Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic Chemistry 32 (2004) 326–340
BIOORGANIC CHEMISTRY
www.elsevier.com/locate/bioorg

Mini-review

S-Adenosylmethionine radical enzymes

E. Neil G. Marsh,* Anjali Patwardhan, and Marja S. Huhta

Department of Chemistry, University of Michigan, Ann Arbor, MI 48109-1055, USA

Received 1 June 2004

Abstract

The role of *S*-adenosylmethionine (SAM) as a precursor to organic radicals, generated by one-electron reduction of SAM and subsequent fission to form 5′-deoxyadenosyl radical and methionine, has been known for some time. Only recently, however, has it become apparent how widespread such enzymes are, and what a wide range of chemical reactions they catalyze. In the last few years several new SAM radical enzymes have been identified. Spectroscopic and kinetic investigations have begun to uncover the mechanism by which an iron sulfur cluster unique to these enzymes reduces SAM to generate adenosyl radical. Most recently, the first X-ray structures of SAM radical enzymes, coproporphyrinogen-III oxidase, and biotin synthase have been solved, providing a structural framework within which to interpret mechanistic studies.

Keywords: *S*-Adenosylmethionine

1. Introduction

S-Adenosylmethionine (SAM) is best known for its role as a biological methylating agent [1,2], although examples are also known in which the carboxy-amino-propyl side chain serves as the alkylating group [3]. Recently, though, there has been increasing interest in the role of SAM in generating organic radicals [4–10]. These are required in a variety of enzyme reactions and formed by single-electron reduction of

* Corresponding author. Fax: 1-734-615-3790.
E-mail address: nmarsh@umich.edu (E.N.G. Marsh).

0045-2068/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bioorg.2004.06.001

SAM and subsequent homolysis to give 5′-deoxyadenosyl radical (Ado·) and methionine.

The first SAM radical enzyme to be identified was lysine-2,3-aminomutase (LAM), discovered by Barker and co-workers in 1970 [11,12]. This enzyme catalyzes the reversible transformation of L-lysine to L-β-lysine, an unusual reaction in which a hydrogen on C-3 exchanges places with the aminogroup on C-2. This reaction bears many mechanistic similarities to the rearrangements catalyzed by adenosylcobalamin(AdoCbl)-dependent enzymes [13–17], and indeed both SAM and AdoCbl function as a source of Ado· radicals that serve as the intermediate carrier of hydrogen in these rearrangements (Fig. 1).

The similarity of the reactions catalyzed by LAM and AdoCbl-dependent isomerases led to SAM being dubbed "a poor man's B_{12}" [18]. However, it is now becoming clear that this epithet is inappropriate [10], as SAM-radical enzymes catalyze a much wider variety of reactions than do AdoCbl-dependent enzymes. These reactions, summarized in Fig. 2, include the generation of a glycyl radical in a group of enzymes that catalyze a diverse range of radical-requiring reactions under anaerobic conditions [19]; oxidation of a serine residue to formylglycine in certain sulfatase enzymes [20]; key roles in sulfur insertion reactions that occur during the biosynthesis of biotin and lipoate [21]; oxidative decarboxylation steps in the anaerobic pathway of haem biosynthesis [22]; and the repair of thymine dimers in photo-damaged DNA

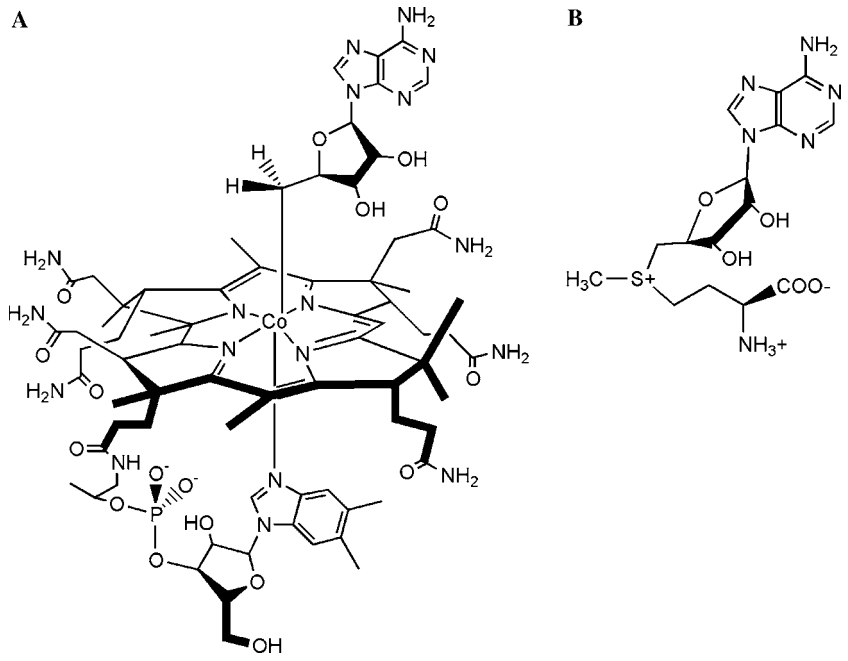

Fig. 1. Structures of adenosylcobalamin (A) and *S*-adenosylmethionine (B). Adenosylcobalamin generates 5′-deoxyadenosyl radical by homolytic fission of the Co–C bond whereas *S*-adenosylmethionine generates 5′-deoxyadenosyl radical by single-electron reductive cleavage of the C–S bond.

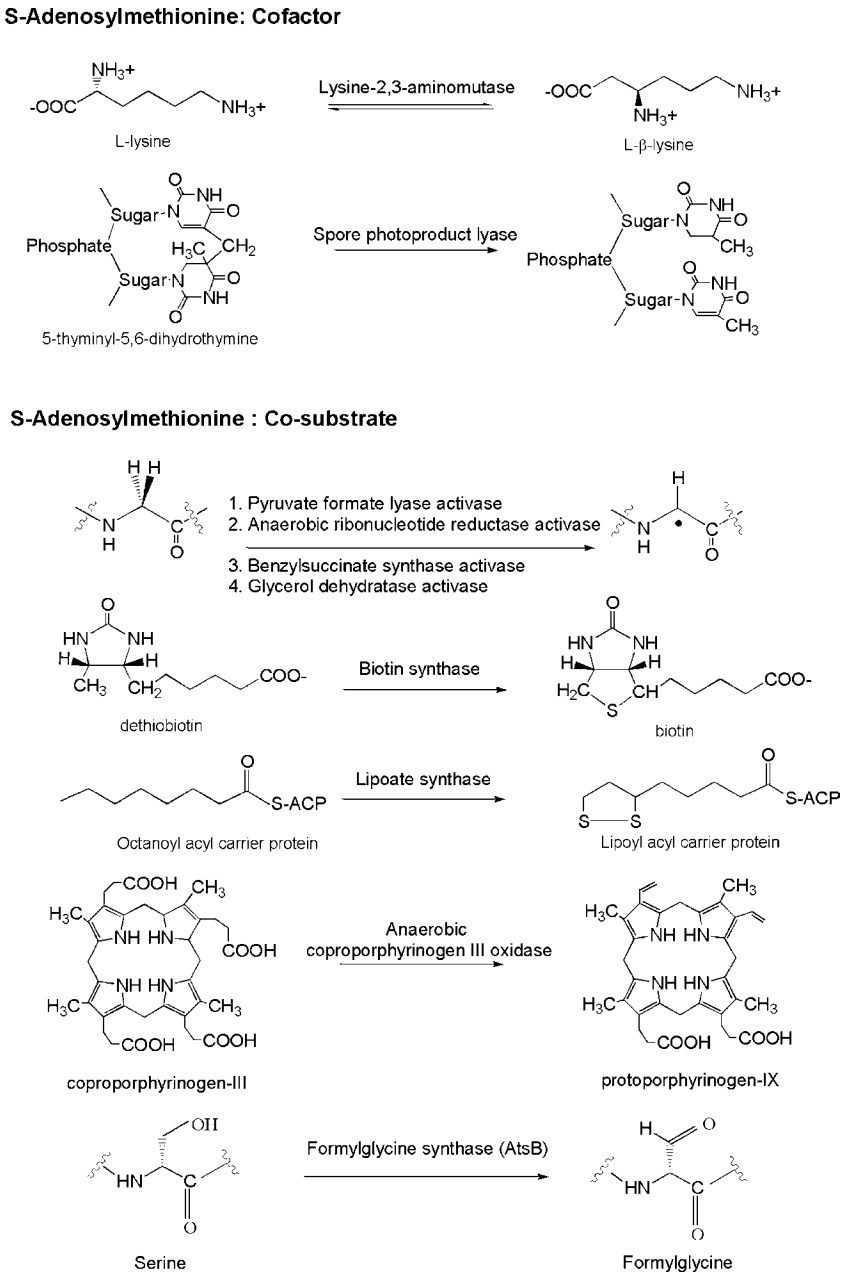

Fig. 2. Reactions catalyzed by SAM radical enzymes. In some enzymes, SAM serves as a true co-factor, i.e., Ado· is formed reversibly from SAM, in others SAM serves as a co-substrate, i.e., Ado· is formed irreversibly from SAM and the radical is used to oxidize the substrate. This results in one equivalent of methionine and 5′-deoxyadenosine being formed at each turn over.

[23]. Genomic sequence analysis [24] hints at the possibility of many more SAM radical enzymes awaiting discovery!

Although these reactions appear very different, all the enzymes characterized so far share a requirement for SAM, a one-electron reductant, and contain a unique $[4Fe–4S]^{+}$ cluster [9,10]. This cluster is extremely oxygen labile and consequently the enzymes only remain active under strictly anaerobic conditions, a factor that has made characterization of these enzymes a challenging endeavor. In this review, we first discuss what is known of the mechanism by which Ado· is generated from SAM, focusing on LAM and pyruvate formate-lyase activase, which are the two most extensively characterized enzymes, and then survey the diverse array of reactions that utilize SAM-generated Ado· radicals.

2. Generation of Ado· from SAM

Although no longer the poor relation to AdoCbl, the comparison between the two co-factors is informative. In AdoCbl, Ado· is generated through enzyme-catalyzed homolysis of the cobalt–carbon bond, and in all cases this is a reversible process [16,17]. The cobalt–carbon bond is relatively weak with bond dissociation energy of $\sim 30\,\mathrm{kcal\,mol^{-1}}$ and can readily undergo homolytic cleavage in response to substrate binding to form the 5′-deoxyadenosyl radical [25]. In contrast, the sulfur–carbon bond of SAM is much stronger ($\geqslant 60\,\mathrm{kcal\,mol^{-1}}$) and cannot be broken homolytically in the active site. Rather, reductive cleavage must occur to produce methionine and the 5′-deoxyadenosyl radical. [10,12]. In general, this is an irreversible process and Ado· radicals generated this way are subsequently used to oxidize the substrate. Interesting, however, two enzymes, LAM and spore photoproduct-lyase [26,27], appear to generate Ado· reversibly; the reactions catalyzed by these enzymes involve no overall change in the oxidation state of the substrate.

3. Role of the iron–sulfur cluster

Iron–sulfur clusters function as redox co-factors in a wide variety of proteins [28,29]. Although different cluster geometries are known, such as 2Fe–2S, 3Fe–4S, and 4Fe–4S, in general, each iron is ligated by at least one cysteine residue from the protein backbone. In contrast, the 4Fe–4S cluster found in SAM radical proteins is coordinated by only three cysteines that are found in the conserved "CxxxCxxC" iron–sulfur cluster-binding sequence motif [24]. The fourth iron is not coordinated by the protein, and this, presumably, is the reason for the extreme lability of the cluster and its ready decomposition under mild oxidizing conditions [30,31].

Electron paramagnetic resonance (EPR) spectroscopy on several of the enzymes has identified the $[4Fe–4S]^{1+}$ form of the cluster as the catalytically active species [32–35]. During the course of the reaction oxidation to a $[4Fe–4S]^{2+}$ cluster occurs, which is EPR silent. Experiments on LAM demonstrated that the rates of formation of the organic substrate radical and the rate of decomposition of the $[4Fe–4S]^{1+}$ signal were

the same, as were the rates of formation of 5′-deoxyadenosine and methionine (the cleavage products of SAM) [36]. Similar experiments on pyruvate formate-lyase activase, demonstrate the role of the $[4Fe–4S]^{1+}$ cluster in generating the glycyl radical on its partner protein, pyruvate formate-lyase (PFL) [37]. In this case, the active $[4Fe–4S]^{1+}$ cluster was prepared by photoreduction of PFL activase with deazaflavin, followed by the addition of SAM and then PFL. This resulted in an EPR signal characteristic of the PFL glycyl radical, disappearance of the signal due to the $[4Fe–4S]^{1+}$ cluster, and the formation of stoichiometric amounts of 5′-deoxyadenosine and methionine. These experiments provide firm support for the role of the iron–sulfur cluster as the source of electrons necessary for the reduction of SAM and the formation of radicals.

The unligated iron is crucial for the interaction between the cluster and SAM, and EPR experiments demonstrate that addition of SAM (in the absence of substrate) to the protein changes the spectral properties of the cluster [8–10,23]. Recent spectroscopic studies have resulted in two detailed proposals for the interaction between SAM and the Fe–S cluster, resulting in two different proposed mechanisms for SAM cleavage (Fig. 3).

Selenium X-ray absorption spectroscopy studies on LAM support direct ligation of the SAM sulfonium sulfur to the unligated iron of the cluster [38], and further electron double nuclear resonance experiments [39] demonstrated that the α-amino

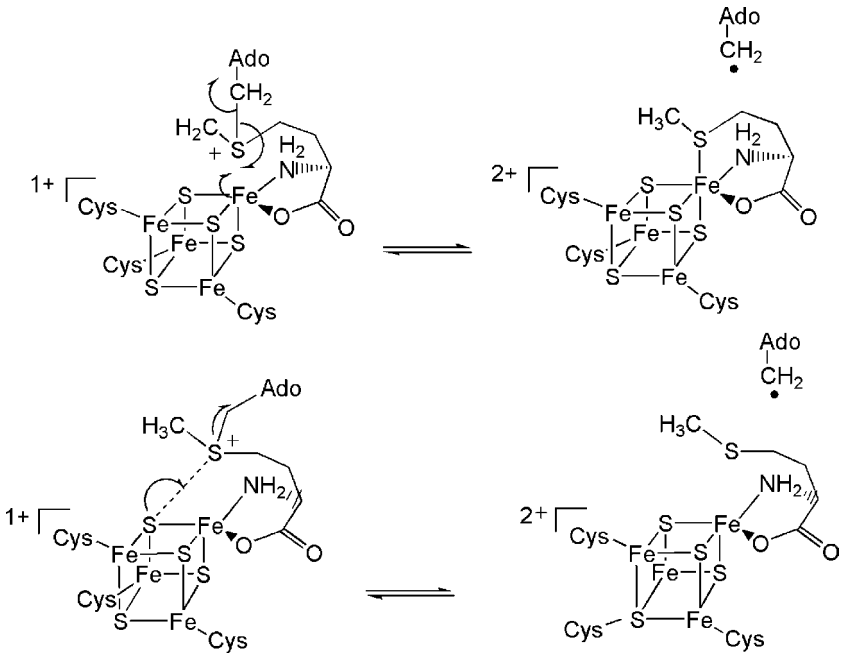

Fig. 3. Different binding modes proposed for SAM binding to the $[4Fe–4S]^{+}$ cluster in LAM (upper scheme) and PFL or biotin synthase (lower scheme). In LAM, electron transfer to the sulfonium ion of SAM is proposed to proceed through the methionine-coordinated iron, whereas in PFL, it is proposed to take place through a sulfur atom in the $[4Fe–4S]^{+}$ cluster.

and α-carboxyl groups of SAM also coordinate to the unligated iron of the cluster. The proposed mechanism involves inner-sphere electron transfer from the $[4Fe–4S]^+$ cluster to the sulfonium of SAM, accompanied by ligation of the fourth iron to the sulfur of SAM and simultaneous cleavage of the S–C bond to form the adenosyl radical (Fig. 3). This would result in methionine coordinating the iron atom of the cluster through sulfur as well as the α-amino and carboxyl groups. [10,38]

In contrast, EPR and Mossbauer spectroscopy on PFL activase and biotin synthase suggested that the sulfonium of SAM is more closely associated with one of the sulfides rather than the unligated iron of the $[4Fe–4S]^+$ [33–35,40]. For these enzymes a mechanism is proposed in which inner-sphere electron transfer occurs from the $[4Fe–4S]^+$ cluster to SAM via a sulfide–sulfonium interaction, resulting in homolytic cleavage of the sulfonium–adenosyl bond to form the adenosyl radical (Fig. 3). This mechanism predicts that methionine would only coordinate to the cluster through the amino and carboxyl groups [33]. The recent crystal structure of biotin synthase (discussed below) supports the spectroscopic data obtained for PFL activase and biotin synthase [41]. The α-amino and α-carboxyl groups of SAM provide ligands to the "free" iron of the cluster, but the sulfonium of SAM is 4.0 Å away from the nearest iron.

The apparently different modes of SAM-binding observed for LAM and PFL activase and biotin synthase may reflect the slightly different roles that SAM plays in each reaction. In the LAM reaction SAM is regenerated after each turnover, whereas during the reactions of both PFL activase and biotin synthase, SAM is a co-substrate and is consumed during each turnover. Coordination between the iron ion of the cluster and the sulfur of methionine may modulate the reactivity of the sulfur and be important in maintaining the proper positioning of methionine necessary for reformation of SAM [42].

4. Emergence of a SAM radical enzyme superfamily

Although they share the CxxxCxxC iron–sulfur cluster-binding sequence [23], very little sequence similarity was apparent between the initially identified SAM radical enzymes. However, using sophisticated iterative sequence profiling methods, Sofia et al. [24] identified distant sequence similarities between the known SAM radical enzymes to generate a profile for SAM radical enzymes. By comparing the protein sequence database against this profile, an intriguingly large, putative superfamily of more than 600 potential SAM radical enzymes was identified. This superfamily includes proteins found at unresolved steps in familiar biosynthetic pathways, including the biosynthesis of thiamin, haem, bacteriochlorophyll, molybdopterin, and nitrogenase co-factor, among others [9,24]. This suggested that SAM radical enzymes were much more widely distributed than previously thought, even allowing for the fact that some of the proteins identified in the analysis are probably not SAM radical enzymes [9].

Based on this sequence analysis, three new SAM radical enzymes: anaerobic coproporphyrinogen-III oxidase [43], anaerobic formylglycine generating enzyme [20], and cobalamin-independent glycerol dehydrase—glycerol dehydrase activase system [44]

have recently been identified and subsequently characterized. This provides an impressive demonstration of the utility of this "post-genomic" approach to enzymology.

5. Biochemically characterized SAM radical enzymes

The discussion above points to a potentially large and mechanistically diverse collection of enzymes, however relatively few SAM radical enzymes have been characterized biochemically. This is undoubtedly because the extreme oxygen sensitivity of all the enzymes examined so far makes these technically challenging systems to assay and characterize. For the purposes of this review, we divide the known enzymes into two classes (Fig. 2). First, those utilizing SAM as a true co-factor of which LAM and spore photoproduct-lyase are currently the only known members. Second, those using SAM as a co-substrate to oxidize either small substrates or proteins by hydrogen abstraction, this second group includes a subgroup of activase enzymes that generate glycyl radicals on a range of partner enzymes.

6. Lysine-2,3-aminomutase and spore photoproduct-lyase

Lysine-2,3-aminomutase catalyzes the inter-conversion of L-lysine and L-β-lysine, in which the 3-*proR* hydrogen migrates to the 2-*proR* position of β-lysine with inversion of configuration. The enzyme, which also requires pyridoxal phosphate as a co-factor, is one of the most extensively characterized SAM radical enzymes [10,45]. More recently identified is spore photoproduct-lyase, which catalyzes the repair of methylene-bridged thymine dimers formed in DNA [46,47]. In neither of these reactions is the redox state of the substrate altered, and, consistent with this, SAM is used as a true co-factor, rather than an oxidizing agent, by these enzymes.

Experiments with tritium-labeled substrate and 5′-tritium-labeled SAM have established that SAM is the intermediate carrier of the migrating hydrogen in LAM. In this respect it functions in a manner exactly analogous to AdoCbl [26,48]. A series of elegant EPR experiments employing isotopically labeled substrates, and substrate analogs of lysine that preferentially stabilize the different radials formed during the course of the rearrangement, have allowed various radical species that are proposed to be intermediates in the mechanism to be identified [49–52]. During the reaction, the α-amino group of lysine forms an external aldimine with pyridoxal phosphate [53], rendering the nitrogen sp^2 hybridized. This allows the 1,2 nitrogen migration to occur through a cyclic azacyclopropylcarbinyl radical intermediate transition state in which nitrogen is bonded to both C-2 and C-3 of lysine and the unpaired electron is situated on the 4′-carbon of pyridoxal and stabilized by the adjacent π system.

For spore photoproduct-lyase both the formation of 5′-deoxyadenosine and methionine, and the transfer of tritium from SAM to thymine have been demonstrated, strongly implicating a role for Ado· in this reaction [46,47]. A plausible mechanism, shown in Fig. 4, and which is supported by chemical model experiments [54], involves

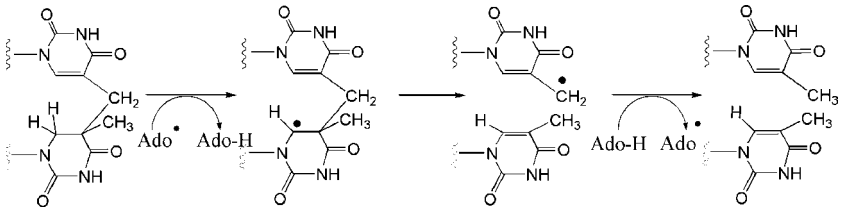

Fig. 4. Proposed mechanism for the spore photoproduct-lyase-catalyzed reaction. In this case Ado• is regenerated at the end of the reaction as there is no overall change in oxidation state.

abstraction of the C-6 hydrogen from the thymine dimer, which then undergoes fragmentation to generate one thymine and the thymine monomer radical. Transfer of hydrogen back from 5′-dA forms the second thymine and regenerates Ado•.

7. SAM radical activases of glycyl radical enzymes

So far, four enzymes are known that require a protein-based glycyl radical for activity, all of which are active only under strictly anaerobic conditions. These enzymes are pyruvate formate-lyase (PFL) [19], anaerobic ribonucleotide reductase (anRR) [55], benzylsuccinate synthase (BSS) [56–58], and glycerol dehydratase [44]. This radical is situated on a specific glycine residue located on a flexible region of the enzyme close to the C-terminus, where it is stabilized by delocalization onto the amide nitrogen and carbonyl groups of the protein back bone and easily observed by EPR. [59–63]. The glycyl radical does not participate directly in the reactions catalyzed by these enzymes, rather, the radical is transferred to a cysteine residue at the active sites of these enzymes and thence onto the substrate. As such, the relatively stable glycyl may be seen as a way of storing the radical—in contrast, the adenosyl radical is too highly reactive to exist for any length of time.

PFL catalyzes the oxidative decarboxylation of pyruvate to form acetyl-CoA and formate. This reaction plays a central role in the anaerobic metabolism of glucose to acetyl-CoA in *Escherichia coli*, where PFL replaces the pyruvate dehydrogenase complex, which functions under aerobic conditions, as the primary means of making acetyl-CoA. anRR catalyses the reduction of ribonucleotides to deoxyribonucleotides necessary for DNA synthesis, and as such is, of course, essential for the growth of bacteria such as *E. coli* under anaerobic conditions. These enzymes are the most well characterized members of this group, and several reviews have appeared recently [10,19,55,64,65].

The least understood, structurally and mechanistically, enzyme in this group is BSS, which catalyzes the initial step in the anaerobic catabolism of toluene in several denitrifying, metal ion and sulfate-reducing bacteria. This extremely unusual reaction involves the addition of toluene across the double bond of fumarate to give (*R*)-benzylsuccinate [66,67]. A potential mechanism for this most unusual reaction is shown in Fig. 5. BSS has been purified from *Thauera aromatica* and shown to posses an

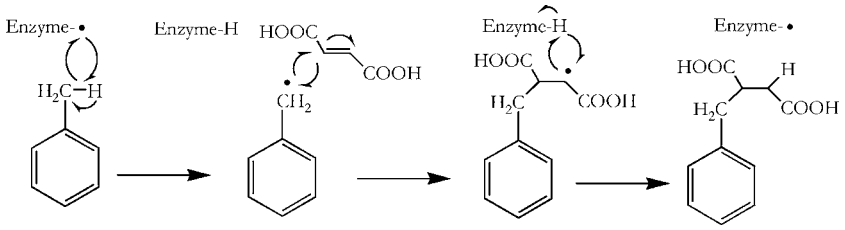

Fig. 5. Proposed mechanism for the glycyl radical enzyme, benzylsuccinate synthase. The glycyl radical is generated on the enzyme by oxidation by Ado•; a reaction catalyzed by a specific SAM radical activase enzyme.

$\alpha_2\beta_2\gamma_2$ structure [68]; in other experiments the glycyl radical has been observed by EPR [62]. The genes encoding BSS and BSS activase have been cloned from several organisms [56,68,69]. The α-subunit shows sequence similarity to the better-characterized glycyl radical enzymes PFL and anRR; the functions of the other subunits are unknown, but genetic analysis has shown them to be required for *T. aromatica* to grow on toluene [70].

Cobalamin-independent glycerol dehydrase from *Clostridium butyricum*, which catalyzes the conversion of glycerol to 3-hydroxy-propionaldehyde, is the most recently discovered member of this group [71]. Despite this, its crystal structure is already determined, which shows the enzyme to adopt the same 10-stranded β/α barrel motif as PFL and anRR [44]. The mechanism has not been investigated as nearly extensively as the cobalamin-dependent enzyme [72–74], and although the overall reactions are clearly similar, the active sites are quite different and the anaerobic enzyme does not require potassium ion for activity.

For each of these enzymes a SAM radical-dependent activase is responsible for introducing the glycyl radical into the protein. The activases are specific to their corresponding enzymes—the PFL and anRR enzymes from *E. coli* do not show cross-reactivity—and as such the activases also play a role in regulating the activity of the parent enzyme. PFL activase is perhaps the best-understood enzyme, in this case glycine-734 is oxidized [59]. The abstraction of hydrogen by Ado• from glycine has been demonstrated by observing the incorporation of deuterium from PFL-labeled with d_2-glycine into 5′-deoxyadenosine. Small peptides have been shown to be substrates for the enzyme. This allowed the adenosyl radical to be trapped as an adduct formed by addition of the radical to the double bond of a peptide incorporating anhydroalanine in place of glycine [75]. The stereochemical course of the reaction has been investigated with peptide substrates to provide evidence that it is the *pro-S* hydrogen of glycine that is abstracted [76].

8. Biotin and lipoate synthases

Biotin synthase and lipoyl synthase catalyze similar reactions involving the insertion of sulfur atom(s) into C–H bonds during the biosynthesis of biotin and lipoic acid [21]. Biotin synthase catalyzes the formation of biotin from dethiobiotin

as the last step in biotin biosynthesis [9,41,77], whereas lipoyl synthase catalyzes the formation of lipoyl acyl carrier protein from octanoyl acyl carrier protein [78]. Of the two enzymes biotin synthase is, so far, better characterized. These reactions are thought to take place in two steps whereby adenosyl radicals generated from SAM are used to oxidize the two unreactive carbon atoms of dethiobiotin to generate an organic radical that subsequently reacts with sulfur to form a carbon–sulfur bond (Fig. 6) [79].

One long-standing question has been the source of sulfur in these reactions. Experiments with isotopically labeled inorganic sulfide demonstrated high levels of incorporation into biotin [80], and Jarrett and co-workers [10,77,81,82] have demonstrated that a 2Fe–2S cluster is also present in biotin synthase, and proposed that this cluster supplies the sulfur. The 2Fe–2S cluster appears to be destroyed during the reaction [83], and recently reported crystal structure [41] (discussed below) reveals the substrates and co-factors in the active site to be appropriately set up for the proposed mechanism. Many mechanistic details (for example which carbon is oxidized first?) remain unknown, in part because the enzyme has never been shown to perform more than a single turn-over during *in vitro* assay. A mechanism involving the destruction of the second iron–sulfur co-factor during the reaction would explain this result. Whether the cluster is rebuilt by intrinsic cysteine desulfurases, in the cell, or whether the enzyme is truly a single-turn-over enzyme (only a few molecules of biotin are required by an *E. coli* cell), is unknown.

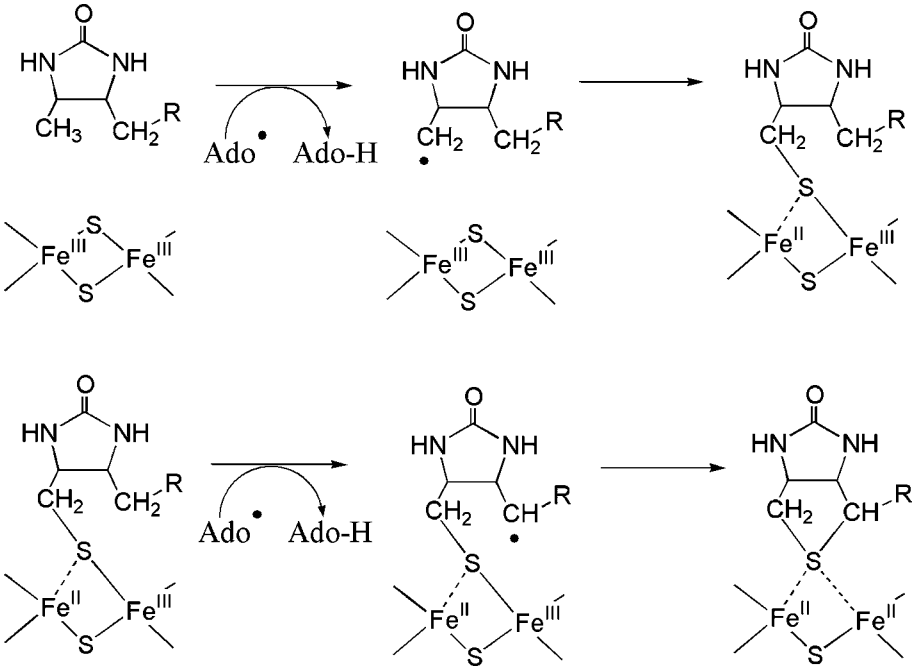

Fig. 6. Proposed mechanism for the sulfur insertion reactions catalyzed by biotin synthase.

9. Coproporphyrinogen III oxidase

This enzyme catalyzes the oxidative decarboxylation of the two propionate groups of the A and B rings of coproporphyrinogen III to form protoporphyrinogen IX under anaerobic conditions in *E. coli* [43]. The enzyme is the product of the *hemN* gene, and its biological function was only deduced after it was identified as a member of the SAM radical superfamily. The mechanism is proposed to involve abstraction of hydrogen from the β-position of the propionate side chain to form a radical that is stabilized by conjugation to the pi system of the pyrrole ring. Further oxidation of this radical by a one-electron acceptor (the physiological electron acceptor is as yet unknown) yields an allylic carbocation that facilitates the decarboxylation and formation of the vinyl group (Fig. 7). Interestingly, the crystal structure (discussed below) shows two molecules of SAM bound in the active site, and it has been suggested that this may be mechanistically significant given that two separate propionate side-chains are oxidized in the overall reaction [22].

10. Formylglycine synthase

The most recently discovered enzyme to be characterized as a SAM radical enzyme is formylglycine synthase (AtsB protein) from *Klebsiella pneumoniae* [20]. Again, the enzyme was identified on the basis of sequence similarities that place it in the SAM radical superfamily. Formylglycine is so far found only in sulfatase enzymes that hydrolyze sulfate esters. It is formed post-translationally by oxidation of either a conserved cysteine or serine to the aldehyde, which in solution is predominately hydrated. Nucleophilic attack of one of the hydroxyl groups on the sulfate ester results in transfer of the sulfate group to the enzyme; subsequent elimination of the sulfate occurs to regenerate the formyl group. It appears only the serine-modifying enzyme is SAM-dependent. The mechanism is proposed to involve hydrogen abstraction from the β-carbon of serine by Ado· and subsequent one electron oxidation to the aldehyde, although the electron acceptor for the second step is not known.

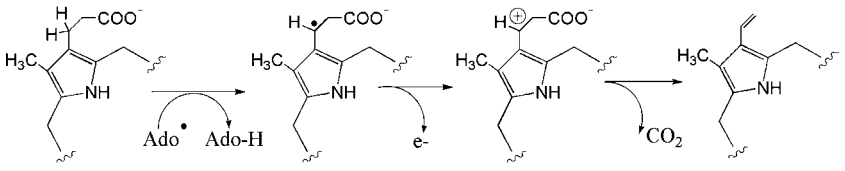

Fig. 7. Proposed mechanism for the oxidative decarboxylation of coproporphyrinogen-III to protoporphyrinogen IX catalyzed by coproporphyrinogen-III oxidase.

11. Structures of SAM radical enzymes: biotin synthase and coproporphyrinogen-III oxidase

Crystal structures for two SAM radical enzymes, biotin synthase and oxygen-independent coproporphyrinogen-III oxidase, have recently been determined, providing an informative picture of the active site, that will presumably be similar for other radical SAM enzymes. The structures also provide support to the proposed mechanism of Ado· generation deduced from spectroscopic and kinetic experiments. Not surprisingly, the active sites of these enzymes are in many ways very similar, although some major structural differences are also apparent that probably represent adaptations to the rather different overall reactions catalyzed by the two enzymes.

Biotin synthase is a homodimer in solution and each monomer comprises an $(\alpha/\beta)_8$ TIM (triosphosphaste isomerase) barrel (Fig. 8A) [41]. The active site is located

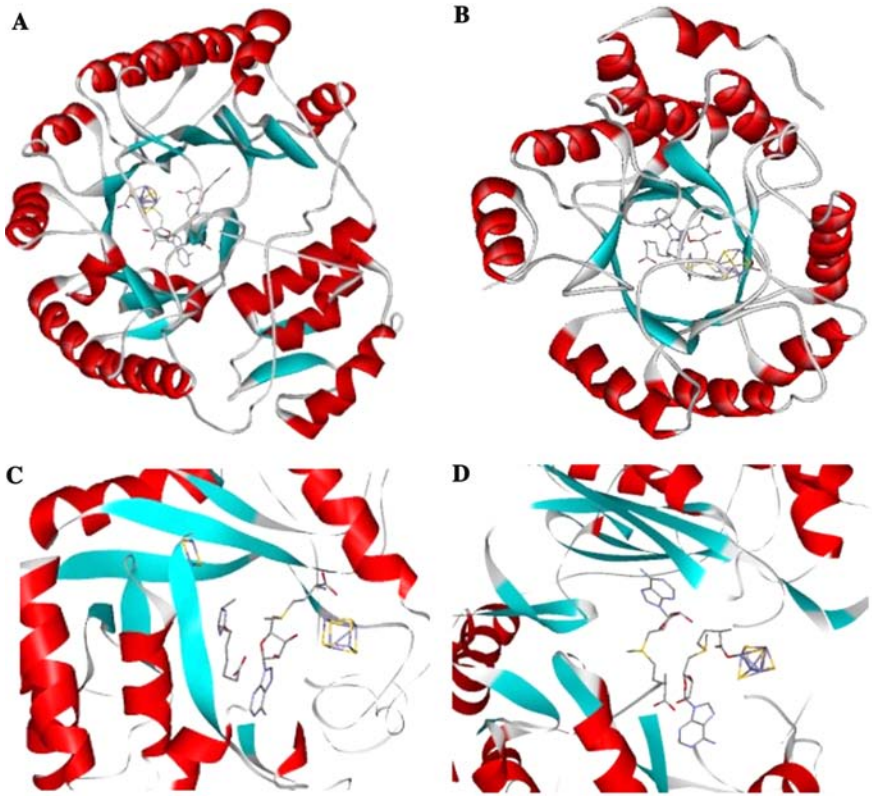

Fig. 8. Structures of biotin synthase and coproporphyrinogen-III oxidase. Views of (A) biotin synthase and (B) coproporphyrinogen-III oxidase illustrating the similarities in tertiary structure between the two enzymes. Views of the active sites of (C) biotin synthase and (D) coproporphyrinogen-III oxidase. For biotin synthase the 4Fe–4S cluster, SAM, dethiobiotin and the 2Fe–2S cluster are depicted in stick form. In the active site of coproporphyrinogen-III oxidase two molecules of SAM are present, only one of which coordinates the 4Fe–4S cluster.

deep within the barrel, juxtaposed between the $[4Fe–4S]^+$ cluster and the [2Fe–2S] cluster that acts as the sulfur source during the reaction (Fig. 8C). Interestingly, the second iron–sulfur cluster has an arginine residue as one of the ligands to iron, which is most unusual and might reflect the equally unusual role of the iron–sulfur cluster in this enzyme. Oxygen-independent coproporphyrinogen-III oxidase is a monomer containing two distinct domains (Fig. 8B). The catalytic domain comprises a 12-stranded β-sheet surrounded by α-helices. The core of this domain contains the active site (Fig. 8D) and is a three-quarter barrel, a $(\alpha/\beta)_6$ variation of the $(\alpha/\beta)_8$ TIM barrel [22]. One reason for this unusual variation of the TIM barrel fold may be that this is an adaptation of the basic fold to accommodate the bulky tetrapyrrole substrate [22]. Interestingly, three AdoCbl-dependent isomerases whose structures are known also adopt the TIM barrel fold. Indeed the TIM barrel of biotin synthase can be superimposed on the TIM barrel of AdoCbl-dependent diol dehydrase, with the [4Fe–4S] cluster occupying the same space as the corrin ring of AdoCbl. The TIM barrel fold, which allows radicals to be sequestered deep within the protein, is apparently well adapted to radical catalysis.

12. Conclusions

It is now clear that SAM radical enzymes comprise a large class of enzymes that catalyze a wide range of radical reaction under anaerobic conditions. Several enzymes have only very recently been discovered and undoubtedly more will follow soon. In general, Ado·, generated from SAM, is used as a powerful oxidizing agent, capable of removing hydrogen from the least reactive of carbon atoms. In this respect, these enzymes may be compared with cytochrome P450 enzymes, or non-haem iron oxidases that catalyze similarly hard oxidative reactions using O_2 as the oxidant. Interestingly, in both cases the redox chemistry of iron is crucial to the activation of the oxidizing agent. Less often, SAM is used to generate Ado· reversibly, and here there are clear mechanistic and structural comparisons with AdoCbl-dependent enzymes, in which Ado· is always formed reversibly. SAM radical enzymes are probably of ancient evolution. In an anaerobic world, before the advent of photosynthesis, the oxygen-dependent mechanisms for functionalizing unreactive carbons would obviously not be available, and by the same token, scavenging of free radicals by oxygen—the major pathway by which radicals cause damage in cells—would not be a problem. AdoCbl-dependent enzymes, which share the same basic fold, may have evolved from SAM radical enzymes because this co-factor allows radicals to be generated reversibly and is oxygen stable.

Acknowledgments

Work in the authors' laboratory on radical enzymes is funded by NIH Grant GM 59227 to E.N.G.M.

References

[1] P.K. Chiang, R.K. Gordon, J. Tal, G.C. Zeng, B.P. Doctor, K. Pardhasaradhi, P.P. McCann, FASEB J. 10 (1996) 471–480.
[2] G. Cantoni, Annu. Rev. Biochem. 44 (1975) 435–451.
[3] A.M. Reeve, S.D. Breazeale, C.A. Townsend, J. Biol. Chem. 273 (1998) 30695–30703.
[4] E.N.G. Marsh, Bioessays 17 (1995) 431–441.
[5] J. Stubbe, W. Van der Donk, Chem. Rev. 98 (1998) 705–762.
[6] P.A. Frey, Annu. Rev. Biochem. 70 (2001) 121–148.
[7] J. Cheek, J.B. Broderick, J. Biol. Inorg. Chem. 6 (2001) 209–226.
[8] M. Fontecave, E. Mulliez, S. Ollagnier-de-Choudens, Curr. Opin. Chem. Biol. 5 (2001) 505–511.
[9] J.T. Jarrett, Curr. Opin. Chem. Biol. 7 (2003) 174–182.
[10] P. Frey, O. Magnusson, Chem. Rev. 103 (2003) 2129–2148.
[11] T. Chirpich, V. Zappia, R. Costilow, H.A. Barker, J. Biol. Chem. (1970) 1778–1789.
[12] P. Frey, G. Reed, Arch. Biochem. Biophys. 382 (2000) 6–14.
[13] E.N.G. Marsh, Essays Biochem. 34 (1999) 139–154.
[14] E.N.G. Marsh, Bioorg. Chem. 28 (2000) 176–189.
[15] R. Banerjee, Biochemistry 40 (2001) 6191–6198.
[16] E.N.G. Marsh, C. Drennan, Curr. Opin. Chem Biol. 5 (2001) 499–505.
[17] R. Banerjee, Chem. Rev. 103 (2003) 2083–2094.
[18] P. Frey, FASEB J. 7 (1993) 662–670.
[19] J. Knappe, A.F.V. Wagner, Adv. Prot. Chem. 58 (2001) 277–315.
[20] Q.H. Fang, J.H. Peng, T. Dierks, J. Biol. Chem. 279 (2004) 14570–14578.
[21] A. Marquet, Curr. Opin. Chem. Biol. 5 (2001) 541–549.
[22] G. Layer, J. Moser, D. Heinz, D. Jahn, W-D. Schubert, EMBO J. 22 (2003) 6214–6224.
[23] J. Cheek, J. Broderick, J. Biol. Inorg. Chem. 6 (2001) 209–226.
[24] H.J. Sofia, G. Chen, B.G. Hetzler, J.F. Reyes-Spindola, N. Miller, Nucleic Acids Res. 29 (2001) 1097–1106.
[25] B.P. Hay, R.G. Finke, Polyhedron 7 (1988) 1469–1481.
[26] J. Baraniak, M. Moss, P. Frey, J. Biol. Chem. (1989) 1357–1360.
[27] J. Cheek, J.B. Broderick, J. Am. Chem. Soc. 124 (2002) 2860–2861.
[28] H. Beinert, R. Holm, E. Munck, Science 277 (1997) 653–659.
[29] H. Beinert, J. Biol. Inorg. Chem. 5 (2000) 2–15.
[30] C. Krebs, T.F. Henshaw, J. Cheek, B.H. Huynh, J.B. Broderick, J. Am. Chem. Soc. 122 (2000) 12497–12506.
[31] J.B. Broderick, T.F. Henshaw, J. Cheek, K. Wojtuszewski, S.R. Smith, M.R. Trojan, R.M. McGhan, A. Kopf, M. Kibbey, W.E. Broderick, Biochem. Biophys. Res. Commun. 269 (2000) 451–456.
[32] K.W. Lieder, S. Booker, F.J. Ruzicka, H. Beinert, G.H. Reed, P.A. Frey, Biochemistry 37 (1998) 2578–2585.
[33] C. Walsby, W. Hong, W. Broderick, J. Cheek, D. Ortillo, J. Broderick, B. Hoffman, J. Am. Chem. Soc. 124 (2002) 3143–3151.
[34] C. Walsby, D. Ortillo, W. Broderick, J. Broderick, B. Hoffman, J. Am. Chem. Soc. 124 (2002) 11270–11271.
[35] M. Cosper, G. Jameson, R. Davydov, M. Eidsness, B. Hoffman, B. Huynh, M. Johnson, J. Am. Chem. Soc. 124 (2002) 14006–14007.
[36] W. Wu, S. Booker, K. Lieder, V. Bandarian, G. Reed, P. Frey, Biochemistry 39 (2000) 9561–9570.
[37] T. Henshaw, J. Cheek, J. Broderick, J. Am. Chem. Soc. 122 (2000) 8331–8332.
[38] N. Cosper, S. Booker, F. Ruzicka, P. Frey, R. Scott, Biochemistry 39 (2000) 15668–15673.
[39] D.W. Chen, C. Walsby, B.M. Hoffman, P.A. Frey, J. Am. Chem. Soc. 125 (2003) 11788–11789.
[40] C. Krebs, W. Broderick, T. Henshaw, J. Broderick, B. Huynh, J. Am. Chem. Soc. 124 (2002) 912–913.
[41] F. Berkovitch, Y. Nicolet, J. Wan, J.T. Jarrett, C.L. Drennan, Science 303 (2004) 76–79.
[42] M. Cosper, N. Cosper, W. Hong, J. Shokes, W. Broderick, J. Broderick, M. Johnson, R. Scott, Protein Sci. 12 (2003) 1573–1577.

[43] G. Layer, K. Verfurth, E. Mahlitz, D. Jahn, J. Biol. Chem. 277 (2002) 34136–34142.
[44] J.R.R. O'Brien, C. Croux, L. Girbal, P. Soucaille, W.N. Lanzilotta, Biochemistry 43 (2004) 4635–4645.
[45] P.A. Frey, M.D. Ballinger, G.H. Reed, Biochem. Soc. Trans. 26 (1998) 304–310.
[46] R. Rebeil, W. Nicholson, Proc. Natl. Acad. Sci. 98 (2001) 9038–9043.
[47] J. Cheek, J. Broderick, J. Am. Chem. Soc. 124 (2002) 2860–2861.
[48] M. Moss, P. Frey, J. Biol. Chem. (1987) 14859–14862.
[49] M.D. Ballinger, P.A. Frey, G.H. Reed, Biochemistry 31 (1992) 10782–10789.
[50] W.M. Wu, K.W. Lieder, G.H. Reed, P.A. Frey, Biochemistry 34 (1995) 10532–10537.
[51] W.M. Wu, S. Booker, K.W. Lieder, V. Bandarian, G.H. Reed, P.A. Frey, Biochemistry 39 (2000) 9561–9570.
[52] P.A. Frey, C.H. Chang, M.D. Ballinger, G.H. Rees, Methods Enzymol. 354 (2002) 426–435.
[53] K.H. Tang, A. Harms, P.A. Frey, Biochemistry 41 (2002) 8767–8776.
[54] R.A. Mehl, T.P. Begley, Org. Lett. 1 (1999) 1065–1066.
[55] M. Fontecave, Cell. Mol. Life Sci. 54 (1998) 684–695.
[56] P. Coschigano, T. Wehrman, L. Young, Appl. Environ. Microbiol. 64 (1998) 1650–1656.
[57] J. Heider, A.M. Spormann, H.R. Beller, F. Widdel, Microbiol. Rev. 22 (1998) 459–473.
[58] C.J. Krieger, W. Roseboom, S.P.J. Albracht, A.M. Spormann, J. Biol. Chem. 276 (2001) 12924–12927.
[59] A. Wagner, M. Frey, F.A. Neugebauer, W. Schager, J. Knappe, Proc. Natl. Acad. Sci. USA 89 (1992) 996–1000.
[60] P. Young, J. Andersson, M. Sahlin, B. Sjoberg, J. Biol. Chem. 271 (1996) 20770.
[61] X. Sun, S. Ollagnier, P. Schmidt, M. Atta, E. Mulliez, L. Lepape, R. Eliasson, A. Gräslund, M. Fontecave, P. Reichard, B-M. Sjöberg, J. Biol. Chem. 271 (1996) 6827–6831.
[62] C. Krieger, W. Roseboom, S. Albracht, A.M. Spormann, J. Biol. Chem. 276 (2001) 12924–12927.
[63] C. Duboc-Toia, A. Hassan, E. Mulliez, S. Ollagnier-de Choudens, M. Fontecave, C. Leutwein, J. Heider, J. Am. Chem. Soc. 125 (2003) 38–39.
[64] J. Stubbe, W.A. van der Donk, Chem. Rev. 98 (1998) 705–762.
[65] A. Becker, K. Firtz-Wolf, W. Kabsch, J. Knappe, S. Schultz, A. Wagner, Nat. Struct. Bio. 6 (1999) 969–975.
[66] T. Biegert, G. Fuchs, F. Heider, Eur. J. Biochem. 238 (1996) 661–668.
[67] R. Rabus, J. Heider, Arch. Microbiol. 170 (1998) 377–384.
[68] B. Leuthner, C. Leutwein, H. Schulz, P. Horth, W. Haehnel, E. Schiltz, H. Schagger, J. Heider, Mol. Microbiol. 28 (1998) 615–628.
[69] G.R. Achong, A.M. Rodriguez, A.M. Spormann, J. Bacteriol. 183 (2001) 6763–6770.
[70] P. Coschigano, FEMS Microbiol. Lett. 217 (2002) 37–42.
[71] C. Raynaud, P. Sarcabal, I. Meynial-Salles, C. Croux, P. Soucaille, Proc. Nat. Acad. Sci. USA 100 (2003) 5010–5015.
[72] T. Toraya, Chem. Rev. 103 (2003) 2095–2127.
[73] M. Yamanishi, M. Yunoki, T. Tobimatsu, H. Sato, J. Matsui, A. Dokiya, Y. Iuchi, K. Oe, K. Suto, N. Shibata, Y. Morimoto, N. Yasuoka, T. Toraya, Eur. J. Biochem. 269 (2002) 4484–4494.
[74] T. Toraya, Cell. Mol. Life Sci. 57 (2000) 106–127.
[75] A. Wagner, J. Demand, G. Schilling, T. Pils, J. Knappe, Biochem. Biophys. Res. Commun. 254 (1999) 306–310.
[76] M. Frey, M. Rothe, A. Wagner, J. Knappe, J. Biol. Chem. 269 (1994) 12432–12437.
[77] N.B. Ugulava, K.K. Frederick, J.T. Jarrett, Biochemistry 42 (2003) 2708–2719.
[78] J. Miller, R. Busby, S. Jordan, J. Cheek, T. Henshaw, G. Ashley, J. Broderick, J. Cronan, M. Marletta, Biochemistry 39 (2000) 15166–15178.
[79] F. Escalettes, D. Florentin, B. Tse Sum Bui, D. Lesage, A. Marquet, J. Am. Chem. Soc. 121 (1999) 3571.
[80] B.T.S. Bui, D. Florentin, F. Fournier, O. Ploux, A. Mejean, A. Marquet, FEBS. Lett. 440 (1998) 226–230.
[81] N.B. Ugulava, B.R. Gibney, J.T. Jarrett, Biochemistry 40 (2001) 8343–8351.
[82] N.B. Ugulava, C.J. Sacanell, J.T. Jarrett, Biochemistry 40 (2001) 8352–8358.
[83] B.T.S. Bui, R. Benda, V. Schunemann, D. Florentin, A.X. Trautwein, A. Marquet, Biochemistry 42 (2003) 8791–8798