# BIOTIN CARBOXYLASES

A thesis submitted

by

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CHAPTER 3

## SUMMARY

1. A method has been developed for purifying propionyl-CoA carboxylase from sheep liver mitochondria. The method involves fractionation with ammonium sulphate and polyethylene glycol, followed by chromatography on DEAE-Sephacel and phenyl-Sepharose. Hexagonal bipyramidal enzyme crystals of 0.1 mm diameter have been obtained. The enzyme has been shown to contain two subunit types, with molecular weights of 58,000 and 72,000. The biotin prosthetic group resides in the larger subunit. The propensity of the enzyme for aggregating thwarted attempts to accurately measure its molecular weight, but "apparent" molecular weights in the range 670,000 to 840,000 were obtained using a variety of techniques.

2. Chemical modification experiments were undertaken in an attempt to determine to which subunit of propionyl-CoA carboxylase the substrates MgATP and propionyl-CoA bind. N-ethylmaleimide and the periodate oxidation products of ATP and dephosphopropionyl-CoA each bound to both subunits. In each case there was some protection against modification by both MgATP and propionyl-CoA, indicating that the two substrates bind at each other's binding sites. The results obtained were consistent with the hypothesis that the substrates bind in a crevice between two subunits of different type.

3. An assessment of the evolutionary relationships between 5 different biotin-dependent enzymes from various organisms

was made by comparing amino acid compositions. The β-methylcrotonyl-CoA carboxylase of *Achromobacter* was found to be closely related to the vertebrate pyruvate carboxylases. Pyruvate carboxylase from *Pseudomonas citronellolis* was found to be more distantly related to the vertebrate pyruvate carboxylases and to β-methyl-crotonyl-CoA carboxylase. There was no evidence of any relationships between acetyl-CoA carboxylase, propionyl-CoA carboxylase, transcarboxylase or pyruvate carboxylase. However, the two subunits of propionyl-CoA carboxylase have very similar compositions. The same is true of the subunits of the pyruvate carboxylase from *Pseudomonas citronellolis* and the two larger subunits of transcarboxy-lase. There was no indication that subunits from different enzymes might be related.

4. Some of the factors that influence the location and rate of movement of the N-carboxybiotin moiety between the two sub-sites of pyruvate carboxylase were studied. The rate of carboxylation of the alternative substrate, 2-oxo-butyrate, was measured at 0°C in an assay system where the isolated enzyme-[$^{14}$C]-carboxybiotin was the carboxyl group donor. The results are consistent with the hypothesis that the location of the carboxybiotin in the active site is determined by the presence of $Mg^{2+}$, acetyl-CoA and the oxo acid substrate. The presence of $Mg^{2+}$ favours the holding of the complex at the first sub-site, whereas α-oxo acids induce the complex to move to the second sub-site. The allosteric activator acetyl-CoA exerts only a slight stimulation on the rate of translocation to the second

sub-site, and this stimulation arises from an increase in the dissociation constant for $Mg^{2+}$.

5. The mechanism of carboxyl group transfer, as catalysed by pyruvate carboxylase and propionyl-CoA carboxylase, was studied. It was found that oxo acids such as oxamate, glyoxylate and hydroxypyruvate induce the movement of carboxybiotin into the second subsite of pyruvate carboxylase, where hydrolysis of the carboxy-biotin occurs. This was interpreted to be inconsistent with the mechanisms previously proposed for the carboxyl transfer reaction. It was shown that, unlike the pyruvate carboxylase reaction, there was no abortive hydrolytic pathway in the reaction catalysed by propionyl-CoA carb-oxylase, indicating that the two enzymes might use different catalytic mechanisms. However, a mechanism that is consistent with all the information available on biotin-dependent carboxyl group transfer reactions was proposed.