CoA biosynthesis in archaea

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Abstract

CoA is a ubiquitous molecule in all three domains of life and is involved in various metabolic pathways. The enzymes and reactions involved in CoA biosynthesis in eukaryotes and bacteria have been identified. By contrast, the proteins geness involved in CoA biosynthesis in archaea have not been fully clarified, and much has to be learned before we obtain a general understanding of how this molecule is synthesized. In the present paper, we review the current status of the research on CoA biosynthesis in the archaea, and discuss important questions that should be addressed in the near future.

Introduction

CoA is a ubiquitous coenzyme involved in a wide range of metabolic pathways, which include the TCA (tricarboxylic acid) cycle, β-oxidation and fatty acid biosynthesis [1–3]. Acyl-CoA compounds, with their thioester bonds, play an important role in conserving energy in catabolic pathways and are also precursors in a variety of biosynthetic pathways for molecules such as fatty acids and isoprenoids. The biosynthesis of CoA itself in bacteria and eukaryotes has been studied extensively, and eight common enzymatic steps converting 2-oxoisovalerate into CoA have been identified [2,3] (Figure 1). 2-Oxoisovalerate is converted into pantothenate via three reactions, which are catalysed by KPHMT (ketopantoate hydroxymethyltransferase), KPR (ketopantoate reductase) and PS (pantothenate synthetase). Pantothenate is then converted into CoA by five enzymes: PanK (pantothenate kinase), PPCS (phosphopantothenoylcysteine synthetase), PPCDC (phosphopantothenoylcysteine decarboxylase), PPAT (phosphopantetheine adenylyltransferase) and DPCCK (dephospho-CoA kinase). In order to synthesize one molecule of CoA, one molecule each of 2-oxoisovalerate, β-alanine, cysteine and ATP are needed as starting material. In addition, four reactions require the cleavage of NTPs (ATP/CTP) and one reaction requires the oxidation of NADPH. Although bacteria, eukaryotic micro-organisms such as yeast and plants harbour a range of metabolic pathways, which include the TCA cycle, β-oxidation and fatty acid biosynthesis, the CoA biosynthesis pathway is common in all three domains of life [1]. However, the enzymes genes responsible for the biosynthesis of 4'-phosphopantothenate in archaea cannot be clearly identified on the basis of primary structure alone. Candidates for KPHMT and KPR genes are present in a number of archaeal genomes, but are not found in others. The genes display limited similarity to their eukaryotic/bacterial counterparts, and the products of these genes have not been studied. Moreover, PS and PanK genes are not found in almost all of the archaeal genomes [1]. The only exceptions are in members of the Thermoplasmatasales, which harbour genes encoding proteins distantly related to classical PanK. In the present paper, we describe our understanding of the mechanisms for CoA biosynthesis in the archaea, focusing mainly on the enzymes involved in the conversion of pantoate into 4'-phosphopantothenate.

Conversion of pantoate into 4'-phosphopantothenate in Thermococcus kodakarensis

T. kodakarensis is a hyperthermophilic archaeon and a member of the Thermococcales [4,5]. The complete genome sequence has been determined [6], and a genetic system has been established [7–11], which allows one to examine the physiological roles of genes in vivo. As in the case of most archaea, genes with similarity to classical PS and PanK genes are not found in the T. kodakarensis genome. Using a comparative genomics approach to search for a structurally novel PanK gene, four candidate genes were identified. The genes (i) encoded putative kinases, (ii) displayed limited similarity to previously characterized kinase genes and thus their substrates were ambiguous, (iii) were present in a wide range of archaeal genomes, and (iv) were not present in any of the bacterial and eukaryotic genomes. Characterization of the four gene products revealed that the TK2141 protein encoded a protein with PanK activity. Further examination of substrate specificity led to the discovery

Key words: Archaea, CoA, genome; pantoate kinase, phosphopantothenate synthase.

Abbreviations used: DPCCK, dephospho-CoA kinase; GHMP, galactokinase/homoserine kinase/hexosamine kinase; PanK, pantoate kinase/phosphopantothenate synthase; PPCS, phosphopantothenoylcysteine synthetase; PPCDC, phosphopantothenoylcysteine decarboxylase; PPAT, phosphopantetheine adenylyltransferase; POK, pantoate kinase/mevalonate kinase/phosphomevalonate kinase; KPHMT, ketopantoate hydroxymethyltransferase; KPR, ketopantoate reductase; LUCA, last universal common ancestor; PanK, pantoate kinase; PS, pantothenate synthetase; TK2141, protein encoded a protein with PanK activity.

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that the TK2141 protein preferred pantoate as a substrate, and was thus designated PoK (pantoate kinase) [12]. As the phosphorylation of pantoate leads to a novel metabolite, 4-phosphopantoate, a second novel enzyme activity, designated PPS (phosphopantothenate synthetase), is necessary to direct the conversion of 4-phosphopantoate into 4′-phosphopantothenate. This is due to the fact that PPCS homologues are present in the genome of \textit{T. kodakarensis} and other archaea. The protein from \textit{Methanocaldococcus jannaschii} has been biochemically proven to harbour the expected PPCS activity [13,14], and the substrate of this enzyme is 4′-phosphopantothenate. In a search for genes that displayed a similar distribution among the published genomes, TK1686 was identified. This gene, as in the case of TK2141, was present in a wide range of archaeal genomes and was not found in bacterial and eukaryotic genomes. The function of the gene could not be predicted on the basis of primary structure and was annotated as an uncharacterized protein conserved in the archaea. Biochemical examination of the gene product confirmed that the protein exhibited PPS activity, and did not display the classical PS activity [12]. Individual gene disruption of TK2141 and TK1686 led to mutant strains that could not grow in a medium based on yeast extract and tryptone, but displayed growth in the same medium supplemented with CoA. These strains could also grow when 4′-phosphopantothenate was supplemented to the medium, but could not grow with pantothenate, strongly supporting the hypothesis that \textit{T. kodakarensis} does not utilize pantothenate for the biosynthesis of CoA and utilizes the PoK/PPS system instead of the PS/PanK system found in bacteria and eukaryotes. As most archaeal genomes harbour PoK and PPS homologues in their genomes, it can be presumed that the majority of archaea utilize the PoK/PPS system for the biosynthesis of CoA (Figure 2).

**Pantoate kinase from \textit{T. kodakarensis}**

PoK from \textit{T. kodakarensis} is a dimeric protein consisting of 300 amino acid residues and is classified as arCOG04263 and COG01829. It is annotated as a member of the GHMP (galactokinase/homoserine kinase/mevalonate kinase/phosphomevalonate kinase) family. Biochemical analyses indicated that PoK displayed broad nucleotide specificity and utilized ATP, GTP, UTP and CTP with comparable $k_{cat}/K_m$ values. The PoK-catalysed reaction displayed Michaelis–Menten kinetics towards these NTPs, whereas substrate inhibition was observed with pantoate. Product inhibition by 4-phosphopantoate was also observed in a non-competitive manner. Whereas bacterial and eukaryotic PanK is inhibited by CoA, PoK activity was not affected by the addition of CoA or acetyl-CoA [12,15]. Sequence alignment of archaeal PoK homologues revealed the presence of seven residues with reactive side chains highly conserved among these proteins. Examination of variant proteins with mutations in individual residues indicated that Ser$^{124}$, Glu$^{134}$ and Asp$^{143}$ play essential roles in PoK catalysis. These three residues are also conserved in other members of the GHMP family, and sequence comparisons suggest that Ser$^{124}$ and Glu$^{134}$ are involved in binding with the phosphate groups of ATP and Mg$^{2+}$ respectively, whereas Asp$^{143}$ is the general base responsible for proton abstraction from the pantoate hydroxy group. Kinetic analysis of the other variant proteins with mutations in Ser$^{28}$, His$^{131}$, Arg$^{155}$ and Thr$^{186}$
Figure 2 | An illustration of the eukaryotic/bacterial and archaeal pathways responsible for the conversion of pantoate into 4′-phosphopantothenate

Note that among the archaea, members of the Thermoplasmatales do not harbour PoK and PPS genes, and instead harbour an archaeal PanK.

indicated that all four residues are involved in recognizing the substrate pantoate and that the last two residues play important roles in PoK catalysis. Gel-filtration analyses of the variant proteins indicated that Thr136 is also involved in dimer assembly [15].

PPS from *T. kodakarensis*

PPS from *T. kodakarensis* is a dimeric protein consisting of 261 amino acid residues and is classified as arCOG04262 and COG01701. It is annotated as an uncharacterized protein conserved in the archaea, and is not structurally related to the bacterial and eukaryotic PS. Among NTPs, the enzyme displayed a strict preference for ATP. In terms of the amine substrate, condensation with 4-phosphopantoate was observed with β-alanine, but could not be detected with structurally related compounds such as γ-aminobutyrate, glycine or aspartate. The PPS-catalysed reaction followed Michaelis–Menten kinetics towards β-alanine, whereas a relatively moderate substrate inhibition was observed with 4-phosphopantoate and ATP. Feedback inhibition by CoA or acetyl-CoA and product inhibition by 4′-phosphopantothenate was not observed [16].

Conversion of pantoate into 4′-phosphopantothenate in *Picrophilus torridus*

*P. torridus* is a thermoacidophilic archaeon and is a member of the Thermoplasmatales. The complete genome sequence has been determined [17], and a number of enzymes and metabolic pathways have been studied [18–20]. In the *P. torridus* genome, a gene distantly related to the bacterial type I PanK genes was identified [21]. The gene, PTO0232, was shown to complement the poor growth of a mutant strain ts9 of *Escherichia coli* that harbours a temperature-sensitive defect in its PanK gene. Biochemical analysis of the PTO0232 protein clearly demonstrated that the protein exhibits PanK activity. The enzyme could utilize GTP, CTP and UTP in addition to ATP as the phosphate donor. Most interestingly, whereas the bacterial PanK from *E. coli* is inhibited by CoA, resulting in feedback inhibition of CoA biosynthesis, the PanK from *P. torridus* was not affected. The gene that encodes a PS, necessary to provide the PanK substrate pantothenate, has not been identified yet.

Evolution of the CoA biosynthesis pathway

The different pathways (PS/PanK compared with PoK/PPS) utilized to convert pantoate into 4′-phosphopantothenate between the bacteria/eukaryotes and archaea, and their wide distribution within the respective domains, suggest that a complete CoA biosynthesis pathway had not evolved before the divergence of the three domains of life. Supporting this, although the bacteria and eukaryotes utilize common reactions, the structures of the proteins responsible for reactions such as those catalysed by PanK, PPCS and PPAT are not related. Therefore there is a possibility that the CoA molecule, as we know it, was not present in the LUCA (last universal common ancestor). Interestingly, the only enzymes among the archael and bacterial pathways that display structural relatedness are PPCS and PPCDC. PPCS catalyses the condensation reaction between 4′-phosphopantothenate and cysteine, and PPCDC catalyses the decarboxylation of the PPCS reaction product, 4′-phosphopantothenoylcysteine. From a chemical point of view, the product of these two reactions, 4′-phosphopantetheine, should be able to meet the functional demands of a CoA molecule with its thiol group and absence...
of a nearby anionic group. Furthermore, the carboxy group donor of the PPCS-catalysed reaction does not necessarily have to be a 4′-phosphopantothenate molecule to maintain the minimal structural requirements to function as a CoA-like compound (Figure 3). Taken together, we can speculate that, in the LUCA, a relatively simple molecule generated from a condensation reaction between a cysteine residue and a carboxylic acid, followed by removal of the cysteine-derived carboxy group may have functioned as an ancestral CoA molecule.

Future aims in research

Although advances have been made in our understanding of archaeal CoA biosynthesis, a number of important questions remain. Other than the PoK, PPS from *T. kodakarensis* and PPCS from *M. jannaschii* described above, the PPCDC from *M. jannaschii*, which is actually a domain of a fusion protein with PPCS, and the PPAT from *Pyrococcus abyssi*, are the only proteins that have clearly been demonstrated to exhibit the expected activities [14,22]. Therefore there are still several enzymatic reactions in which the proteins/genes involved have not been identified or experimentally verified in any archaeal species. These include the first two reactions starting from 2-oxoisovalerate, catalysed by KPHMT and KPR, and the final reaction, catalysed by DPCK. As mentioned above, KPHMT and KPR homologues are absent from many archaeal genomes, including those of a number of methanogens, which surely should be able to synthesize CoA. Examining the activities of the homologues in archaeal species that harbour these homologues and searching for structurally novel enzymes responsible for these activities in those that lack these homologues will be some of the primary goals of research in this field. Differences in the donor compound of the hydroxymethyl group in the KPHMT-catalysed reaction may be related to the absence of classical KPHMT homologues from some archaea. Another interesting target is the identification of the enzymes responsible for pantothenate synthesis in members of the Thermoplasmatales.

An important point that should be addressed is the regulation of CoA biosynthesis. As described above, the biosynthesis of a CoA molecule requires a number of precursor compounds, energy and reducing equivalents. In bacteria and eukaryotes, a major factor in preventing the excess generation of CoA is made possible by the enzymatic properties of PanK. PanK activity is strongly inhibited in a competitive manner in the presence of CoA or acetyl-CoA [23–25]. Intracellular accumulation of CoA would thus shut down the biosynthesis of CoA via feedback inhibition on PanK. In terms of the archaea that utilize the PoK/PPS system, PanK is not present, and it has been shown that both PoK and PPS are not inhibited by CoA or acetyl-CoA [12,15,16]. Substrate and/or product inhibition have been observed in both PoK and PPS from *T. kodakarensis*, but the effects were moderate compared with the strong inhibition observed in bacterial and eukaryotic PanK, and observed only at relatively high concentrations of the substrate/product [15,16]. It can thus be expected that other mechanisms exist in this organism in order to regulate CoA biosynthesis. Even in *P. torridus*, which harbours a PanK that is structurally related
to the bacterial type I enzyme, it has been shown that the activity of the enzyme is not affected by the presence of CoA [21]. This raises the possibilities of a novel mechanism of regulation in the biosynthesis of CoA in archaea.

References


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