glucose was retained at C-3 of calditol, without any apparent partial transfer to other positions, as deduced from the ^{2}H NMR spectrum (Fig. 1e).

The above results indicate that the cyclopentanoid portion of calditol is biosynthesized by the formation of a 1,5 carbon bond from D-glucose, involving inversion of configuration at C-4 of glucose and without loss of the C-4 proton. In addition, the results seem to suggest an 'inositol like' pathway to calditol, ruling out a 'shikimate like' pathway (pathway C, Scheme 1). In fact, in the latter case, oxidation at both C-3 and C-4 occurs along the pathway followed by reduction. As a consequence, if the pathway C was operative, the experiments with C-3 and C-4 deuterated glucose would involve a ca. 50% reintroduction of deuterium at both C-3 and C-4 positions of calditol. Among the 'inositol like' pathways A and B, pathway A should be preferred. In fact, a C-4 oxidation is likely, since inversion of configuration at this carbon could involve an oxidation–reduction step. In addition pathway B, which is operative in the biosynthesis of the cyclopentane ring formation of allosamizoline,[8] involves stereospecific loss of one of the two C-6 protons which was not found in the calditol experiment with fully deuterated D-glucose as precursor. The choice of pathway A is not in conflict with the finding that deuterium is retained at this carbon almost completely. This result is largely precedented by similar findings reported in the literature. To remain confined to the five-membered cyclitols see, for example, Ref. 8. A likely explanation would be that oxidation–cyclization–reduction steps are carried out on a single enzyme containing a compartmentalized, tightly linked pool of NAD(P) which in the oxidation step is transformed in NAD(P)^{2}H, promptly giving the ^{2}H atom in the reduction step.

References

1. Gambacorta, A.; Gliozzi, A.; De Rosa, M. *World J. Microbiol. Biotechnol.* **1995**, *11*, 115.
2. Koga, Y.; Nishihara, M.; Morii, H.; Akagawa-Matsushita, M. *Microb. Rev.* **1993**, *57*, 164.
3. Sugai, A.; Sakuma, R.; Fukuda, I.; Kurosawa, N.; Itoh, Y. K.; Kon, K.; Ando, S.; Itoh, T. *Lipids* **1995**, *30*, 339.
4. Schouten, S.; Hopmans, E. C.; Pancost, R. D.; Simminghe Damsté, J. S. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 14421.
5. Untersteller, E.; Fritz, B.; Blériot, Y.; Sinay, P. *C.R. Acad. Sci. Paris Ser. II* **1999**, 429.
6. Nicolaus, B.; Trincone, A.; Esposito, E.; Vaccaro, M. R.; Gambacorta, A.; De Rosa, M. *Biochem. J.* **1990**, *266*, 785.
7. (a) Flesch, G.; Rohmer, M. *J. Chem. Soc., Chem. Commun.* **1988**, 868; (b) Rohmer, M.; Sutter, B.; Sahm, H. *J. Chem. Soc., Chem. Commun.* **1989**, 1471.
8. Jenkins, G. N.; Turner, N. J. *Chem. Soc. Rev.* **1995**, 169.
9. Sakuda, S.; Zhou, Z.-Y.; Takao, H.; Yamada, Y. *Tetrahedron Lett.* **1996**, *37*, 5711.
10. Nicolaus, B.; Trincone, A.; Lama, L.; Romano, I.; Marsiglia, F.; Gambacorta, A. *Biotech. Lett.* **1991**, *13*, 667.
11. D-Glucose-C-d7, >97 atom % D, was purchased from ISOTEC, inc.
12. Yu, Y.; Russell, R. N.; Thorson, J. S.; Liu, L.-d.; Liu, H.-w. *J. Biol. Chem.* **1992**, *267*, 5868.