Reductive Cleavage of S-Adenosylmethionine by Biotin Synthase from Escherichia coli*

Received for publication, November 28, 2001, and in revised form, January 30, 2002
Published, JBC Papers in Press, February 7, 2002, DOI 10.1074/jbc.M111324200

Sandra Ollagnier-de Choudens‡, Yiannis Sanakis§§, Kirsty S. Hewitson¶¶, Eckard Münch§, and Marc Fontecave††
From the ‡Laboratoire de Chimie et Biochimie des Centres Réseaux Biologiques, Département de Biologie Moléculaire et Structurale-Chimie Biologique, CEA/CNRS/Université Joseph Fourier, UMR 5047, 17 Avenue des Martyrs, 38054 Grenoble Cedex 09, France, ¶¶Carnegie Mellon University, Pittsburgh, Pennsylvania 15213, the Oxford Center for Molecular Sciences, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY, United Kingdom, and the ‡‡Department of Chemistry, University of Southampton, Highfield, Southampton SO17 1BJ, United Kingdom

Biotin synthase (BioB) catalyzes the insertion of a sulfur atom between the C6 and C9 carbons of dethiobiotin. Reconstituted BioB from Escherichia coli contains a [4Fe-4S]2+/1+ cluster thought to be involved in the reduction and cleavage of S-adenosylmethionine (AdoMet), generating methionine and the reactive 5'-deoxyadenosyl radical responsible for dethiobiotin H-abstraction. Using EPR and Mössbauer spectroscopy as well as methionine quantitation we demonstrate that the reduced S = 1/2 [4Fe-4S]1+ cluster is indeed capable of injecting one electron into AdoMet, generating one equivalent of both methionine and S = 0 [4Fe-4S]2+ cluster. Dethiobiotin is not required for the reaction. Using site-directed mutagenesis we show also that, among the eight cysteines of BioB, only three (Cys-53, Cys-57, Cys-60) are essential for AdoMet reductive cleavage, suggesting that these cysteines are involved in chelation of the [4Fe-4S]2+/1+ cluster.

A new class of iron-sulfur enzymes has recently emerged. They are defined on the basis of their absolute requirement for S-adenosylmethionine (AdoMet) (1). The combination of a reduced iron-sulfur cluster and AdoMet is generally considered to result in the reductive cleavage of AdoMet and the generation of a 5'-deoxyadenosyl radical essential for initiation of catalysis. The prototypes for this class of enzymes are the activating components of anaerobic ribonucleotide reductase (RNR) and pyruvate formate lyase (PFL), lysine aminomutase (LAM), and biotin synthase (BioB) (2–5). The proteins of these systems are the monomer mainly in the form of a [4Fe-4S]1+ cluster, probably chelated by the three cysteines in the CXXX-CXXC sequence that is conserved among all enzymes and a fourth, not yet identified ligand (6–15). For RNR, PFL, and LAM, the [4Fe-4S]1+ cluster in Jarrett’s enzyme preparations (25). It is a protein-bound glycyl radical in RNR and PFL and a lysine-derived radical in LAM (19–21). In all cases, electrons are not transferred from the cluster to AdoMet in the absence of the substrate, showing that the reaction is thermodynamically unfavorable. In this report we present studies of the same reaction for BioB.

Biotin synthase (BioB) catalyzes the incorporation of a sulfur atom into dethiobiotin to form biotin (see Scheme 1 below) (22). In vitro, this reaction is very inefficient and the reported yields of biotin never exceed 1–2 equivalents per protein after several hours reaction, raising the possibility that BioB is not an enzyme but a reactant (23–25). The protein from Escherichia coli is a 76-kDa homodimer that binds a [4Fe-4S] cluster on each polypeptide chain (14, 26). Recently, Jarrett and co-workers (27) suggested that the active form of BioB contained two clusters, one [4Fe-4S] and one [2Fe-2S], per polypeptide chain. On the other hand, using a different procedure for reconstitution of iron centers in BioB, we are able to generate enzyme of similar activity with no more than 4 Fe and 4 S atoms per monomer mainly in the form of a [4Fe-4S] cluster (14, 15).

In addition to an Fe-S cluster and AdoMet, several proteins and cofactors are necessary for biotin formation: namely NADPH, DTT, iron, sulfide, cysteine, flavodoxin, and flavodoxin reductase (23, 24). The flavodoxin system is thought to effect in the reduction of the cluster. Requirement of iron, sulfide, and DTT suggests that the cluster is labile and has to be reconstituted during catalysis. Finally, the source of the sulfur for the biosynthesis of dethiobiotin has not been firmly established, although it has been suggested to be the cluster itself (28). This function is proposed to specifically reside in the [2Fe-2S] cluster in Jarrett’s enzyme preparations (25). It should be noted that, in vivo, the sulfur atom of biotin is derived from cysteine (24). Finally, studies of site-directed mutants have demonstrated the requirement of six cysteines for activity. Cys-53, Cys-57, and Cys-60 (numbers referred to the Escherichia coli enzyme) are thought to be important for chelation of the [4Fe-4S] cluster (13, 15). The function of Cys-97, Cys-128, and Cys-188, which are also absolutely required, is still unknown (24). It has been suggested that these cysteines might provide the site for a second cluster, namely the [2Fe-2S] center proposed by Jarrett and collaborators (25).

As for RNR, PFL, and LAM, the [4Fe-4S] cluster in BioB is suggested to play a key role in the reductive cleavage of AdoMet. The formation of biotin from dethiobiotin requires abstraction of two hydrogen atoms. Removal of each hydrogen atom, sequentially, is presumably accomplished by the product
of the reductive cleavage of AdoMet, the reactive 5'-deoxyadenosyl radical. This is consistent with the observation that formation of biotin requires at least two equivalents of AdoMet (29, 30). Radicals at C-9 or C-6 are thus thought to react with a sulfur source to form the tetrahydrothiophene moiety of biotin.

In this report we specifically address the question of the role of the [4Fe-4S] cluster during AdoMet activation. We show that in BioB from E. coli only the reduced [4Fe-4S]1+ cluster is able to reduce AdoMet into methionine and that the substrate dethiobiotin is not required for the reaction. Mutations at Cys-97, Cys-128, and Cys-188 do not affect AdoMet reduction activity of BioB. In contrast, mutations at Cys-53, Cys-57, and Cys-60 abolished this activity.

EXPERIMENTAL PROCEDURES

Materials—All chemicals were of reagent grade and obtained from Sigma-Aldrich Chemical Co. or Fluka unless otherwise stated. 57FeO2 (99.9% purity) was converted into ferric chloride by dissolving it in a hot concentrated (35%) hydrochloric acid of analytical grade (Carlo Erba) and repeatedly concentrated in water. 5-Deaza-7,8-dimethyl-10-methyl-isoselaloxazine (5-DAF) was prepared according to Ashton et al (31).

Preparation of Reconstituted Biotin Synthase—Mutants and wild-type proteins were prepared as described previously (12, 15). Reconstitution of apoproteins was achieved with either 56Fe or 57Fe, as described (14, 15). In both cases the reconstituted proteins were desalted over Sephadex G-25 to remove adventitiously bound iron.

Preparation of Reduced Samples—Reconstituted wild-type and mutated proteins were reduced inside an anaerobic glove box. 5-DAF was dissolved in Me2SO, diluted to a final concentration of 1 mM with water. Reduced proteins were reduced inside an anaerobic glove box. 5-DAF was converted into ferric chloride by dissolving it in a hot concentrated (0.05 T) was applied parallel to the observed γ-rays.

RESULTS

Reductive Cleavage of AdoMet by Reduced BioB—In the following experiments, all carried out inside an anaerobic glove box, BioB designates purified preparations of anaerobically reconstituted biotin synthase, containing 3.3–3.9 iron and 3.4–4.0 sulfide per monomer, mainly in the form of [4Fe-4S]2+ clusters as shown by Mössbauer spectroscopy (see Ref. 14 and below). Purification and reconstitution have been described previously (12, 14, 15). Anaerobic reaction of reduced BioB, containing mainly [4Fe-4S]1+ clusters, with AdoMet, was assayed for the formation of methionine, one of the products of the reductive cleavage of AdoMet. The concentration of S = 1/2 [4Fe-4S]1+ cluster was determined by quantifying its characteristic EPR signal (Fig. 1, inset); the [4Fe-4S]1+ cluster form is EPR-silent, and the EPR spectra of the S = 3/2 [4Fe-4S]1+ cluster, generally a minor species, are difficult to quantify because of their broad features and small signal amplitudes.

In a typical experiment, BioB (0.1–0.16 mM) was first reduced by illuminating in the presence of substoichiometric amounts of deazaflavin (5-DAF), using a 0.1 M Tris-HCl buffer pH 8.0 (buffer A). This procedure afforded a maximum of 50–60% of polypeptides containing a [4Fe-4S]1+ cluster as determined from the intensity of the EPR signal. When reduced BioB was reacted with an excess of AdoMet (0.5 mM) in buffer A, the EPR signal of the cluster slowly declined following first-order kinetics with a rate constant of 0.025 ± 0.005 min−1, and the reaction was completed within about 1 h (Fig. 1). As shown below, the EPR-silent reaction product has been identified as the corresponding [4Fe-4S]2+ cluster form.

In the course of the reaction, methionine was formed with the same rate, showing that methionine formation is associated with cluster oxidation. All along the reaction, 0.9–1.0 mol of methionine was formed per mol of cluster converted to the [4Fe-4S]2+ form. In three experiments the same 1:1 ratio was obtained with different preparations having widely different amounts of reduced cluster (data not shown). Furthermore, when the final EPR-silent form of BioB, obtained after reaction with AdoMet, was desalted, reduced a second time and then exposed again to AdoMet, a second and comparable burst of methionine formation was observed (0.8 mol of methionine per mol of reoxidized cluster). Finally, the reaction proceeded with similar rates and yields in the presence of an excess of either dethiobiotin (2.5 mM) or DTT (5 mM). No formation of methionine was observed when either BioB or AdoMet was omitted from the reaction mixture. Furthermore, in the absence of AdoMet the S = 1/2 signal of the 1+ state did not decline...
The Role of the [Fe-S] Center in AdoMet Reduction

13451

TABLE I
AdoMet reductase activity of reconstituted wild-type and mutants BioB

| Protein       | [4Fe-4S]1+ | Methionine | Activity |
|---------------|------------|------------|----------|
| Wild-type     |            |            |          |
| Sample A      | <5         | <0.01      | <0.04    |
| Sample B      | 52         | <0.01      | <0.04    |
| Sample C      | 35         | 0.8–1.0    | 0.24     |
| C55A          | <5         | <0.01      | <0.04    |
| C57A          | <5         | <0.01      | <0.04    |
| C60A          | <5         | <0.01      | <0.04    |
| C97A          | 64         | 0.7        | <0.04    |
| C128A         | 51         | 0.8        | <0.04    |
| C188A         | 70         | 0.4        | <0.04    |

a Percentage (±5%) of total iron in the S = 1/2 [4Fe-4S]1+ state as quantitated by EPR spectroscopy after reduction with photoactivated deazaflavin; percentage does not include [4Fe-4S]1+ clusters in the S = 3/2 state. BioB mutant data are from preparations such as sample B of the wild-type enzyme (photoreducted reconstituted protein).

b Nanomoles of methionine formed/nmol of oxidized (Fe-S) cluster (±0.1) after 120-min reaction of reduced protein with 0.5 mM AdoMet in darkness.

c Nanomoles of biotin formed/nmol of enzyme monomer (±0.04) under the conditions of the assay described previously (13).

doublet drawn above the experimental spectrum) with isomer shift \( \delta = 0.45 \text{ mm/s} \) and quadrupole splitting \( \Delta E_Q = 1.13 \text{ mm/s} \), accounting for ~60% of the total iron. The parameters of this doublet are the same as those reported for the [4Fe-4S]2+ cluster of BioB (14). The spectrum also contains the contributions of four minority species. Two doublets, each representing approximately 6% of the iron, exhibit \( \Delta E_Q \approx 3.1 \text{ mm/s} \), \( \delta \approx 1.2 \text{ mm/s} \) and \( \Delta E_Q \approx 3.1 \text{ mm/s} \), \( \delta \approx 0.7 \text{ mm/s} \) (see also Fig. 3 of Ref. 14). The two broad lines between ~2 mm/s and ~3 mm/s Doppler velocity are the high energy features of these doublets. Most likely both doublets represent adventitiously bound Fe3+. Isomer shifts of ~0.7 mm/s are typical of tetrahedral high spin Fe3+ complexes suggesting that this species represents mononuclear Fe3+ bound to cysteinyl sulfurs of sites lacking a cluster. A third minority species, contributing 10–15% of the absorption in Fig. 2A, exhibits a doublet with \( \Delta E_Q \approx 0.55 \text{ mm/s} \) and \( \delta \approx 0.27 \text{ mm/s} \), parameters identical to those of the [2Fe-2S]2+ cluster in oxygen-exposed BioB samples (14). Finally, the spectrum contains an unresolved paramagnetic component (approximately 10% of Fe), recognizable by some shallow absorption between ~1 mm/s and ~3 mm/s Doppler velocity. This paramagnetic component, or at least a fraction of it, might belong to a [4Fe-4S]1+ cluster, even though it is difficult to see in the EPR spectrum (Table I).

A sample similar to the one shown in Fig. 2A, was incubated for 15 min with 0.5 mM AdoMet before freezing at liquid nitrogen. Its Mössbauer spectrum (not shown) was the same as that of Fig. 2A, suggesting that under these conditions the presence of AdoMet does not affect the [4Fe-4S]1+ cluster.

The 4.2 K Mössbauer spectrum of a photoreduced sample (sample B), shown in Fig. 2B, exhibits a broad paramagnetic component very similar to the spectrum reported for the [4Fe-4S]1+ clusters of BioB (14). It is not possible to obtain for this sample a precise estimate of the fraction of iron associated with the [4Fe-4S]1+ state. Our analysis, however, indicates that 60–80% of the iron is associated with the 1+ state and that possibly 10% of the iron belongs to [4Fe-4S]2+ clusters. The lower limit would suggest that all [4Fe-4S]2+ clusters have been reduced. The upper limit is not inconsistent with our observations for sample A if we take into account our previous observation that, upon photoreduction, the iron of the [2Fe-2S] cluster is used to build clusters with [4Fe-4S] cores (14). Accordingly no [2Fe-2S] cluster could be detected in sample B. Moreover, some of the adventitiously bound iron is likely to be recruited for cluster reconstitution during the extended (45

FIG. 1. Correlation between decay of the EPR signal of reduced BioB and methionine formation. BioB (180 μM, 3.3 iron/polypeptide chain) in 0.1 M Tris-HCl, pH 8.0, was first reduced with 5-DAF under argon. Then illumination was stopped, 500 μM AdoMet was added anaerobically, and the solution was kept in darkness. At the indicated times, the reduced iron-sulfur cluster (●) and methionine (○) were assayed as described under “Experimental Procedures.” Insert, EPR spectrum of photoreduced BioB (200 μM). Temperature, 10 K; microwave power, 0.160 milliwatts; modulation amplitude, 10 G; receiver gain, 2.105; frequency, 9.447 GHz.

measurably within the same time range. We conclude that the [4Fe-4S]1+ cluster is competent for AdoMet cleavage, even in the absence of the dethiobiotin substrate. The reaction can be described with the following reaction,

\[ [4Fe-4S]^{1+} + 1 \text{AdoMet} \rightarrow 1 \text{methionine} + \text{Ado}^- + [4Fe-4S]^{2+} \]

**REACTION 1**

In a control experiment reconstituted BioB lacking EPR-active clusters and containing mainly EPR-silent [4Fe-4S]2+ clusters was incubated with an excess of AdoMet (0.5 mM) in buffer A. After 1-h incubation no significant production of methionine (less than 0.01 mol/mol of monomer BioB) was observed, demonstrating that the [4Fe-4S]2+ center is not competent for AdoMet cleavage to methionine.

The same type of experiment was performed with each of the eight Cys-to-Ala mutant proteins available in our laboratory. As reported earlier, among the five mutated enzymes that still exhibit upon anaerobic reduction the S = 1/2 EPR signal of the [4Fe-4S]1+ center, only two, namely C276A and C288A, were fully enzymatically active, whereas the three others, C97A, C128A, and C188A, were inactive (Table I). The other mutant proteins, C55A, C57A, and C60A, were both EPR-silent after incubation with photoactivated deazaflavin and inactive (Table I). Determination of methionine for each EPR-active mutant after 1 h reaction with an excess of AdoMet gave the same results as the wild-type protein (Table I). Only for the C188A mutant was a decreased production of methionine observed. No methionine was detected with the three EPR-silent C55A, C57A, and C60A mutants, treated exactly under the same conditions.

**AdoMet Oxidizes the Reduced [4Fe-4S]1+ Cluster of BioB into [4Fe-4S]2+.** To characterize the EPR-silent iron center after reaction with AdoMet, similar experiments were carried out with a preparation of wild-type BioB reconstituted with 57Fe (sample A), reduced with photoactivated DAF (sample B), and incubated for 15 min with AdoMet (sample C), as described above. Aliquots of each sample (0.83 mM Fe) were transferred into Mössbauer cups and EPR tubes, and a fraction was set aside for analysis of methionine formation. The Mössbauer spectrum of sample A (Fig. 2A) exhibits a major species (the...
The distribution of iron within different species in samples A, B, and C as derived from Mössbauer analysis is reported in Table II. From these numbers and those from the EPR analysis, a reasonably good correlation, within the uncertainties, is observed between the amount of $S = 1/2$ [4Fe-4S]$^{1+}$ (35 $\mu$m as determined by EPR spectroscopy) being oxidized by AdoMet, the amount of [4Fe-4S]$^{2+}$ being formed (40 $\mu$m as determined by Mössbauer spectroscopy), and that of methionine formed (30 $\mu$m) after a 15-min reaction in this experiment. The Mössbauer data show clearly that the loss of the EPR signal in the course of incubation with AdoMet is due to a one-electron oxidation of the [4Fe-4S]$^{1+}$ cluster and not due to the destruction of the iron-sulfur cluster, as witnessed by the observation that no mononuclear Fe was generated during the reaction.

A Mössbauer spectrum identical within the noise to that shown in Fig. 2C was obtained when the incubation with AdoMet was done in the presence of the dethiobiotin substrate.

**DISCUSSION**

Formation of biotin from dethiobiotin (DTB) is a complex enzymatic reaction that involves radical chemistry (22). Biotin synthase, a member of the radical-SAM superfamily, contains at least one iron-sulfur cluster, which is thought to catalyze the reduction of AdoMet by reduced flavodoxin and its cleavage into methionine and the 5'-deoxyadenosyl radical. The latter is suggested to serve for initiating C-H to C-S bond formation by abstraction of H atoms at position 6 and 9 of DTB. In this work we report studies of the first step of the reaction, namely the reductive cleavage of AdoMet.

The type of iron-sulfur cluster(s) in BioB is still a matter of controversy. One proposal, based on spectroscopic studies (11), envisioned a [2Fe-2S] center that could, under reducing conditions, convert into one [4Fe-4S] center bridging the two subunits of the dimer. This proposal was then challenged when we (14, 15) and others (26, 28) demonstrated that anaerobic preparations of BioB could bind as much as 4 Fe and 4 S per monomer in the form of [4Fe-4S]$^{2+}$ clusters that undergo degradation into [2Fe-2S] clusters upon exposure to air (14, 26, 28). However, very recently Ugulava and co-workers (25, 27), on the basis of optical and electrochemical studies, reported that each monomer of active BioB could bind one [4Fe-4S] and one [2Fe-2S] cluster. From this study it was suggested that the former was involved in the cleavage of AdoMet and that the latter served as a sulfur donor during dethiobiotin to biotin conversion. Using a different procedure, anaerobic reconstitution of BioB in our hands resulted in an active protein containing 3.5 to 4 Fe per polypeptide chain and equivalent amounts of sulfide. Characterization of our preparations by Mössbauer spectroscopy generally shows the presence of a mixture of [4Fe-4S]$^{2+}$ and [2Fe-2S]$^{2+}$ clusters and mononuclear ferrous iron impurities. The proportions of the different species vary from one preparation to another, but more that 60% (up to 90% in previous samples) of the iron is always associated with [4Fe-
The Role of the [Fe-S] Center in AdoMet Reduction

4S] clusters (14, 15). The sample used in the present study, for example, had about 60% of its total iron in the form of a [4Fe-4S]2+ cluster, i.e. approximately 0.55 cluster/polypeptide. The incomplete [4Fe-4S] assembly or lack of clusters altogether in some monomers probably reflects the liability of the cluster and its sensitivity to oxygen, as previously noted (14, 15, 28). In particular, the presence of [2Fe-2S]2+ clusters (perhaps 0.2 cluster/polypeptide in sample A) is usually an indication for contamination of the sample by air. On the other hand, whether some of the observed [2Fe-2S]2+ clusters could be located in a second metal binding site is an opened possibility, as suggested by Ugulava and collaborators (25). Under the reducing conditions employed here, iron in [2Fe-2S] clusters are mobilized to form almost exclusively [4Fe-4S]2+ clusters. For sample B described here 60–80% of the iron belongs to [4Fe-4S]1+ clusters, and no evidence for [2Fe-2S] centers could be obtained.

The data presented here provide the first direct spectroscopic evidence that the [4Fe-4S]1+ cluster, but not [4Fe-4S]2+, is competent for AdoMet reduction and cleavage. In single turnover experiments, the EPR signal of the reduced cluster was shown to decay after addition of an excess of AdoMet (Fig. 1). This is in marked contrast to all studied members of the radical-SAM superfamily of enzymes. Indeed, for the β2-activating component of the anaerobic ribonucleotide reductase (RNR), the activase of the pyruvate formate lyase (PFL), and, for lysine aminomutase, the reaction between the reduced cluster and AdoMet occurs only in the presence of the substrate (the α2 component of the RNR, PFL, and lysine aminomutase, respectively, (16, 18, 21)). Reduction of AdoMet is a thermodynamically unfavorable reaction, as a consequence of the extremely low redox potential of sulfonium in general. The required presence of substrate has been attributed to the required coupling of this reaction to thermodynamically favorable reactions of radical formation. For BioB we show that the oxidation of the cluster by AdoMet occurs even in the absence of substrate and thus seems to be thermodynamically more favorable. In fact, the addition of DTB substrate did not affect the reaction.

Our Mössbauer study shows that the product of [4Fe-4S]1+ cluster oxidation was exclusively the [4Fe-4S]2+ form, with parameters identical to those of the 2+ cluster before reduction. We have no evidence for the destruction of the cluster even when the reaction was carried out in the presence of the dethiobiotin substrate. This result does not lend support to the hypothesis that the [4Fe-4S] cluster is the source of sulfur during biotin formation (28). It rather shows that it is stable under anaerobic conditions, only shuttling between the 2+ and 1+ oxidation states. The observation that several identical cycles of cluster reduction and oxidation by AdoMet could be achieved with the same preparation is indeed consistent with a stable cluster under these conditions. However, some caution is advised regarding this conclusion because our reaction did not include all the components required for biotin formation.

During the course of the reaction we could not detect any significant changes of the shape and saturation properties of the EPR signal of the cluster. This is also in contrast with RNR and PFL whose clusters were shown to be affected by the presence of AdoMet, as shown by EPR and Mössbauer spectroscopy (6, 16, 18). We thus have not obtained direct spectroscopic evidence here for an intermediate biotin synthase-AmoAc-SAM superfamily of enzymes. Indeed, for the BioB have also been shown to be essential for enzyme activity. However, the corresponding Cys-to-Ala mutants were shown to be able to access the [4Fe-4S]1+ state similar to that of the wild-type enzyme (15). This led us to suggest that these cysteines were not involved in the chelation of the [4Fe-4S] center but were important for other functions not yet identified. Whether they could serve as ligands for an additional cluster as suggested by Ugulava et al. (25) remains open at this stage. It should be noted that only Cys-97 and Cys-188, but not Cys-128, are fully conserved among 15 BioB proteins from different species. The results reported here show that these three cysteines are not required for AdoMet reduction and cleavage by the [4Fe-4S]1+ cluster, providing further indication that they are not ligands of the [4Fe-4S] cluster. Accordingly, the corresponding mutants could react with AdoMet with rates and yields of methionine production roughly similar to those for the wild-type enzyme (Table I).

On the basis of the present studies we conclude that the [4Fe-4S]1+ center bound to cysteines 53, 57, and 60 is responsible for the one-electron reductive cleavage of AdoMet. This reaction is essential because it provides the primary one-electron donor. When compared with RNR and PFL, the reductive cleavage of AdoMet by reduced BioB is much slower even in the presence of substrate (16, 18). Considering that two molecules of AdoMet must be cleaved for the production of one molecule of biotin, the sluggishness of this reaction step raises the question whether, at least in vitro, the very low enzymatic activity generally obtained is partly due to such an inefficient electron transfer to and cleavage of AdoMet. Why this reaction is so slow is presently not clear. Further experiments are required to learn whether and how this reaction step can be accelerated. Nevertheless, the rate constant from methionine formation is significantly larger than that for biotin formation with our preparations (14, 15), showing that reductive cleavage of AdoMet is kinetically competent.

Finally, the effects of mutations of the cysteine residues of BioB on the reaction have revealed interesting features. Recent site-directed mutagenesis experiments on BioB led us to propose that Cys-53, Cys-57, and Cys-60, which belong to the CXXCXXC triad characteristic of the members of the radical-SAM family, coordinate to the cluster (15). The corresponding Cys-to-Ala mutants are unable to access the 1+ state of the [4Fe-4S] cluster. We have shown here that these mutants are also not able to catalyze the reductive cleavage of AdoMet, further confirming the role of the cysteines of the triad as ligands of the cluster.

Three additional cysteines, Cys-97, Cys-128, Cys-188 in E. coli BioB have also been shown to be essential for enzyme activity. However, the corresponding Cys-to-Ala mutants were shown to be able to access the [4Fe-4S]1+ state similar to that of the wild-type enzyme (15). This led us to suggest that these cysteines were not involved in the chelation of the [4Fe-4S] center but were important for other functions not yet identified. Whether they could serve as ligands for an additional cluster as suggested by Ugulava et al. (25) remains open at this stage. It should be noted that only Cys-97 and Cys-188, but not Cys-128, are fully conserved among 15 BioB proteins from different species. The results reported here show that these three cysteines are not required for AdoMet reduction and cleavage by the [4Fe-4S]1+ cluster, providing further indication that they are not ligands of the [4Fe-4S] cluster. Accordingly, the corresponding mutants could react with AdoMet with rates and yields of methionine production roughly similar to those for the wild-type enzyme (Table I).

On the basis of the present studies we conclude that the [4Fe-4S]1+ center bound to cysteines 53, 57, and 60 is responsible for the one-electron reductive cleavage of AdoMet. This reaction is essential because it provides the primary one-electron donor for dethiobiotin activation. This conclusion is not inconsistent with Jarrett’s recent hypothesis (25, 27) according to which the [4Fe-4S] cluster functions as a redox catalyst for AdoMet activation whereas the [2Fe-2S] cluster serves only in the later phases of the reaction. The function of Cys-97, Cys-128, and Cys-188 remains a question that is currently being addressed in our laboratory.

REFERENCES
1. Sofia, H. J., Chen, G., Hetschler, E. G., Reyes-Spindola, J. F., and Miller, N. E. (2001) Nucleic Acids Res. 29, 1097–1106
2. Cheek, J., and Broderick, J. B. (2001) J. Biol. Inorg. Chem. 6, 209–226
3. Fontecave, M., Mulliez, E., and Ollagnier-de Choudens, S. (2001) Curr. Opin. Chem. Biol. 5, 506–511
4. Frey, P. A. (2001) Annu. Rev. Biochem. 70, 121–148
5. Frey, P. A., and Booker, S. J. (2001) Adv. Protein Chem. 58, 1–45
6. Ollagnier, S., Mulliez, E., Schmidt, P. P., Eliasson, R., Gaillard, J., Denzner, C., Bergman, T., Graislund, A., Reichard, P., and Fontecave, M. (1997) J. Biol. Chem. 272, 24216–24223
7. Tamarit, J., Gerez, C., Moer, C., Mulliez, E., Trautwein, A., and Fontecave, M. (2000) J. Biol. Chem. 275, 15669–15675
8. Kulzer, R., Pils, T., Kappl, R., Huttermann, J., Knappe, J. (1998) J. Biol. Chem. 273, 4987–4993
9. Petrovich, R. M., Ruzicka, F. J., Reed, G. H., and Frey, P. (1992) Biochemistry 31, 11061–11066
10. Sokol, K., and Frey, P. A. (2001) Annu. Rev. Biochem. 70, 121–148
11. Tamarit, J., Gerez, C., Moer, C., Mulliez, E., Trautwein, A., and Fontecave, M. (2000) J. Biol. Chem. 275, 15669–15675
12. Kulzer, R., Pils, T., Kappl, R., Huttermann, J., Knappe, J. (1998) J. Biol. Chem. 273, 4987–4993
The Role of the [Fe-S] Center in AdoMet Reduction

10. Ruzicka, F. J., Lieder, W., and Frey, P. A (2000) J. Bacteriol. 182, 469–476
11. Duin, E. C., Lafferty, M. E., Crouse, B. R., Allen, R. M., Sanyal, I., Flint, D. H., and Johnson, M. K. (1997) Biochemistry 36, 11811–11820
12. Hewitson, K. S., Baldwin, J. E., Shaw, N. M., and Roach, P. L. (2000) FEBS Lett. 466, 372–376
13. McVver, L., Baxter, R. L., and Campopiano, D. J. (2000) J. Biol. Chem. 275, 13888–13894
14. Ollagnier-de Choudens, S., Sanakis, Y., Hewitson, K. S., Roach, P. L., Baldwin, J. E., Munch, E., and Fontecave, M. (2000) Biochemistry 39, 4165–4173
15. Hewitson, K., Ollagnier-de Choudens, S., Sanakis, Y., Shaw, N. M., Baldwin, J. E., Munch, E., Roach, P. L., and Fontecave, M. (2002) J. Biol. Inorg. Chem. 7, 83–93
16. Henshaw, T. F., Cheek, J., and Broderick, J. B. (2000) J. Am. Chem. Soc. 122, 8331–8332
17. Lieder, K. W., Booker, L. S., Ruzicka, F. J., Beinert, H., K. W., Reed, G. H., and Frey, P. A. (1996) Biochemistry 35, 2578–2585
18. Padovani, D., Thomas, F., Trautwein, A. X., Mulliez, E., and Fontecave, M. (2001) Biochemistry 40, 6713–6719
19. Wagner, A. F. V., Frey, M., Neugebauer, F. A., and Schäfer, W. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 996–1000
20. Sun, X., Ollagnier, S., Schmidt, P. P., Atta, M., Mulliez, E., LePape, L., Eliasson, R., Gräslund, A., Fontecave, M., Reichard, P., and Sjöberg, B.-M. (1997) J. Biol. Chem. 271, 6827–6831
21. Wu, W., Booker, S., Lieder, K. W., Bandarian, V., Reed, G. H., and Frey, P. A. (2000) Biochemistry 39, 9561–9570
22. Marquet, A., Tse Sum Bui, B., and Florentin, D. (2001) Vitamins and Hormones 61, 51–85
23. Sanyal, I., Cohen, G., and Flint, D. H (1994) Biochemistry 33, 3625–3631
24. Birch, O. M., Furchmann, M., Shaw, N. M (1995) J. Biol. Chem. 270, 19158–19165
25. Ugulava, N. B., Sucanell, C. J., and Jarrett, J. T. (2001) Biochemistry 40, 8352–8358
26. Ugulava, N. B., Gibney, B. R., and Jarrett, J. T. (2000) Biochemistry 39, 5206–5214
27. Ugulava, N. B., Gibney, B. R., and Jarrett, J. T. (2001) Biochemistry 40, 8343–8351
28. Tse Sum Bui, B., Florentin, D., Fournier, F., Ploux, O., Mejan, A., and Marquet, A. (1998) FEBS Lett. 440, 226–230
29. Shaw, N. M., Birch, O. M., Tinschert, A., Venetz, V., Dietrich, R., Savoy, L. A. (1998) Biochem. J. 330, 1079–1085
30. Guivanvarc’h, D., Florentin, D., Bui, B. T. S., Nunzi, F., and Marquet, A. (1997) Biochem. Biophys. Res. Commun. 236, 402–406
31. Ashton, W. T., Brown, R., and Tolman, R. L. (1978) J. Heterocycl. Chem. 15, 489–491
32. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
33. Nozaki, Y. (1986) Arch. Biochem. Biophys. 249, 437–446
34. Fish, W. W. (1988) Methods Enzymol. 158, 357–364
35. Beinert, H. (1983) Anal. Biochem. 131, 373–378
Reductive Cleavage of S-Adenosylmethionine by Biotin Synthase from *Escherichia coli*

Sandrine Ollagnier-de Choudens, Yiannis Sanakis, Kirsty S. Hewitson, Peter Roach, Eckard Münck and Marc Fontecave

*J. Biol. Chem.* 2002, 277:13449-13454.

doi: 10.1074/jbc.M111324200 originally published online February 7, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M111324200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 35 references, 9 of which can be accessed free at http://www.jbc.org/content/277/16/13449.full.html#ref-list-1