A Novel Enzymatic Rearrangement

Danica Galonić Fujimori[1,*]
[1]Department of Cellular and Molecular Pharmacology and Department of Pharmaceutical Chemistry, University of California, San Francisco, 600 16th Street, San Francisco, California 94158, USA
*Correspondence: fujimori@cmp.ucsf.edu
DOI 10.1016/j.chembiol.2010.12.003

Human Fe(II)-α-ketoglutarate-dependent dioxygenases are a diverse enzyme family involved in a number of biological processes, from collagen biosynthesis to transcription. In this issue, Leung et al. (2010) add a new reaction to their ever-expanding catalytic repertoire.

Carnitine, C3-hydroxylated trimethylaminobutyrate, is an essential metabolite required for the transport of fatty acids and products of their β-oxidation into mitochondria (Kerner and Hoppel, 2000). Carnitine is biosynthetically derived from *N*-trimethyllysine in four enzymatic steps. The final step is catalyzed by an Fe(II) and α-ketoglutarate (α-KG)-dependant γ-butyrobetaine hydroxylase (BBOX), an enzyme that uses the canonical reactivity of the family to hydroxylate the β-carbon in the substrate (Figure 1) (Hulse et al., 1978; Lindstedt et al., 1977). This enzyme is inhibited by 3-(2,2,2-trimethylhydrazine)-propionate (THP), a drug clinically used for the treatment of myocardial infarction by interfering with carnitine-mediated transport (Dambrova et al., 2002). By studying the mechanism of action of the inhibitor, Leung et al. (2010) demonstrate that this compound acts as a competitive inhibitor of the enzyme, occupying a nearly identical position in the active site, and that BBOX catalyzes *N*-methyl demethylation and an unprecedented rearrangement involving cleavage of the hydrazine N-N bond and the formation of a new C-C linkage.

BBOX is a dimeric dioxygenase where the N-terminal zinc-binding domain of each subunit provides the dimerization interface. The crystal structure of the enzyme, obtained in the presence of zinc in place of the oxygen-sensitive Fe(II) and *N*-oxalylglycine instead of α-KG substrate, shows metal coordination by the dihistidine-carboxylate motif characteristic of a majority of Fe(II)-α-KG-dependent enzymes. The *pro-R* hydrogen atom of the C3 carbon of the substrate γ-butyrobetaine is adjacent to the metal, indicating the feasibility of the required hydrogen atom abstraction and subsequent oxygen rebound needed for hydroxylation of the prime substrate. To accommodate the trimethylammonium functionality, the enzyme harbors an aromatic cage, reminiscent of that present in chromatin-binding PHD and Tudor domains (Adams-Cioaba and Min, 2009).

BBOX exibits remarkable substrate promiscuity and it processes, albeit with diminished rates, a number of modified substrates, including betaines containing both longer and shorter carbon chains, as well as γ-butyrobetaine possessing either a 3*S* (L-carnitine) or 3*R* (D-carnitine) hydroxyl moiety. Additionally, both *N*-*tert*-butyl and *N*-isopropyl β-alanine derivatives are substrates for the enzyme, yielding malonic acid semialdehyde and either *tert*-butyl or isopropylamine, respectively.

THP acts as a competitive inhibitor as well as the substrate for BBOX, in agreement with previous observations (Spaniol et al., 2001). Careful NMR analysis revealed that, upon incubation with the enzyme and the required cofactors—Fe(II), α-KG, and oxygen—THP is converted into the malonic acid semialdehyde (**VII**) (Figure 2), dimethylamine, and formaldehyde. Release of formaldehyde is a hallmark of oxidative demethylation characteristics of jumonji family of histone demethylases (Cloos et al., 2006; Tsukada et al., 2006; Whetstine et al., 2006). Additionally, NMR analysis of the incubation products revealed the presence of an unexpected rearranged β,γ-diamino product **X**, where the γ-nitrogen is substituted with both methyl and hydroxymethyl groups. To account for the formation of these products, the authors propose a mechanism initiated by the canonical iron(IV)-oxo species-mediated hydrogen atom abstraction from the β-carbon of THP, analogous to the first step of hydroxylation of the γ-butyrobetaine substrate. Rather than undergoing subsequent oxygen rebound, the THP-derived radical **II** undergoes a fragmentation to form an imine **III** and a trimethylamine radical cation **IV**, which is then converted to a methylene radical **V** via a 1,2-hydrogen shift. This is a branching point in the formation of the observed reaction products. The oxygen rebound between the enzymatic Fe(III)-hydroxo species (**I**) and the methylene radical **V** result in a dimethylated hemiaminal intermediate **VI**, which subsequently fragments to form formaldehyde and dimethylamine. Aqueous hydrolysis of the imine **III** provides malonate semialdehyde (**VII**, Path A) (Figure 2). A competing pathway uses the methylene-centered radical **V** and the imine intermediate **III** in an alternative manner: addition of the radical into the imine carbon provides an adduct with an unpaired electron on nitrogen (**VIII**, Path B) (Figure 2). A 1,5-hydrogen shift, followed by hydroxylation of the resultant carbon-centered radical **IX** gives the rearranged product **X**. Further support of this mechanism is derived from the analysis of the reaction of *N*-[^{13}C-methyl]-labeled THP

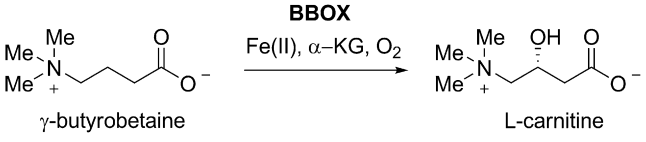

Figure 1. Final Step in Carnitine Biosynthesis Is Catalyzed by γ-Butyrobetaine Hydroxylase, BBOX, an Fe(II)- and α-Ketoglutarate-Dependent Dioxygenase

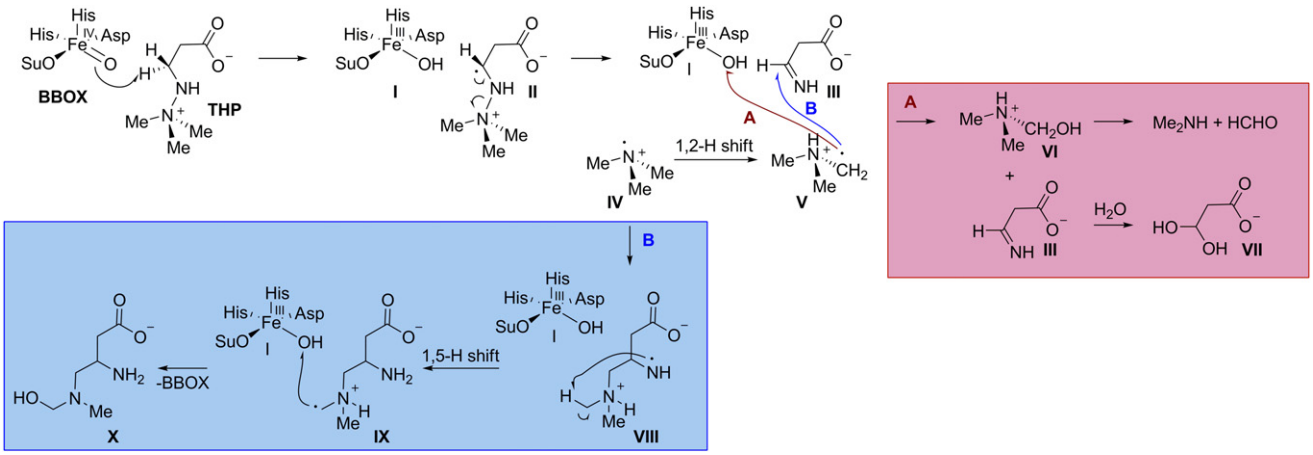

Figure 2. THP, 3-(2,2,2-Trimethylhydrazine)-Propionate, Is a Competitive Inhibitor and a Substrate of BBOX
Upon incubation, THP undergoes a novel rearrangement, resulting in the formation of hydroxymethyl diamine (**X**). Su, succinate.

with the enzyme, showing incorporation of the C13 label in all three carbons attached to the γ-nitrogen in the rearranged product, as all three methyl groups in the trimethylamine radical cation **IV** can participate in the 1,2-hydride shift to generate methylene-centered radical **V**.

These findings have several important implications. By catalyzing both the hydroxylation of the substrate to give a stable C-OH linkage and by catalyzing demethylation via hydroxylation of an amine-appended methyl group, BBOX provides a mechanistic link between two of the most common enzymatic functions in Fe(II)-αKG family: hydroxylation and demethylation. THP, an inhibitor of the enzyme, also serves as its substrate to give several products, including one resulting from a novel rearrangement enabled by radical fragmentation, rearrangement, and subsequent recombination of two fragments. Finally, the detailed insight into the mode of inhibition and the structure of the inhibitor and substrate-bound forms of the enzyme provide a starting point for the design of novel inhibitors of BBOX with cardioprotective effects.

REFERENCES

Adams-Cioaba, M.A., and Min, J. (2009). Biochem. Cell Biol. *87*, 93–105.

Cloos, P.A., Christensen, J., Agger, K., Maiolica, A., Rappsilber, J., Antal, T., Hansen, K.H., and Helin, K. (2006). Nature *442*, 307–311.

Dambrova, M., Liepinsh, E., and Kalvinsh, I. (2002). Trends Cardiovasc. Med. *12*, 275–279.

Hulse, J.D., Ellis, S.R., and Henderson, L.M. (1978). J. Biol. Chem. *253*, 1654–1659.

Kerner, J., and Hoppel, C. (2000). Biochim. Biophys. Acta *1486*, 1–17.

Leung, I.K.H., Krojer, T.J., Kochan, G.T., Henry, L., von Delft, F., Claridge, T.D.W., Opperman, U., McDonough, M.A., and Schofield, C.J. (2010). Chem. Biol. *17*, this issue, 1316–1324.

Lindstedt, G., Lindstedt, S., and Nordin, I. (1977). Biochemistry *16*, 2181–2188.

Spaniol, M., Brooks, H., Auer, L., Zimmermann, A., Solioz, M., Stieger, B., and Krahenbuhl, S. (2001). Eur. J. Biochem. *268*, 1876–1887.

Tsukada, Y., Fang, J., Erdjument-Bromage, H., Warren, M.E., Borchers, C.H., Tempst, P., and Zhang, Y. (2006). Nature *439*, 811–816.

Whetstine, J.R., Nottke, A., Lan, F., Huarte, M., Smolikov, S., Chen, Z., Spooner, E., Li, E., Zhang, G., Colaiacovo, M., et al. (2006). Cell *125*, 467–481.

