P001 Allosteric regulation of the biotin-dependent enzyme pyruvate carboxylase by acetyl-coenzyme A

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The recent publication of a number of structures of pyruvate carboxylase has enabled us to start to explore the detailed structure-function relationships in the allosteric activation of the enzyme by the physiological regulator, acetyl CoA. The structure of the enzyme has revealed that there is a separate allosteric domain, where acetyl CoA binds, that lies at the junction of the biotin carboxylation (BC) and carboxyltransfer (CT) domain and the junction of the carboxyltransfer domain and the biotin carboxyl carrier protein (BCCP) domain. In addition, the mechanism of action of the homo-tetrameric enzyme is much more complex than previously thought. The enzymic tetramer appears to be inherently asymmetrical with only two of the four subunits configured to be catalytically active, thus indicating half of the sites reactivity. These two subunits in the R. etli pyruvate carboxylase structure have the allosteric activator bound to them and they are configured to perform inter-subunit catalysis, where the biotin cofactor is carboxylated in the BC domain of one subunit and transfers its carboxyl group to pyruvate in the CT domain its partner subunit. In the present work we present initial studies of the role of acetyl CoA in coordinating the activities of the different parts of the enzyme.