THE SUBUNIT STRUCTURE OF THE PYRUVATE DECARBOXYLASE OF *ESCHERICHIA COLI* K12

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1. Introduction

It has been shown that so called 0°-type mutations, which affect the synthesis of all three components (decarboxylase, lipoic reductase transacetylase and dihydrolipoic dehydrogenase) of the pyruvate dehydrogenase enzyme complex can be located in the structural gene of the decarboxylase component [1,2]. The polarity of these mutations is so strong that no enzyme can be detected by immunological or enzymical methods [3]. Two features are interesting: first these mutations are very common and comprise about 28% of 100 ace-mutants isolated and second they belong mainly to the am- or oc-type [4] of nonsense mutations. Preliminary mapping indicated that they are scattered over a large segment of the decarboxylase cistron. This finding differs from the situation in other systems where extremely polar mutations are restricted to small regions of the operon [5–7]. These observations led to the hypothesis that a segment of the decarboxylase gene codes for an activator-protein, which is also an integral part of the decarboxylase enzyme and is necessary for the synthesis of the whole pyruvate dehydrogenase complex [3,8]. As pyruvate seems to be the natural inducer of the pyruvate dehydrogenase [8] it was speculated that it activates the activator substance and thereby stimulates the synthesis of the three enzymes. One approach to the regulation problem is to analyse the subunit structure of the decarboxylase and to localize the 0°-type mutations in one of the subunit, if there are different ones. It is shown in this paper that the decarboxylase probably consists of two identical subunits.

2. Materials and methods

The enzymatic activity of the pyruvate dehydrogenase complex was assayed as described elsewhere [9]. The activity of the decarboxylase component was determined by adding the lipoic reductase transacetylase-dihydrolipoic dehydrogenase partial complex and assaying the overall reaction. Separation of the decarboxylase component from the complex was performed as described by Reed and Willms [9]. Acrylamide gel electrophoresis was done as described by Davis [10]. Some gels were prerun for 1–2 hr before use, without altering the results. Urea was included in both the stacking and the separation gels. Gels were always prerun for 2 hr for electrophoresis in mercaptoethanol, as this cannot be added to the gel.

3. Results

3.1. *Dissociation of the pyruvate decarboxylase*

The decarboxylase component separated from the purified dehydrogenase complex [11] showed no activity in the overall reaction and was therefore essentially free of the other two enzymes. The specific activities of the different decarboxylase preparations were about 1500. Sedimentation runs in the analytical ultracentrifuge indicated that these preparations consist of two components with different sedimentation constants (fig. 1). Both components could be separated preparatively by either sucrose gradient centrifugation

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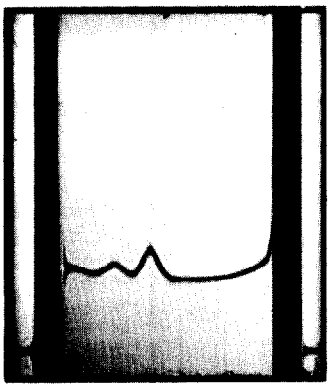

Fig. 1. Schlieren pattern of decarboxylase. The sample was run at a protein concentration of 2.8 mg/ml in 0.05 phosphate buffer pH 7 at 59, 780 rpm.

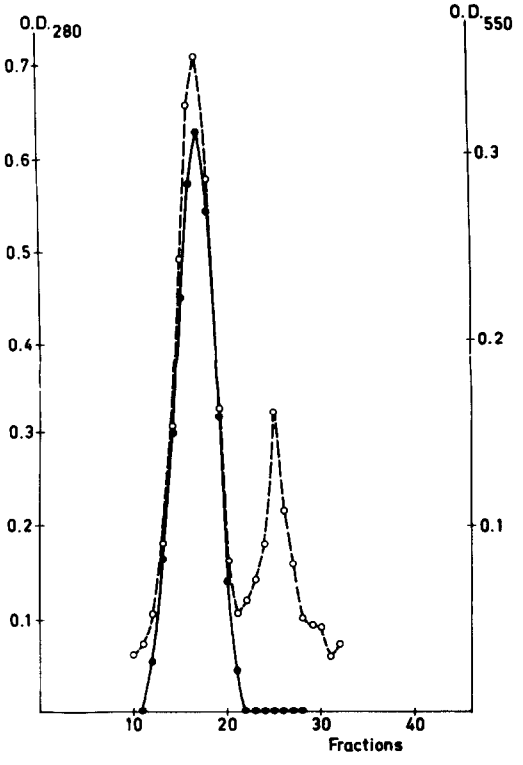

Fig. 2. Sucrose gradient centrifugation of decarboxylase 1.4 mg of decarboxylase was applied to a 5–20% linear sucrose gradient and centrifuged 5 hr at 50,000 rpm in a SW 50 Spinco rotor. 8 Drops were collected and assayed for absorption at 280 nm, ●—●; specific activity is plotted as OD_{550}, o - - - o.

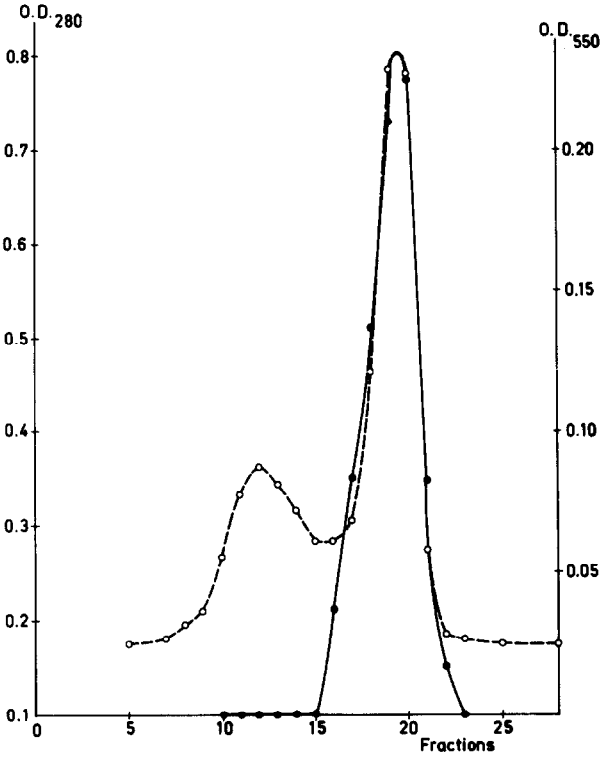

Fig. 3. Agarose electrophoresis of decarboxylase. 0.5 ml of decarboxylase (2.5 mg) was applied to the electrophoresis column stabilized with 0.14% agarose in 0.02 M phosphate buffer pH 7.5. Electrophoresis was run for 20 hr at 8 mA and 900 V. 0.6 ml Fractions were collected and assayed as in fig. 2.

or zone-electrophoresis on agarose columns [12]. Fig. 2 shows that the fast sedimenting component is enzymatically active. This component has also a faster electrophoretic mobility (fig. 3), The first indication that the slowly sedimenting component might be a subunit of the fast sedimenting one came from the observation that incubation of the decarboxylase for 3–5 hr at pH 9.5 resulted in the disappearance of all enzymatic activity. In such preparations, the fast sedimenting material decreased and the slow sedimenting material increased. This indicates that at high pH, the active decarboxylase dissociates into smaller inactive subunits. As the separation of the decarboxylase from the complex is done at pH 9.5, degraded material is always found depending on how long the enzyme is on the column. Acrylamide gel electrophoresis showed that both components consist of the same

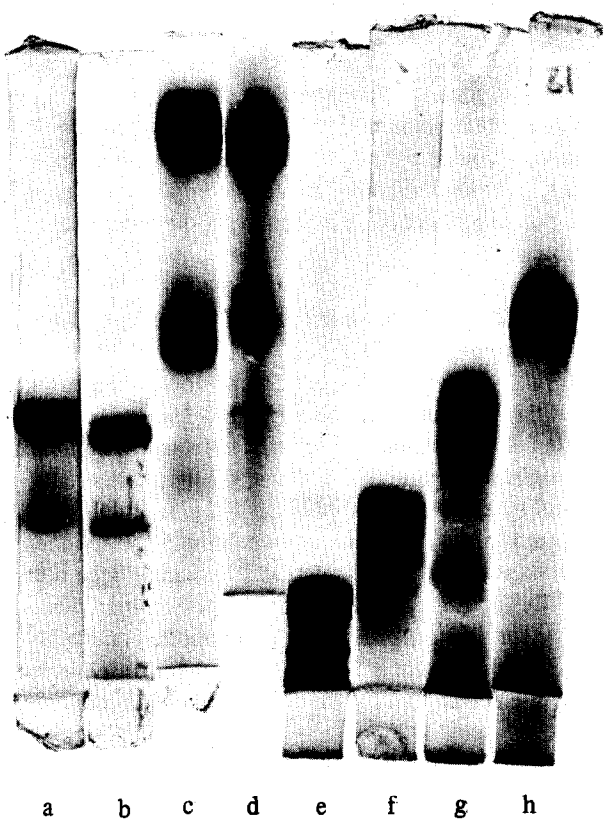

Fig. 4. Acrylamide gel electrophoreses of decarboxylase in 7% gel a–f and 4% gel g–h; gels a–d were run without urea, gels e, f, g in 5 M urea and gel h in 5 M urea. 0.3 Mercaptoethanol. All runs were performed at room temperature at 3 mA per tube. a. 40 μg decarboxylase, 2 hr; b. 50 μg dimer fraction from agarose electrophoresis, 2 hr; c. 70 μg dimer fraction from sucrose gradient, 4 hr; d. 35 μg monomer fraction from sucrose gradient, 4 hr; e. 60 μg decarboxylase in 7% gel 5 M urea, 6 hr; f. 60 μg decarboxylase in 7% gel 5 M urea, 18 hr; g. 60 μg decarboxylase in 4% gel 5 M urea, 4 hr; h. 60 μg decarboxylase in 4% gel 5 M urea, 0.3 M mercaptoethanol, 6 hr.

material. Fig. 4a shows an electrophoresis pattern of the original decarboxylase preparation. Fig. 4b and 4c show acrylamide gels of the active fraction from the sucrose-gradient centrifugation and agarose electrophoresis respectively. Fig. 4d shows the electrophoresis pattern of the inactive component.

All preparations separate into two bands. The obvious interpretation is that the fast sedimenting peak is a polymer of the slow sedimenting one and only the former is enzymatically active.

3.2. *Molecular weight and sedimentation constants of the monomer and dimer molecule*

To determine whether the decarboxylase is a dimer or a higher polymer, the sedimentation coefficients were determined at free sedimentation for both components at different protein concentrations and extrapolated to infinite dilution. For the first peak $S_{20,w}$ = 9.6×10^{-13} sec and for the second peak $S_{20,w}$ = 5.6×10^{-13} sec. Assuming that both molecules are perfect spheres and assuming that the partial specific volume of the protein is 0.75 ml/g, the molecular weight can be calculated from Stoke's law. After correction for the hydration of the molecule assumed to be S_1 = 0.2 g/g, the molecular weights were estimated to be 175,000 and 76,000 respectively. The molecular weights of the two components were also estimated by gel filtration on a calibrated Sephadex G-200 column [13]. Fig. 5 shows that the ratio of the elution volumes for the first peak is V/Vo = 1.56 corresponding to a molecular weight of 190,000 and that of the second peak is V/Vo = 1.71 corresponding to a molecular weight of 140,000. The data from ultracentrifugation suggests that the active decarboxylase is a dimer if the inactive subunit is the monomer. The similar molecular weights of 176,000 and 190,000 estimated by both procedures would also indicate that the dimer molecule has a more or less spherical shape. The discrepancy of the molecular weights of the monomer subunit 79,000 and 140,000 respectively is unexplained. One possibility is that the monomer is an elongated

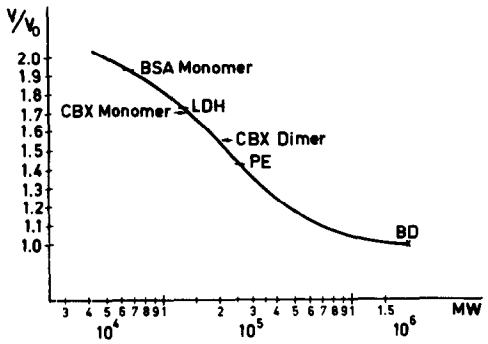

Fig. 5. Log-linear plot of the ratio of elution volumes (V/Vo) versus molecular weight of different proteins: BSA monomer, Bovine serum albumin monomer; LDH, Lactate dehydrogenase, PE, Phycoerythrin; CBX monomer and CBX dimer; decarboxylase monomer and dimer.

molecule. The molecular weights of the dimer correspond perfectly to the value reported by Reed and Willms [9] of 184,000 by centrifugation ($S_{20,w}$ = 9.2 X 10^{-13} sec) and diffusion experiments.

3.3. *Subunit structure of the monomer component*

Disc electrophoresis of dimer plus monomer fractions (figs. 4b, c, d) always gives two bands. The relative amounts of both are not constant in different runs. Several explanations are possible, for example: 1) One band could represents the native, protein and the other the denatured protein 2) One band could be a monomer and the other an aggregation product of it.

To decide between these possibilities, the preparations were run in 5 M urea on acrylamide gels. Surprisingly, at least 6 bands were be seen on 7% as well as on 4% gels (figs. 4e, f, g). If the decarboxylase is completely denatured by dissolution in 10 M urea and 0.3 M mercaptoethanol and the electrophoretic run performed in 5 M urea with 0.3 M mercaptoethanol, only one band, the fast moving band, can be seen (fig. 4h). This indicates, that the different slow moving bands on the 5 M urea gels are aggregation products, possibly mixed disulfides, which can be dissociated by mercaptoethanol. Furthermore, this finding shows that the slow moving band in figs. 4a, b, c, d is probably also an aggregation product. Since the dimer as well as the monomer molecule show only the fast moving band if completely denatured, it is concluded, that the decarboxylase consits of only one type of polypeptide chain, identical with the monomer subunit.

3.4. *Amino acid composition of the decarboxylase and end group analyses*

Table 1 shows the amino composition of the decarboxylase. Values in column 1, 2 and 3 represent the data for the decarboxylase isolated from the complex, as well as the monomer and the dimer-fraction separated by agarose electrophoresis [12]. In column 4, average values are given and in column 5 the residues per 90,000 daltons are calculated. It can be seen that the Cys-content of the molecule is rather low. The value for ammonia suggests that about half of the Glu and Asp found are amides. This would correspond to the isoelectric point of pH 6.5 roughly estimated by

Table 1
Amino acid analyses.

	Protein (μmole/mg)				Residues 90,000 daltons
	Decarboxylase unseparated	Decarboxylase monomer	Decarboxylase dimer	Average value	
Try	0.1138	0.0941	0.1197	0.1092	9.8
Lys	0.4672	0.4806	0.5058	0.4845	43.6
His	0.1883	0.2391	0.2418	0.2231	20.0
NH_4	0.1057	–	0.9731	1.0150	91.4
Arg	0.4356	0.4078	0.4423	0.4286	38.6
Cys *ac*	–	0.0411	0.0676	0.0676	6.1
Asp	0.9148	0.9631	0.9680	0.9486	85.4
Met	0.1755	0.1031	0.1610	0.1465	13.2
Thr	0.4088	0.4727	0.4576	0.4464	40.2
Ser	0.4853	0.5304	0.4963	0.5040	45.4
Glu	1.1157	1.155	1.1681	1.1463	103.2
Pro	0.4364	0.4288	0.3465	0.4039	36.4
Gly	0.7987	0.7694	0.8587	0.8089	72.8
Ala	0.8088	0.7702	0.7763	0.7851	70.7
Val	0.5234	0.5090	0.5014	0.5112	46.0
Ile	0.5279	0.5549	0.5502	0.5443	49.0
Leu	0.6674	0.7434	0.7256	0.7121	64.1
Tyr	0.3599	0.3063	0.3786	0.3483	31.4
Phe	0.3158	0.3343	0.3274	0.3258	29.3

isoelectric focussing on acrylamide gels [14]. The Tyr/Try ratio was determined by measuring the ultraviolet spectrum in 0.1 M alkali and calculation was done according to Bencze and Schmidt [15]. The ratio was found to be Tyr/Try = 1.63. The ultraviolet absorption maximum was at 278 nm. Qualitative end group analysis was done by the dansylation-method of Gray and Hartley as described by Gros and Labouesse [16]. 5 nmoles of decarboxylase were dansylated for 30 min at 20°C, precipitated with 10% trichloroacetic acid and the precipitate washed with 1 M HCl. Hydrolyses were performed at 110°C for 4 hr. Aliquots of 0.025 nmole decarboxylase were applied to polyamide-sheets and run as described by Woods and Wang [17]. In preparations of both the monomer and the dimer, only serine was found, indicating that serine is the *N*-terminal amino acid. Quantitative analysis was performed by the carbamylation procedure as described by Stark [18]. 10 mg decarboxylase were carbamylated and 10 mg were used as blank. The yield of serine was 30% in this procedure. 2.11 serine residues per 180,000 daltons were found.

5. Discussion

Both the monomer and the dimer decarboxylase bind to the lipoic reductase transacetylase-dihydrolipoic dehydrogenase partial complex (Dennert, unpublished results). It is shown here that only the dimer decarboxylase molecule can recombine with the other two enzymes to stimulate the overall enzymatic reaction. Acrylamide gel-electrophoreses as well as amino acid-analyses show that monomer and dimer consist of the same material, i.e. the two subunits are identical. Electrophoretic runs in 5 M urea and 0.3 M mercaptoethanol show only one band which indicates that the decarboxylase consists of only one type of polypeptide chain, which seems to be identical with the monomer. That only one serine residue per 90,000 daltons is found in quantitative end group determinations further supports this interpretation. It cannot be completely excluded that the two peptide chains, although having the same *N*-terminus and the same electrophoretic mobility, are not identical. Some differences in amino acid composition would not be detected in such a large polypeptide by these methods. It is therefore only probable that the two chains are identical. These results do not support the model drawn from genetic data [3, 8] that perhaps one subunit of the decarboxylase plays some role in the regulation of the pyruvate dehydrogenase. This finding and the lack of regulatory mutants leaves the mechanism of regulation of this enzyme open to discussion.

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