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A Nucleosidase Required for In Vivo Function of the *S*-Adenosyl-L-Methionine Radical Enzyme, Biotin Synthase

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Summary

Biotin synthase is an *S*-adenosyl-L-methionine (SAM) radical enzyme that inserts sulfur into dethiobiotin to produce biotin. The reaction proceeds through 5′-deoxyadenosyl radical intermediates that become reduced during the sulfur insertion step to give another product of the reaction, 5′-deoxyadenosine. We report that *Escherichia coli* strains lacking the 5′-methylthioadenosine/*S*-adenosylhomocysteine nucleosidase encoded by the *pfs* gene are deficient in biotin synthase activity due to accumulation of 5′-deoxyadenosine, a new substrate of the *pfs*-encoded nucleosidase. Physiological experiments indicate that lipoic acid synthase, another SAM radical enzyme, is also inhibited by 5′-deoxyadenosine accumulation.

Introduction

S-adenosyl-L-methionine (SAM) radical enzymes reductively cleave SAM to form the highly reactive 5′-deoxyadenosyl radical (DOA*) [1]. The radical, in turn, abstracts a hydrogen atom from an appropriately positioned carbon atom. The reaction steps that follow hydrogen atom abstraction are unique to each enzyme. In biotin synthase (BioB), the formation of one molecule of biotin (Figure 1A) requires abstraction of two hydrogen atoms, one each from C-9 and C-6 of the substrate, dethiobiotin (DTB), followed by insertion of a sulfur atom between these two carbon atoms [2–4] (Figure 1). The sulfur atom is probably derived from the [2Fe-2S] center of the protein [3, 5, 6]. The hydrogen atom abstraction step results in reduction of the 5′-deoxyadenosyl radicals to 5′-deoxyadenosine (5′-DOA) [7] (Figure 1B). It is generally believed that two 5′-deoxyadenosyl radicals are required for synthesis of each molecule of biotin, and hence two molecules of 5′-DOA would also be formed [2, 8]. We report that hydrolysis of 5′-DOA must occur for full function of BioB in vivo. The function of lipoic acid synthase (LipA) was also inhibited by accumulation of 5′-DOA in vivo.

Results and Discussion

We explored the possibility that 5′-DOA is a physiologically important inhibitor of *Escherichia coli* BioB based on two reports. First, 5′-DOA was reported to be an extremely potent inhibitor of the BioB reaction [9]. One molecule of 5′-DOA per molecule of BioB was reported to give complete inhibition of biotin synthesis in a defined in vitro system (note that conflicting results have been reported very recently [10], see below). Second, *E. coli* mutant strains carrying a deletion of the *pfs* gene that encodes 5′-methylthioadenosine (MTA)/*S*-adenosylhomocysteine (SAH) nucleosidase [11] grow very poorly unless supplemented with biotin [12]. Since the known Pfs substrates differ markedly in the size of the moiety attached to the ribose 5′ position (Figure 1C), and the X-ray crystal structures of Pfs [13, 14] suggested that the active site would bind 5′-DOA, it seemed possible that Pfs could cleave 5′-DOA to adenine plus 5′-deoxyribose (Figure 1B). Thus, in this hypothesis, the lack of Pfs would result in accumulation of inhibitory concentrations of 5′-DOA that would inhibit BioB function and thereby cause the reported biotin requirement [12].

Pfs Cleaves 5′-DOA In Vitro and In Vivo

Our hypothesis predicted that 5′-DOA was a substrate of the *pfs*-encoded nucleosidase. A hexahistidine-tagged version of Pfs was purified to homogeneity and tested for the ability to cleave 5′-DOA (Figure 2). The products of 5′-DOA cleavage were adenine and 5′-deoxyribose, the same products that resulted from cleavage by acid hydrolysis. Moreover, 5′-DOA inhibited Pfs-catalyzed hydrolysis of SAH (and vice versa), whereas MTA inhibited 5′-DOA cleavage (Figure 2C). These data indicate that MTA is the preferred Pfs substrate, whereas 5′-DOA, like SAH [15], is a poorer substrate. (Note that because adenine is produced by cleavage of all three substrates, the competition data of Figure 2C must be evaluated by loss of a given substrate.) We also assayed dialyzed crude extracts of the wild-type strain and the *Δpfs* strain ER105 for 5′-DOA cleavage activity. HPLC analyses showed that the *Δpfs* extract had <0.15% of the 5′-DOA cleavage activity of the wild-type extract, suggesting that Pfs may be the sole 5′-DOA cleavage enzyme of *E. coli*. It should be noted that the *Δpfs* strains carried a genetically well characterized deletion-insertion *pfs* allele [12] in which residues 8–226 of the 232 residue coding sequence had been replaced with a kanamycin resistance cassette [12]. We found that strain ER105, which carries this allele, lacked detectable Pfs activity (<0.01% of the activity of the isogenic wild-type strain) upon assay [15] of the ability to cleave [^{3}H-*methyl*]MTA (made by mild acid treatment of [^{3}H-*methyl*]SAM [16] and purified by reverse-phase chromatography).

E. coli Δpfs Strains Cannot Convert DTB to Biotin

Our hypothesis also predicted that *Δpfs* strains would be deficient in BioB activity. This was readily tested by supplementation of the medium with DTB in place of biotin. A deficiency in BioB activity would not allow DTB to replace biotin in supporting growth of these strains. This prediction was satisfied; a *Δpfs* strain was unable to grow on DTB, whereas an isogenic strain having a functional *bioB* gene (but unable to make DTB) grew well on DTB (Figure 3). (The *Δpfs* strain was also unable to grow on 7,8-diaminopelargonic acid, the bio-

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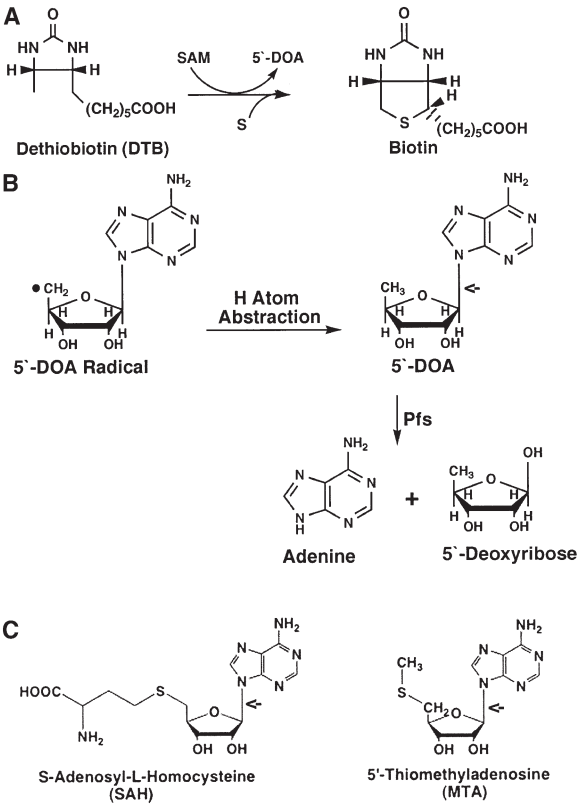

Figure 1. The Biotin Synthase and Pfs Reactions

(A) The biotin synthase (BioB) reaction.

(B) The proposed interrelationship between the functions of BioB and Pfs.

(C) The known substrates of Pfs. The bond cleaved by Pfs is designated by arrows in (B) and (C).

tin synthetic intermediate that precedes DTB). Moreover, like *bioB* strains [17], *Δpfs* strains should excrete DTB. Excretion of DTB was observed by the ability of the *Δpfs* strains to crossfeed strains (*bioD*) that lacked dethiobiotin synthase, whereas *bioB* strains were not crossfed [17] (Figure 3). Other crossfeeding experiments showed that a *Δpfs* strain crossfed a *bioD* strain as efficiently as did a *bioB* strain and that crossfeeding was blocked when plates spread with avidin (which binds DTB tightly) were used (data not shown). Bioassays showed that the concentrated medium of a biotin-starved overnight culture (about 4×10^8 cells/ml) of the *Δpfs* strain ER105 supported growth of a *bioD* null mutant strain, but failed to support growth of a *bioB* null mutant strain (as expected for accumulation of DTB). Comparison of the *bioD* strain growth response to that given by known concentrations of DTB indicated that the medium contained about 8 nM DTB. The *Δpfs* strains had phenotypic properties identical to those of authentic *bioB* mutants, except that biotin supplementation failed to restore normal growth to *Δpfs* strains [12].

Exogeneous 5′-DOA Inhibits Growth of *E. coli*

5′-DOA was cleaved by Pfs in vitro (Figure 2), and, therefore (if exogenous 5′-DOA could enter *E. coli*), we expected that the addition of 5′-DOA to the growth medium would inhibit growth of *Δpfs* strains, whereas wild-type strains should be appreciably less sensitive. Indeed, addition of exogenous 5′-DOA (80–800 mM) together with biotin (800 pM) to cultures of wild-type and *Δpfs* strains resulted in marked growth inhibition of the mutant strain, whereas growth of the wild-type strain was only slightly inhibited (Figure 3). The observed preferential inhibition of the mutant strain demonstrated that 5′-DOA was a Pfs substrate in vivo as well as in vitro.

Supplementation with Lipoic Acid and Biotin Restores Almost Normal Growth to *Δpfs* Strains

Lipoic acid synthase (LipA) is a SAM radical enzyme required for aerobic metabolism [18–20]. Since LipA and BioB are the only *E. coli* SAM radical proteins known to be required for aerobic growth, we tested if the addition of lipoic acid plus biotin would result in improved growth over that seen with biotin supplementation alone. Indeed, the presence of both cofactors improved the growth rate of the *Δpfs* strain to almost that of the parental wild-type strain (Figure 3). Moreover, the combination of biotin and lipoic acid largely overcame growth inhibition of *Δpfs* strains by exogenous 5′-DOA (Figure 3).

Testing Other Possible *Δpfs* Phenotypes

A formal possibility for the biotin requirement of *Δpfs* strains was that MTA produced by spermidine biosynthesis might inhibit BioB due to its resemblance to 5′-DOA. We therefore constructed a strain that carried a total deletion of the coding sequence of *speE*, the gene encoding the enzyme responsible for MTA synthesis [21, 22], and then constructed a *Δpfs ΔspeE* strain by transduction. The *Δpfs ΔspeE* strain had the same biotin requirement as its *Δpfs* parental strain (Figure 3). Therefore, MTA seems to play no role in the inhibition of biotin synthesis seen in *Δpfs* strains.

Most of the known and suspected *E. coli* SAM radical enzymes [23, 24] function in anaerobic metabolism (e.g., pyruvate formate lyase activase), and, thus, we tested if supplementation with biotin allows anaerobic growth of *Δpfs* strains on a minimal glucose medium containing casein hydrolysate. We found that anaerobic growth proceeded normally (lipoic acid is not required for anaerobic growth [25]). Therefore, BioB and LipA may bind 5′-DOA with appreciably higher affinities than do other SAM radical enzymes. This is interesting since bioinformatics criteria place BioB and LipA proteins in their own subclass of the SAM radical enzyme family [24]. In the presence of 5′-DOA, growth of the wild-type strain is slightly inhibited even when biotin and lipoate are added (Figure 3). Hence, there may also be another SAM radical enzyme active under aerobic conditions that is sensitive to inhibition by 5′-DOA. However, it is also possible that the accumulated 5′-deoxyribose weakly inhibits an unrelated metabolic reaction (i.e., nucleoside synthesis).

Conclusions

Our results indicate that 5′-DOA is a physiologically relevant inhibitor of *E. coli* BioB activity. This agrees with the prior report by Fontecave and coworkers [9] that

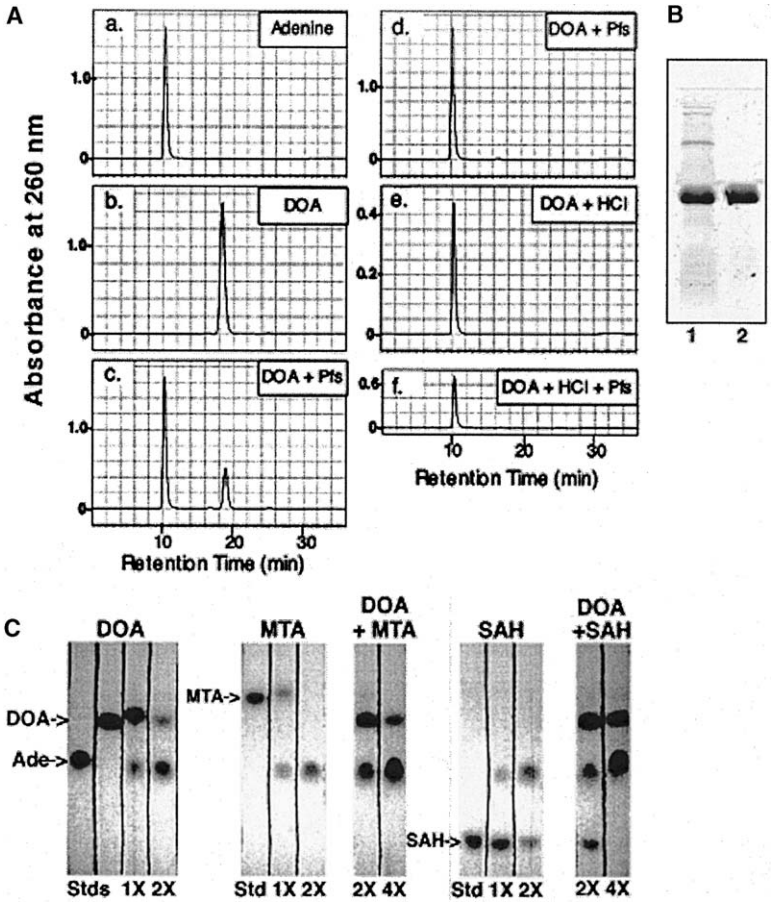

Figure 2. Hydrolysis of 5′-DOA by Pfs

(A–C) (A) contains HPLC profiles of standards and the products of 5′-DOA hydrolysis (detection by absorbance at 260 nm). Profiles (a) and (b) are standards of adenine and 5′-DOA, respectively. Profiles (c) and (d) are the results of (c) partial and (d) total cleavage of 5′-DOA by purified Pfs. Profile (e) shows the products of acid hydrolysis (0.1 M HCl, 100°C, 1 hr) of 5′-DOA, whereas, in profile (f), equal absorbances of the products of 5′-DOA by acid and Pfs were mixed before injection onto the HPLC column. (B) shows an SDS-polyacrylamide gel analysis [27] of Pfs expression and purification. Lane 1 is the crude extract of the induced cells, whereas lane 2 is the enzyme eluted from a nickel-cheleate column. (C) The lower left part of the figure shows segments of a fluorescent indicator silica gel, 60 thin-layer plate developed in n-butanol:acetic acid:water (60:15:25) that have been arranged to facilitate presentation. The adenine-containing compounds were visualized under 254 nm UV light. 1× denotes 50 ng of His-tagged Pfs, and Std denotes the standard. The plate was subsequently sprayed with p-anisidine-phthalic acid to visualize the released monosaccharides. In each case, the products released by Pfs and by acid hydrolysis comigrated. DOA, 5′-DOA; Ade, adenine; MTA, 5′-methylthioadenosine; SAH, *S*-adenosylhomocysteine. Note that all three Pfs substrates give rise to the same UV-absorbing product (adenine), and thus competition between the substrates must be followed by decreases in the intensities of the substrate spots. A system using fluorescent indicator cellulose, thin-layer plates developed in water also showed cleavage of 5′-DOA plus adenine (data not shown). The *pfs* coding sequence was amplified from *E. coli* genomic DNA with Pfu Turbo DNA polymerase (Stratagene) with primers 5′-CCATGGGAATGAAAATCGGCATCATTGGTGC-3′ and 5′-AAGCTTAATGATGATGATGATGATGGCCA TGTGCAAGTTTCTGC-3′. The gene was inserted into pQE60 by using the NcoI and HindIII sites of the primers to encode a Pfs having a C-terminal hexahistidine tag. The protein was purified as described previously [27] and dialyzed against 50 mM sodium N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) buffer (pH 7.0). For assay of Pfs activity, a substrate (800 pmol) or a mixture of substrates (800 pmol each) in 0.1 ml of 50 mM HEPES (pH 7.0) was incubated with various amounts of purified Pfs protein for 1 hr at 37°C. For HPLC analysis, 10 μl of the reaction mixtures was injected onto a Waters C18 reversed-phase column (4.6 × 250 mm) equilibrated with eluent A (50 mM ammonium acetate [pH 3.5]). The column was eluted at a flow rate of 0.7 ml/min for 5 min with 70% eluent A/30% eluent B (eluent B is 50 mM ammonium acetate:methanol, 90:10), followed by a 13 min linear gradient from 30%–100% eluent B in eluent A, and finally with a 18 min elution with 100% eluent B.

suggests that 5′-DOA is a very potent inhibitor of *E. coli* biotin synthase in vitro. However, immediately prior to submission of this manuscript for publication, Marquet and coworkers [10] reported that 5′-DOA was not an inhibitor of their in vitro *E. coli* biotin synthase system (although no data were given). One trivial explanation for the conflict is that the BioB preparations examined by Marquet were contaminated with Pfs, a rather abundant activity in *E. coli* cell extracts. It also seems possible that the differing responses to 5′-DOA are related to the sequence of events that follows destruction of the BioB [2Fe-2S] cluster thought to donate the biotin sulfur atom. Marquet and coworkers [2] reported that cluster destruction is accompanied by biotin formation, whereas Huynh and coworkers [4] report that biotin formation is 10- to 1000-fold slower than cluster destruction and is biphasic. It therefore seems that there may be several steps in the formation of biotin by BioB and that different enzyme preparations may have different rate-limiting steps. If so, and if 5′-DOA inhibits a discrete step in biotin formation, BioB preparations that are limited at another step might not show inhibition. It seems likely that most of the conflicting results obtained in vitro involve subtle details in the preparation and assay of this extraordinarily demanding enzyme.

The results we have obtained in vivo indicate that forms of the enzyme that are inhibited by 5′-DOA reflect the in vivo function of BioB. Application of such in vivo tests seems especially advisable for biotin synthase given the controversial nature of the literature on this enzyme (e.g., [10, 26]).

Significance

The mechanism of biotin synthase, the last step in the biosynthesis of the essential metabolic cofactor, biotin (vitamin H), has remained elusive despite inten-

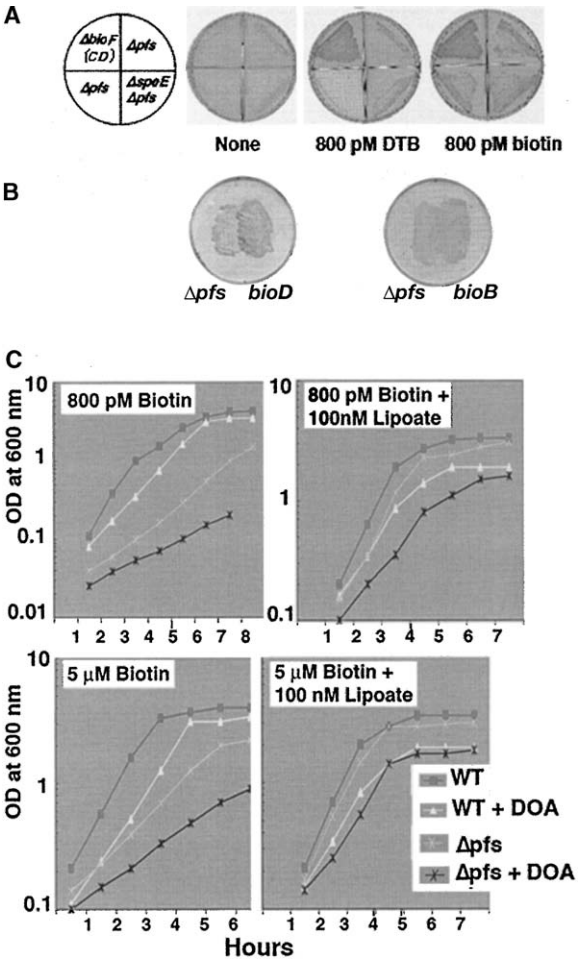

Figure 3. Growth Properties of *E. coli Δpfs* Strains in the Presence and Absence of Exogenous 5′-DOA

(A) *E. coli Δpfs* strains fail to grow on DTB and phenotypically mimic *bioB* strains. The plates are divided into quadrants (see plating scheme, each is separated from the next by a plastic wall). In the upper quadrants, the strains were (left quadrant) strain ER90, which has a functional *bioB* gene, but lacks function of the biotin operon genes downstream of *bioB*, and (right quadrant) the *Δpfs* derivative of ER90. The strains of the lower quadrants are (left quadrant) ER105, a *Δpfs* derivative of the wild-type strain MC1061, and (right quadrant) ER109, an *ΔspeE* derivative of ER105. The minimal medium was M9-glucose-leucine (10 μg/ml) supplemented as given in the figure.

(B) The two plates indicate crossfeeding tests done on biotin-free medium. Heavy suspensions of washed cells were spread side by side, and the plates were incubated for several days. Diffusion of intermediates from strains blocked late in the pathway allows growth of strains blocked in earlier steps of the pathway. Note that the ridge of growth of the *bioD* strain that is not seen with the *bioB* strain. Bioassays [29] confirmed accumulation of DTB by ER105 cultures starved of biotin (see text).

(C) Growth of the wild-type (WT) strain MC1061 and its *Δpfs* derivative, ER105, in glucose-leucine minimal medium (supplemented as given in the figure) in the presence or absence of 80 mM 5′-DOA (DOA). Growth was followed by turbidity at 600 nm. The *Δpfs::Km* allele of *E. coli* strain NC13 [12] was transduced into the wild-type *E. coli* strain MC1061(WT) with phage P1*vir*. Strain ER90 encodes a functional *bioB* gene and an inactivated *bioF* gene in which the first 22 codons of *bioF* were replaced with a chloramphenicol resistance cassette [28]. The downstream *bioD* gene (and probably *bioC*, which lies between *bioF* and *bioD*) was also inactivated (presumably due to classical Rho-dependent transcriptional polarity), as shown by the inability of the strain to grow on 7,8-diaminopelargonic acid [29]. The *ΔspeE* deletion strain was constructed by using the one-step procedure of Datsenko and Wanner [27]. The primers used were 5′-TCCCTGATATTTTTTTACGGGTGTTAACAAAG GAGGTATCAACCCTGTGTAGGCTGGAGCTGCTTCG-3′ and 5′-ATT AAAGCCATGCAGTTTCAGTTTTTTCAATTTCTTATCTTCTCCCATAT GAATATCCTCCTTAG-3′). The deletion event was verified by colony PCR, followed by sequencing of the PCR product.

sive investigations. Capable laboratories have reported forms of the protein having very different properties as well as data that support three different reaction mechanisms. For example, one laboratory has reported that the reaction product, 5′-deoxyadenosine, is a potent biotin synthase inhibitor in vitro, whereas a second laboratory observed no such inhibition. Given the strong disagreements among these and many other published results, it seems clear that tests of physiological relevance are needed to guide in vitro studies of biotin synthase. We report one such test. Mutants of *E. coli* lacking a nucleosidase that hydrolyzes 5′-deoxyadenosine were shown to be deficient in biotin synthase activity in vivo, indicating that 5′-deoxyadenosine is a physiologically important synthase inhibitor. Therefore, biotin synthase preparations that show 5′-deoxyadenosine inhibition in vitro are those likely to be physiologically relevant and hence those most deserving of mechanistic study. We also show that 5′-deoxyadenosine accumulation also inhibits lipoic acid synthesis, which requires a protein (LipA) having both sequence similarities and marked mechanistic analogies with biotin synthase.

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References

1. Frey, P.A., and Magnusson, O.T. (2003). S-Adenosylmethionine: a wolf in sheep's clothing, or a rich man's adenosylcobalamin? Chem. Rev. *103*, 2129–2148.
2. Tse Sum Bui, B., Benda, R., Schunemann, V., Florentin, D., Trautwein, A.X., and Marquet, A. (2003). Fate of the (2Fe-2S)(2+) cluster of *Escherichia coli* biotin synthase during reaction: a Mossbauer characterization. Biochemistry *42*, 8791–8798.
3. Berkovitch, F., Nicolet, Y., Wan, J.T., Jarrett, J.T., and Drennan, C.L. (2004). Crystal structure of biotin synthase, an S-adenosylmethionine-dependent radical enzyme. Science *303*, 76–79.
4. Jameson, G.N., Cosper, M.M., Hernandez, H.L., Johnson, M.K., and Huynh, B.H. (2004). Role of the [2Fe-2S] cluster in recombinant *Escherichia coli* biotin synthase. Biochemistry *43*, 2022–2031.
5. Tse Sum Bui, B., Florentin, D., Fournier, F., Ploux, O., Mejean, A., and Marquet, A. (1998). Biotin synthase mechanism: on the origin of sulphur. FEBS Lett. *440*, 226–230.
6. Gibson, K.J., Pelletier, D.A., and Turner, I.M., Sr. (1999). Transfer

of sulfur to biotin from biotin synthase (BioB protein). Biochem. Biophys. Res. Commun. *254*, 632–635.
7. Escalettes, F., Florentin, D., Tse Sum Bui, B., Lesage, D., and Marquet, A. (1999). Biotin synthase mechanism: evidence for hydrogen transfer from the substrate into deoxyadenosine. J. Am. Chem. Soc. *121*, 3571–3578.
8. Ugulava, N.B., Frederick, K.K., and Jarrett, J.T. (2003). Control of adenosylmethionine-dependent radical generation in biotin synthase: a kinetic and thermodynamic analysis of substrate binding to active and inactive forms of BioB. Biochemistry *42*, 2708–2719.
9. Ollagnier-de-Choudens, S., Mulliez, E., and Fontecave, M. (2002). The PLP-dependent biotin synthase from *Escherichia coli*: mechanistic studies. FEBS Lett. *532*, 465–468.
10. Tse Sum Bui, B., Lotierzo, M., Escalettes, F., Florentin, D., and Marquet, A. (2004). Further investigation on the turnover of *Escherichia coli* biotin synthase with dethiobiotin and 9-mercaptodethiobiotin as substrates. Biochemistry *43*, 16432–16441.
11. Cornell, K.A., and Riscoe, M.K. (1998). Cloning and expression of *Escherichia coli* 5′-methylthioadenosine/S-adenosylhomocysteine nucleosidase: identification of the *pfs* gene product. Biochim. Biophys. Acta *1396*, 8–14.
12. Cadieux, N., Bradbeer, C., Reeger-Schneider, E., Koster, W., Mohanty, A.K., Wiener, M.C., and Kadner, R.J. (2002). Identification of the periplasmic cobalamin-binding protein BtuF of *Escherichia coli*. J. Bacteriol. *184*, 706–717.
13. Lee, J.E., Cornell, K.A., Riscoe, M.K., and Howell, P.L. (2003). Structure of *Escherichia coli* 5′-methylthioadenosine/S-adenosylhomocysteine nucleosidase inhibitor complexes provide insight into the conformational changes required for substrate binding and catalysis. J. Biol. Chem. *278*, 8761–8770.
14. Lee, J.E., Settembre, E.C., Cornell, K.A., Riscoe, M.K., Sufrin, J.R., Ealick, S.E., and Howell, P.L. (2004). Structural comparison of MTA phosphorylase and MTA/AdoHcy nucleosidase explains substrate preferences and identifies regions exploitable for inhibitor design. Biochemistry *43*, 5159–5169.
15. Della Ragione, F., Porcelli, M., Carteni-Farina, M., Zappia, V., and Pegg, A.E. (1985). *Escherichia coli* S-adenosylhomocysteine/5′-methylthioadenosine nucleosidase. Purification, substrate specificity and mechanism of action. Biochem. J. *232*, 335–341.
16. Schlenk, F., and Zydek-Cwick, C.R. (1969). Stability of the glycosidic bond of S-adenosylsulfonium compounds toward acid. Arch. Biochem. Biophys. *134*, 414–422.
17. Cleary, P.P., and Campbell, A. (1972). Deletion and complementation analysis of biotin gene cluster of *Escherichia coli*. J. Bacteriol. *112*, 830–839.
18. Cicchillo, R.M., Iwig, D.F., Jones, A.D., Nesbitt, N.M., Baleanu-Gogonea, C., Souder, M.G., Tu, L., and Booker, S.J. (2004). Lipoyl synthase requires two equivalents of S-adenosyl-L-methionine to synthesize one equivalent of lipoic acid. Biochemistry *43*, 6378–6386.
19. Miller, J.R., Busby, R.W., Jordan, S.W., Cheek, J., Henshaw, T.F., Ashley, G.W., Broderick, J.B., Cronan, J.E., Jr., and Marletta, M.A. (2000). *Escherichia coli* LipA is a lipoyl synthase: in vitro biosynthesis of lipoylated pyruvate dehydrogenase complex from octanoyl-acyl carrier protein. Biochemistry *39*, 15166–15178.
20. Zhao, X., Miller, J.R., Jiang, Y., Marletta, M.A., and Cronan, J.E. (2003). Assembly of the covalent linkage between lipoic acid and its cognate enzymes. Chem. Biol. *10*, 1293–1302.
21. Xie, Q.W., Tabor, C.W., and Tabor, H. (1993). Deletion mutations in the *speED* operon: spermidine is not essential for the growth of *Escherichia coli*. Gene *126*, 115–117.
22. Tabor, C.W., Tabor, H., and Xie, Q.W. (1986). Spermidine synthase of *Escherichia coli*: localization of the *speE* gene. Proc. Natl. Acad. Sci. USA *83*, 6040–6044.
23. Nicolet, Y., and Drennan, C.L. (2004). AdoMet radical proteins–from structure to evolution–alignment of divergent protein sequences reveals strong secondary structure element conservation. Nucleic Acids Res. *32*, 4015–4025.
24. Sofia, H.J., Chen, G., Hetzler, B.G., Reyes-Spindola, J.F., and Miller, N.E. (2001). Radical SAM, a novel protein superfamily linking unresolved steps in familiar biosynthetic pathways with radical mechanisms: functional characterization using new analysis and information visualization methods. Nucleic Acids Res. *29*, 1097–1106.
25. Reed, K.E., and Cronan, J.E., Jr. (1993). Lipoic acid metabolism in *Escherichia coli*: sequencing and functional characterization of the *lipA* and *lipB* genes. J. Bacteriol. *175*, 1325–1336.
26. Jarrett, J.T. (2005). The novel structure and chemistry of iron-sulfur clusters in the adenosylmethionine-dependent radical enzyme biotin synthase. Arch. Biochem. Biophys. *433*, 312–321.
27. Datsenko, K.A., and Wanner, B.L. (2000). One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA *97*, 6640–6645.
28. Choi-Rhee, E., and Cronan, J.E. (2005). Biotin synthase is catalytic *in vivo*, but catalysis engenders destruction of the protein. Chem. Biol. *12*, 461–468.
29. Eisenberg, M.A., and Krell, K. (1969). Dethiobiotin synthesis from 7,8-diaminolargonic acid in cell-free extracts of a biotin auxotroph of *Escherichia coli* K-12. J. Biol. Chem. *244*, 5503–5509.