Pyruvate Carboxylase: Relating Structure to Function

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SUMMARY

Pyruvate carboxylase (PC) [EC. 6.4.1.1] is a large tetrameric enzyme, found in a wide variety of organisms where it catalyses the conversion of pyruvate to oxaloacetate. PC is a member of the enzyme family known as the biotin-dependent carboxylases, where the covalently attached prosthetic group biotin is an absolute requirement for activity. The reaction mechanism involves the carboxylation of biotin, followed by transfer of this carboxylate moiety to pyruvate. These two reactions occur at spatially distinct subsites and are known as the 1st and 2nd partial reactions respectively. Although the catalytic mechanism of the above reactions has been extensively studied [see Knowles (1989): *Annu. Rev. Biochem.* 58, p195-221], far less is known of the 3-dimensional structure of the tetrameric molecule and how this structure is involved in the various functions of the enzyme. This is due in part, to the absence of PC crystals suitable for X-ray diffraction studies and the size of the tetramer (approximately Mr 520,000) effectively precluding it from analysis by NMR.

Due to the lack of a detailed 3-D structural model, the study reported in this thesis was aimed at providing structural information on the functional aspects of PC, by the combination of a number of techniques including partial proteolysis, chemical modification, immunochemistry, electron microscopy, and molecular biology.

Partial cDNA and genomic clones encoding rat PC were sequenced, providing 753 amino acids of sequence data from the C-terminus of the protein. These are encoded by ten exons over approximately 5 kb of DNA. Significant sequence homologies were observed between PC from rat and yeast both at the protein and nucleotide levels.

Limited treatment of sheep, rat, yeast and chicken PC with chymotrypsin demonstrated the existence of a large C-terminal fragment which is stable to further proteolysis, and sequence analysis by Edman degradation, showed the N-termini of the corresponding fragments to be identical in all four species. Similar treatment of sheep PC with proteinase K resulted in cleavage at a site identical to chymotrypsin, but in addition a second fragment was produced which lacked approximately Mr 6,000 from the C-terminus. These results together with the sequence similarities between rat and yeast PC confirm the proposed ligand binding regions reported by Lim *et al.* [(1988): *J. Biol Chem.* 263, p11493-11497].

The effects of chymotrypsin and proteinase K treatment on the tetrameric structure

truncated by 10 - 13% of the length of the molecule, although 40% of the total mass of the protein had been removed. A structural model has been proposed to explain this difference. Tetrameric particles could also be observed in samples where treatment with proteinase K had truncated the protein at positions Gly-17 and Arg-978. This indicates that the residues important for intersubunit binding are not contained in regions of the protein assigned to binding ATP or biotin.

Western analysis of partially proteolysed sheep PC, allowed the localisation of 5 monoclonal antibody epitopes within the length of the polypeptide. Monoclonal antibodies #12 and #42 bind to a region within approximately Mr 6,000 of the C-terminus, #6 and #18 bind to a region within approximately Mr 75,000 of the C-terminus while monoclonal antibody #60 binds to a region between Mr 75,000 and Mr 6,000 from the C-terminus of PC.

Limited treatment of PC with trinitrobenzene sulphonate (TNBS) results in modification of two lysine residues, one of which (Lys-A) is important for mediating the allosteric effect of acetyl-CoA. (Ashman *et al.* (1973) *Biochem. Biophys. Res Commun.* 51, p924-931).

Acetyl-CoA and 3´5´ADP were shown to protect yeast PC from inhibition by TNBS as is the case for PC from sheep . A [^{14}C]acetyl-CoA binding assay was developed, and used to demonstrate that modification of Lys-A inhibits the binding of acetyl-CoA to yeast PC, indicating that Lys-A is at or near the acetyl-CoA binding site. Localisation of Lys-A within the protein sequence was attempted using peptide mapping techniques. Fab fragments of anti-TNP IgG were produced and observed as complexes with TNBS-treated yeast PC, using electron microscopy. Lys-A and hence the acetyl-CoA binding site was localisėd to the central region of the tetramer, near the inter-subunit junction using this technique.