FEBS 25565 FEBS Letters 509 (2001) 345–349

Minireview

A new family of CoA-transferases

Johann Heider*

Mikrobiologie, Institut für Biologie II, Universität Freiburg, Schänzlestr. 1, 79104 Freiburg, Germany

Received 19 September 2001; revised 5 November 2001; accepted 5 November 2001

First published online 20 November 2001

Edited by Hans Eklund

Abstract CoA-transferases are found in organisms from all lines of descent. Most of these enzymes belong to two well-known enzyme families, but recent work on unusual biochemical pathways of anaerobic bacteria has revealed the existence of a third family of CoA-transferases. The members of this enzyme family differ in sequence and reaction mechanism from CoA-transferases of the other families. Currently known enzymes of the new family are a formyl-CoA: oxalate CoA-transferase, a succinyl-CoA: (*R*)-benzylsuccinate CoA-transferase, an (*E*)-cinnamoyl-CoA: (*R*)-phenyllactate CoA-transferase, and a butyrobetainyl-CoA: (*R*)-carnitine CoA-transferase. In addition, a large number of proteins of unknown or differently annotated function from Bacteria, Archaea and Eukarya apparently belong to this enzyme family. Properties and reaction mechanisms of the CoA-transferases of family III are described and compared to those of the previously known CoA-transferases.

Key words: Succinyl-CoA: (*R*)-benzylsuccinate CoA-transferase; Formyl-CoA: oxalate CoA-transferase; (*E*)-Cinnamoyl-CoA: (*R*)-phenyllactate CoA-transferase; Butyrobetainyl-CoA: (*R*)-carnitine CoA-transferase; 2-Methylacyl-CoA racemase; Ternary complex mechanism

1. Introduction

CoA-transferases catalyse reversible transfer reactions of coenzyme A groups from CoA-thioesters to free acids. Most characterised CoA-transferases are grouped into two enzyme families. Family I contains CoA-transferases for 3-oxoacids (EC 2.8.3.5; EC 2.8.3.6) [1–3], short-chain fatty acids (EC 2.8.3.8; EC 2.8.3.9) [4–6] and glutaconate (EC 2.8.3.12) [7–9]. Most family I enzymes operate with succinyl-CoA or acetyl-CoA as possible CoA donors and contain two dissimilar subunits in different aggregation states ($\alpha_2\beta_2$ or $\alpha_4\beta_4$). An exception is mammalian succinyl-CoA: acetoacetate CoA-transferase (EC 2.8.3.5): it consists of only one subunit, which contains subdomains orthologous to the respective α- and β-subunits of other enzymes [1,2]. Two conserved amino acid sequence motives, one in either subunit, are known as signatures for family I enzymes (Prosite entries PS01273 and PS01274). The small family II of CoA-transferases consists of the homodimeric α-subunits of citrate lyase and citramalate lyase (EC 2.8.3.10, EC 2.8.3.11). These enzymes actually catalyse the transfer of an acyl carrier protein (ACP), which contains a covalently bound CoA derivative, but also accept free CoA-thioesters in vitro [10–12] (see below for further details). The reaction mechanisms of enzymes of families I and II are known [11–13]. In both families of enzymes, the reactions involve alternative formation of thioester and anhydride intermediates, but the mechanistic details are very different.

Recently, some related CoA-transferases involved in anaerobic metabolic pathways were identified, whose amino acid sequences do not resemble those of CoA-transferases of families I and II. These enzymes apparently belong to a new family of CoA-transferases. The currently characterised members are from Bacteria, but genes for similar enzymes are also found in Archaea and Eukarya. Most of the known enzymes of this family III catalyse CoA-transfer reactions in a highly substrate- and stereospecific manner. The current knowledge on the members of this enzyme family is presented in this review.

2. Reaction mechanisms of CoA-transferase families

2.1. Family I

In CoA-transferases of family I, the reaction proceeds via a ping-pong mechanism, involving a glutamate residue of the enzyme as acceptor of covalently bound intermediates. The first partial reaction is a nucleophilic attack of the active site glutamate at the CoA-thioester substrate to yield an enzyme-bound acyl-glutamyl anhydride (Fig. 1) [7,13]. The liberated $CoAS^-$ anion does not dissociate from the enzyme, but is engaged in a second nucleophilic attack at the mixed anhydride. This produces an enzyme-bound glutamyl-CoA-thioester and liberates the acid of the donor thioester (Fig. 1) [7,13]. The CoA-accepting acid then attacks the glutamyl-CoA to form another mixed anhydride with the enzyme, and the liberated $CoAS^-$ anion finally reacts with the anhydride to form the product thioester (Fig. 1). The process is fully reversible, and the enzyme-bound free acids and thioesters exchange with the medium [13].

2.2. Family II

CoA-transferases of family II catalyse a partial reaction in the mechanism of the complex citrate or citramalate lyases [10–12]. These lyases consist of three subcomplexes with different functions, namely a CoA-transferase (α-subunit), a lyase (β-subunit) and an acyl carrier protein (γ-subunit). The γ-subunit contains a covalently bound $2' \rightarrow 1''$-(5″-phosphori-

*Fax: (49)-761-203 2626.
E-mail address: heiderj@uni-freiburg.de (J. Heider).

PII: S0014-5793(01)03178-7

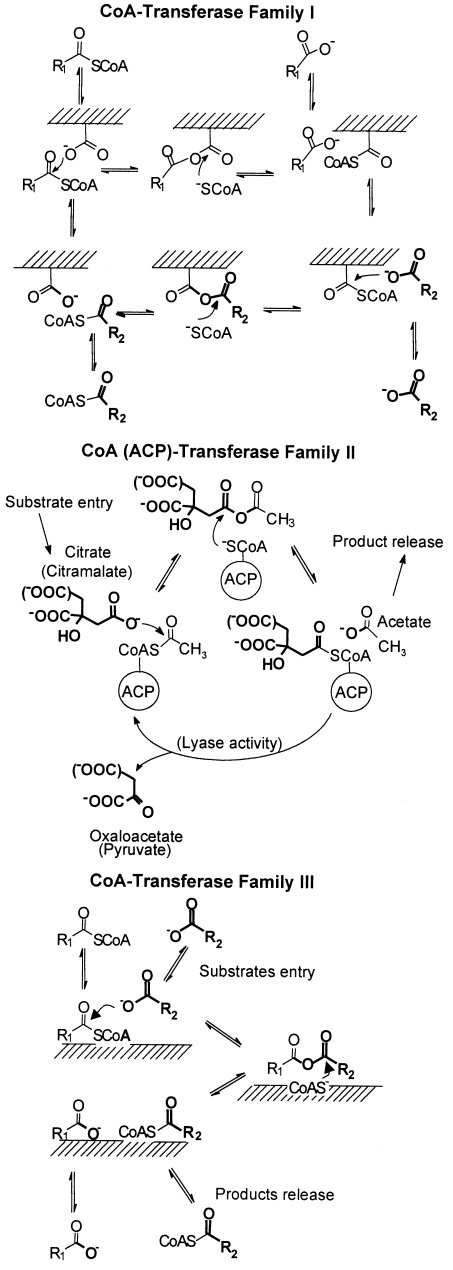

Fig. 1. Mechanisms of CoA-transferases of families I, II and III. Enzymes of family I operate via a ping-pong mechanism, forming a CoA-thioester intermediate with a glutamate of the active site. Enzymes of family II actually transfer an acyl carrier protein (ACP) subunit of the citrate or citramalate lyase enzyme complex, which also regenerates the acetyl-ACP donor compound. However, these enzymes also catalyse CoA-transfer reactions via a ternary complex mechanism. Enzymes of family III are genuine CoA-transferases operating via a ternary complex mechanism. The substrates to be activated are printed in bold to indicate the fate of the transferred atoms. For further description of the mechanisms see the text.

bosyl)-3′-dephospho-CoA moiety, which differs from the 4′-phosphopantetheine group usually present in other acyl carrier proteins [14,15]. The thiol of the prosthetic group is converted to an acetyl-thioester to activate the enzyme [10,12]. The CoA-transferase subcomplex then catalyses the exchange of free citrate (or citramalate) against the acetyl group of ACP. In contrast to the mechanism of family I enzymes, this reaction proceeds via a ternary complex mechanism without covalently bound intermediates at the enzyme. Both substrates bind to the enzyme simultaneously, and a mixed anhydride of the two acids is formed during the transition state (Fig. 1) [10–12]. The generated intermediate, citryl- (or citramalyl)-*S*-acyl carrier protein, serves as activated substrate for the lyase subcomplex, which cleaves off oxaloacetate (respectively pyruvate) and thereby regenerates the acetyl-*S*-acyl carrier protein for another round of catalysis (Fig. 1) [10–12].

2.3. Family III

The amino acid sequences of the CoA-transferases of this family are completely different from those of families I and II. Moreover, kinetic analysis of two enzymes of family III revealed an apparent reaction mechanism via a ternary complex [16,17], differentiating them from family I enzymes. It may be assumed that the reaction mechanism of family III enzymes is analogous to that found in family II enzymes, but with CoA-thioesters instead of ACP-thioesters as physiological substrates (Fig. 1, lower panel). (*R*)-Phenyllactate CoA-transferase also resembles family II enzymes in forming a complex with the following enzyme of the metabolic pathway, (*R*)-phenyllactyl-CoA dehydratase [16]. This results in recycling of the CoA donor, (*E*)-cinnamoyl-CoA, within the enzyme complex [16], similar to the acetyl-ACP recycling of citrate and citramalate lyases (Fig. 1, middle panel). However, the other known members of family III seem to operate with freely diffusible CoA donor and acceptor compounds. In addition to the four characterised enzymes, many proteins of unknown functions seem to belong to the enzyme family, as derived from the amount of genes coding for similar proteins in the database (see below).

3. Members of family III of CoA-transferases

The first CoA-transferase of the new family to be characterised was formyl-CoA: oxalate CoA-transferase from *Oxalobacter formigenes* [18,19], a monomer of 45 kDa, which is important in the energy metabolism of *O. formigenes* (Fig. 2). It is specific for oxalate and formyl-CoA, exhibiting partial activity only with succinyl-CoA and none with acetyl-CoA as alternative CoA donor [18,19]. Details of the reaction mechanism of this enzyme were not studied so far.

A second enzyme with considerable similarity to oxalate CoA-transferase is from the anaerobic toluene-degrading bac-

Table 1
Identity matrix of some members of CoA-transferase family III

	BbsE	BbsF	Frc	PLCT	CaiB	BaiF
BbsF	26					
Frc	23	28				
PLCT	24	21	25			
CaiB	25	18	24	24		
BaiF	22	23	27	24	37	
Epi	26	21	25	21	22	22

The values represent identical amino acids (in %) between the individual sequences of the subunits of (*R*)-benzylsuccinate CoA-transferase (BbsE, AAF89840; and BbsF, AAF89841), oxalate CoA-transferase (Frc, AAC45398), (*R*)-phenyllactate CoA-transferase (PLCT, from preliminary genome sequence of *C. difficile*), (*R*)-carnitine CoA-transferase (CaiB, CAA52112), putative cholate CoA-transferase (BaiF, AAC45415), and 2-methylacyl-CoA racemase (Epi, XP043772). Accession numbers are given in parentheses.

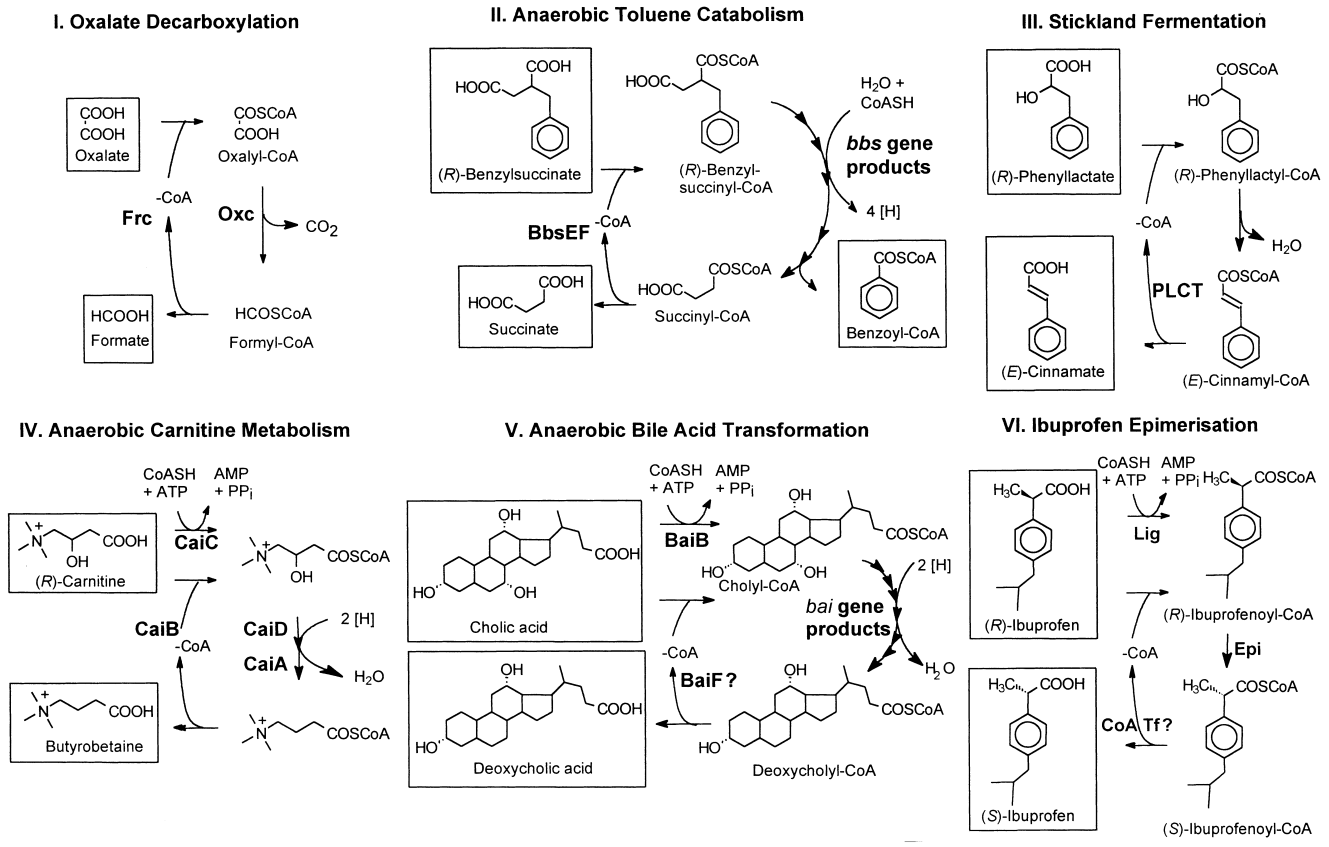

Fig. 2. Metabolic pathways involving CoA-transferases and proposed CoA-transferases of family III. (I) Formyl-CoA: oxalate CoA-transferase (*frc* gene product). The oxalyl-CoA formed is decarboxylated to formyl-CoA, the CoA donor of the enzyme. Proton motive force for energy conservation is generated via electrogenic antiport of oxalate and formate. (II) Succinyl-CoA: (*R*)-benzylsuccinate CoA-transferase (*bbsEF* gene products). Benzylsuccinyl-CoA formed by the enzyme is further degraded by β-oxidation, yielding benzoyl-CoA and the CoA donor, succinyl-CoA. (III) (*E*)-cinnamoyl-CoA: (*R*)-phenyllactate CoA-transferase (PLCT). Subsequent dehydration of the produced (*R*)-phenyllactyl-CoA to the CoA donor cinnamoyl-CoA is catalysed by another compound of a common enzyme complex. (IV) Butyrobetainyl-CoA: (*R*)-carnitine CoA-transferase (CaiB). The enoyl-CoA hydratase CaiD dehydrates (*R*)-carnitinyl-CoA to crotonobetainyl-CoA, which is then reduced to butyrobetainyl-CoA by the acyl-CoA dehydrogenase CaiA. Both intermediates serve as CoA donor for (*R*)-carnitine activation. The proposed function of (*R*)-carnitine CoA ligase (CaiC) is to prevent depletion of the CoA-thioester pool by unspecific hydrolysis. (V) Proposed deoxycholyl-CoA: cholate CoA-transferase (BaiF). Cholate needs to be activated to the CoA-thioester to enter a complex reductive pathway to deoxycholate. This reaction is proposed to be catalysed in parallel by a CoA-transferase (BaiF) for the normal reaction cycle and by a CoA ligase (BaiB) for repleting hydrolysed CoA-thioesters. See text for further details. (VI) Proposed reaction of 2-methylacyl-CoA racemase with the anti-inflammatory drug ibuprofen. The known epimerase activity of the enzyme (Epi) may either be present in addition to CoA-transferase activity (CoA Tf), or the epimerase may have evolved from a CoA-transferase. Question marks in (V) and (VI) indicate the hypothetical status of the reactions.

terium *Thauera aromatica* and was characterised as a succinyl-CoA: (*R*)-benzylsuccinate CoA-transferase [17,20]. It activates (*R*)-benzylsuccinate, the first intermediate of anaerobic toluene catabolism [21,22], to 2-(*R*)-benzylsuccinyl-CoA and thereby initiates β-oxidation of benzylsuccinate (Fig. 2) [17,20]. (*R*)-Benzylsuccinate is formed by addition of toluene to the double bond of a fumarate cosubstrate via a new glycyl radical enzyme [23,24]. The new CoA-transferase initiates the further degradation of (*R*)-benzylsuccinate by a specific β-oxidation pathway [23] (Fig. 2). The enzyme consists of two very similar subunits in an $\alpha_2\beta_2$ conformation, which are encoded by the *bbsE* and *bbsF* genes [17,25]. Both subunits share similarity to the subunit of oxalate CoA-transferase (Table 1) [25].

The third member of family III is a recently characterised (*E*)-cinnamoyl-CoA: (*R*)-phenyllactate CoA-transferase of Stickland-fermenting *Clostridia*, as evident from the N-terminal sequence of the enzyme from *Clostridium sporogenes* [16] and the sequence of the ortholog from the preliminary genomic sequence of *Clostridium difficile* (Sanger centre; Table 1). This enzyme is part of a larger enzyme complex catalysing the mechanistically difficult α-dehydration of (*R*)-phenyllactate to (*E*)-cinnamate (Fig. 2), which is involved in reductive conversion of phenylalanine to phenylpropionate during Stickland fermentation [16]. The CoA-transferase subunit apparently activates (*R*)-phenyllactate to the CoA-thioester at the expense of an (*E*)-cinnamoyl-CoA cosubstrate, which is subsequently regenerated by a (*R*)-phenyllactyl-CoA dehydratase present in a common enzyme complex [16].

Finally, a homodimeric butyrobetainyl-CoA: (*R*)-carnitine CoA-transferase (CaiB) from *Escherichia coli* and *Proteus* sp. was recently recognised as member of the enzyme family (Table 1) [26,27]. This enzyme was previously described as carnitine dehydratase [26,28], but a recent re-investigation showed that it actually catalyses the reversible transfer of CoA between (*R*)-carnitine, crotonobetaine and butyrobetaine, rather

than dehydration of carnitine [26,27]. A revised pathway of (*R*)-carnitine reduction is suggested, in which (*R*)-carnitine is activated to the CoA-thioester at the expense of the reduced intermediate, butyrobetainyl-CoA (Fig. 2) [26]. Further steps of the pathway are dehydration of (*R*)-carnitinyl-CoA to crotonobetainyl-CoA by a recently identified enoyl-CoA hydratase (CaiD) [26] and reduction to butyrobetainyl-CoA by an acyl-CoA dehydrogenase (CaiA; Fig. 2). Even if reduction of (*R*)-carnitine is not directly coupled to generation of proton motive force, the reaction may still lead to increased energy yields under fermentative growth conditions; the oxidation of one NADH per external electron acceptor should allow to conserve more energy from acetyl-CoA via phosphotransacetylase and acetate kinase (expected 0.5 mol ATP per mol carnitine reduced). Notably, increased energy conservation is only possible, if the thioester intermediates are recycled by CoA-transferases. Activation of the reducible substrates via an AMP-producing CoA ligase (at the expense of two phospho-anhydride bonds in every cycle) would even result in a loss of energy. The presence of a gene coding for such a CoA ligase (CaiC, Fig. 2) in the same operon may be explained by the requirement to restore the pool of CoA-thioesters, which would otherwise be slowly depleted by thioester hydrolysis.

In addition to the four characterised CoA-transferases of family III, a large number of similar hypothetical enzymes are found in the sequence database. Many of these proteins are already listed as 'CaiB/BaiF family' in the paralogous protein family database (accession number PF02515). Sequence identities between the subunits of the enzymes discussed in detail are listed in Table 1, and the most similar regions from a multiple alignment of selected proteins of the enzyme family is shown as accessory material in the web version of this article (http://www.elsevier.com/PII/S0014579301031787). The function of most members of the protein family is unknown, even of those that were annotated as 'similar to CaiB'. In addition to the four CoA-transferases listed above, functions are only assigned to two other members of the enzyme family, BaiF and 2-methylacyl-CoA racemase. However, neither of the latter enzymes is currently known as CoA-transferase.

3.1. BaiF

The BaiF protein (for bile acid-induced) plays a role in cholic acid reduction to deoxycholate by an *Eubacterium* sp. and is encoded together with genes for other enzymes of the pathway in the *bai* operon [29]. The first committed step, activation of cholic acid to the CoA-thioester, was proposed to be catalysed by a CoA ligase encoded in the operon (BaiB; Fig. 2) [29]. Cholyl-CoA is then reduced to deoxycholyl-CoA by a complex pathway, which involves several oxidation and reduction steps and a dehydration reaction (Fig. 2) [29]. The *baiF* gene product was proposed to catalyse hydrolysis of deoxycholyl-CoA as the last step of the pathway, because the purified enzyme had thioesterase activity [30]. However, the enzyme was not tested for CoA-transferase activity, and thioesterase activity is a known side reaction of glutaconate CoA-transferase [13]. Therefore, the actual role of BaiF may be CoA-transfer between deoxycholyl-CoA and cholate, and a pathway of cholic acid reduction may be proposed in analogy to that of (*R*)-carnitine (Fig. 2). In both pathways, CoA-thioester synthesis would principally be catalysed by CoA-transferases (BaiF and CaiB, respectively), but the presence of additional CoA ligases (BaiB and CaiC, respectively) is still required to replete hydrolysed thioesters.

3.2. 2-Methylacyl-CoA racemase

Another protein related to CoA-transferases of family III is a mammalian enzyme involved in metabolism of 2-methyl-branched fatty acids and anti-inflammatory drugs, such as ibuprofen [2-(4-isobutylphenyl)-propionate]. It has been purified from rat liver and was characterised as a monomeric 2-methylacyl-CoA racemase, which reversibly converts the two enantiomers [31–33]. cDNAs for the same enzyme are also known from mouse, human and other animals, and genes coding for similar enzymes were found in some bacterial genomes, e.g. those of *Mycobacterium tuberculosis* or *Streptomyces coelicolor*. The overall epimerisation reaction is currently believed to be initiated by stereospecific activation of the (*R*)-enantiomers of 2-methyl fatty acids by long-chain fatty acid CoA ligase [34], followed by reversible epimerisation of the CoA-thioesters by 2-methylacyl-CoA racemase and unspecific thioester hydrolysis [31]. Regarding the strong sequence similarity of 2-methylacyl-CoA racemase with CoA-transferases of family III (Table 1), the question arises whether this enzyme also catalyses CoA-transfer reactions. CoA-transferase activity would not be detected in the enzyme assays used so far [31–33]. Therefore, a possible new role is suggested for 2-methylacyl-CoA racemase as a bifunctional enzyme that activates and epimerises its substrates (Fig. 2).

4. Conclusions

Evidence has accumulated that the enzymes of the 'CaiB/BaiF' protein family are CoA-transferases of a novel type. The currently characterised CoA-transferases of this family are involved in special anaerobic biochemical pathways, which activate specific organic acids for subsequent decarboxylation, β-oxidation, or elimination of α- or β-hydroxy groups. Two enzymes of the family, which are significantly similar with those of proven CoA-transferases (Table 1), are currently described with different functions, namely a thioesterase (BaiF) and an acyl-CoA epimerase (Epi). Therefore, these enzymes may either have additional CoA-transferase activities or may have evolved to their current functions from CoA-transferases. All members of the enzyme family share considerable amino acid sequence similarity of their subunits, and a similar ternary complex reaction mechanism was identified for two family III members. The largest differences between the enzymes relate to their quaternary structures, which vary considerably. The known enzymes of the family are either monomers (oxalate CoA-transferase [18]), homodimers (CaiB [26–28]) or form more complex structures, such as the $\alpha_2\beta_2$ structure of benzylsuccinate CoA-transferase [17] or the enzyme complex of phenyllactate CoA-transferase and phenyllactyl-CoA dehydratase [16]. One of the related proteins of unknown function from *M. tuberculosis* (accession number CAB06121) even seems to be a fusion of two subdomains that are both similar to CoA-transferases of the new family.

Acknowledgements: The author thanks his coworkers Dr. Christina Leutwein and Dr. Birgitta Leuthner for their contributions to this work. Research was supported by grants of the Deutsche Forschungsgemeinschaft.

References

[1] Corthésy-Theulaz, I.E., Bergonzelli, G.E., Henry, H., Bachmann, D., Schorderet, D.F., Blum, A.L. and Ornston, N. (1997) J. Biol. Chem. 272, 25659–25667.
[2] Parales, R.E. and Harwood, C.S. (1992) J. Bacteriol. 174, 4657–4666.
[3] White, H. and Jencks, W.P. (1976) J. Biol. Chem. 251, 1708–1711.
[4] Sramek, S.J. and Frerman, F.E. (1975) Arch. Biochem. Biophys. 171, 14–26.
[5] Wiesenborn, D.P., Rudolph, F.B. and Papoutsakis, E.T. (1989) Appl. Environ. Microbiol. 55, 329–823.
[6] Barker, H.A., Jeng, I.M., Neff, N., Robertson, J.M., Tam, F.K. and Hosaka, S. (1978) J. Biol. Chem. 253, 1219–1225.
[7] Buckel, W., Dorn, U. and Semmler, R. (1981) Eur. J. Biochem. 118, 315–321.
[8] Jacob, U., Mack, M., Clausen, T., Huber, R., Buckel, W. and Messerschmidt, A. (1997) Structure 5, 415–426.
[9] Mack, M. and Buckel, W. (1995) FEBS Lett. 357, 145–148.
[10] Buckel, W. and Bobi, A. (1975) Eur. J. Biochem. 64, 255–262.
[11] Buckel, W., Buschmeier, V. and Eggerer, H. (1971) Hoppe-Seylers Z. Physiol. Chem. 352, 1195–1205.
[12] Dimroth, P. and Eggerer, H. (1975) Proc. Natl. Acad. Sci. USA 72, 3458–3462.
[13] Selmer, T. and Buckel, W. (1999) J. Biol. Chem. 274, 20772–20778.
[14] Dimroth, P. (1976) Eur. J. Biochem. 64, 269–281.
[15] Robinson Jr., J.B., Singh, M. and Srere, P.A. (1976) Proc. Natl. Acad. Sci. USA 73, 1872–1876.
[16] Dickert, S., Pierik, A.J., Linder, D. and Buckel, W. (2000) Eur. J. Biochem. 267, 3874–3884.
[17] Leutwein, C. and Heider, J. (2001) J. Bacteriol. 183, 4288–4295.
[18] Baetz, A.L. and Allison, M.J. (1990) J. Bacteriol. 172, 3537–3540.
[19] Sidhu, H., Ogden, S.D., Lung, H.-Y., Luttge, B.G., Baetz, A.L. and Peck, A.B. (1997) J. Bacteriol. 179, 3378–3381.
[20] Leutwein, C. and Heider, J. (1999) Microbiology 145, 3265–3271.
[21] Heider, J. and Fuchs, G. (1997) Anaerobe 3, 1–22.
[22] Heider, J., Spormann, A.M., Beller, H.R. and Widdel, F. (1999) FEMS Microbiol. Rev. 22, 459–473.
[23] Biegert, T., Fuchs, G. and Heider, J. (1996) Eur. J. Biochem. 238, 661–668.
[24] Leuthner, B., Leutwein, C., Schulz, H., Hörth, P., Haehnel, W., Schiltz, E., Schägger, H. and Heider, J. (1998) Mol. Microbiol. 28, 615–628.
[25] Leuthner, B. and Heider, J. (2000) J. Bacteriol. 182, 272–277.
[26] Elssner, T., Engemann, C., Baumgart, K. and Kleber, H.-P. (2001) Biochemistry 40, 11140–11148.
[27] Engemann, C., Elssner, T. and Kleber, H.-P. (2001) Arch. Microbiol. 175, 353–359.
[28] Eichler, K., Schunck, W.H., Kleber, H.-P. and Mandrand-Berthelot, M.-A. (1994) J. Bacteriol. 176, 2075–2970.
[29] Hylemon, P.B. and Harder, J. (1999) FEMS Microbiol. Rev. 22, 475–488.
[30] Ye, H.-Q., Mallonee, H., Wells, J.E., Björkhem, I. and Hylemon, P.B. (1999) J. Lipid Res. 40, 17–23.
[31] Reichel, C., Brugger, R., Bang, H., Geislinger, G. and Brune, K. (1997) Mol. Pharmacol. 51, 576–582.
[32] Schmitz, W., Helander, H.M., Hiltunen, J.K. and Conzelmann, E. (1997) Biochem. J. 326, 883–889.
[33] Shieh, W.-R. and Chen, C.-S. (1993) J. Biol. Chem. 268, 3487–3493.
[34] Brugger, R., Reichel, C., Garcia Alia, B., Brune, K., Yamamoto, T., Tegeder, I. and Geisslinger, G. (2001) Biochem. Pharmacol. 61, 651–656.