

On the origin of mitochondria: a reexamination of the molecular structure and kinetic properties of pyruvate dehydrogenase complex from brewer's yeast

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Received 9 July 1982

Mitochondria, origin of

Pyruvate dehydrogenase complex, type II
Endosymbiosis (Brewer's yeast)

Gram-positive bacteria

1. INTRODUCTION

Pyruvate dehydrogenase complex (PDC), consisting of the three enzyme components pyruvate dehydrogenase (E1), lipoate acetyltransferase (E2), and lipoamide dehydrogenase (E3), has been isolated from many organisms (review [1]). In the course of these studies, it has increasingly become clear that there are principally two structural types of PDC: one type (type I) found in Gram-negative bacteria, and another type (type II) present in mammalian mitochondria. The main distinctive features are: in type I complexes, the E2 core is built from 24 subunits with octahedral symmetry [1,2] whereas in type II complexes, E2 forms an icosahedral core with presumably 60 subunits [1,3]; E1, in type I PDC, possesses one large subunit (M_r 89 000–100 000) [1,4,5], but in type II complexes, two essential subunits of E1 (α , M_r 40 000–43 000; β , M_r 36 000–38 000) are present [1,3]. Finally, type II complexes generally are larger (M_r 7–9 $\times$ 10⁶ [1,6]) than type I complexes although there is considerable uncertainty about the M_r values and

their significance [7,8]. Further differences were found in the kinetic and regulatory properties of the two PDC types.

Eukaryotic mitochondria, according to the endosymbiont hypothesis [9,19], are thought to have arisen from prokaryotes which established an endosymbiotic relationship within another 'proto-eukaryotic' cell. Based on the observation that Gram-positive bacteria such as *Bacillus stearothermophilus* [11,12] and *Bacillus subtilis* [13] turned out to have pyruvate dehydrogenase complexes which are structurally similar to type II but not type I PDC, Henderson et al. [11] proposed that the forerunner of mitochondria may have possessed Gram-positive rather than Gram-negative characteristics. This hypothesis is in agreement with comparative structural studies of other enzymes such as citrate synthase and succinate thio-kinase [14], and was further supported when the structure of PDC from the lower eukaryote, baker's yeast (*Saccharomyces cerevisiae*) was found to be quite similar to that reported for *Bacillus* PDC [15,16]. Surprisingly, however, PDC from brewer's yeast (*Saccharomyces carlsbergensis*) had been reported in a previous study [17] to possess one large E1 chain as type I complexes as well as a number of kinetic properties which differed from the baker's yeast complex but were similar to type I complexes. This paper reports a reexamination of the subunit M_r values and kinetic properties of PDC from brewer's yeast. The properties of this complex were found to be essentially analogous to PDC from baker's yeast.

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Abbreviations: PDC, pyruvate dehydrogenase complex, consisting of pyruvate dehydrogenase (E1) (EC 1.2.4.1), lipoate acetyltransferase (E2) (EC 2.3.1.12), and lipoamide dehydrogenase (E3) (EC 1.6.4.3); SDS, sodium dodecylsulfate; M_r , relative molecular mass

2. EXPERIMENTAL

Bottom-fermenting brewer's yeast (*S. carlsbergensis*) was a gift from Löwenbräu AG, München, FRG. PDC was purified from brewer's yeast in the presence of the protease inhibitors phenylmethanesulfonyl fluoride, leupeptin and pepstatin A (Peptide Institute, Osaka, Japan) as described in [15] for the baker's yeast enzyme with the following modifications. After the protamine sulfate fractionation step, the supernatant (containing PDC) was directly subjected to ultracentrifugation without prior isoelectric precipitation. The complex was then further purified by two successive ultracentrifugations (4.5 h at $153\,000 \times g_{\max}$), sucrose density gradient centrifugation, and a third ultracentrifugation as above [15]. Starting from 500 g (wet weight) of yeast, about 11 mg PDC were obtained (specific activity up to 11 units/mg; yield as referred to the first poly(ethylene glycol) supernatant 20–25%).

Lipoamide dehydrogenase (E3) was purified from heat-treated homogenates of brewer's yeast by the method of [18] with some modifications (P. Heinrich and G.-B. Kresze, unpublished results). From 500 g (wet weight) of yeast, 4–5 mg of E3 were obtained (specific activity, 450–740 units/mg; yield 48–62%).

PDC and E3 were assayed at 30°C under the conditions given in [15]. Substrate concentrations were determined and kinetic data were analyzed as in [15].

Dodecylsulfate gel electrophoresis was performed in slab gels containing 12.5% (v/v) acrylamide in the system of Laemmli [19]. Elastase treatment of PDC was done under the conditions described in [16].

3. RESULTS

3.1. Subunit M_r values

Figure 1 compares the dodecylsulfate gel pattern of PDC from brewer's yeast (lane 4) with the complex from baker's yeast (lane 2). Although the *S. carlsbergensis* complex was not free from impurities, it is clear that the enzyme shows essentially the same gel pattern as the *S. cerevisiae* complex. This is also evident from the sample in lane 3

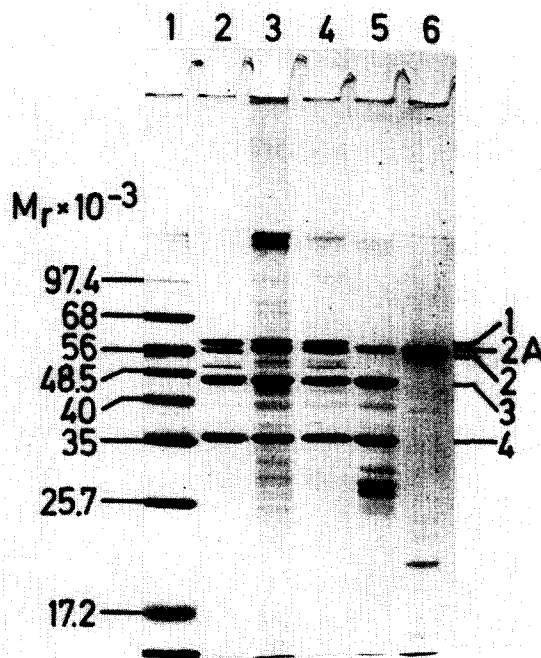


Fig.1. Dodecylsulfate gel pattern of pyruvate dehydrogenase complex. Lane 1, M_r marker proteins [15]; lane 2, PDC from baker's yeast [15]; lane 3, PDC from brewer's yeast before sucrose density gradient centrifugation (spec. act. 3.6 units/mg); lane 4, PDC from brewer's yeast (spec. act. 10.7 units/mg); lane 5, brewer's yeast PDC after treatment with elastase (28 μ g elastase/ml, 30 min at 22°C; residual PDC activity 0.15%); lane 6, lipoamide dehydrogenase from brewer's yeast (spec. act. 735 units/mg).

representing brewer's yeast PDC in an earlier stage of the purification (after the second ultracentrifugation step) where the total yield, starting from the first poly(ethylene glycol) precipitate, was 45%. This high yield excludes the possibility that the four-banded complex seen in lane 4 was a minor component purified from any material contaminating the commercial yeast used in this study. Apparent M_r values for the four bands were: band 1, 58 000; band 2, 56 000; band 3, 45 000; and band 4, 35 000.

To examine whether the enzyme components can be assigned to the same gel bands as in PDC from baker's yeast, the gel pattern of brewer's yeast PDC was compared with elastase-treated complex (lane 5) and with E3 isolated from heat-

Table 1
Kinetic properties of pyruvate dehydrogenase complexes from yeast

		<i>S. carlsbergensis</i> PDC	<i>S. carlsbergensis</i> PDC [17]	<i>S. cerevisiae</i> PDC [15]
Pyruvate	K_m	250 μ M (pH 8.1) 180 μ M (pH 6.5)	290 μ M (pH 7.5)	650 μ M (pH 8.1) 130 μ M (pH 6.5)
	Hill coefficient:	1.01 (pH 8.1)	n.d.	0.97 (pH 8.1)
Coenzyme A Acetyl-CoA	K_m	11 μ M	6.7 μ M	14 μ M
	K_i	39 μ M	2.1 μ M	18 μ M
	Type of inhibition:	Competitive with CoA; Uncompetitive with pyruvate	Competitive with pyruvate	Competitive with CoA; Uncompetitive with pyruvate
NAD ⁺ NADH	K_m	104 μ M	250 μ M	72 μ M
	K_i	54 μ M	70 μ M	23 μ M
	Type of inhibition:	Competitive with NAD ⁺	Competitive with NAD ⁺	Competitive with NAD ⁺

treated homogenates of brewer's yeast (lane 6). In the pyruvate dehydrogenase complexes of baker's yeast [16] and bovine kidney [20], elastase treatment cleaves the E2 component while leaving the other bands unaffected. Evidently, band 1 corresponds to the E2 component. Furthermore, comparison of lanes 4 and 6 identifies band 2 as the E3 component. These assignments agree with those found for PDC from baker's yeast [16].

In contrast to the baker's yeast enzymes, all our preparations of PDC and E3 from brewer's yeast contained a protein with apparent M_r 57 000 (band 2A in fig.1) in SDS gel electrophoresis which appears either to bind to E3 or to have very similar properties as E3 since it was not removed during purification of either PDC or E3 although these enzymes were isolated by very different procedures. The isolation method used for E3 gives a single band when applied to baker's yeast (P. Heinrich and G.-B. Kresze, unpublished results). Lipoamide dehydrogenases from all sources so far investigated [21] are thought to possess two identical subunits, so it appears unlikely that band 2A represents a second subunit of E3. However, the identity and function (if any) of band 2A protein are as yet unknown. Apart from this band, the subunit M_r values of PDC from brewer's yeast, in contradiction to [17], were found to be identical with those of baker's yeast PDC.

3.2. Kinetic and regulatory properties

A number of kinetic properties of brewer's yeast PDC are summarized in table 1 and compared with the values found for baker's yeast PDC [15] and with those reported previously for the brewer's yeast complex [17]. It can be seen that the properties of the *S. carlsbergensis* complex are essentially similar to those of the *S. cerevisiae* enzyme. In contrast to the results reported in [17], acetyl-CoA was found to be a competitive inhibitor with respect to CoA but not pyruvate. This is analogous to type II complexes but differs from type I PDC. Furthermore, pyruvate gave normal (hyperbolic) saturation kinetics (Hill coefficient $h = 1.01$) just as in the *S. cerevisiae* [15] and bovine kidney [22] complexes but differently from *E. coli* PDC [22] where a positive homotropic effect of pyruvate was reported. K_m for pyruvate was lower at pH 6.5 than at pH 8.1 although at pH 6.5 V_{max} was only 48% of the value determined at pH 8.1. This again agrees with the behaviour of other 2-oxoacid dehydrogenase complexes [15,23].

Brewer's yeast PDC was not inactivated when incubated with ATP (conditions: 0.5 mM ATP; 1.25 mM Mg^{2+} ; pH 7.5; 60 min at 23°C in the presence or absence of 25 mM NaF). This agrees with earlier results obtained with the pyruvate dehydrogenase complexes of *S. carlsbergensis* [17], *S. cerevisiae* [15], and *B. stearothermophilus* [12].

4. DISCUSSION

PDC from brewer's yeast was found to possess essentially analogous structural, kinetic, and regulatory properties as PDC from baker's yeast. This lends further support to the hypothesis of Henderson et al. [11] that the structural resemblance of type II PDC with the complexes from Gram-positive bacteria (as well as lower eukaryotes) may indicate that the 'protomitochondrion' has evolved from a Gram-positive rather than a Gram-negative bacterium. It has been reported earlier [24] that *S. carlsbergensis* PDC possesses an icosahedral E2 core as type II complexes.

The reasons for the differences between our results and those of [17] concerning the subunit M_r values as well as some regulatory properties of the brewer's yeast complex are not clear. However, if one disregards the possibility that these major differences may result from the use of different *S. carlsbergensis* strains, one should be aware that in the earlier work [17] specific protease inhibitors such as leupeptin and pepstatin A were not available. Since pyruvate dehydrogenase complexes are very sensitive against proteolysis [15,20] the complex was obtained with only moderate specific activity (6.15 units/mg), very low yield (5%), and high instability in [17]. Thus, some of the earlier results may be caused by artefacts due to proteolytic degradation.

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft, Bonn, FRG.

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