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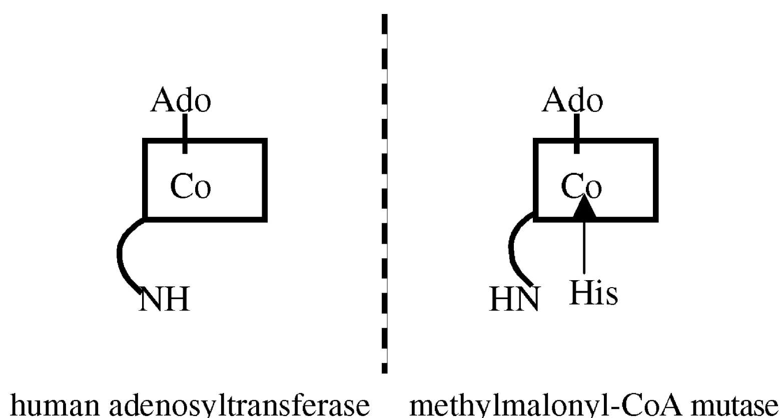

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Mirror "Base-off" Conformation of Coenzyme B_{12} in Human Adenosyltransferase and Its Downstream Target, Methylmalonyl-CoA Mutase

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In humans, B_{12} serves as a cofactor in two known enzymes with different intracellular residences: methionine synthase, in the cytosol, and methylmalonyl-CoA mutase, in the mitochondrion. Correspondingly, the proteins involved in intracellular cobalamin metabolism are housed in the two compartments.[1] The proteins involved in mitochondrial B_{12} processing have been identified recently. They include adenosyltransferase, which converts cob(II)alamin to coenzyme B_{12} or adenosylcobalamin (AdoCbl),[2,3] and MMAA, a protein with GTPase activity but of otherwise unknown function.[4] The importance of these proteins to mitochondrial B_{12} metabolism is revealed by patient mutations that lead to isolated dysfunction of methylmalonyl-CoA mutase but spare the cytosolic B_{12} enzyme, methionine synthase.[1] Two major conformations of B_{12} have been found in proteins: the "base-on" form in which the endogenous corrin base, dimethylbenzimidazole, is the lower axial ligand and the "base-off/His-on" conformation in which a histidine residue in the protein replaces dimethylbenzimidazole.[5] In the latter subclass, the coordinating histidine is embedded in a conserved primary sequence motif, DXHXXG,[6] which is absent in bacterial and human adenosyltransferases. This has led to the prediction that adenosyltransferases bind B_{12} in the "base-on" conformation. Indeed, the crystal structure of CobA, an adenosyltransferase involved in the de novo biosynthesis of B_{12} in *Salmonella*, confirmed this prediction.[7] A modified histidine-containing B_{12} binding motif is seen in the corrinoid protein, MtrA, found in methanogens.[8]

However, spectroscopic studies on human adenosyltransferase reveal unexpectedly that the cofactor is bound in the "base-off" conformation in both the Co^{3+} and Co^{2+} oxidation states. Binding of AdoCbl to human adenosyltransferase results in a large blue shift in the UV−visible spectrum from 524 to 456 nm, revealing loss of axial nitrogen coordination (Figure 1). The spectrum is identical to the spectrum of the truncated cofactor analogue, adenosylcobinamide that, lacking the dimethylbenzimidazole ligand, is perforce, in the "base-off" state (not shown).

Binding of cob(II)alamin, in which cobalt is in the 2^+ oxidation state, is accompanied by substantial changes in the electron paramagnetic resonance (EPR) spectrum.[9] Cob(II)alamin in solution exhibits an EPR spectrum that is diagnostic of the "base-on" species in which the octet of hyperfine lines resulting from interactions between the unpaired electron and the cobalt nucleus ($I = 7/2$) is further split into triplets due to superhyperfine interaction with a nitrogen ($I = 1$) ligand (Figure 2). The spacing of the hyperfine lines is 109 G, which is typical for "base-on" cobalamin. In contrast, the spectrum of the bound cofactor reveals significant differences in the low- and high-field regions. Notably, the superhyperfine triplet splittings due to nitrogen coordination are absent, and the high-field lines appear as singlets with a spacing of 137 G, typical for "base-off" cobalamin.[9]

Curiously, the "base-off" mode of cobalamin binding in human adenosyltransferase that generates AdoCbl is mirrored in the enzyme that utilizes AdoCbl, methylmalonyl-CoA mutase. However, the coordination environments for B_{12} in the two proteins are distinct. Thus, in human adenosyltransferase, the lower axial ligand site appears to be vacant, while in human methylmalonyl-CoA mutase, the site vacated by the endogenous ligand, dimethylbenzimidazole, is occupied by a histidine residue, H626. However, the presence of a weak ligand in human adenosyltransferase, viz., H_2O, cannot be ruled out by these data. A similar situation is seen in the copper chaperone, Atx1 which has an unusually low coordination environment for Cu(I) as compared to its target protein.[11]The presence of

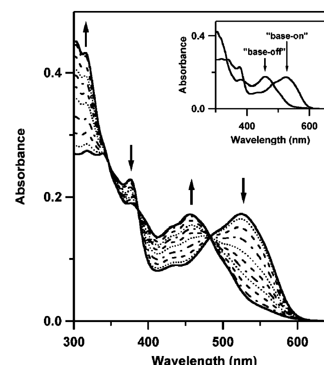

Figure 1. UV−visible electronic spectrum of AdoCbl bound to human adenosyltransferase. Adenosyltransferase (in 50 mM Tris-HCl buffer, pH 8.0), was added in aliquots to a solution of 23 μM AdoCbl in the same buffer. The final spectrum contained 96 μM adenosyltransferase which was purified as described.[10] (Inset) Comparison of free AdoCbl spectra in the "base-off" and "base-on" states.

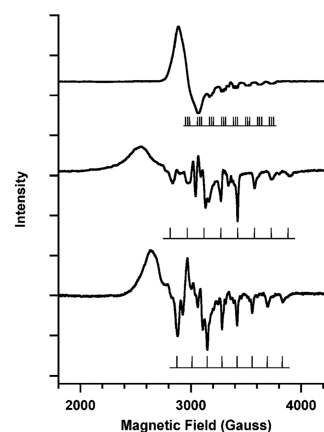

Figure 2. Comparison of the EPR spectra of 100 μM cob(II)alamin generated by photolysis of AdoCbl under anaerobic conditions in 10 mM Tris-HCl, pH 8 (upper), bound to 1.6 mM human adenosyltransferase in the same buffer (lower), and of the free photolyzed AdoCbl in 1% H_3PO_4 (middle). The spectra were recorded at 100 K, modulation amplitude 10 G, microwave power 40 mW, and microwave frequency, 9.383 GHz. The octet of hyperfine lines appear as singlets in the middle and lower spectra and exhibit superhyperfine triplet splittings in the upper spectrum as indicated by the line markings.

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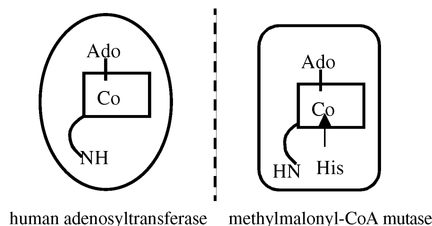

Figure 3. Mirror "base-off" conformation of AdoCbl in human adenosyltransferase and methylmalonyl-CoA mutase.

the "base-off" conformer in human adenosyltransferase can be rationalized from a thermodynamic standpoint. In the adenosyltransferase-catalyzed reaction, cob(II)alamin is reduced to cob(I)-alamin for subsequent nucleophilic attack on ATP to generate AdoCbl. The redox potential for the cob(II)alamin/cob(I)alamin couple is ∼110 mV more favorable for the "base-off" versus the "base-on" conformer.[12] Methionine synthase, an enzyme that also needs to reduce cob(II)alamin to cob(I)alamin during reductive reactivation solves this problem by transient conversion of bound cob(II)alamin from the "base-off/His-on" state to a four-coordinate "base-off" state in complex with the reducing partner, flavodoxin.[13] In contrast, the B_{12} derivative associated with the corrinoid/iron−sulfur protein in acetogenic bacteria is always in the "base-off" state,[14] although the presence of a weak lower axial ligand cannot be excluded. The "base-off" conformation in this protein is similarly expected to facilitate reduction of the cobalt during reductive activation.[15]

In solution the distribution of the "base-on" and "base-off" conformers is governed by the pK_a (3.7) of dimethylbenzimidazole in AdoCbl.[16] Thus, at physiological pH, the concentration of the "base-off" species is exceedingly low. We have examined the efficacy with which human adenosyltransferase binds AdoCbl versus the "base-off" truncated analogue, adenosylcobinamide (Table 1). The K_d's differ by 5-fold indicating that the nucleotide tail contributes modestly to the strength of cofactor binding. Moreover, the enzyme exhibits relatively broad specificity and does not appear to discriminate substantially between analogues containing upper axial ligands with widely varying bulk. In comparison to human methylmalonyl-CoA mutase, which binds AdoCbl tightly ($K_d = 0.04\ \mu$M), human adenosyltransferase binds with an ∼40-fold lower affinity ($K_d = 1.7\ \mu$M).

In conclusion, spectroscopic analysis of human adenosyltransferase, the locus of the *cbl*B set of patient mutations, reveals an unusual coordination environment for the bound B_{12} cofactor. The mirror "base-off" conformation of cobalamin in the enzyme that synthesizes the active coenzyme form and the enzyme that utilizes it is striking. Interestingly, the coordination environment for cobalt is distinct in the two proteins which is reflected in an ∼40-fold difference in their affinity for B_{12}.

Table 1. Comparison of Dissociation Constants for Adenosyltransferase for Different B_{12} Analogues

analogue	K_d (μM)[a]
AdoCbl	1.7 ± 0.4
AdoCbi	12.0 ± 2.5
OHCbl	8.5 ± 1.1
MeCbl	8.2 ± 1.2
CNCbl	7.8 ± 0.5
CN, aq Cbi	27.9 ± 9.3

[a] K_d's were determined fluorimetrically as described previously[17] and represent the average of five to six independent determinations.

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