The biotin-carboxylation reaction of pyruvate carboxylase: the roles of acetyl CoA, Mg2+ and biotin.

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Pyruvate carboxylase [EC 6.4.1.1] is a biotin-dependent enzyme that catalyses the pyruvate carboxylation of pyruvate to two partial reactions. In the first partial reaction (reaction 1), MgATP and HCO3− are used to carboxylate biotin, which then acts as a mobile group carrier to transport the -CO2H to the site of the second partial reaction in which pyruvate is carboxylated to form oxaloacetate [1]. In reaction 1 it is thought that HCO3− is activated by phosphorylation to form carboxyphosphate and it is this intermediate that actually transfers a carboxy group to biotin in formation of carboxybiotin [1,2]. Both Mg2+ and acetyl CoA are required in reaction 1 and it has been shown that acetyl CoA is required for the transfer of the carboxy group carrier to transport the -CO2H to the site of the pyruvate carboxylase-catalysed dephosphorylation of MgATP in the absence of pyruvate or by measuring the rate of the enzyme-catalysed phosphorylation of MgADP by carboxybiotin, which is a structural analogue of carboxyphosphate [3].

Fig. 1 shows that although acetyl CoA is not absolutely required for the transfer of Pi from carbamyl phosphate to MgADP, it greatly enhances both Vmax and V/K. Although Kmax for carbamyl phosphate in the presence of acetyl CoA was about twice that in its absence, this increase in Kmax may reflect a decreased affinity for carbamyl phosphate due to the conformation changes induced in the enzyme by acetyl CoA [4]. In both the absence and presence of acetyl CoA, substrate inhibition was apparent, with a K1 in both cases estimated at about 32mM. One possible explanation for this phenomenon is that the carbamyl phosphate is able to bind weakly to the MgADP binding site and at high carbamyl phosphate concentrations this site competes with MgADP. Another possibility is that carbamyl phosphate can form a dead-end complex with MgATP.

The effects of [Mg2+] on the kinetic parameters of the phosphorylation of MgADP by carbamyl phosphate in the absence of acetyl CoA were also examined. By going from 5mM Mg2+ to 40mM Mg2+, Vmax increased by a factor of four, but over the same range of [Mg2+], Kmax also increased by a factor of three. Again, like acetyl CoA, increasing [Mg2+] increases Vmax, but also increases Kmax for carbamyl phosphate. These effects of Mg2+ may be due to a conformation change in the enzyme induced by this ion [5] which has a similar effect to acetyl CoA in that this conformation of the enzyme may be optimal for catalysis, the access to the active site by carbamyl phosphate may be restricted and hence produce the increase in Kmax.

The reaction system contained 20mM NaHCO3, 0.1-2mM ATP, 10μCi [32P]ATP and 4.5mM free Mg2+ in 0.1M Tris-Cl pH 7.8 at 25°C with 0mM (0), 3mM (△) or 6mM (□) oxamate. Released [32P]Pi was measured as described by Reimann and Umfleet [8].

Fig. 2 shows the effects of increasing [oxamate] on the enzyme-catalysed dephosphorylation of MgATP in the absence of acetyl CoA and pyruvate. Oxamate is an inhibitor-analogue of pyruvate and binds at the pyruvate binding site on the enzyme to act as a signal for the movement of biotin from the site of reaction 1 to that of the second partial reaction [9]. The inhibition of MgATP-dephosphorylation by oxamate shown in Fig. 2 demonstrates that the transfer of Pi from MgATP to HCO3− has a dependency on the presence of biotin at the site of reaction 1. This complements the finding of Attwood and Graneri [6] that the transfer of Pi from carbamyl phosphate to MgADP is dependent on the presence of biotin at that part of the active site of the enzyme.

Our results suggest that although there is no absolute requirement for acetyl CoA, the rate of Pi transfer between carboxybiotin and MgADP is enhanced by this cofactor. Mg2+ also enhances this reaction as does the presence of biotin, which although it does not participate directly in MgATP-dephosphorylation, also enhances the rate of this forward reaction. It is likely that all of these rate enhancements are due to conformation changes in the site of reaction 1 induced by Mg2+ and acetyl CoA or due to the presence of biotin.

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