SUBUNIT SIZE OF CYTOPLASMIC YEAST PYRUVATE DECARBOXYLASE

Johannes ULLRICH and Manfred KEMPFLE
Biochemisches Institut der Universität Freiburg, and Physiologisch-Chemisches Institut der Universität Bonn, Germany

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1. Introduction

From the number of thiamine pyrophosphate (TPP) binding sites estimated earlier [1], cytoplasmic yeast pyruvate decarboxylase (PDC) (EC 4.1.1.1) was hitherto expected to contain 4 polypeptide chains of a molecular weight of 40,000–45,000, each of them carrying one active site. For the decarboxylase moiety in the pyruvate dehydrogenase complex of *E. coli*, Reed et al. found a similar molecular weight of the intact enzyme [2] and an $\alpha,\alpha,\beta,\beta$-structure [3]. In contrast, cytoplasmic yeast pyruvate decarboxylase was found to be separable into only two polypeptide chains with a molecular weight of circa 90,000 each, which are probably identical.

2. Materials and methods

Pyruvate decarboxylase was prepared as essentially described [1] from dried brewer's yeast (*Saccharomyces carlsbergensis*, Weihenstephan strain T 34) which was a gift of Ganter-Brauerei, Freiburg. The sample used here had a specific activity of 46 units/mg at TPP and Mg^{++} saturation, pH 6.0, and 30° [1] and contained less than 5% of impurities. Guanidine hydrochloride was purchased from Sigma Chemical Company, St. Louis, Mo., USA; all other chemicals were products of E.Merck A.G., Darmstadt, or Riedel de Haën, Seelze.

Denaturation and separation into subunits were achieved by treatment of the enzyme with 6 M guanidinium chloride for 2–4 days at room temperature. For ensuring complete cleavage of disulfide bridges, 10 mM or 100 mM β-mercaptoethanol was added to several samples. The values for specific gravity (1.143) and viscosity (1.63 × viscosity of water) of the 6 M guanidinium chloride solution were taken from [4] and the same values used for the solutions containing mercaptoethanol. The partial specific volume of PDC was calculated [5,6] to be 0.742 from the amino-acid composition [7]. The value obtained was assumed to be true for solutions in diluted buffer as well as in 6 M guanidinium chloride [8,9] and used for all the evaluations. For the determination of the molecular weights of native and denatured pyruvate decarboxylase, a Phywe model U 60 L analytical ultracentrifuge was used.

Sedimentation runs were performed at 50,000 rpm (180,000 × *g*), 20°, 12 mm light path, and protein concentrations of 0.2–3.0 mg/ml in 50 mM phosphate buffer, pH 6.8, using the UV scanner and the Schlieren diaphragm. Diffusion measurements were performed in a synthetic boundary cell [10] under otherwise the same conditions. In this cell, the enzyme solution was layered underneath buffer or a less concentrated enzyme solution during the initial acceleration of the centrifuge.

Sedimentation equilibrium runs were performed in a cesium chloride density gradient at 25° and 50,000 rpm for 14–16 hr, using initial concentrations of 2.5 M CsCl and 0.26 mg/ml protein in 50 mM phosphate, pH 6.8. The 253.7 nm scanning curves of these runs were evaluated by the method described by Dirkx [11] and by Kempfle [12]. The denatured enzyme could not be measured by this method because of high content of UV-absorbing impurities in the guanidine hydrochloride used. For the denatured enzyme, Archibald runs in the transient state [13] were performed at 15,000 rpm (17,000 × *g*), 20° and 0.5–3.0 mg/ml protein in 50 mM phosphate and 6 M guanidinium chloride pH 6.8 and evaluated as described [9].

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Table 1
Sedimentation and diffusion constants and molecular weights of natuve and denatured yeast pyruvate decarboxylase (conditions are given in the text).

Guanidine-HCl	Mercaptoethanol	Protein mg/ml	No. of runs	$S^0_{20} \times 10^{13}$	$D_0 \times 10^7$	Molecular weight
–	–	0.5–3.0	2 X 4*	9.95†	5.55	170,200††
6 M	–	0.5–2.5	2 X 4**	1.31	2.30	91,800†††
6 M	10 mM	0.5–2.5	4**	1.37	2.46	89,500
6 M	100 mM	0.5–2.5	3**	1.37	2.54	86,800

* Sedimentation starting with the uniformly filled cell and with 2 layers in a synthetic boundary cell.
** Sedimentation in a synthetic boundary cell only.
† The same value had been found earlier [1].
†† CsCl density gradient centrifugation gave M = 176,500.
††† Archibald method gave M_{app} = 13,700 and M = 84,500.

In 20 mM sodium dodecyl sulfate as denaturing agent at pH 7, in the absence or presence of β-mercaptoethanol, the sedimentation of pyruvate decarboxylase could not be measured because of the formation of detergent micells migrating in the gravity field and disturbing the picture.

3. Results and discussion

The data obtained are compiled in table 1. The molecular weight determination of the intact protein confirms the earlier measurements by sucrose density gradient centrifugation with internal reference standards of known molecular weights [1]. A moderate concentration dependence of the sedimentation constant found in the experiments suggests that the enzyme molecule is somewhat different from an exactly globular shape. Denaturation in 6 M guanidinium chloride results in the formation of subunits which migrate in the ultracentrifuge as a single and symmetric peak with a much lower sedimentation coefficient and a somewhat lower diffusion coefficient. Their molecular weight was found to be half that of the intact protein. This means that yeast pyruvate decarboxylase consists of two subunits instead of the expected four. These polypeptide chains must contain 770–780 amino-acyl residues and seem to be at least identical in size. Experiments to prove their identity in structure and to better elucidate their nature are in progress.

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