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My Favourite Enzyme:

Pyruvate Carboxylase

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Introduction

Having completed a PhD in molecular and cellular biology in mammalian reproduction at the University of Sydney, my first postdoctoral appointment began early in 1965 in the laboratory of Sir Hans Krebs at Oxford University where I had the pleasure of working with Dr Eric Newsholme on the regulation of gluconeogenesis and glycolysis during fetal development. My introduction to Pyruvate Carboxylase (PC) came early in 1966 when Michael Scrutton arrived back in Oxford to submit his D.Phil. thesis after spending a remarkably productive time working on PC in the laboratory of its discoverer, Professor Merton Utter, at Western Reserve University Medical School, Cleveland, Ohio. Not surprisingly, Michael bubbled with infectious enthusiasm about PC, and presented an outstanding seminar to the Department.

In late 1966 I moved to Professor Utter's group and had an invaluable and enjoyable experience broadening my skills and filling some of the many gaps in my knowledge, particularly in biophysical techniques. The co-discoverer of PC, Professor Bruce Keech, returned to Cleveland in 1968 on sabbatical leave from the University of Adelaide, Australia, and we soon established an effective and agreeable collaboration. This collaboration was renewed in late 1969 when I arrived in Adelaide, where I secured a Faculty position in 1970, and continued until Bruce retired in 1983.

The occurrence and biological roles of Pyruvate Carboxylase

PC was discovered in the context of defining the pathway of gluconeogenesis in liver and kidney (1,2), but was soon recognised to play important roles also in lipogenesis (3), in glyceroneogenesis in liver and adipose tissue (4) as well as in the functions of brain (5) and pancreatic islets (5,6). The central anaplerotic role of this mitochondrial enzyme in various mammalian tissues has been summarised in recent reviews (8,9).

PC is also found in many, though not all, prokaryotes, and has been shown to play essential roles in several important pathogens (10,11). PC is found in most fungi, generally in the cytoplasm, and in many invertebrates where its functions are equally significant.

The reaction for carboxylating pyruvate

PC catalyses the ATP-dependent formation of oxaloacetate from pyruvate, and in most species this enzyme is stimulated allosterically by acetyl-CoA. The overall reaction [Eq. I] is composed of two partial reactions [Eq. II & III] catalysed at separate sub-sites located on

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different subunits. The major effect of the allosteric activator is to enhance the rate of carboxylation of the biotin prosthetic group in the first partial reaction.

$$\text{Pyruvate}+\text{HCO}_3^-+\text{ATP} \xleftrightarrow{\text{Mg}^{2+},\text{acetyl-CoA}} \text{Oxaloacetate}+\text{ADP}+\text{P}_i \quad \text{(Eq. I)}$$

$$\text{E-biotin}+\text{ATP}+\text{HCO}_3^- \xleftrightarrow{\text{Mg}^{2+},\text{acetyl-CoA}} \text{E-biotin-CO}_2^-+\text{ADP}+\text{P}_i \quad \text{(Eq. II)}$$

$$\text{E-biotin-CO}_2^-+\text{Pyruvate} \leftrightarrow \text{E-biotin}+\text{Oxaloacetate} \quad \text{(Eq. III)}$$

The structure of PC

In mammals and most microbes PC is a homotetramer composed of identical subunits of 120-130 kDa, and is active only in its tetrameric form. Analysis of the first complete gene and protein sequence of PC in *S. cerevisiae* (12) revealed 3 regions with strong sequence identity to component subunits of other biotin-dependent enzymes. An N-terminal domain that includes the Biotin Carboxylation (BC) sub-site shares ~45% identity with the ATP-binding subunit of *E. coli* acetyl-CoA carboxylase. A centrally located domain accommodating the Carboxyltransferase (CT) sub-site shares 32% identity with the pyruvate-binding 5S subunit of methyl-malonyl-CoA transcarboxylase in *Propionibacterium shermanii*. The biotin prosthetic group in PC is attached covalently to the epsilon-amino group of a specific lysine residue in a highly-conserved motif ~35 residues from the enzyme's C-terminus in a domain that shares a high degree of identity with the Biotin Carboxyl Carrier Protein (BCCP) subunit of *E. coli* acetyl-CoA carboxylase and the 1.3S biotinylated subunit of transcarboxylase in *P. shermanii*. High resolution structures of PC from *Rhizobium etli* by St. Maurice *et al* (13) and *Staphylococcus aureus* by Xiang & Tong (14) showed the subunits are arranged in rhombohedral geometry within the quaternary structure of the tetramer as a dimer of dimers. The monomers within each dimeric pair are arranged anti-parallel so that the BC sub-site of one is opposite and ~75 Angstroms from the CT sub-site of the other [see Fig. 1], but there is minimal direct contact between them. Within the dimeric pair of subunits 1 and 2 the BCCP domain of subunit 1 is located between the BC sub-site of subunit 1 and the CT sub-site of subunit 2, and vice versa. Subunits 3 and 4 combine to form another dimeric pair arranged orthogonal to and on another plane to the dimer of subunits 1 and 2. The BCCP domains of each dimer are located on opposite sides of the tetramer, as revealed by electron microscopy of 1:1 complexes of PC and avidin (15).

The reaction mechanism

In the first partial reaction (Eqn II) bicarbonate is activated by ATP to form a carboxyphosphate intermediate (16) that then directly, or more likely indirectly, transfers CO_2 onto the 1′-nitrogen of the covalently attached biotin in the BCCP domain. The biotin prosthetic group then serves to transport this carboxyl group from the BC sub-site to the opposing CT sub-site where pyruvate accepts it and is converted to oxaloacetate. The separation of the partial reactions was inferred from kinetic studies (17) and by analogy to the situation for acetyl-CoA carboxylase in *E. coli* (18) and methyl-malonyl-CoA transcarboxylase in *P. shermanii* (19). Both are biotin-dependent enzymes composed of three classes of subunits that are all required for catalysis of the overall reaction. Proof of this separation of sub-sites came with the determination of PC structures from *R. etli* (13)

with ATP bound in the BC sub-site and from *S. aureus* (14) with pyruvate bound in the CT sub-site. The crucial roles in substrate binding and catalysis of particular amino acid side chains in the BC and CT sub-sites are becoming clearer as site-directed mutagenesis studies have been focused by the recent structural advances (9,13,20,21).

Activation by acetyl-CoA

It has long been known that acetyl-CoA activates PC *in vitro* (22) and *in vivo* (23,24) and that its major effect is exerted on the first partial reaction (9,22). However, apart from the demonstration that its action was allosteric (25,26), its binding site proved to be elusive until revealed recently when an analog of acetyl-CoA, viz. ethyl-CoA, was shown to be bound in a novel fold composed of residues located in the polypeptide chain between the BC and CT domains and also between the CT and BCCP domains (13). Whilst the conformational change induced by ethyl-CoA on the two subunits *R. etli* PC to which it was bound appeared to explain its activating effect, this does not seem to be the case with a weaker activator, Coenzyme A bound to *S. aureus* PC (21). Some microbial PCs, eg. *Pseudomonas citronellolis* (27) and *Azotobacter vinelandii* (28), have 4 protomers composed of two polypeptide chains, a biotinylated 75kDa (α)subunit and a 52 kDa (β)subunit arranged in an $(\alpha\beta)_4$ structure. These bacterial PCs do not respond to the addition of acetyl-CoA, presumably because they lack the highly conserved residues involved in binding the nucleotide portion of acetyl-CoA that are located between the BC domain [= β-subunit] and CT domain [= α-subunit].

Regulation of expression and activity of PC

A single gene encoding PC resides on chromosome 11q13.4 in humans and chromosome 1q43 in rat. Both are composed of 19 coding exons arranged similarly (29). The hormones and other factors regulating gluconeogenesis, lipogenesis, glycerogenesis, neurotransmitter secretion and insulin secretion in specific tissues are all clearly different, and accordingly PC exhibits tissue-specific regulation (9,29). In rat, alternative transcription from two promoters is responsible for producing five different mature transcripts expressed in a tissue-specific manner with all containing the same coding region but differing in their 5′ non-coding sequences (30).

Fasting and diabetes have been shown to induce 2-3-fold increases in hepatic PC activity in several species, most probably mediated via the effects of increased glucocorticoid secretion, and augmented by indirect actions of glucagon. Conversely, insulin has been shown to down-regulate PC expression in diabetic rats [reviewed in (29)].

Expression of PC has also been shown to be increased 2-5-fold at the onset of obesity in Zucker fatty rats (fa/fa) (31). The lipogenic role of PC has been further demonstrated by evidence of a functional PPARγ response element in the murine PC gene both *in vitro* and *in vivo* (32).

Unlike acetyl-CoA carboxylase, which is regulated by phosphorylation (33), PC activity does not appear to be controlled this way even under conditions when pyruvate dehydrogenase was phosphorylated (34).

PC deficiency

Given the range of tissues and major metabolic functions in which PC participates, it′s hardly surprising that a deficiency of PC in humans usually leads to severe clinical symptoms. PC deficiency (OMIM, 266150) is a rare autosomal recessive disease with three almost distinct phenotypes (35). Type A is characterised by hypoglycemia accompanied by

mild to moderate lactic acidemia and sometimes elevated ketone body levels. Apart from the acute symptoms these patients also suffer from psychomotor retardation, though they rarely die at a young age. Type B, having no detectable PC protein in any tissues, is the most severe form which leads to death generally within three months from lactic acidemia accompanied by hyperammonemia, citrullinemia and hyperlysinemia. Type C has a benign phenotype associated with episodes of lactic acidemia and no psychomotor disorders.

Concluding remarks

Fifty years after the discovery of PC, and with only a little over 2000 papers listed by PubMed in response to a search for“pyruvate carboxylase”, it is not surprising that there remain many questions to be answered about this complex enzyme and its diverse functions. This quest will be challenging but also potentially very rewarding for an improved understanding and treatment of diseases involving PC activity.

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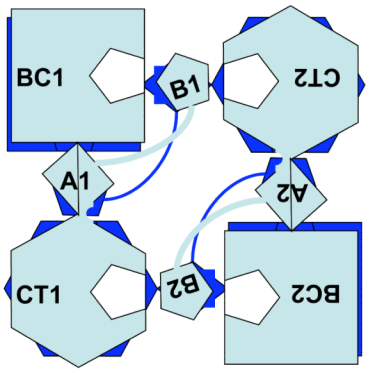

Figure 1. Schematic representation of the arrangement of the individual monomers making up the *R.etli* PC tetramer

Light grey coloured shapes represent the uppermost dimer pair composed of subunits 1 and 2. Black shapes below represent the other dimer pair composed of subunits 3 and 4.

BC1 & BC2, Biotin carboxylase domains (residues 1-465) of subunits 1 & 2 respectively

A1 & A2, Allosteric domains (residues 471-489 + 1002-1073) of subunits 1 & 2 respectively

CT1 & CT2, Carboxyltransferase domains (residues 515-1000) of subunits 1 & 2 respectively

B1 & B2, Biotin carboxyl carrier domains (residues 1084-1154) of subunits 1 & 2 respectively

White pentagonal shapes within BC1 and CT2, and within BC2 and CT1 indicate notional positions of catalytic sub-sites served by B1 and B2 respectively.