Limited proteolysis and proton n.m.r. spectroscopy of the 2-oxoglutarate dehydrogenase multienzyme complex of *Escherichia coli*

Richard N. PERHAM* and Gordon C. K. ROBERTS†

*Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, U.K. and

†Division of Molecular Pharmacology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.

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The 2-oxoglutarate dehydrogenase multienzyme complex of *Escherichia coli* was treated with trypsin at pH 7.0 at 0°C. Loss of the overall catalytic activity was accompanied by rapid cleavage of the lipoate succinyltransferase polypeptide chains, their apparent M_r falling from 50 000 to 36 000 as judged by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. A slower shortening of the 2-oxoglutarate decarboxylase chains was also observed, whereas the lipoamide dehydrogenase chains were unaffected. The inactive trypsin-treated enzyme had lost the lipoic acid-containing regions of the lipoate succinyltransferase polypeptide chains, yet remained a highly assembled structure, as judged by gel filtration and electron microscopy. The lipoic acid-containing regions are therefore likely to be physically exposed in the complex, protruding from the structural core formed by the lipoate succinyltransferase component between the subunits of the other component enzymes. Proton nuclear magnetic resonance spectroscopy of the 2-oxoglutarate dehydrogenase complex revealed the existence of substantial regions of polypeptide chain with remarkable intramolecular mobility, most of which were retained after removal of the lipoic acid-containing regions by treatment of the complex with trypsin. By analogy with the comparably mobile regions of the pyruvate dehydrogenase complex of *E. coli*, it is likely that the highly mobile regions of polypeptide chain in the 2-oxoglutarate complex are in the lipoate succinyltransferase component and encompass the lipoyl-lysine residues. It is clear, however, that the mobility of this polypeptide chain is not restricted to the immediate vicinity of these residues.

The 2-oxoglutarate dehydrogenase multienzyme complex of *Escherichia coli* consists of multiple copies of three enzymes that function successively in the mechanism: 2-oxoglutarate decarboxylase (E1, EC 1.2.4.2), lipoate succinyltransferase (E2) and lipoamide dehydrogenase (E3, EC 1.6.4.3). The E2 component is composed of 24 polypeptide chains arranged with octahedral symmetry (DeRosier & Oliver, 1972) and forms a structural core to which the other enzymes are bound tightly but non-covalently (Reed, 1974). The enzyme particle is very large, ultracentrifugation studies indicating an M_r of about 2.5×10^6 (Reed, 1974). The substrate is carried in thioester linkage by lipoyl-lysine residues of the E2 core, whereby it is thought to be able to move between the catalytic centres of the three enzymes. The most recent evidence is that there is one such lipoyl-lysine swinging arm per E2 poly-

peptide chain (Collins & Reed, 1977; Angelides & Hammes, 1979). These lipoic acid residues are involved in an extensive system of intramolecular trans-succinylation reactions (Collins & Reed, 1977) resembling the intramolecular transacetylation reactions that provide active-site coupling in the closely related pyruvate dehydrogenase complexes of *E. coli* (Collins & Reed, 1977; Bates *et al.*, 1977; Danson *et al.*, 1978), *Bacillus stearothermophilus* (Stanley *et al.*, 1981), ox and pig heart (Stanley *et al.*, 1981) and ox kidney (Cate & Roche, 1979).

Studies of the pyruvate dehydrogenase complexes from *E. coli* (Hale & Perham, 1979*a,b*; Bleile *et al.*, 1979), from *B. stearothermophilus* (Perham & Wilkie, 1980) and from ox kidney (Kresze & Ronft, 1980) and ox heart (Bleile *et al.*, 1981) by means of limited proteolysis and electron microscopy suggested that the lipoic acid-containing regions of the

E2 chains are exposed, protruding from the core between the E1 and E3 components. It was conjectured that these regions of polypeptide chain might be flexible (Hale & Perham, 1979b; Bleile *et al.*, 1979). A surprisingly large degree of intramolecular mobility of large segments of polypeptide chain encompassing the lipoyl-lysine residues was revealed by proton n.m.r. spectroscopy of the *E. coli* complex (Perham *et al.*, 1981). This intrinsic mobility, akin to that of a random coil, greatly extends the potential distance (2.8 nm) that can be spanned by a single lipoyl-lysine swinging arm and provides a rational structural basis for understanding the systems of active-site coupling which these enzymes possess (Perham *et al.*, 1981).

In the present paper we apply the techniques of limited proteolysis, electron microscopy and proton n.m.r. spectroscopy to the 2-oxoglutarate dehydrogenase multienzyme complex from *E. coli*. We demonstrate the same highly distinctive features of subunit structure and conformational mobility for this enzyme complex as were found for the *E. coli* pyruvate dehydrogenase complex. Intramolecular protein mobility of a novel kind is evidently common to the 2-oxo acid dehydrogenase multienzyme complexes.

Materials and methods

Reagents

2-Oxoglutarate, NAD⁺ (free acid, grade 2), coenzyme A (grade 1) and soya bean trypsin inhibitor were obtained from Boehringer Mannheim. Thiamin diphosphate was from Sigma. Dihydrolipoamide was synthesized by the method of Reed *et al.* (1958). *N*-Ethyl[2,3-¹⁴C]maleimide (CFA. 293) was purchased from The Radiochemical Centre, Amersham. Trypsin (TPCK-treated) was from the Millipore Corp.

Enzymes and enzyme assays

2-Oxoglutarate dehydrogenase complex was purified by the method of Reed & Mukherjee (1969) from *E. coli* Crookes strain. The NAD⁺-reduction activities of the whole 2-oxoglutarate dehydrogenase complex and of the lipoamide dehydrogenase (E3) component were assayed spectrophotometrically (Danson & Perham, 1976).

Radiolabelling of lipoic acid residues with *N*-ethyl[2,3-¹⁴C]maleimide

Lipoic acid residues in the 2-oxoglutarate dehydrogenase complex can be selectively labelled with *N*-ethyl[2,3-¹⁴C]maleimide in the presence of substrate by the method of Brown & Perham (1976). 2-Oxoglutarate dehydrogenase complex was dissolved in 50 mM-potassium phosphate buffer, pH 7.0, containing 1 mM-NAD⁺, 5 mM-MgCl₂ and 0.5 mM-

thiamin diphosphate. The protein concentration was 3.3 mg/ml. 2-Oxoglutaric acid was added (final concn. 1 mM) and the mixture was incubated under N₂ at 0°C for 2 min. *N*-Ethyl[2,3-¹⁴C]maleimide (15 Ci/mol, final concn. 0.33 mM) was introduced and samples were removed for enzyme assay at various times. After about 25 min the enzyme complex was almost fully inactivated and the reaction was quenched by adding 2-mercaptoethanol (final concn. 0.14 M). The enzyme complex was separated from excess reagent by gel filtration at 4°C on Sepharose 6B (column size 560 mm × 15 mm) in 20 mM-sodium phosphate buffer, pH 7.0, containing 2 mM-EDTA and 0.02% NaN₃.

Radioactivity measurements

Radioactivity was measured in an LKB Rackbeta liquid-scintillation counter as described by Danson & Perham (1976).

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

Electrophoresis in 7.5% (w/v) polyacrylamide gels containing 0.1% sodium dodecyl sulphate was carried out and the gels were stained with Coomassie Brilliant Blue as described previously (Perham & Thomas, 1971). Subunit molecular weights were estimated by comparison of the mobilities of the stained bands with those of suitable marker proteins (Perham & Thomas, 1971). Radioactively labelled proteins were excised and quantified by liquid-scintillation counting and gels were sliced for autoradiography as described before (Brown & Perham, 1976).

Ultracentrifugation

Protein samples (about 3 mg/ml) were dialysed against a buffer containing 13 mM-Na₂HPO₄, 12 mM-KH₂PO₄ and 50 mM-NaCl, adjusted to a pH of 7.0 (*I* 0.1 M). Centrifugation was in a Beckman Model E ultracentrifuge at 33450 rev./min and 17°C. Sedimentation was followed by Schlieren optics.

Electron microscopy

Protein solutions (0.1 mg/ml) were treated with 1% glutaraldehyde, stained with 1% phosphotungstic acid and examined in a Philips EM 301 electron microscope, as described by Henderson *et al.* (1979).

N.m.r. spectroscopy

The enzyme complex was pelleted in the ultracentrifuge (3 h, 45000 rev./min, Beckman 50 Ti rotor), dissolved in ²H₂O containing 20 mM-potassium phosphate and 2 mM-sodium EDTA, apparent p²H 7.6, and dialysed exhaustively against the same

buffer. Spectra were obtained at 270 MHz using a Bruker WH270 spectrometer with quadrature phase detection, a 6 kHz spectral width and pulse interval of 0.34 s, as described by Perham *et al.* (1981). Sample temperature was $25(\pm 1)^{\circ}\text{C}$.

Results

Radiolabelling of lipoic acid residues with N-ethyl[2,3- ^{14}C]maleimide

Lipoic acid residues in the 2-oxoglutarate dehydrogenase were selectively labelled with *N*-ethyl[2,3- ^{14}C]maleimide in the presence of substrate (Brown & Perham, 1976), as described in the Materials and methods section.

Samples of the radioactive enzyme were analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Almost all (95%) of the radioactivity was found to be associated with the E2 polypeptide chain (Brown & Perham, 1976; Collins & Reed, 1977). The specific radioactivity of the enzyme complex was found to be 210 000 d.p.m./mg of protein. Assuming M_r values for the E1, E2 and E3 polypeptide chains to be 95 000, 50 000 and 56 000, respectively (Perham & Thomas, 1971; Reed, 1974) and that the molar ratios of these polypeptide chains (E1:E2:E3) in the complex are 0.5:1:0.5 (Reed, 1974), this specific radioactivity corresponds to 0.8 mol of lipoic acid/mol of E2 chain. This is in reasonable agreement with the value of one lipoic acid residue per E2 chain reported by Collins & Reed (1977).

Limited digestion with trypsin

2-Oxoglutarate dehydrogenase complex (1 mg/ml) was incubated with trypsin ($5\text{ }\mu\text{g/ml}$) in 20 mM-sodium phosphate buffer containing 2 mM-EDTA and 0.02% NaN_3 at pH 7.0 and 0°C . After various times samples were removed for enzyme assays and for sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The overall activity of the 2-oxoglutarate dehydrogenase complex was observed to be rapidly lost, whereas that of the lipoamide dehydrogenase (E3) component was unaffected. The loss of the overall activity was accompanied by rapid degradation of the E2 polypeptide chain, apparent M_r 50 000, the principal fragment derived from it having an apparent M_r of approx. 36 000 (Fig. 1). The E3 polypeptide chain was unchanged, as expected, whereas the E1 polypeptide chain appeared to suffer a slow partial fragmentation, a new band of only slightly smaller M_r gradually being formed. There was little change in the gel electrophoresis pattern between 10 min and 30 min treatment with trypsin, suggesting that a relatively stable tryptic product was being formed.

When the experiment was repeated with 2-oxoglutarate dehydrogenase complex in which the lipoic

Fig. 1. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of 2-oxoglutarate dehydrogenase complex from *E. coli* and the products of limited digestion with trypsin

Enzyme complex (1 mg/ml) was incubated with trypsin ($5\text{ }\mu\text{g/ml}$) in 20 mM-sodium phosphate buffer containing 2 mM-EDTA and 0.02% NaN_3 at pH 7.0 and 0°C . Samples were submitted to gel electrophoresis after 0, 1 min, 2 min, 10 min, 15 min and 30 min, respectively. E1, 2-oxoglutarate decarboxylase; E2, lipoate succinyltransferase; E3, lipoamide dehydrogenase.

acid residues were labelled with *N*-ethyl[2,3- ^{14}C]maleimide, the same gel electrophoretic pattern was observed. Autoradiography of the gels indicated that none of the Coomassie-staining bands contained radioactivity after the treatment with trypsin, a result confirmed by liquid-scintillation counting of the excised gel bands. Radioactive peptides of low M_r were found towards the bottom of the gel. At the end of the digestion (30 min) an excess of soya bean trypsin inhibitor (final concn. $25\text{ }\mu\text{g/ml}$) was added and the mixture (2 ml) was gel filtered on Sepharose 6B in the same buffer. Samples of the column effluent were examined for A_{280} , for radioactivity, and for lipoamide dehydrogenase activity (Fig. 2). Most of the protein was eluted in the high- M_r region of the column effluent, the E3 activity coinciding with this peak. On the other hand, the radioactivity was restricted to the low- M_r region of the effluent.

Portions of the two peaks were analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Fig. 2). The high- M_r peak was found to be composed of E1 and E3 polypeptide chains, together with the fragment of apparent M_r 36 000 derived from the E2 polypeptide chains. This was evidently still an assembled structure from which lipoic acid-containing regions of the E2 polypeptide chains had been cleaved by trypsin. The small amount of

Fig. 2. Gel filtration of *N*-ethyl[2,3- 14 C]maleimide-labelled 2-oxoglutarate dehydrogenase complex from *E. coli* after trypsin treatment

Enzyme complex was digested with trypsin for 30 min as in Fig. 1 and the reaction was terminated by adding soya bean trypsin inhibitor (25 μ g/ml). The mixture was gel filtered on a column (50 cm $\times$ 1.5 cm) of Sepharose 6B equilibrated with 20 mM-sodium phosphate buffer, pH 7.0, containing 2 mM-EDTA and 0.02% NaN_3 . Fractions (approx 1.5 ml) were collected. O, A_{280} ; ●, E3 activity (arbitrary units); Δ , radioactivity. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis patterns are shown alongside. (a) Trypsin digest of enzyme complex applied to Sepharose 6B column; (b) sample taken from fraction 15.

slightly shortened E1 polypeptide chain in the trypsin digest applied to the column (cf. track 6 in Fig. 1) was no longer bound to the complex after gel filtration (Fig. 2). The radioactive lipoic acid-containing regions of the E2 polypeptide chains were not detected as Coomassie-staining bands in the gel electrophoresis patterns. They are obviously of relatively low M_r , migrating rapidly in the gel system.

Electron microscopy

Samples of native 2-oxoglutarate dehydrogenase complex and of the inactive product of tryptic digestion (fraction 16 from the Sepharose 6B column in Fig. 2) were treated with 1% glutaraldehyde, negatively stained with phosphotungstic acid and photographed in an electron microscope (Plate 1). Despite the loss of the lipoic acid-containing regions of its E2 polypeptide chains, the trypsin-treated enzyme complex closely resembled the native enzyme. The retention of the assembled structure of the complex after treatment with trypsin, which had been inferred from the gel filtration analysis, was thus amply confirmed. The underlying octahedral E2 core can be seen in some favourable images of the native and trypsin-treated enzyme complex (Plate 1).

N.m.r. spectroscopy

The 270 MHz proton n.m.r. spectrum of the native 2-oxoglutarate dehydrogenase complex is shown in Fig. 3(a). In addition to the underlying broad envelope of resonances expected in the spectrum of such a large protein complex, theoretical linewidths being of the order of 8 kHz, there are several sharp resonance lines between -0.6 and -3 p.p.m. attributable to the protein. The existence of these sharp resonances, with linewidths of only about 50 Hz, clearly shows that some amino acid residues have substantial freedom to move rapidly with respect to the enzyme complex, as explained elsewhere (Perham *et al.*, 1981).

The most prominent sharp resonance in the spectrum, at -2.34 p.p.m. from dioxan, can be attributed to the methyl resonances of alanine and threonine residues, and the smaller peak at -2.77 p.p.m. is likely to be derived from the methyl protons of valine, leucine and isoleucine residues (Perham *et al.*, 1981). Other sharp resonances can similarly be assigned to methionine *S*-methyl protons (-1.55 p.p.m.) and ϵ -methylene protons of lysine residues (-0.7 p.p.m.). No sharp signals were observed to low-field of the HO^2H peak (2-8 p.p.m.) where resonances of aromatic protons would be expected. The chemical shifts of the sharp resonances

EXPLANATION OF PLATE 1

Electron microscopy of the 2-oxoglutarate dehydrogenase complex from E. coli

Samples were fixed by treatment with 1% glutaraldehyde, negatively stained with 1% sodium phosphotungstate and photographed in a Philips EM 301 electron microscope. (a) Intact enzyme complex; (b) enzyme complex after treatment with trypsin (fraction 16 from the Sepharose column in Fig. 2). Magnification (for both parts) is approx. $\times 400\,000$.

Fig. 3. 270 MHz proton n.m.r. spectra of 2-oxoglutarate dehydrogenase complex from *E. coli* and the tryptic core complex (a) Intact complex, 62 mg/ml, $s_{20,w}$ of a portion of this sample, diluted to 3 mg/ml, was 32 S. (b) Tryptic core complex, 29 mg/ml, $s_{20,w}$ of a portion of this sample, diluted to 4 mg/ml, was 25 S. Deconvoluted spectra are shown as insets. Peaks marked E arise from the 2 mM-EDTA present in the buffer. Chemical shifts are expressed relative to internal dioxan.

ances are consistent with a random coil-like structure for the highly mobile regions of polypeptide chain, though small parts of these regions could have defined three-dimensional structure.

It is not easy to integrate this spectrum accurately because it is difficult to draw an appropriate baseline. However, from an approximate integration of spectra obtained with a 5 s pulse interval based on the 2 mM-EDTA as an internal standard and by analogy with the corresponding spectrum of the *E. coli* pyruvate dehydrogenase complex (Perham *et al.*, 1981), it appears that some 50–100 protons per enzyme protomer are contributing to the sharp lines. We define a protomer as 1/24th part of the enzyme complex because the particle is based on octahedral (24-fold repeat) symmetry (Reed, 1974). Quite large regions of polypeptide chain must therefore have high mobility with respect to the enzyme complex.

On the other hand, the absence of sharp aromatic resonances indicates that the appearance of the spectrum is not due to general mobility of the polypeptide chain; the sharp lines must arise from defined regions of the primary structure.

The spectrum of the tryptic core of the 2-oxoglutarate dehydrogenase complex (from which the low-*M*, lipoic acid-containing peptides had been removed by gel filtration, as in Fig. 2) is shown in Fig. 3(b). It is generally very similar to that of the intact complex (Fig. 3a) although there are significant differences between Figs. 3(a) and 3(b). In the trypsin-treated enzyme the overall intensities of the sharp components of the spectrum appear to be somewhat reduced, and their relative intensities are also altered: the peak at -2.77 p.p.m. assigned to valine, leucine and isoleucine methyl protons is more pronounced. As can be seen more clearly in the

deconvoluted spectra shown as inserts in Fig. 3, there are distinct, though small, differences between the two spectra at -2.48 and -1.44 p.p.m. Notwithstanding these differences, the existence of a very similar group of sharp resonances in the proton n.m.r. spectrum of the tryptic core of the 2-oxoglutarate dehydrogenase complex indicates that substantial regions of highly mobile polypeptide chain have been retained in the structure.

Discussion

The principal effect of trypsin on the native 2-oxoglutarate dehydrogenase complex of *E. coli* at pH 7.0 was to degrade the lipoate succinyltransferase (E2) polypeptide chain, lowering its apparent M_r from 50 000 to 36 000 as judged by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Fig. 1). A slower shortening of the E1 polypeptide chain was also apparent, whereas the E3 chain was unaffected. Inhibition of the catalytic activity of the complex accompanied the degradation of the E2 chain. The inactive trypsin-treated enzyme had lost the lipoic acid-containing regions of its E2 polypeptide chains yet remained a highly assembled structure, as judged by gel filtration (Fig. 2) and electron microscopy (Plate 1). The E2 component provides binding sites for the E1 and E3 components in the native enzyme (Reed, 1974). Our results indicate that the octahedral symmetry of the structure is conferred by the association of the tryptic fragments of the E2 chains with apparent M_r 36 000, these fragments or folding domains also providing the binding sites for the E1 and E3 components. This conclusion is in agreement with the unpublished work of D. K. McRorie, D. M. Bleile & L. J. Reed, quoted by Bleile *et al.* (1981). The lipoic acid-containing regions of the E2 chains are highly susceptible to removal by trypsin, presumably therefore being physically exposed and protruding between the E1 and E3 subunits bound to an inner region of the E2 core.

These results are identical with those obtained with the pyruvate dehydrogenase complex of *E. coli* (Hale & Perham, 1979a,b, 1980; Bleile *et al.*, 1979) in which the inner octahedral core of the complex is also provided by tryptic fragments of apparent M_r 36 000 that bind the E1 and E3 subunits but contain no lipoic acid. The lipoic acid-containing regions of the E2 core are readily excised by trypsin without causing substantial dissociation of the complex, although some shortening of the E1 chains also takes place. Similar results have been observed with pyruvate dehydrogenase complexes based on icosahedral symmetry, e.g. those from *B. stearothermophilus* (Perham & Wilkie, 1980) and ox kidney (Kresze & Ronft, 1980) and ox heart (Bleile *et al.*, 1981). With these complexes, however, release of the

lipoic acid-containing regions of the E2 chains by tryptic digestion is accompanied by release of the E1 and E3 subunits from the structure. The inner E2 core generated by trypsin evidently does not contain intact binding sites for the E1 and E3 components, some contribution from the lost part or parts of the E2 chains presumably being necessary. This idea is supported by the observation that chymotrypsin can release smaller lipoic acid-containing regions from the E2 chains of the *B. stearothermophilus* complex without inducing dissociation of the E1 and E3 components (H. W. Duckworth, R. Jaenicke, R. N. Perham & A. O. M. Wilkie, unpublished work). Although the sites of attachment of the lipoyl groups were not firmly identified, digestion of the 2-oxoglutarate dehydrogenase complex of ox kidney with elastase or papain has also been found to cleave the E2 chains with accompanying disassembly of the complex (Kresze *et al.*, 1981). Whether or not disassembly of a 2-oxo acid dehydrogenase complex accompanies limited proteolysis of the structure therefore depends on the sites of cleavage of the E2 chains.

The proton n.m.r. spectrum of the 2-oxoglutarate dehydrogenase complex of *E. coli* (Fig. 3a) clearly reveals the existence of substantial regions of polypeptide chain with marked intramolecular mobility. The spectrum closely resembles that obtained from the pyruvate dehydrogenase complex of *E. coli* (Perham *et al.*, 1981). Spectra indicating the existence of similarly mobile regions of polypeptide chain have also been observed for the pyruvate dehydrogenase complexes of *B. stearothermophilus* and ox heart and the 2-oxoglutarate dehydrogenase complex of ox heart (Wawrzynczak *et al.*, 1981; H. W. Duckworth, R. Jaenicke, R. N. Perham & G. C. K. Roberts, unpublished work). In the pyruvate dehydrogenase complex of *E. coli*, the highly mobile regions were identified as being parts of the lipoate acetyltransferase polypeptide chains. After treatment of the *E. coli* complex with trypsin, all of the most mobile regions were found to have been lost. These results suggested that the highly mobile regions are connected with the lipoyl-lysine residues that provide the swinging arms in the enzyme mechanism (Perham *et al.*, 1981). The close similarity of the proton n.m.r. spectra of the 2-oxoglutarate and pyruvate dehydrogenase complexes, with the characteristic prominence of the resonance of alanine and threonine methyl protons and the notable absence of peaks from aromatic side-chain protons, argues for a similar assignment of the highly mobile regions to the lipoate succinyltransferase (E2) component of the 2-oxoglutarate dehydrogenase complexes.

After treatment of the 2-oxoglutarate dehydrogenase complex of *E. coli* with trypsin, which removes the lipoic acid-containing regions of the E2

core, the residual enzyme complex was found to retain most of the highly mobile regions ascribed to the E2 polypeptide chains (Fig. 3b), although the treatment had clearly had some effect on these regions as judged by the changes in intensity of some of the sharp signals in the proton n.m.r. spectrum. This result is identical with that obtained when the lipoic acid-containing regions of the E2 core of the pyruvate dehydrogenase complex of *B. stearrowthermophilus* are removed by treatment with chymotrypsin (H. W. Duckworth, R. Jaenicke, R. N. Perham & G. C. K. Roberts, unpublished work). It is clear that high conformational mobility is not restricted to the immediate attachment site of the lipoic acid in each E2 chain. It could well be that there is a small region of organized structure encompassing the lipoyl-lysine residue which serves to promote its interaction with the active sites of E1 and E3 components and which is linked to the inner part of the E2 core by means of the highly mobile region of polypeptide chain detected by proton n.m.r. spectroscopy.

The E2 cores of the pyruvate and 2-oxoglutarate dehydrogenase multienzyme complexes are very uncommon proteins. The symmetry of the complexes, octahedral or icosahedral, is dictated by the multiple association of a part of each E2 chain to form an inner core devoid of lipoic acid residues but containing the lipoate acyltransferase activity (Hale & Perham, 1979a,b; Bleile *et al.*, 1979; Perham & Wilkie, 1980; Kresze & Ronft, 1980; Bleile *et al.*, 1981; Kresze *et al.*, 1981). The lipoyl-lysine residues are incorporated in regions of the E2 chains with unusually high conformational mobility with respect to the rest of the complex. The amino acid sequences of these regions, apart from the immediate attachment sites of the lipoyl groups (Reed, 1974; Hale & Perham, 1980) are unknown, but it may be significant that they are thought to be of low isoelectric point and to migrate anomalously on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Bleile *et al.*, 1979, 1981; Kresze & Ronft, 1980). The high value for their frictional ratio (f/f_0), which has reasonably been taken to indicate a swollen or extended structure (Bleile *et al.*, 1979, 1981), would equally be consistent with the more random coil-like structure we infer from the proton n.m.r. spectra.

The additional freedom to move conferred on the lipoyl groups by virtue of this polypeptide chain mobility is likely to facilitate the coupling of active sites that are physically distant in the complexes. It can account for the ability of the pyruvate dehydrogenase complex of *E. coli* to lose lipoic acid residues by proteolysis (Bleile *et al.*, 1979) or by chemical modification (Ambrose-Griffin *et al.*, 1980; Berman *et al.*, 1981) without corresponding loss of overall catalytic activity, as discussed elsewhere (Perham *et*

al., 1981). For the 2-oxoglutarate dehydrogenase complex of *E. coli*, however, it has been reported that the loss of catalytic activity is directly proportional to the modification of lipoic acid residues and that the distance between neighbouring active sites of enzymes E1 and E3 can be spanned by a single lipoyl-lysine swinging arm (Angelides & Hammes, 1979). The intramolecular coupling of active sites by transacylation reactions in the E2 core of these enzymes (Bates *et al.*, 1977; Collins & Reed, 1977; Danson *et al.*, 1978; Cate & Roche, 1979; Stanley *et al.*, 1981) and this novel polypeptide chain mobility are therefore unlikely to be primarily concerned with physically spanning the gaps between E1 and E3 subunits but rather with providing a mechanism for the efficient kinetic linkage of the three different chemical reactions catalysed by the complexes. Such a mechanism is restricted to a highly aggregated multienzyme complex and provides a plausible explanation for the rare quaternary structure of these enzymes (Danson *et al.*, 1978; Berman *et al.*, 1981). It can accommodate the otherwise serious problem that the three types of active site in these enzyme particles are not present in equivalent numbers (Reed, 1974; Bates *et al.*, 1975; Henderson & Perham, 1980). For example, the fact that the E3:E2 chain ratio is often much less than 1, as in the 2-oxoglutarate dehydrogenase complex studied here, means that there cannot be a simple transfer of substrate between three successive enzymes rigidly arranged. A system of active-site coupling and polypeptide chain mobility appears to be mandatory in the E2 core.

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