EVIDENCE FOR TWO-DOMAIN SUBUNIT STRUCTURE OF KIDNEY LIPOATE ACETYLTRANSFERASE

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1. Introduction

Earlier studies on the inactivation of the mammalian pyruvate dehydrogenase (EC 1.2.4.1) complex (PHD complex) by a thiol protease from rat liver [1] had shown that the lipoate acetyltransferase (EC 2.3.1.12) was cleaved into two smaller fragments detectable on SDS–polyacrylamide gel electrophoresis [1,2]. Here we have studied the subunit structure of kidney lipoate acetyltransferase using trypsin (EC 3.4.21.4) which was found to yield also two fragments with app. mol. wt 36 000 and 28 000. As will be shown here both fragments possess about equal catalytic activity for acetyl transfer, and each apparently contains protein-bound lipoamide suggesting that the subunit of the mammalian lipoate acetyltransferase consists of two homologous if not identical domains.

2. Materials and methods

Bovine kidney pyruvate dehydrogenase complex (spec. act. 16–20 units/mg protein) was prepared by poly(ethylene glycol) precipitation [2] with some modifications to be detailed elsewhere. Lipoate acetyltransferase was resolved according to [2]. Marker proteins for molecular weight determination (LMW protein standards) were from Bio-Rad Labs., CA and poly(ethylene glycol) 6000 from Serva, Heidelberg. Bovine pancreatic trypsin (30 U/mg), other enzymes or coenzymes came from Boehringer, Mannheim. Radiochemicals were purchased from New England Nuclear, Chicago. Laboratory chemicals, analytical grade, were from Merck, Darmstadt.

Enzyme activities were measured as follows: PHD complex [3], lipoate acetyltransferase [4], dihydrolipoamide dehydrogenase (EC 1.6.4.3) [4]. SDS–polyacrylamide gel electrophoresis was done in 10% gels according to [5]. Lipoyl groups of lipoate acetyltransferase were acetylated with [1-^{14}C]acetyl-CoA as in [6]. Protein was determined by the biuret method [7] or by the modified Lowry procedure [8] using Lab-Trol E® (Merz and Dade, Munich) as standard.

3. Results

In fig.1 limited tryptic digestion of lipoate acetyltransferase resulted in the complete disappearance of the native protein band (app. mol. wt 70 000) in favour of two new polypeptides (F_1, F_2) with app. mol. wt 36 000 and 28 000. Like in the studies of the liver protease [1,2] there was no change in acetyltransferase activity after trypsin treatment.

After extraction from SDS gels each of the tryptic fragments was shown to possess enzymatic activity for acetyl transfer, which amounted to ~1/3rd of that of the nicked acetyltransferase (table 1). This is probably an underestimation as the SDS gel electrophoretic separation and extraction procedure may well have led to some inactivation of the fragments. Disregarding this possibility it appears that the specific activity is about the same in F_1 and F_2.

In fig.2 the lipoyl groups of lipoate acetyltransferase were labelled by incorporation of [^{14}C]acetyl residues. The acetylated protein was then subjected to limited tryptic digestion followed by SDS–polyacrylamide gel electrophoresis. As can be seen the acetyltransferase-bound radioactivity after trypsin treatment was localized exclusively on two regions of

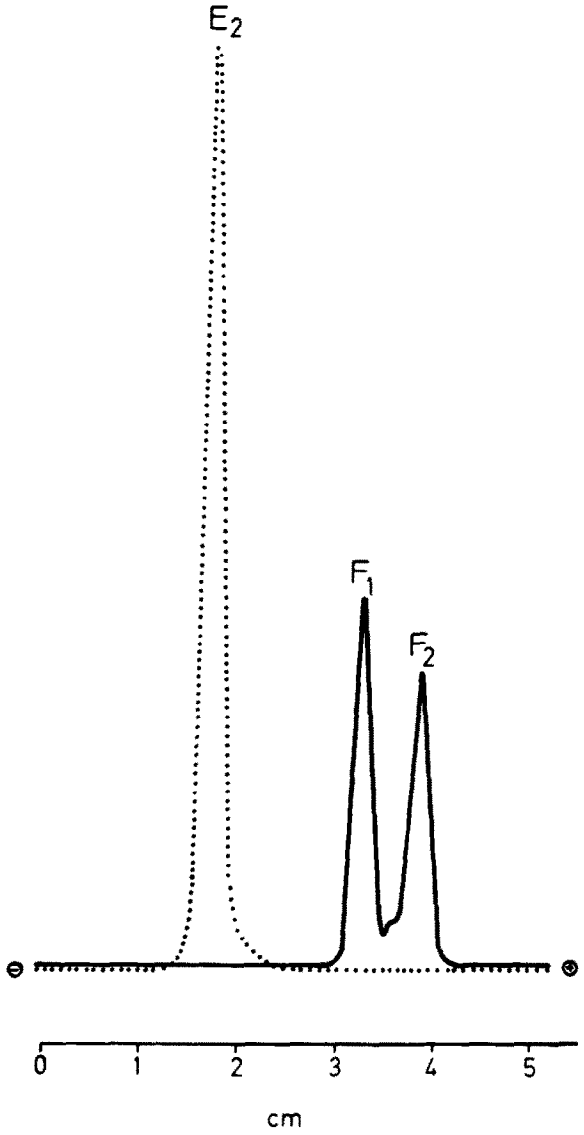

Fig.1. Limited tryptic proteolysis of kidney lipoate acetyltransferase. Lipoate acetyltransferase was incubated at 25°C with trypsin (2.25 μg/mg) in 50 mM Mops-Na containing 2 mM dithiotreitol. After 30 min proteolysis was stopped with 8 M urea, 1% SDS and 1% mercaptoethanol, and samples corresponding to 19.2 μg protein analyzed by SDS–polyacrylamide gel electrophoresis [5]. After staining with Coomassie brilliant blue G-250 the gels were scanned (Gilford spectrophotometer, λ = 603 nm). Marker proteins for molecular weight determination were bovine serum albumin (67 000), ovalbumin (45 000), carbonic anhydrase (30 000), soybean trypsin inhibitor (21 000). (. . .) (E_2), native lipoate acetyltransferase (70 000 daltons); (——) trypsin-treated lipoate acetyltransferase; apparent molecular weights of fragments designated F_1 and F_2 were 36 000 and 28 000. The sum of the peak areas F_1 and F_2 corresponds closely to the peak area for the native polypeptide.

Table 1
Enzymatic activity of tryptic lipoate acetyltransferase fragments after extraction from SDS–polyacrylamide gels (units/mg protein)

	Exp. I	Exp. II
Nicked enzyme (before electrophoresis)	3.3.	4.0
Fragment F_1	1.48	1.4
Fragment F_2	1.48	1.4

Lipoate acetyltransferase after limited tryptic digestion was separated on SDS–polyacrylamide gel electrophoresis. The regions corresponding to the bands of F_1 and F_2 were cut out from unstained gels run in parallel, and extracted by homogenisation with 50 mM Mops-Na buffer (pH 7.0) containing 2 mM dithiotreitol, and keeping the samples for 12 h at 10°C. After centrifugation the eluates were dialyzed overnight against the elution buffer, lyophilized and redissolved in an appropriate volume of the elution buffer. For further details see section 2

the gel corresponding to the position of the stained protein bands of fragments F_1 and F_2. It appears therefore that protein-bound lipoamide is present in each of the fragments. The radioactivity of the two peaks together was ~90% of that of the undigested enzyme.

4. Discussion

Our results suggest that the kidney lipoate acetyltransferase contains two homologous domains very similar in catalytic activity as well as in lipoyl content which probably corresponds to 1 residue/fragment chain. This is compatible with preliminary results suggesting a stoichiometry of 2 mol lipoamide/mol native acetyltransferase subunit.

Limited tryptic proteolysis has been applied by others for studies of the subunit structure of the lipoate acetyltransferase from *Escherichia coli* yet has led to contradictory results. A structure of two homologous domains, similar to that proposed here, has been described [9–11]. This, however has been questioned by more recent work indicating that the bacterial enzyme consists of two heterologous domains one containing the catalytic site, and the other the lipoyl moieties [12].

Concerning the structure of the lipoate acetyltransferase core of the mammalian PHD complex a composition of 60 subunits [13] as well of 24 sub-

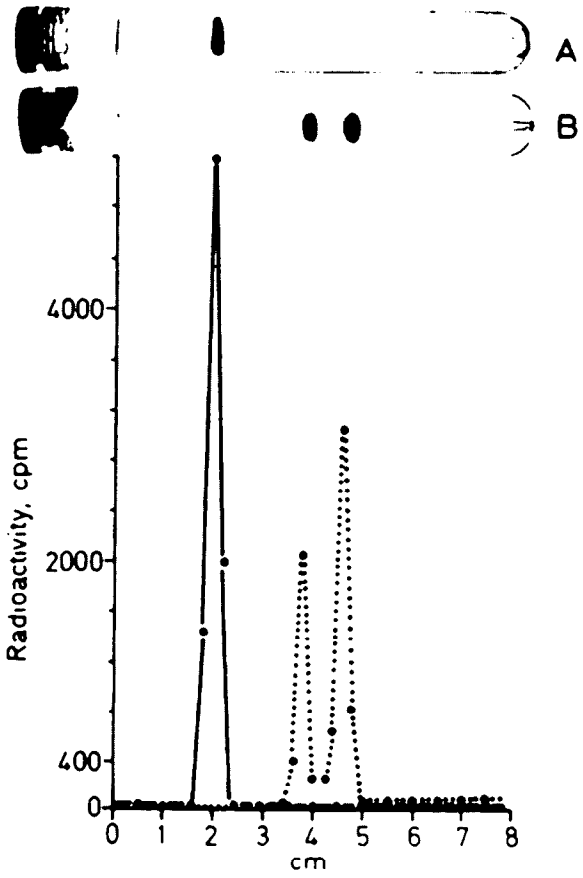

Fig.2. Limited tryptic proteolysis of acetylated lipoate acetyltransferase. Acetylation of lipoyl residues was performed with [1-^{14}C]acetyl-CoA [6]. The labelled enzyme was analyzed by SDS polyacrylamide gel electrophoresis [5] before (gel A) and after (gel B) treatment with trypsin as indicated in fig.1. Unstained gels run in parallel were cut into 1-2 mm slices which were kept overnight at 50°C in Bray scintillation fluid containing 0.1 vol. NCS tissue solubilizer (Amersham/Searle, IL) and 0.02 vol. 4 M ammonia. The curves represent the distribution of protein-bound radioactivity before (——) and after (. . .) tryptic digestion.

units [14,15] has been proposed. From our results an alternate model may be suggested implying that the core is made up of 30 polypeptide chains whose 60 domains are arranged by trimer clustering at the 20 vertices of a pentagonal dodecahedron. This structure would be stereometrically comparable with the 60 subunit model proposed in [16]. It would also seem compatible with the pyruvate dehydrogenase subunit stoichiometry assuming that the 60 ($\alpha\beta$) chains [13] are bound as ($\alpha_2\beta_2$) tetramers to the interdomain regions of the acetyltransferase which may form the 30 edges of the pentagonal dodecahedron.

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