KINETIC STUDIES ON PROPIONYL-CoA CARBOXYLASE FROM PIG HEART

A thesis submitted by John Brian Edwards B.Sc. (Hons.),
to the University of Adelaide, South Australia,
for the degree of Doctor of Philosophy.

Department of Biochemistry,
University of Adelaide, S.A.
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SUMMARY.

Propionyl-CoA carboxylase which catalyses the reaction:

\[
\text{CH}_3\text{CH}_2\text{CO}\cdot\text{SCoA} + \text{ATP} + \text{HCO}_3^- \xrightarrow{\text{Mg}^{2+}} \text{CH}_3\text{CH}_2\text{CO}\cdot\text{SCoA} + \text{ADP} + \text{Pi}
\]

was purified from pig heart and a series of experiments were carried out to investigate some of the chemical and kinetic properties of the enzyme.

The function of the essential thiol group in propionyl-CoA carboxylase was investigated using N-ethyl maleimide as the thiol modifying reagent. The inhibition of the enzyme was first order with respect to time and inhibitor concentration. From an analysis of the data it was concluded that only one molecule of N-ethyl maleimide reacted with the enzyme. The rate of inhibition remained constant between pH 7.0 and 8.0 then increased very rapidly with increasing pH. The inflection point at pH 8.2 in the pH/pK plots indicated that the thiol group was involved in the formation of the enzyme-propionyl-CoA complex. This was confirmed by a comparison of the kinetic constants of the native and the chemically modified enzyme which showed that the apparent K_m value for propionyl-CoA increased while the values for ATP and HCO_3^- remained constant. By measuring the rate constant of the inactivation process in the presence of varying propionyl-CoA concentrations, it was
concluded that the inhibitor could not react with the enzymepropionyl-CoA complex. The inhibited amino acid was identified as cysteine by reaction with $^{14}C$-N-ethyl maleimide and the subsequent isolation of the radio-active cysteine-inhibitor complex. From this evidence it was concluded that a cysteine residue was involved in binding propionyl-CoA to the active site.

The enzyme is also stimulated in the presence of certain univalent cations. In the presence of $K^+$ ions the $V_{\text{max}}$ values for all substrates increased. In addition, the apparent $K_m$ value for $\text{HCO}_3^-$ was decreased although the affinity for the other substrates was unaffected. Kinetic evidence thus suggested that $K^+$ caused a conformational change on the enzyme, a result that was supported by the calculation of the entropy changes induced in the protein by this univalent cation.

This hypothesis was further substantiated by showing an increase in the rate of inhibition by N-ethyl maleimide in the presence of $K^+$ ions. This accumulated data provided strong evidence that a conformational change accompanied the reaction of alkali metals with propionyl-CoA carboxylase.

The allosteric activation of propionyl-CoA carboxylase by Mg$^{2+}$ and MgATP$^{2-}$ was also examined. The results suggest
that Mg$^{2+}$ has at least two roles in the reaction mechanism. Firstly, it forms a complex with ATP$^{3-}$ to form the true MgATP$^{2-}$ substrate and secondly, it forms a complex with the enzyme to activate the enzymic reaction. The MgATP$^{2-}$ complex deviates from Michaelis & Menten kinetics in such a way as to indicate a hysteric co-operative effect involving at least two molecules of ligand. Free Mg$^{2+}$ reduces the slope of the Hill plot for MgATP$^{2-}$ thus suggesting either a change in the order of the reaction with respect to MgATP$^{2-}$ or a reduction in the interaction between the MgATP$^{2-}$ sites. It is proposed that one site acts as an effector site and the other, a catalytic site.

Furthermore, ATP$^{4-}$ is a competitive inhibitor with respect to MgATP$^{2-}$ and since Mg$^{2+}$ has been shown to alter the kinetic properties, it could be suggested that this cation is binding at the effector site for MgATP$^{2-}$ and mimics the action of MgATP$^{2-}$ at this site. On the other hand, ATP$^{4-}$ could be binding at the catalytic site.

Two substrate kinetics were also carried out in an endeavour to substantiate previous reaction mechanism proposals. Although the evidence is only preliminary, the data obtained does not conform to the simple reaction scheme proposed by other investigators.