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Mechanistic Studies on Phosphopantothenoylcysteine Decarboxylase

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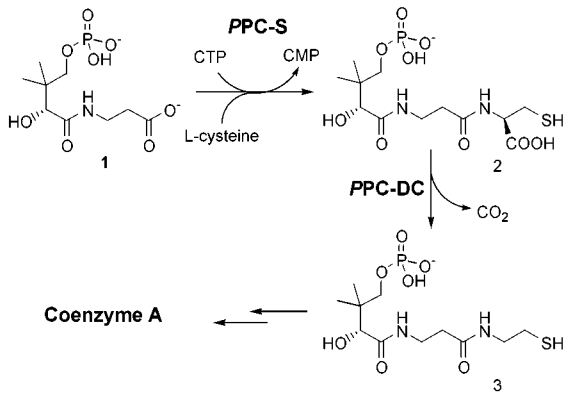
Phosphopantothenoylcysteine decarboxylase (*P*PC-DC) catalyzes the decarboxylation of the cysteine moiety of 4′-phosphopantothenoylcysteine (**2**, *P*PC) to form 4′-phosphopantetheine (**3**). The activity was recently shown to reside on a bifunctional enzyme in *E. coli* (CoaBC, previously Dfp) which also harbors phosphopantothenoylcysteine synthetase (*P*PC-S) activity (Scheme 1).[1,2] Both of these activities form part of the biosynthetic machinery that converts pantothenate (Vitamin B_5) into Coenzyme A, an essential cofactor in all biological systems.[3]

Prior to the characterization of the enzyme as a flavoprotein there was no obvious mechanism for the stabilization of the carbanion intermediate resulting from the decarboxylation of **2**.[4] Scheme 2 outlines three mechanistic proposals for this reaction, all of which depend on mediation by the flavin cofactor. In pathway **a**, which is analogous to the mechanism of general acyl-CoA dehydrogenase (GAD),[5] deprotonation of the cysteine moiety at C_α followed by formal hydride transfer to the flavin would give **4**. This hydride transfer could be direct or proceed by a sequential single electron transfer (SET) mechanism.[6] Tautomerization of **4** to **5** activates the substrate to undergo decarboxylation in a manner similar to that of β-keto acids. Alternatively, the thioaldehyde **5** could be formed by direct oxidation of the thiol in a mechanism that is analogous to the mechanism of NAD^+-dependent alcohol dehydrogenase (ADH), as in pathway **b**.[7] Decarboxylation via pathway **c** relies on the formation of the charge-transfer complex **8** between oxidized flavin and the thiolate anion of **2**, which mediates the subsequent formation of a thiol-flavin adduct at C(4a) (**9**). The activation of the thiol provided by such an adduct allows facile oxidization to the thioaldehyde, which then undergoes decarboxylation as for the other pathways.[8] However, pathway **c** can also proceed by oxidation of the thiol by an SET mechanism analogous to the mechanism of monoamine oxidase.[9]

In all three pathways the reaction is completed by reduction of the intermediate formed after decarboxylation. However, depending on the pathway followed, this can either occur by tautomerization of the intermediate **6** to the thioaldehyde **7**, which is reduced directly by hydride transfer or by an SET mechanism, or via the thiol-C(4a) adduct depicted in **10**.

Scheme 1



Enzyme-catalyzed solvent exchange reactions have been observed in a number of systems in which the mechanism involves abstraction of the α-proton.[10] This suggests a strategy to differentiate between pathway **a** which might show this exchange, as is the case in GAD,[11] and pathways **b** and **c**, where enzyme-catalyzed exchange at C_α would not be possible. When we performed solvent exchange reactions in which **1** is allowed to react with L-cysteine in D_2O (pD 8.0) in the presence of cytidine 5′-triphosphate (CTP) and the enzyme, we did not observe any exchange except for the replacement of the carboxyl group as judged by ESI-MS analysis of the resulting reaction mixtures. While this result supports pathways **b** and **c**, it does not exclude the possibility that the reaction proceeds via pathway **a** if the enzymatic base is monoprotic and not accessible to solvent.

The activation of *P*PC for the decarboxylation reaction involves the cleavage of C−H bonds. This suggests that primary deuterium isotope effects might allow differentiation between the proposed mechanisms. If deprotonation at C_α is kinetically significant in pathway **a**, one should see an isotope effect at this position while there is no possibility of observing a primary isotope effect at this center in either pathways **b** or **c**. All three mechanisms predict a possible primary deuterium isotope effect at C_β.[12]

We have measured the $^D(V/K)$ isotope effects at C_α and C_β for the decarboxylation reaction by means of direct competition reactions, exploiting the fact that the enzyme has both *P*PC-S and *P*PC-DC activities. A $^D(V/K)$ isotope effect on an enzymatic reaction will be observed only if the isotopically sensitive step occurs before or at the first irreversible step in the reaction sequence.[13] The observation of a $^D(V/K)$ isotope effect using cysteine as the substrate therefore requires that the intermediate **2** is first released from the enzyme before being decarboxylated, as the formation of an amide bond is highly unlikely to be reversible. We confirmed this by the detection of **2** using ESI-MS analysis of partially converted reaction mixtures.[14] Thus a mixture of racemic cysteine and racemic 2-[^{2}H]cysteine or racemic cysteine and racemic 3,3-[$^2H]_2$cysteine was treated with the enzyme in the presence of CTP and **1** and the relative deuterium content of the product **3** measured by ESI-MS analysis after both

(1) Strauss, E.; Kinsland, C.; Ge, Y.; McLafferty, F. W.; Begley, T. P. *J. Biol. Chem.* **2001,** *276*, 13513−13516.
(2) Kupke, T.; Uebele, M.; Schmid, D.; Jung, G.; Blaesse, M.; Steinbacher, S. *J. Biol. Chem.* **2000**, *275*, 31838−31846.
(3) Begley, T. P.; Kinsland, C.; Strauss, E. In *Vitamins and Hormones*; Litwack, G., Begley, T. P., Eds.; Academic Press: San Diego, CA, 2001; Vol. 61, pp 157−171.
(4) Yang, H.; Abeles, R. H. *Biochemistry* **1987**, *26*, 4076−4081.
(5) Palfey, B. A.; Massey, V. In *Comprehensive Biological Catalysis*; Sinnott, M., Ed.; Academic Press: San Diego, CA, 1998; Vol. 3, pp 83−154.
(6) Lai, M.-T.; Oh, E.; Liu, H.-W. *J. Am. Chem. Soc.* **1993**, *115*, 1619−1628.
(7) Clarke, A. R.; Dafforn, T. R. In *Comprehensive Biological Catalysis*; Sinnott, M., Ed.; Academic Press: San Diego, CA, 1998; Vol. 3, pp 1−76.
(8) For a detailed study on the reduction of flavins by thiols, see: (a) Loechler, E. L.; Hollocher, T. C. *J. Am. Chem. Soc.* **1980**, *102*, 7312−7321. (b) Loechler, E. L.; Hollocher, T. C. *J. Am. Chem. Soc.* **1980**, *102*, 7322−7327.
(9) Silverman, R. B. *Acc. Chem. Res.* **1995**, *28*, 335−342.
(10) For a discussion of solvent exchange in enzyme-catalyzed β-elimination reactions, see: Gerlt, J. A.; Gassman, P. G. *J. Am. Chem. Soc.* **1992**, *114*, 5928−5934.
(11) Ghisla, S.; Thorpe, C.; Massey, V. *Biochemistry* **1984**, *23*, 3154−3161.
(12) Primary deuterium isotope effects have also been used to study both GAD and ADH. (a) For GAD, the value for k_H/k_D was reported as 2.5 for H_α and 14 for H_β. See: Pohl, B.; Raichle, T.; Ghisla, S. *Eur. J. Biochem.* **1986**, *160*, 109−115. (b) For ADH, $^D(V/K)$ was found to be 3.0. See: Damgaard, S. E. *Biochemistry* **1981**, *20*, 5662−5669.
(13) For a discussion of $^D(V/K)$, see: Cleland., W. W. *CRC Crit. Rev. Biochem.* **1982**, *13*, 385−428.
(14) Strauss, E.; Begley, T. P. Unpublished observation.

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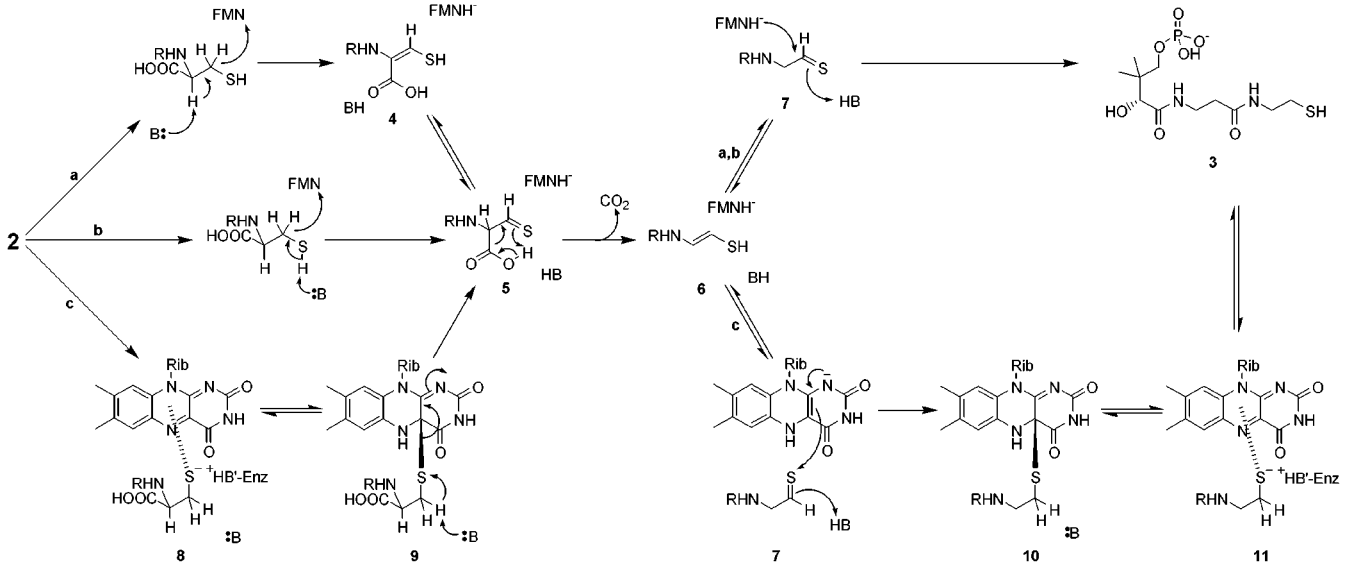
Scheme 2



partial (<5%) and complete conversion.[15,16] The isotope effects were calculated by dividing the ratio of deuterated and nondeuterated products at partial conversion by the ratio of the same at complete conversion, as previously described.[13] The results demonstrated a very small isotope effect at C_α (1.07 ± 0.03) and a primary isotope effect at C_β (1.81 ± 0.04). While the data are consistent with pathways **b** and **c**, it is possible that the reaction could proceed by pathway **a** if the deprotonation at C_α is fast.

To determine the extent of release of **2** from the enzyme, we synthesized β,β-dideuterated **2** by published methods[1] using L-3,3-[^{2}H]$_2$cysteine and determined the value of ^{D}(V/K) at C_β to be 2.54 ± 0.03.[17] This is substantially larger than the value of 1.81 ± 0.04 determined by synthesizing **2** enzymatically and indicates that there is significant channeling of **2** to product.

The presence of a thiolate anion in close proximity to C(4a) of flavin (as depicted in pathway **c**) should result in the formation of a thiolate−flavin charge-transfer complex, which may be observable in the UV/visible spectra of the enzyme−substrate or enzyme−product complexes. Such charge-transfer complexes have been detected as long-wavelength absorptions in the flavin spectra of certain members of the family of disulfide oxidoreductases, specifically mercuric reductase and lipoamide dehydrogenase.[5,18] In contrast, for pathways **a** and **b**, hydride transfer from the substrate to the flavin will require that the thiol is oriented away from the flavin, in which case it will be unable to form such a charge-transfer complex.

The UV/visible absorption spectrum of pure enzyme and in the presence of substrate (**2**) and product (**3**) is shown in Figure 1, and clearly demonstrates the formation of an induced charge-transfer band at 560 nm. This observation is consistent with the presence of the charge-transfer complexes **8** and **11** and suggests that the decarboxylation of *P*PC proceeds via pathway **c**.[19]

This study is the first to provide mechanistic insight into the novel decarboxylation reaction catalyzed by *P*PC-DC. We show that solvent exchange of the α-proton is not observed and that the ^{D}(V/K) isotope effect at C_α is small: these results suggest that the reaction does not proceed via pathway **a**. The isotope effect at C_β, in combination with the presence of substrate and product induced charge-transfer complexes as observed in the UV/visible spectrum of the enzyme, support pathway **c** as the means by which the substrate activating thioaldehyde is formed, whether by an SET mechanism or via a thiol-C(4a) flavin adduct.

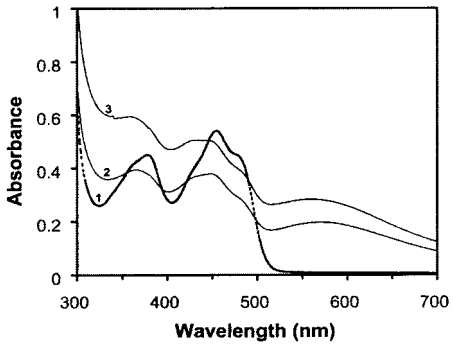


Figure 1. Spectra of pure CoaBC (line 1) and in the presence of 4.5 mM substrate (**2**, line 2) and 4.5 mM product (**3**, line 3) at pH 8.0.

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Supporting Information Available: Detailed description of all experimental procedures and tables of results (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) While L-3,3-[^{2}H]$_2$-cysteine was commercially available, only racemic DL-2-[^{2}H]-cysteine could be obtained. So as not to bias the results of the experiment, only racemic mixtures of cysteine were used.

(16) The amount of conversion was determined to be well below 5% by a $^{14}CO_2$-release assay (ref 1) with identical samples in which the mixture of cysteines was replaced by a solution of L-1-[^{14}C]cysteine. This makes it unnecessary to correct for extent of conversion. See: Bigeleisen, J.; Wolfsberg, M. *Adv. Chem. Phys.* **1958**, *1*, 15−76.

(17) The decarboxylation of **2** does not proceed to completion if it is directly introduced to reaction mixtures, even after prolonged exposure to the enzyme. Therefore the isotope effect was calculated by dividing the ratio of deuterated and nondeuterated products after partial (<5%) conversion by the ratio of deuterated and nondeuterated substrates.

(18) (a) Thorpe, C.; Williams, C. H. *Biochemistry* **1981**, *20*, 1507−1513. (b) Miller, S. M.; Massey, V.; Ballou, D.; Williams, C. H.; Distefano, M. D.; Moore, M. J.; Walsh, C. T. *Biochemistry* **1990**, *29*, 2831−2841.

(19) The structure of EpiD, which catalyzes the oxidative decarboxylation of a peptidyl-cysteine to the corresponding enethiol as part of the biosynthesis of lantibiotics, shows that the substrate thiol is close to the flavin and supports our mechanistic analysis. See: Blaesse, M.; Kupke, T.; Huber, R.; Steinbacher, S. *EMBO J.* **2000**, *19*, 6299−6310, and the Supporting Information for a full discussion of this enzyme.