Substrate channelling in 2-oxo acid dehydrogenase multienzyme complexes
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Abstract
Heteronuclear NMR spectroscopy and other experiments indicate that the true substrate of the E1 component of 2-oxo acid dehydrogenase complexes is not lipoic acid but the lipoyl domain of the E2 component. E1 can recognize the lipoyl-lysine residue as such, but reductive acylation ensues only if the domain to which the lipoyl group is attached is additionally recognized by virtue of a mosaic of contacts distributed chiefly over the half of the domain that contains the lipoyl-lysine residue. The lipoyl-lysine residue may not be freely swinging, as supposed hitherto, but may adopt a preferred orientation pointing towards a nearby loop on the surface of the lipoyl domain. This in turn may facilitate the insertion of the lipoyl group into the active site of E1, where reductive acylation is to occur. The results throw new light on the concept of substrate channelling and active-site coupling in these giant multifunctional catalytic machines.

Introduction
The 2-oxo acid dehydrogenase multienzyme complexes constitute a family of related enzymes that includes the pyruvate dehydrogenase (PDH), 2-oxoglutarate dehydrogenase (OGDH) and branched-chain 2-oxo acid dehydrogenase (BCDH) complexes. They catalyse the oxidative decarboxylation of the relevant 2-oxo acid, transferring the resultant acyl group to CoA and generating NADH in the process. In the PDH complex, the three component enzymes are pyruvate decarboxylase (E1p; EC 1.2.4.1), dihydrolipoyl acetyltransferase (E2p; EC 2.3.1.12) and dihydrolipoyl dehydrogenase (E3; EC 1.8.1.4) (reviewed in [1–4]). Corresponding enzymes make up the OGDH and BCDH complexes; in each case, the E1 and E2 are specific for the particular 2-oxo acid undergoing decarboxylation. E1 catalyses the initial decarboxylation, utilizing thiamin diphosphate as a cofactor, and the subsequent reductive acetylation of a lipoyl group covalently attached to a lysine residue in E2. E2 catalyses the transfer of the acyl group to CoA and E3, which carries out an identical reaction in each complex and is normally the same enzyme in all three instances, concludes the process by reoxidizing the dihydrolipoyl group and regenerating the dihydrolipoyl ring at the expense of NAD+.

In the PDH complex from Escherichia coli and most Gram-negative bacteria, E2p forms a cubic core consisting of 24 polypeptide chains arranged with octahedral symmetry, whereas in Bacillus stearothermophilus and most Gram-positive bacteria, the E2p core is icosahedral and comprises 60 E2p chains [1,2]. E1 and E3 are bound tightly but non-covalently in peripheral positions around the E2 core. The OGDH and BCDH complexes follow the same structural pattern, with E2 cores [dihydrolipoyl succinyltransferase (E2o) and dihydrolipoyl branched chain acyltransferase (E2b)] of octahedral symmetry. The intact complexes are thus of giant size, with molecular masses of (5–10) × 106 Da and diameters of up to 50 nm, significantly bigger than a ribosome.

In this article we review some of the structural features of the 2-oxo acid dehydrogenase complexes that are of particular relevance to the mechanisms of active-site coupling and substrate channelling in such vast catalytic machines [4].

The lipoyl domain and active-site coupling
The lipoyl group is covalently attached in an amide linkage to the Nε-amino group of a specific lysine residue of an independently folded domain (about 80 residues) that forms the N-terminal part
of the E2 chain. The number of lipoyl domains per E2 chain can vary, e.g. from one in the E2p chain of *B. stearothermophilus* and the E2o chain of *E. coli* to three in the E2p chain of *E. coli* [1,2]. The lipoyl domain plays a vital role in coupling the reactions within the complex in an organized and specific manner. Acting as a 'swinging arm' [5], the lipoyl-lysine group visits each of the three active sites of the complex, carried by a lipoyl domain that is itself rendered mobile by virtue of the conformational flexibility in the segment of polypeptide chain that links it to the inner E2 core of the complex.

The NMR solution structures of the single *E. coli* E2o [6] and the innermost of the three *E. coli* E2p [7] lipoyl domains have been solved, as have those of others from various other organisms [8–10], including that of the *B. stearothermophilus* E2p chain [11]. Their overall backbone structures are virtually identical. They consist of a β-barrel formed from two four-stranded β-sheets, arranged with a 2-fold axis of quasi-symmetry. The lipoyl-lysine is located at the tip of a tight, type 1 β-turn in one β-sheet, and the N- and C-termini are close in space in the other β-sheet on the opposite side of the domain (Figure 1). Exact positioning of the target lysine within the protruding β-turn is essential for correct post-translational modification by the lipoylating system(s) of the *E. coli* cell [12].

**Substrate channelling**

As shown first with the PDH complex of *E. coli*, free lipoate can act as the substrate for E2p and E3 but is a very poor substrate for Elp. However, the E2p lipoyl domain is an excellent substrate (*k_{cat}/K_m* raised by a factor of 10⁴); moreover, the E2p and E2o lipoyl domains from *E. coli* function as substrates only for their cognate Els ([13,14] and references therein). Similar results have been reported for the lipoyl domains of *Azotobacter vinelandii* E2p and E2o [15]. Thus the true substrate in these complexes is not lipoic acid or lipoyl-lysine but the lipoyl domain; recognition of the domain by its cognate El provides an elegant mechanism for substrate channelling whereby reductive acylation is confined to a lipoyl group covalently attached to a specific lysine residue of

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**Figure 1**

Structure of the innermost lipoyl domain of *E. coli* E2p

Left-hand panel, schematic structure of the lipoyl domain drawn using MOLSCRIPT [23], with a freely rotating lipoyl-lysine residue at the tip of the β-turn between β-strands 4 and 5. Middle and right-hand panels, two views of the lipoyl domain with the lipoyl-lysine residue in a preferred orientation, close in space to the surface loop between β-strands 1 and 2. Reproduced from *Biochemistry* 2000, 39, 8448–8459. © 2000 Am. Chem. Soc.
the intended E2 component [2–4]. How the E2p and E2o lipoyl domains are recognized by their correct partner E1s yet have the same structural scaffold thus becomes an important question.

**Interaction of the lipoyl domain with E1**

The binding of the lipoyl domain to *E. coli* E1p is both weak (*K_\text{d}*, not less than 1 mM, despite a *K_{ass} of \approx 20 \mu M) and transient [16]. Thus co-crystallization of the lipoyl domain with E1 is likely to be impossible. However, NMR has been used to identify regions on the apo-form of the *B. stearothermophilus* E2p lipoyl domain that provide important contact sites in the interaction with E1p. A prominent surface loop, linking \( \beta \)-strands 1 and 2, is present only in the \( \beta \)-sheet that contains the lipoyl-lysine residue, and lies close in space to the lipoyl-lysine \( \beta \)-turn (Figure 1). Alterations in chemical shift in the presence of E1 indicated that this loop is likely to make contact with E1 during catalysis; likewise, the results of directed mutagenesis indicated that residues flanking the lipoyl-lysine residue in its \( \beta \)-turn are also of critical importance [17].

The crystal structures of the heterotetrameric (\( \alpha_2 \beta_2 \)) E1ps from *Pseudomonas putida* [18] and humans [19] have recently been solved. The thiamin diphosphate in the active site is buried at the bottom of a 2 nm-deep funnel-shaped hole at the interface between the \( \alpha \)- and \( \beta \)-subunits. In order for the lipoyl group to reach the cofactor, and assuming that no major conformational changes are involved, the protruding \( \beta \)-turn housing the lipoyl-lysine residue would have to point into the entrance of the funnel and the lipoyl-lysine side chain (1.4 nm) to become fully extended. The lipoyl domain, and the surface loop region between \( \beta \)-strands 1 and 2 in particular, would by necessity come into close contact with the surface of E1p, as indicated by the earlier NMR experiments [17].

The quaternary structures of the *E. coli* E1p and 2-oxoglutarate decarboxylase (E1o; \( \alpha_2 \)) and *B. stearothermophilus* E1p (\( \alpha_2 \beta_2 \)) differ, and therefore so may their interactions with the lipoyl domain. However, more detailed experiments with heteronuclear NMR spectroscopy, involving studies of both chemical shifts and transverse relaxation times (\( T_2 \)), have indicated that the same general principles apply [20,21]. In the *B. stearothermophilus* E1p, it is clear that the incoming lipoyl domain makes contact with both E1\( \alpha \) and E1\( \beta \) subunits, consistent with the active site lying between them. Moreover, although the surface loop region between \( \beta \)-strands 1 and 2 is a major site of contact, there are other sites distributed

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**Figure 2**

Points of contact between the lipoylated *E. coli* lipoyl domain and its partner E1p

Left-hand and middle panels, two views of the lipoyl domain rotated by 180°, indicating residues (numbered in single-letter code) that exhibit significant changes in chemical shift in the presence of E1p. Right-hand panel, residues that exhibit significant changes in \( T_2 \) values in the presence of E1p. Reproduced from [21] with permission. © (2001) Academic Press.
over the surface of the lipoyl domain physically remote from the lipoyl-lysine residue [20]. These experiments were carried out with the apo-domain, as the lipoylated domain appeared to bind too tightly to the \textit{B. stearothermophilus} Elp and substantial line-broadening was observed. However, with the \textit{E. coli} Elp, the lipoylated domain could be studied [21], and again it was inferred that a significant number of residues on the domain, largely restricted to the lipoyl-lysine-containing half and dominated by the lipoyl-lysine \( \beta \)-turn, come into contact with Elp (Figure 2). Elp does not interact detectably with the apo-domain from \textit{E. coli} E2p, but some interaction was detected with the holo-domain. The conclusion must be that the Elp components can recognize the lipoyl-lysine residue as such, but that reductive acylation ensues only if the domain to which the lipoyl group is attached is additionally recognized [21].

**Implications for catalysis**

In comparing the NMR spectra of \(^{15}N\)-labelled apo- and holo-forms of the lipoyl domain of \textit{E. coli} E2p, it became apparent that the lipoyl-lysine \( \beta \)-turn becomes less flexible following post-translational modification. From the chemical shift data, it appears that, rather than being freely swinging, as hitherto supposed, the lipoyl group may have a preferred orientation (Figure 1), pointing towards the surface loop linking \( \beta \)-strands 1 and 2 [7]. This is the self-same loop that comes into contact with Elp as the lipoyl domain approaches the Elp active site. It has also been observed that limited proteolysis of a surface loop in the Ela chain at the entrance to the active site of the Elp component of the \textit{B. stearothermophilus} PDH complex has a pronounced inhibitory effect on the catalytic activity of the intact complex. At the same time, there was no loss of catalytic activity of Elp itself and no effect on the ability of the proteolysed Elp to bring about the reductive acetylation of a free lipoyl domain [22]. This has raised the interesting possibility that the lipoyl domain may have a restricted trajectory in the intact complex, causing it to approach the Elp active site in a particular way. A preferred orientation of the lipoyl-lysine residue on the lipoyl domain, directed towards the nearby surface loop between \( \beta \)-strands 1 and 2, may contribute to the chance of an efficacious encounter and facilitate the insertion of the lipoyl-lysine arm into the Elp active site, as discussed above.

Given that the true substrate for the 2-oxo acid dehydrogenase complexes is actually the lipoyl domain, much slower to diffuse than lipoic acid itself, even if free, the tethering of the lipoyl domain in the complex assumes a much clearer significance. The local concentration of the domain is raised to millimolar concentrations, way above the \( K_m \) values it exhibits as a substrate for the component enzymes. Recognition of the lipoyl domain by Elp provides the molecular basis of an elegant and efficient form of substrate channelling; and the flexibility in the tethering linker regions and the three-dimensional organization of the enzyme complex, together with a preferred orientation of the classical swinging arm on the surface of the domain, may promote catalytically advantageous trajectories of the domain between the contributing active sites.

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**References**

Conversion of pancreatic cells to hepatocytes

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Abstract

Transdifferentiation is the name used to describe the conversion of one differentiated cell type to another. During development, the liver and pancreas arise from the same region of the endoderm and cells from the two organs can transdifferentiate in the adult under different experimental procedures. We have produced two in vitro models for the transdifferentiation of pancreatic cells to hepatocytes. The first utilizes a pancreatic exocrine cell line AR42J-B13 and the second comprises cultures of mouse embryonic pancreas. We have analysed the pancreatic hepatocytes and they express a range of liver markers including albumin, transferrin and transthyretin. We also present evidence for (i) the molecular mechanism which regulates the conversion between pancreas and liver and (ii) the cellular basis of the switch in phenotype.

Introduction

The ability of one differentiated cell type to convert to another is termed transdifferentiation or metaplasia [1–3]. The process of transdifferentiation is important for two reasons. Firstly, for understanding the molecular and cellular basis of embryonic development, as the conversion of one cell type to another generally occurs between cells which arise from neighbouring regions of the same germ layer (mesoderm, endoderm or ectoderm) [1–3]. Secondly, transdifferentiation leads to a predisposition towards certain neoplastic transformations and therefore elucidating the molecular basis of the conversion will also provide information on the processes underlying the development of cancer [2].

In order to demonstrate that transdifferentiation has occurred in a system, Eguchi and Kodama [4] suggested that two prerequisites be fulfilled. The first involves demonstrating (preferably with molecular evidence) the differentiation state of the two cells types before and after the transdifferentiation event. The second prerequisite involves showing a direct ancestor–descendent relationship between the cells prior to and following transdifferentiation. It is difficult to fulfil these prerequisites under in vitro conditions. However, in vitro culture systems are more amenable to testing these prerequisites. One of the best-studied in vitro models for transdifferentiation is the conversion of pigmented epithelial cells of the retina to lens cells, so-called Wolffian lens regeneration [4]. Developing in vitro models for the transdifferentiation of one cell type to another is crucial as it will allow us to define the molecular and cellular mechanisms which distinguish the two cell types involved in the switch.

Models for the transdifferentiation of pancreas to liver

Numerous examples of transdifferentiation exist but perhaps one of the most interesting is the appearance of hepatocytes in the pancreas. Foci of hepatocytes have been shown to be induced in the pancreas of rats, mice or hamsters following various experimental procedures, e.g. copper...