STRUCTURE AND POST-TRANSLATIONAL MODIFICATION OF THE LIPOYL DOMAIN OF 2-OXO ACID DEHYDROGENASE COMPLEXES: A NEW FAMILY OF PROTEIN DOMAINS

Richard N. Perham, Nicola G. Wallis, Simon M. Brocklehurst, Frederic Dardel, Adrian L. Davis and Ernest D. Laue

Cambridge Centre for Molecular Recognition
Department of Biochemistry, University of Cambridge
Tennis Court Road, Cambridge CB2 1QW, England, U.K.

INTRODUCTION

The 2-oxo acid dehydrogenase multienzyme complexes are among the best examples of multifunctional proteins fashioned from independently-folded protein domains joined by linker sequences of various lengths and degrees of conformational flexibility (Patel and Roche, 1990; Perham, 1991). The structural core of these complexes is provided by the dihydrolipoyl acyltransferase (E2) component, an aggregate of 24 or 60 polypeptide chains arranged with octahedral or icosahedral symmetry, respectively, according to the source. The E2 chains themselves are highly segmented: they comprise, from the N-terminus, up to three lipoyl domains, a peripheral subunit-binding domain, and a large core-forming acyltransferase domain, all linked together by long (25-30 residue) segments of polypeptide chain rich in alanine, proline and charged/hydrophilic amino acids (Reed and Hackert, 1990; Perham, 1991).

The structure of the peripheral subunit-binding (approx. 40 residues) of the E2o chain of the 2-oxoglutarate dehydrogenase complex of Escherichia coli has been determined by means of NMR spectroscopy (Robien et al., 1992) and that of the acetyltransferase domain of the assembled E2p core of the pyruvate dehydrogenase (PDH) complex of Azotobacter vinelandii has been determined at 2.6Å resolution by means of X-ray crystallography (Mattevi et al., 1992). The structure of the lipoyl domain is of particular interest for two reasons: first, despite the fact that the dithiolane ring of the lipoyl group is at the end of a

1Permanent address: Laboratoire de Biochimie, Ecole Polytechnique, 91128 Palaiseau Cedex, France.
lipoxy-l-lysine side chain 1.4 nm long, and is free to rotate with respect to the bulk of the protein, the lipoxy group must be attached to a lipoxy domain if it is to serve as an effective substrate for the 2-oxo acid decarboxylase (E1) component of the complex; and secondly, the lipoxy domain confers specificity on the pendant dithiolane ring for reductive acetylation only by the E1 component of the parent 2-oxo acid dehydrogenase complex (Graham et al., 1989), a beautiful mechanism for substrate channelling (Perham, 1991).

In the present paper, we describe the determination of the structure of the lipoxy domain of the PDH complex of Bacillus stearothermophilus by means of NMR spectroscopy (Dardel et al., 1991; 1992). This has enabled us to initiate experiments designed to unravel the molecular mechanism by which the E1 component selects its cognate lipoxy domain for reductive acetylation of the attached lipoxy group, and also to analyse the specificity of the enzyme(s) in the E.coli cell that selectively lipoxylate the N^6-amino group of the target lysine residue in the lipoxy domain. In addition, we have devised a new method for predicting the three-dimensional structures of proteins from homologous template structures and used it to show that a putative biotinyl domain of yeast pyruvate carboxylase (Lim et al., 1988) and the lipoxylated H-protein of the pea leaf glycine cleavage system (Kim and Oliver, 1990) are likely closely to resemble the lipoxy domain. These structures unexpectedly appear to be representative of a new class of protein domains.

**STRUCTURE OF THE LIPOXY DOMAIN**

A sub-gene encoding the single lipoxy domain (residues 1-79) of the E2p chain of the PDH complex of B. stearothermophilus has been expressed in E. coli and lipoylated and non-lipoylated forms of the domain have been purified (Dardel et al., 1990). It was apparent from the initial analysis by NMR spectroscopy that the post-translational modification was without detectable effect on the conformation of the polypeptide chain (Dardel et al., 1990) and that the predominant secondary structure was "sheet" (Dardel et al., 1991).

A full structural analysis (Dardel et al., 1992) has now revealed that the domain is composed largely of two four-stranded β-sheets forming a flattened β-barrel around a well-defined hydrophobic core (Figure 1). The polypeptide chain weaves backwards and forwards from one sheet to the other, with the exception of a single type-I turn on the corner of one sheet. Significantly, the lipoxy-lysine residue (Lys-42) is prominently displayed in this tight turn and is thus readily accessible to the active site of the E1 component. The N- and C-terminal residues of the domain are located close together in the other β-sheet. A further important feature of the domain is its two-fold quasi-symmetry. The Ca-positions of residues 15-39 can be superposed on those of residues 52-76 after a rotation of 180° about the barrel axis, a symmetry reflected in a weak similarity between the N- and C-terminal halves of the amino acid sequence.

Comparison of the amino acid sequences of lipoxy domains from a wide range of E2 chains from pyruvate, 2-oxoglutarate and branched-chain 2-oxo acid dehydrogenase complexes (Perham, 1991; Russell and Guest, 1991) indicates that amino acids occupying key positions in the lipoxy domain fold are highly conserved. This in turn suggests that all lipoxy domains will exhibit similar structures.

**MOLECULAR RECOGNITION AND THE LIPOXY DOMAIN**

As described above, the structure of the lipoxy domain must hold the key to two important questions of molecular recognition: how does the domain interact