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Delivery of tailor-made cobalamin to methylmalonyl-CoA mutase

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Abstract

Methylmalonyl coenzyme A mutase (MCM) catalyzes the adenosylcobalamin-dependent isomerization of methylmalonyl-CoA to succinyl-CoA. Adenosyltransferase, an enzyme that carries out the final step in biosynthesis of adenosylcobalamin, is shown to be involved in delivery of the cofactor to MCM.

Since its discovery more than half a century ago, the structure, biosynthesis and chemical properties of cobalamin (vitamin B_{12}) have captivated structural biologists, enzymologists and chemists. The adenosylated form of cobalamin, adenosylcobalamin (Fig. 1a), is an enzymatic cofactor for MCM, deficiency of which leads to methylmalonic aciduria. The complete picture of cobalamin trafficking in biology is still far from visible1; however, Padovani *et al.*2 take an important step forward by showing that in the methylotroph *Methylobacterium extorquens*, where the intracellular concentration of cobalamin is low, the protein adenosyltransferse (ATR) can transfer adenosylcobalamin to the MCM.

Cobalamin is an organometallic cofactor in which a cobalt atom is bound in the plane of a corrin macrocycle, which contributes four equatorial nitrogen atoms to coordinate the metal ion. The two faces of the molecule are not equivalent; in solution, the lower axial coordination position of the cofactor is occupied by a dimethylbenzimidazole moiety, which in turn is attached to the corrin macro-cycle. The upper axial face of the molecule is occupied either by a methyl group or an adenosyl ligand. Two modes of binding of cobalamin to target proteins have been demonstrated by X-ray crystallography3,4. In one, the dimethylbenzimidazole remains coordinated to the cobalt (base-on form), whereas in the other the dimethylbenzimidazole is replaced by the imidazole side chain of a histidine residue in the active site of the protein (base-off/His-on form) (Fig. 1a).

MCM catalyzes the adenosylcobalamin-dependent isomerization of methyl-malonyl-CoA to succinyl-CoA. In MCM, adenosylcobalamin is bound in the base-off/His-on form5 (Fig. 1b). Conversion of apo- to holo-MCM presents two significant challenges. First, the cellular concentration of cobalamin is generally low, and simple diffusion would be inefficient for formation of holo-MCM. Second, in solution and under physiological conditions (pH 7.4), adenosylcobalamin exists predominantly in the base-on form, since the pK_a of the dimethylbenzimidazole base is quite low (pK_a ~ 3.7)6. Both of these challenges now appear to be overcome by the ATR protein. ATRs from a number of sources catalyze the reductive adenosylation of cobalamin with ATP to generate adenosylcobalamin. As in MCM, ATR also binds adenosylcobalamin in a base-off conformation, which suggests that in addition to tailoring cobalamin for MCM, it could also deliver it7.

Padovani *et al.*2 show that ATR can indeed deliver adenosylcobalamin to MCM, thus explaining how MCM acquires the cofactor despite its low concentration. The distinct UV-visible spectral features of adenosylcobalamin bound to ATR (base-off form) and MCM (base-off/His-on form) allows monitoring of the kinetics of cofactor transfer from ATR to MCM (Fig. 1b). Simulations of the kinetic traces are most consistent with

adenosylcobalamin bound to ATR being transferred to apo-MCM to generate holo-MCM, in an associative process that does not involve release of the cofactor into solution. This avoids formation of the base-on form, which would be favored otherwise under physiological conditions. Moreover, the transfer is shown to be reversible; interestingly, under the conditions of the experiments, binding to ATR is favored. The imidazole of His596 in MCM, which coordinates adenosylcobalamin, is proposed to play a role in the transfer; site-directed variants of MCM that lack this histidine are drastically impaired in the transfer. These results are of importance because they provide a glimpse of how a cobalamin cofactor, which is specifically tailored with an adenosyl moiety, is delivered to the intended enzyme. These data further illuminate why holo-MCM does not form in *M. extorquens* when ATR is deleted, in spite of presence of adenosylcobalamin in the cell8.

In addition to ATR, *M. extorquens* also encodes MeaB, which is a metallochaper-one that binds MCM and may be involved in GTP-dependent protection of the cofactor from destruction by loss of the adenosyl moiety8›9. The coordinated influences of ATR and MeaB on MCM catalysis represent a new paradigm for delivery and protection of the cobalamin from oxidative damage in cobalamin-dependent enzymes. The X-ray crystal structure of MeaB was solved recently10; as additional structural and biochemical information on the MCM-MeaB-ATR system emerges, it will be particularly interesting to explore how mutations in these proteins, which are known to lead to methylmalonic aciduria, exert their influence on the transformation catalyzed by MCM. Such investigations should provide a molecular correlation between function and disease.

References

1. Banerjee R. ACS Chem. Biol. 2006; 1:149–159. [PubMed: 17163662]
2. Padovani D, Labunska T, Palfey BA, Ballou DP, Banerjee R. Nat. Chem. Biol. 2008; 4:194–196. [PubMed: 18264093]
3. Drennan CL, Huang S, Drummond JT, Matthews RG, Ludwig ML. Science. 1994; 266:1669–1674. [PubMed: 7992050]
4. Shibata N, et al. Structure. 1999; 7:997–1008. [PubMed: 10467140]
5. Padmakumar R, Taoka S, Padmakumar R, Banerjee R. J. Am. Chem. Soc. 1995; 117:7033–7034.
6. Brown KL, Hakimi JM, Jacobsen DW. J. Am. Chem. Soc. 1984; 106:7894–7899.
7. Yamanishi M, Labunska T, Banerjee R. J. Am. Chem. Soc. 2005; 127:526–527. [PubMed: 15643868]
8. Korotkova N, Lidstrom ME. J. Biol. Chem. 2004; 279:13652–13658. [PubMed: 14734568]
9. Padovani D, Banerjee R. Biochemistry. 2006; 45:9300–9306. [PubMed: 16866376]
10. Hubbard PA, et al. J. Biol. Chem. 2007; 282:31308–31316. [PubMed: 17728257]

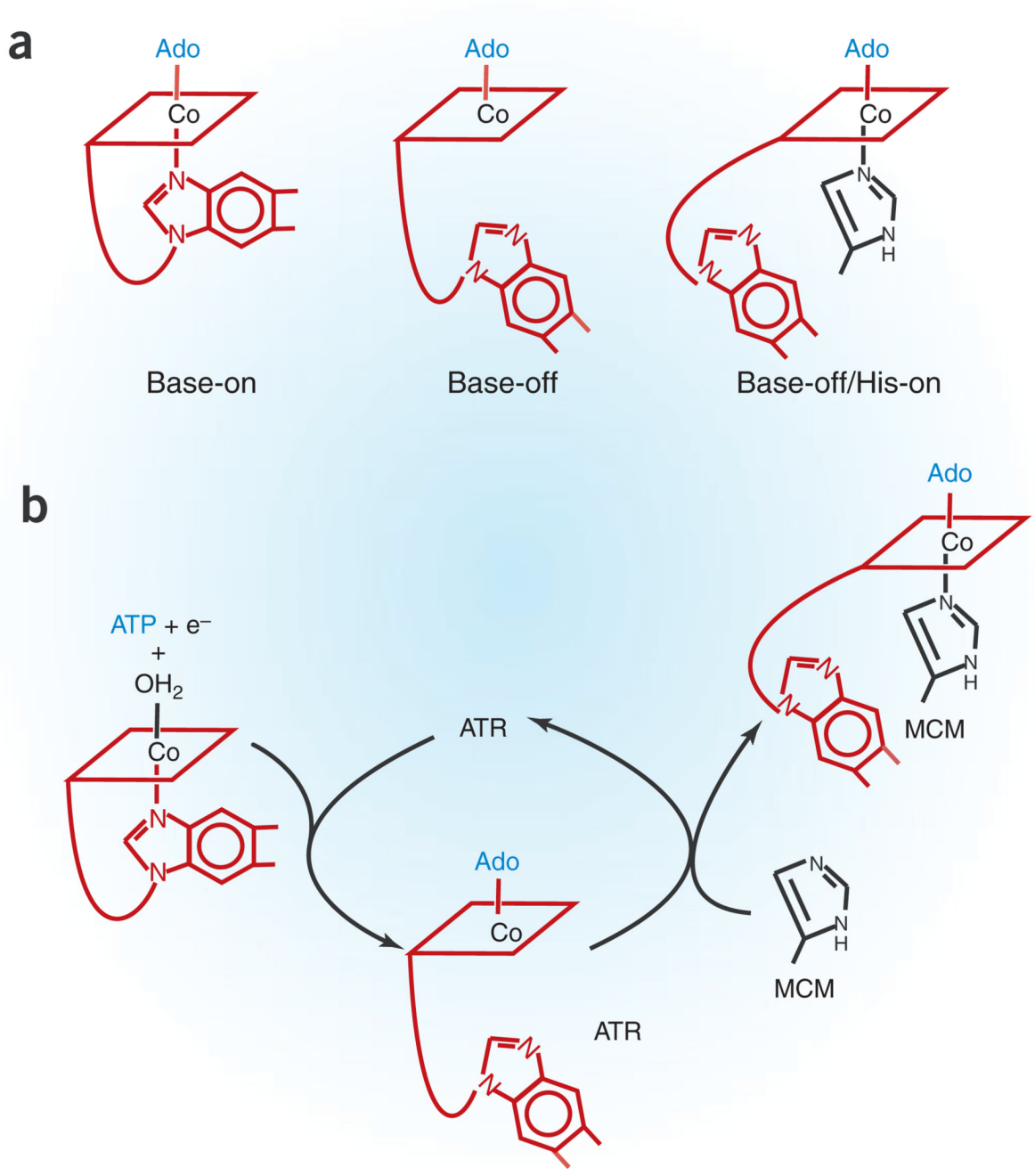

Figure 1.

Coordination states of cobalamin and tailoring delivery of cobalamin from ATR to MCM. (**a**) Cobalamin can exist in base-on, base-off or base-off/His-on forms. (**b**) ATR catalyzes the reductive adenosylation of cobalamin and transfers adenosylcobalamin to MCM. The adenosylcobalamin remains bound to ATR in the base-off conformation. Upon transfer to MCM, the imidazole side chain of His596 serves as the lower axial ligand to the cofactor, forming a base-off/His-on adenosylcobalamin.