CONFORMATIONAL MOBILITY OF POLYPEPTIDE CHAINS IN THE 2-OXO ACID DEHYDROGENASE COMPLEXES FROM OX HEART REVEALED BY PROTON NMR SPECTROSCOPY

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1. Introduction

The lipoate acetyltransferase (E2) component of the pyruvate dehydrogenase multienzyme complex from *Escherichia coli* consists of 24 polypeptide chains arranged with octahedral symmetry [1,2] whereas that of the pyruvate dehydrogenase complex from mammalian mitochondria [1] and from *Bacillus stearothermophilus* [3] contains 60 polypeptide chains arranged with icosahedral symmetry. In each case, multiple copies of the pyruvate decarboxylase (E1) and lipoamide dehydrogenase (E3) components are bound to this E2 core. The substrate is carried in thioester linkage by lipoyl–lysine residues in the E2 chains which act as swinging arms [4,5] with almost unrestricted physical mobility of the side chain [6,7].

Studies of the pyruvate dehydrogenase complexes from *E. coli* [8–10], from *B. stearothermophilus* [11] and from ox kidney [12] and ox heart [13] by limited proteolysis and electron microscopy suggested that lipoic acid-containing regions of the E2 chains protrude from the E2 core between the E1 and E3 subunits. An extraordinary degree of conformational mobility of large segments of the E2 chains encompassing the lipoyl–lysine residues was demonstrated by proton NMR spectroscopy of the *E. coli* complex [14]. It is likely that this mobility greatly extends the working radius of a swinging arm and is intimately connected with the novel system of active site coupling possessed by this enzyme [15–17]. We show here by means of proton NMR spectroscopy that conformational mobility of this unusual kind is also a property of the pyruvate and 2-oxoglutarate dehydrogenase complexes of ox heart.

Abbreviations: NMR, nuclear magnetic resonance; SDS, sodium dodecyl sulphate; M_r, relative molecular mass

2. Materials and methods

The pyruvate and 2-oxoglutarate dehydrogenase complexes were prepared from ox heart as in [18], which involves the addition of Triton X-100 to buffers to promote solubilization of the enzymes. The complexes were assayed and their purity confirmed by SDS–polyacrylamide gel electrophoresis in 11.5% Tris–glycine slab gels [18].

The inactivation of the pyruvate dehydrogenase complex by phosphorylation [1,19] was achieved at 25°C in the buffer used for NMR spectroscopy by adding ATP and $MgCl_2$ to final concentrations of 1 mM and 5 mM, respectively. After 45 min, the complex retained <5% of its original activity (16 IU/mg).

For NMR spectroscopy the enzyme complexes were centrifuged (2 h at 165 000 × *g*), redissolved in ^2H_2O containing 20 mM potassium phosphate, 2 mM sodium EDTA and 0.1% (v/v) Triton X-100 or Tween 80, apparent pD 7.6, and dialysed exhaustively against the same buffer at 4°C. Spectra were obtained at 270 MHz using a Bruker WH270 spectrometer at 25°C as in [14]. A 6 kHz spectral width was employed with quadrature phase detection. The pulse interval was 0.34 s; before transformation the free induction decay was multiplied by an exponential function equivalent to a line broadening of 5 Hz. Chemical shifts are expressed relative to internal dioxan.

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3. Results

3.1. *Pyruvate dehydrogenase complex*

The 270 MHz proton NMR spectrum of the pyruvate dehydrogenase complex prepared from ox heart in the presence of Triton X-100 is shown in fig.1A. Superimposed on the very broad envelope of resonances expected for such a large protein structure (reported $M_r \sim 9 \times 10^6$ [1]) are several sharp resonance lines between −0.6 and −3.0 ppm, and also near 3.4 ppm. The relevant regions of the spectrum of the buffer containing Triton X-100 (*iso*-octylphenoxypolyethoxyethanol) are shown inset. It is apparent that some of the sharp signals in the spectrum of the protein solution arise from the detergent; most obviously those of the phenoxy protons at 3.1−3.6 ppm. In order to make a clear distinction between the resonances of the protein and those of the detergent, we prepared the complex by the same method [18] substituting Tween 80 (polyoxyethylene sorbitan monooleate) for Triton in all the buffers. The spectrum of this preparation (together with that of the detergent) is shown in fig.1B. Comparison of the spectra in fig.1A and B shows clearly that all the resonance at −3.0 ppm in fig.1A arises from the Triton. In the Triton spectrum, the resonance at −2.35 ppm has 50% of the intensity of that at −3.0 ppm, so that it must contribute <50% of the intensity of the resonance at −2.3 ppm in fig.1A, the remainder coming from the protein. The resonance at −2.8 ppm must arise wholly from the protein, while the resonances in the region −1.0 to −2.0 ppm contain only minor contributions from the detergent.

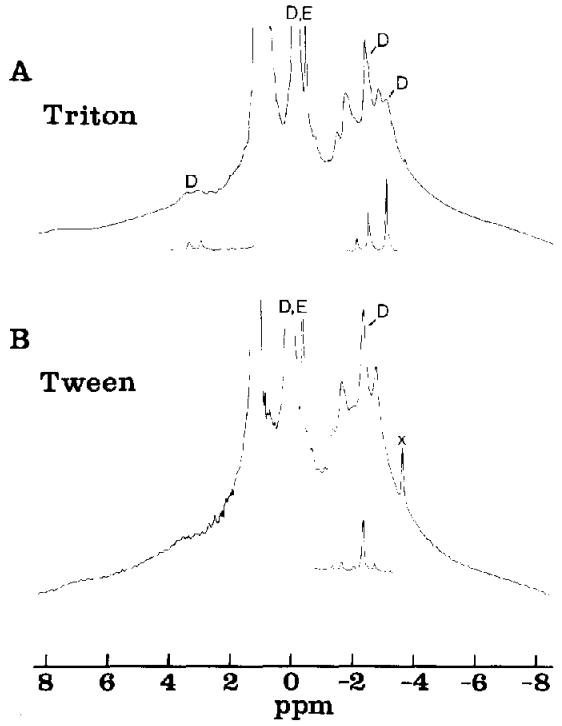

Fig.1. The 270 MHz proton NMR spectra of ox heart pyruvate dehydrogenase complex solubilized with (A) Triton X-100 and (B) Tween 80. The protein concentration in (A) was 32 mg/ml and the apparent $s_{20,w}$ of the major aggregate determined at a single protein concentration was 76 S. The complex concentration in Tween 80 was similar but difficult to determine accurately because of its tendency to aggregate and precipitate under these conditions. In each case, the relevant regions of the spectrum of the detergent are shown inset. The two intense resonances near 0 ppm arise from the detergent and the EDTA in the buffer (marked E). Other resonances which arise wholly or in part from the detergent are labelled D. The resonance marked X arises from the presence of silicone-based antifoam used in the initial extraction of the complex from heart tissue [18].

The sharp resonance in the enzyme spectrum at −2.8 ppm can be assigned to the methyl protons of valine, leucine and isoleucine residues, that at −2.3 ppm to the methyl protons of alanine and threonine residues and that at −1.6 ppm can be tentatively attributed to methione *S*-methyl protons. These sharp resonances have linewidths of only ~50 Hz, whereas a rigid particle with M_r 9 × 10^6 would be expected to have proton linewidths of ~10 kHz. As discussed in [14], the existence of such sharp lines in the proton NMR spectrum must mean that an appreciable number of amino acid residues have considerable mobility with respect to the enzyme complex. The absence of sharp resonances attributable to aromatic protons of the protein indicates that the spectrum is not due to general mobility of polypeptide chains but must arise from defined regions of the primary structure. The chemical shifts of the sharp resonances are consistent with a random coil-like structure for these highly mobile regions of polypeptide chain.

We cannot integrate these spectra accurately because it is obviously difficult to draw an appropriate baseline and to allow quantitatively for the contribution from the detergent resonances. However, by analogy with the corresponding spectrum of the *E. coli* pyruvate dehydrogenase complex [14], which the present spectra closely resemble, it appears that many protons/enzyme protomer are contributing to the sharp lines. We define a protomer as 1/60th part of

the enzyme complex because the particle is based on icosahedral (60-fold repeat) symmetry [1]. Substantial regions of polypeptide chain must therefore enjoy high mobility with respect to the enzyme complex.

3.2. *Phosphorylation of the pyruvate dehydrogenase complex*

On addition of ATP and $MgCl_2$, the complex-bound phosphokinase brings about the phosphorylation of the E1α subunits of the complex and its concomitant inactivation [1,19]. Phosphorylation of the preparation of pyruvate dehydrogenase complex which gave the NMR spectrum of fig.1A had no effect on the protein spectrum.

3.3. *2-Oxoglutarate dehydrogenase complex*

The 270 MHz NMR spectra of the 2-oxoglutarate dehydrogenase complex of ox heart prepared in the presence of Triton X-100 and Tween 80 are shown in fig.2A and B, respectively, with the detergent spectra inset. Comparison of these two spectra, as outlined above for the pyruvate dehydrogenase complex, shows that there are sharp resonances from the protein at −2.8 ppm, −2.3 ppm and between −1.0 and −2.0 ppm, closely resembling those found in the spectrum of the pyruvate dehydrogenase complex (fig.1).

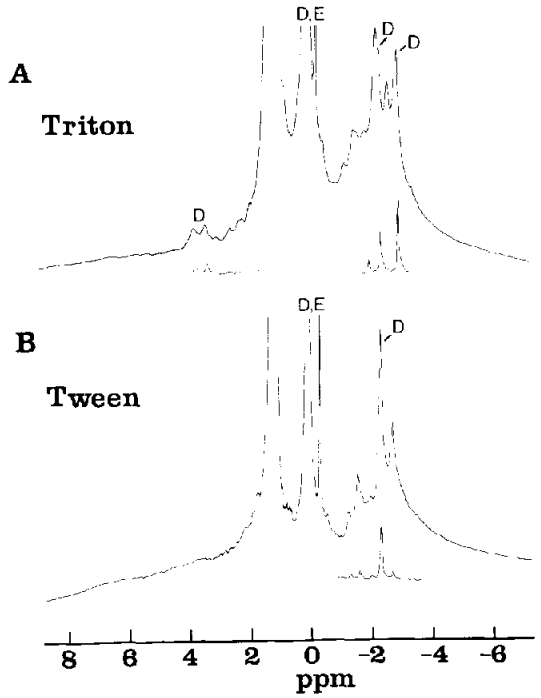

Fig.2. The 270 MHz proton NMR spectra of ox heart 2-oxoglutarate dehydrogenase complex solubilized with (A) Triton X-100 and (B) Tween 80. The protein concentration in (A) was 25 mg/ml and the apparent $s_{20,w}$ of the complex determined at a single protein concentration was 40 S. The protein concentration in (B) was similar, but again some aggregation and precipitation of the complex was observed. Other details as for fig.1.

The intensities of the sharp resonances from the protein in the spectra in fig.2 are very similar to those in fig.1 and to those described in detail for the pyruvate dehydrogenase complex of *E. coli* [14]. We infer that in this complex too, substantial regions of polypeptide chain have high mobility with respect to the enzyme complex.

4. Discussion

The 270 MHz proton NMR spectra of the pyruvate (fig.1) and 2-oxoglutarate (fig.2) dehydrogenase complexes from ox heart demonstrate the existence of considerable regions of polypeptide chain with marked intramolecular mobility. Similar results were obtained with the pyruvate dehydrogenase complex of *E. coli* [14]. For that complex we were able, by limited proteolysis experiments, to identify the highly mobile regions as being associated with the lipoic acid-containing regions of the lipoate acetyltransferase (E2) polypeptide chains. Large lipoic acid-containing regions of the pyruvate dehydrogenase complex from ox kidney [12] and ox heart [13] can be released by limited proteolysis and appear to protrude from the E2 core. The same is probably true of the 2-oxoglutarate dehydrogenase complex from ox kidney [20]. Unfortunately these mammalian complexes become disassembled during such limited proteolysis [12,13,20] and we cannot therefore obtain the proton NMR spectrum of the remaining 'core complex'.

There is a close resemblance between the proton NMR spectra of the *E. coli* and ox heart enzymes, with a characteristic prominence of resonances from alanine and threonine methyl protons and from leucine, isoleucine and valine methyl protons, and with a notable absence of resonances from aromatic side-chain protons. By analogy with the *E. coli* pyruvate dehydrogenase complex [14], it is reasonable to postulate that the highly mobile parts of the 2-oxo acid dehydrogenase complexes from ox heart are in those regions of the lipoate acetyltransferase and succinyl-

transferase components which encompass the lipoyl–lysine residues. Nothing is known yet of the amino acid sequences of these regions but they have been reported to be of low isoelectric point and to migrate anomalously on SDS–polyacrylamide gel electrophoresis [12,13,20]. The high value for their frictional ratio (f/f_0), which could indicate an extended structure [13], would equally be consistent with the more random coil-like structure we infer from the proton NMR spectra. It is possible that the lipoyl–lysine residues are included in a small region of organized structure which acts to promote interaction with the active sites of the other component enzymes and which is linked to the inner part of the E2 core by means of the highly mobile region of polypeptide chain revealed by proton NMR spectroscopy [14]. We note that the mobility is unaffected in the pyruvate dehydrogenase complex of ox heart when that enzyme is inhibited by phosphorylation of its E1α subunits.

The novel conformational mobility we have described above and for the pyruvate dehydrogenase complex of *E. coli* [14], which should not be confused with substrate-induced conformational changes of the kind normally associated with enzymes, is also found in the pyruvate dehydrogenase complex of *B. stearothermophilus* and the 2-oxoglutarate dehydrogenase complex of *E. coli* (H. W. Duckworth, R. Jaenicke, R. N. P., G. C. K. R., unpublished). It is a general property of the 2-oxo acid dehydrogenase complexes, and is most likely to be concerned with the unusual systems of the active site coupling possessed by these multienzyme complexes [15–17,21–23].

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