S-15-4   The Lipoyl Domain and Its Role in Thiamin Diphosphate-Dependent Oxidative Decarboxylation

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I. INTRODUCTION

In most cells the oxidative decarboxylation of 2-oxo acids is carried out by multienzyme complexes of unusually elaborate structure. They comprise three different enzymes and are assembled round a core consisting of multiple copies of the lipoate acyltransferase (E2) subunit arranged with octahedral (24-mer) or icosahedral (60-mer) symmetry (for recent reviews, see [1-3]). The acyltransferase polypeptide chain is highly segmented: it contains one, two or three lipoyl domains (depending on the source), a peripheral (E1/E3) subunit-binding domain, and a large C-terminal domain that houses the acyltransferase active site. This C-terminal domain is also responsible for aggregating with appropriate symmetry (octahedral or icosahedral) to generate the core of the whole complex, leaving the lipoyl domains free to interdigitate between the E1 and E3 subunits. The lipoyl domains are linked to each other and to the inner core by long segments of polypeptide chain (20-25 amino acid residues), often rich in alanine, proline and charged amino acids, that appear to be extended and conformationally flexible, as judged by NMR spectroscopy [3,4].

The thiamin diphosphate (ThDP)-dependent decarboxylation of the 2-oxo acid substrate takes place in the active site of the E1 subunits, leading in turn to the reductive acylation of a lipoyl group bound to a lipoyl domain. The transfer of the acyl group to CoA occurs in the active site of the E2 inner-core domain, and the resultant dihydrolipoyl group is reoxidized to the dithiolane ring by the E3 subunits at the expense of NAD⁺. Given the physical separation of the three contributing active sites (E1, E2 and E3), it appears that the flexibility with which the inter-domain segments of the E2 chain are endowed enables the lipoyl domains to move between these sites as an important part of the mechanism.

II. THE MECHANISM OF OXIDATIVE DECARBOXYLATION

The first step of the reaction, oxidative decarboxylation of the 2-oxo acid, is catalysed by the E1 component in the presence of ThDP as cofactor. 2-Acetyl-ThDP has been detected in the E1 active site of the pyruvate dehydrogenase (PDH) complex of *Escherichia coli*, but it is not clear whether it is a compulsory intermediate in reductive acetylation of the lipoyl group bound to E2 (see Fig. 1). The initial product, 8-S-acetyldihydrolipoamide, can undergo intramolecular acetyl transfer to produce an equilibrium mixture of the S⁶- and S⁸-isomers, but this is too slow to form part of the normal catalytic reaction [6]. The E1 component can recognize only the R-enantiomer of the lipoyl group [7].

Fig. 1. Reductive acetylation of the lipoyl group of E2 by the E1 component of the PDH complex [5].

Why is the lipoic acid cofactor attached to a protein domain in the E2 component? The dithiolane ring is 1.4nm from the polypeptide backbone, and thus is at the end of what is effectively a freely rotating 'swinging arm' [1-3]. Likewise, the chemistry of the reaction (Fig. 1) envisages no need for the dithiolane ring to be covalently linked to a protein. However, the ability of lipoic acid to act as a substrate for the E1 component of the *E. coli* PDH complex is greatly enhanced k_{cat}/K_m raised by a factor of 10,000 by attachment of the cofactor to the lipoyl domain [3,8]. Moreover, only the lipoyl domain from the cognate E2 chain will function with a given E1. Thus, the lipoyl domain both activates the lipoyl group and confers specificity on it. Since a correctly lipoylated synthetic decapeptide is also ineffective as a substrate, it is clear that a folded lipoyl domain is required to present the dithiolane ring to the E1 active site and that the E1 component must have a means of recognizing the relevant lipoyl domain [3,8]. Clues to this molecular recognition event must lie in the structure of the lipoyl domain.

III. STRUCTURE OF THE LIPOYL DOMAIN

To generate enough of the lipoyl domain for detailed structural analysis, a sub-gene encoding residues 1-85 of the E2 chain of the

Bacillus stearothermophilus PDH complex has been over-expressed in *E. coli*. Most (80%) of the product is unlipoylated, some (16%) is correctly lipoylated on Lys-42, and a very small fraction (4%) is aberrantly octanoylated on the same residue [9]. This unexpected mistake in the post-translational modification appears to be an effect of saturating the *E. coli* lipoylating enzymes with excessive amounts of the protein substrate and has no counterpart in the normal cell [10].

Fig. 2. Secondary structure of the lipoyl domain of the E2 chain of the PDH complex of *B. stearothermophilus*. NOESY connectivities used to establish the positions of β -strands are indicated by double-headed arrows. The numbers on the lines connecting the β -sheets indicate the number of amino acids in the connecting segments of polypeptide chain.

The 400 MHz ^1H -NMR spectra of the lipoylated and unlipoylated forms of the *B. stearothermophilus* domain are essentially identical, indicating that lipoylation of the domain is not accompanied by any substantial conformational change [9]. A full structural analysis is now almost complete. The domain has an unusual fold, consisting essentially of two large elements of anti-parallel β -sheet (Fig. 2). The N- and C-terminal ends are close in space in one element, and the lipoyl-lysine residue is prominently displayed on a protruding turn in the other, which is likely to facilitate its interaction with the active site of E1 [11].

IV. THE INTERACTION WITH E1

The structure of the lipoyl domain is nearing completion. Unfortunately, little is yet known about the active site of the E1 component with which it has to interact, nor for that matter about the active site of any ThDP-dependent enzyme. However, an important clue has come from the recognition of a sequence motif of some 30 residues, beginning with a -GDG- sequence and ending with an -NN- sequence, that is common to all ThDP-utilizing enzymes and which may represent part at least of the ThDP-binding site [12]. Directed mutagenesis of this region may throw light on this, but of course a crystallographic analysis of the enzyme will be required to yield a full picture. In the meantime, the interaction of the lipoyl domain with E1 and the chemical basis of its molecular recognition by the cognate enzyme, can be pursued by protein chemical and NMR methods.

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