Molecular architecture of the pyruvate dehydrogenase complex: bridging the gap

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Abstract
The PDC (pyruvate dehydrogenase complex) is a high-molecular-mass (4–11 MDa) complex of critical importance for glucose homoeostasis in mammals. Its multi-enzyme structure allows for substrate channelling and active-site coupling: sequential catalytic reactions proceed through the rapid transfer of intermediates between individual components and without diffusion into the bulk medium due to its ‘swinging arm’ that is able to visit all PDC active sites. Optimal positioning of individual components within this multi-subunit complex further affects the efficiency of the overall reaction and stability of its intermediates. Mammalian PDC comprises a 60-meric pentagonal dodecahedral dihydrolipoamide (E2) core attached to which are 30 pyruvate decarboxylase (E1) heterotetramers and six dihydrolipoamide (E3) homodimers at maximal occupancy. Stable E3 integration is mediated by an accessory E3-binding protein associated with the E2 core. Association of the peripheral E1 and E3 enzymes with the PDC core has been studied intensively in recent years and has yielded some interesting and substantial differences when compared with prokaryotic PDCs.

Introduction
The mitochondrial 2-oxoacid dehydrogenase complexes are a family of large, multi-enzyme assemblies that serve as models for the study of protein–protein interactions and molecular recognition phenomena. They catalyse the oxidative decarboxylation of a vital group of 2-oxoacid intermediates in carbohydrate and amino acid metabolism. Principal members include the PDC (pyruvate dehydrogenase complex), OGDC (2-oxoglutarate dehydrogenase complex) and BCOADC (branched-chain 2-oxoacid dehydrogenase complex).

PDC controls the key committed step in carbohydrate utilization, namely the conversion of pyruvate into acetyl-CoA and NADH, thus linking glycolysis to the citric acid cycle in a three-step reaction. Initially, pyruvate is decarboxylated by the ThDP (thiamin diphosphate)-dependent E1 enzyme that also catalyses the subsequent transfer of the resulting acetyl group from ThDP on to the lipoamide moiety of E2 in a reductive acetylation step, generating a stable intermediate. In the presence of CoASH, E2 catalyses the transacetylation reaction to form acetyl-CoA. Finally, the E2 dihydrolipoamide is reoxidized by E3. E3 transfers electrons via a redox-active pair of cysteine residues to FAD and on to the terminal electron acceptor NADH.

The overall reaction can be summarized as:

\[ \text{pyruvate} + \text{CoASH} + \text{NAD}^+ \rightarrow \text{acetyl-CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+ \]

Genetic and physiological defects in PDC have been implicated (i) in a wide range of diseases including various types of metabolic acidosis and mitochondrial myopathies; (ii) as major autoantigens in the autoimmune disease, primary biliary cirrhosis; (iii) in neurodegenerative disorders, e.g. Alzheimer’s disease; and (iv) as primary targets for modification by environmental toxins and oxidative damage.

Basic core organization
All three 2-oxoacid complexes display a similar mode of quasi-symmetric organization of their subunits E1, E2 and E3. Apart from playing a role in the catalytic mechanism, E2 also forms a central 24-meric or 60-meric core, consistent with octahedral (432) or icosahedral (532) symmetry respectively [1,2]. For OGDC and BCOADC, as well as PDC from Gram-negative bacteria, the E2 core is assembled as an octahedron. Only in Gram-positive bacteria and eukaryotes does the PDC core assume an icosahedral morphology. Furthermore, eukaryotic PDC contains an additional protein, E3BP (E3-binding protein; previously protein X), which is associated with the E2 core [3,4] and responsible for binding E3.

Both E2 and E3BP have similar, modular domain structures: human E2 and E3BP consist of two (E2) or one (E3BP) N-terminal LD(s) [lipoyl domain(s)] of approx. 80 amino

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Abbreviations used: BCOADC, branched-chain 2-oxoacid dehydrogenase complex; cy-o-EM, cryo-electron microscopy; E3BP, E3-binding protein; LD, lipoic domain; OGDC, 2-oxoglutarate dehydrogenase complex; PDC, pyruvate dehydrogenase complex; PDK, PDC kinase; PDP, PDC phosphatase; SAXS, small angle X-ray scattering; SBD, subunit-binding domain; ThDP, thiamin diphosphate.

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acids. Each LD carries a lipoic acid moiety covalently linked to a lysine residue situated at the tip of a type I β-turn. The LD is followed by an SBD (subunit-binding domain) of approx. 35 residues, responsible for interaction with E1 and/or E3, and a C-terminal domain of approx. 250 residues that is essential for core formation. In E2, the C-terminal domain is also the catalytic domain, while the active-site motif is mutated in E3BP, rendering it incapable of catalysing the acetyltransferase reaction. All domains are interconnected by alanine- and proline-rich linker regions of approx. 30 amino acids in length that impart the flexibility necessary for the LD to visit all three active sites during catalysis.

Two models for the eukaryotic PDC core have been proposed: in the ‘addition model’ based on cryo-EM (cryo-electron microscopy) of recombinant PDC cores from Saccharomyces cerevisiae, 60 molecules of E2 are thought to form the edges of the icosahedron, while 12 copies of E3BP are located on the inside of the E2 core, apparently associated with the tips of E2 trimers [5]. More recently, Hiromasa et al. [6] proposed an alternative ‘substitution’ model based on results from analytical ultracentrifugation and SAXS (small angle X-ray scattering) where 12 E3BP molecules are thought to replace an equal number of E2 enzymes within the core.

**Active-site coupling**

The ability of PDC to sustain substrate channelling and active-site coupling is the basis for its efficient and tightly controlled function (see [7] for an excellent review). Fluorescence energy transfer studies revealed that the three different active sites have to be at least 40 Å (1 Å = 0.1 nm) apart, since no energy transfer could be detected for Azotobacter vinelandii PDC [8,9]. Cryo-EM reconstructions of Geobacillus stearothermophilus (formerly Bacillus stearothermophilus) and bovine PDC indicate even larger distances. Milne et al. [10] have shown the existence of an annular gap of approx. 90 Å between the E2 core and deposited E1, while it is somewhat smaller (~75 Å) between E2 and E3 [11]. The distances reported for bovine PDC are shorter with a space approx. 50 Å in length between the core and both E1 and E3 respectively [12]. The difference in spacing observed in Geobacillus and bovine PDC remains unclear. However, taking the LD and its linker into account, bridging
the distances between active sites becomes straightforward, as gaps as large as 200 Å or more could be traversed [7,13].

**PDC architecture**

The PDC core provides the structural and mechanistic framework for the tight but non-covalent association of E1 and E3. In eukaryotes, tight protein–protein interactions are formed specifically between E2-SBD and E1, and E3BP-SBD and E3 respectively. However, in Gram-positive bacteria such as *G. stearothermophilus*, E1 and E3 both associate with E2, as E3BP features only in eukaryotes. Consequently, E1 and E3 have to compete for overlapping binding sites on E2; interaction of E2 with either E1 or E3 prevents complex formation with the other [14]. Interestingly, PDC-deficient patients who totally lack E3BP possess partial complex activity (10–20% of controls) [15], apparently because the SBD of E2 has retained a limited ability to mediate low-affinity E3 binding (S.D. Richards and J.G. Lindsay, unpublished work).

In *G. stearothermophilus* PDC, up to 60 molecules of E1 or E3 can be associated with the E2 core. Furthermore, binding of both E1 and E3 to E2-SBD has been shown to occur at stoichiometries of 1:1 in solution [16–19] as well as by crystallography (Figures 1A and 1B). The crystal structure for the E1–E2-SBD subcomplex shows that the binding site for E2-SBD is located across the E1 2-fold axis [20]. Similarly, in the E3–E2-SBD structure, the binding site on E3 is close to the 2-fold axis of symmetry [21]. Thus the association of a second molecule of E2-SBD with either E1 or E3 is not possible. Occupation of both binding sites would result in steric clashes in one of the E2-SBD loop regions.

In contrast, it has been known for a long time that a total of 30 E1 and six E3 molecules can associate with the mammalian PDC core [22], suggesting the possibility of formation of 2:1 stoichiometric subcomplexes. The crystal structures determined for human E3 complexed with its cognate SBD from E3BP [23,24] resemble those solved for the bacterial subcomplexes. However, when tested in solution using various biochemical and biophysical techniques, including non-denaturing PAGE, isothermal titration calorimetry, analytical ultracentrifugation and SAXS, results indicate the formation of subcomplexes at a ratio of 2:1 rather than 1:1 (E3BP/E3) [25], suggesting the existence of a network of so-called ‘cross-bridges’ on the surface of PDC (Figure 2). Essentially, cross-bridge formation is thought to constrain the mobility of the E3BP (and E2) LD, especially if analogous cross-bridges exist between E1 and E2 as suggested by preliminary evidence from our laboratory. Consequently, this arrangement may impair the ability of any one LD to interact with all three different active sites. However, migration of acetyl groups between the S-6 and S-8 atoms of lipoamide has been documented [26], and experiments using labelled pyruvate have confirmed that acetyl groups and reducing equivalents can be shuttled between different LDs [27–29].

The discrepancy between crystal and solution structures is thought to arise from differences in the conformation of E3 in solution apparent in low-resolution structures of both E3 on its own (M. Smolle, D. Byron and J.G. Lindsay, unpublished work) as well as a complex formed between E3 and two molecules of E3BP-didomain (consisting of the LD and SBD; Figure 1C). Further contributing factors could be differences in buffer composition and/or the effects of crystal packing. Indeed, the solution conformations of other proteins, including yeast pyruvate decarboxylase [30], can differ significantly from their crystal structures [31–34].

Another aspect of PDC function potentially impacted by a network of cross-bridges is its regulation by the tightly bound PDK (PDC kinase) and a loosely bound PDP (PDC phosphatase). It has been reported that only one to three molecules of PDK are bound per complex [35]. Both regulatory enzymes associate with the inner E2 LD whereby the lipoyl cofactor provides an important part of the recognition site. PDP and PDK both act by de-/phosphorylation of E1 on any of three specific E1 α serine residues. Phosphorylation of E1 renders the complex inactive [15]. Since only one to three PDK molecules are present per complex, the enzyme has to migrate around the surface of the complex in order to act on the entire population of bound E1 molecules. A hand-over-hand mechanism has been proposed where the dimeric PDK molecule migrates from one inner LD to its nearest neighbour [36]. Regular spacing of the E2 LDs linked by a system of E1 cross-bridges could greatly facilitate this type of movement and provide convenient access to all E1 molecules.
Concluding remarks
Understanding PDC assembly, subunit organization and the molecular basis for its operational efficiency and precisely regulated activity remain at the forefront of scientific research due to its pivotal role in energy metabolism. Furthermore, increasing recognition of PDC involvement in a wide spectrum of major human diseases and as a prime target for oxidative stress and environmental toxins has served to stimulate renewed interest in this fascinating multi-enzyme assembly as an attractive area for further basic research and drug development.

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References

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