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INVITED ABSTRACTS OF THE 4TH EUROPEAN SYMPOSIUM ON VITAMIN B[SUB 12] AND B[SUB 12]-PROTEINS, SEPTEMBER 2-6, 1996, INNSBRUCK, AUSTRIA

Reductive activation of methanol: 5-hydroxybenzimidazolylcobamide methyltransferase from Methanol sarcina barkeri

The conversion by Methanosarcina barker) of methanol into methylcoenzyme M (2-methyl-thioethanesulfonate, Ch_3S-CoM), the substrate of the methane-forming reaction, is catalyzed by a complex enzyme system including methanol: 5-hydroxybenzimidazolylcobamide methyltransferase (MT_1) (Fig. 35) [1]. MT_1 is a 135 kDa protein that harbours as its prosthetic groups two molecules 5-hydroxybenzimidazolylcobamide (B_{12}HBI). In the reaction cycle MT_1 accepts the methyl group from methanol and binds it as methyl-B_{12}HBI. Subsequently, the methyl group is transferred by a second methyltransferase (MT_2) to coenzyme M (2-mercaptoethanesulfonate, Coenzyme M). In the in vitro reaction an additional set of enzymes and coenzymes are required, notably ATP (in catalytic amounts), Methyltransferase Activating Protein (MAP), hydrogen, hydrogenase, and ferredoxin [1]. The latter play a role in the (re)activation of MT_1, when the enzyme has become inactive as the result of the oxidation of the catalytic competent Co(I)B_{12}HBI.

Inactive MT_1 contained B_{12} as the Co(III) and Co(II) species and incubation with hydrogen, hydrogenase and ferredoxin did not allow a reduction beyond the Co(II) level. EPR experiments demonstrated the 5-hydroxybenzimidazole base to be co-ordinated to the cobalt atom ('base-on'). When both ATP and MAP were supplied, Co (II)-B_{12}HBI was transformed into the 'base-off' state. This has an important implication, since studies with isolated B_{12}HBI showed that the midpoint reduction potential of 'base-off' Co(II)-B_{12}HBI to Co(I)-B_{12}HBI (E_0 = - 500 mV) is about 100 mV more positive as compared to the same reduction of 'base-on' Co(II)B_{12}HBI [2]. Yet, the potential still may be too low for reduction with hydrogen ($E'^{,sub\ 0}(2H^+/H_2)$ = -414 mV). However, when methanol was also present in the reactivation mixture, the corrinoids in MT_1 were methylated and hereafter the enzyme had become fully active. This suggests that methanol is required to trap Co(I) out of the 'base-off'-Co

(II)/Co(I) equilibrium. By using purified MAP it was found that the enzyme was phosphorylated by ATP. MAP-phosphate substituted for ATP in the activation of MT_1 indicating that the phosphorylated enzyme catalyzed the critical step in the activation process: the conformational change of 'base-on' into 'base-off' Co(II)-B_{12}HBI.

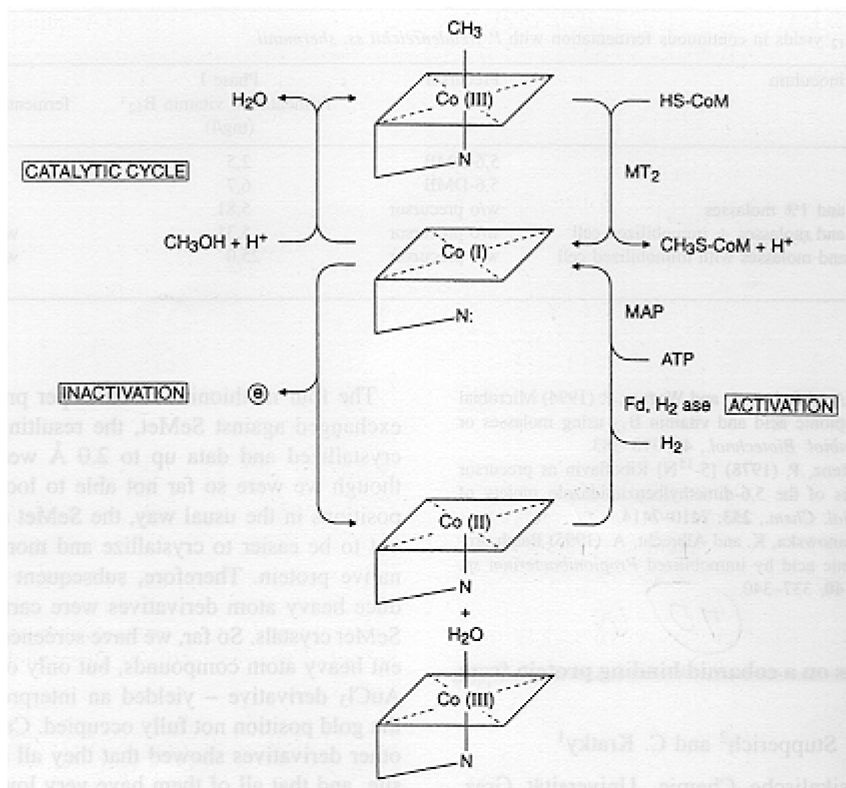

Fig. 35. The methanol: HS-CoM methyltransferase system. Methanol: 5-hydroxybenzimidazolylcobamide methyltransferase (MT_1) is schematically represented by its corrinoid prosthetic group; MT_2, methylcobamide: HS-CoM methyltransferase; MAP, Methyltransferase Activating protein; H_2ase, hydrogenase; Fd, ferredoxin.

References

[1.] Daas, P.J.H., Gerrits, K.A.A., Keltjens, J.T., van der Drift, C. and Vogels, G.D. (1993) J. Bacterial., 175, 1278-1283.

[2.] Daas, RJ.H., Keltjens, J.T., Hagen, W.R. and van der Drift, C. (1995) Arch. Biochem. Biophys., 319, 244-249.

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