REDETERMINATION OF THE MOLECULAR WEIGHTS OF THE COMPONENTS OF THE PYRUVATE DEHYDROGENASE COMPLEX FROM E. COLI K12[+]

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SUMMARY

The validity of molecular weight determination in SDS-polyacrylamide gels for the three components of the pyruvate dehydrogenase complex: pyruvate dehydrogenase, dihydrolipoamide transacetylase, and dihydrolipoamide dehydrogenase has been checked by measuring their free electrophoretic mobilities and their retardation coefficients. A linear relationship between these parameters has been found for all three enzymes as compared with standard proteins. This substantiates earlier molecular weight determinations in SDS-polyacrylamide gels for the components of the pyruvate dehydrogenase complex which are confirmed by this study for different acrylamide gel concentrations.

INTRODUCTION

The molecular composition of a multienzyme complex is given by the molecular weight of the complex as a whole and by the number ratio and the molecular weights of the components' polypeptide chains. We have shown that for a "core" pyruvate dehydrogenase complex (PDHC) from Escherichia coli the ratio of the polypeptide chains of the components is unity (1). We have also reported that their molecular weights as determined by SDS-polyacrylamide gel electrophoresis (2) are 100 000 daltons for the pyruvate dehydrogenase (3), 80 000 daltons for the dihydrolipoamide transacetylase

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("transacetylase") (4), and 56 000 daltons for the dihydrolipoamide dehydrogenase ("flavoprotein") (5). These values have independently also been found by Perham & Thomas (6). Others (7), using different methods, have reported somewhat lower values.

The exact molecular weights of the monomers of the components are crucial for the evaluation of their ratio in the PDHC. It has become clear that molecular weight determination on SDS-polyacrylamide gels is reliable only for polypeptides which exhibit a linear relationship between free electrophoretic mobility and retardation coefficient as compared with standard proteins used for the calibration curve (8).

To reexamine our earlier molecular weight determinations, I have determined the change of these parameters in different polyacrylamide gel concentrations for the components of the PDHC as compared with standard proteins.

MATERIALS AND METHODS

PDHC was prepared from E. coli K1-1 LR 8-16 (9) as described elsewhere (4).

Myoglobin, ovalbumin, bovine serum albumin were purchased as protein markers from Schwarz/Mann, Orangeburg, N.Y., USA; lactate dehydrogenase, phosphorylase a, and ß-galactosidase came from Boehringer, Mannheim. Acrylamide, N,N'-methylene bisacrylamide, sodium dodecyl sulfate came from BDH Chemicals Ltd, Poole, England. Coomassie brilliant blue came from Schwarz/Mann, Orangeburg, N.Y., USA.

The molecular weight determinations were carried out in SDS polyacrylamide slab gels (10), following the method of Weber and Osborn (2). The well had a cross section area of 1.5 x 6 mm. Comparability between different samples is much improved by using slab gels instead of tube gels.

The samples were dissolved in 4% SDS, 10 mM dithiothreitol, 1 mM EDTA, and 10 mM sodium phosphate buffer pH 7.0. Gels were prepared from a stock solution which contained acrylamide and N,N'-methylene bisacrylamide in a ratio of C[1] = 2.63. The dilutions were such as to give acrylamide-bisacrylamide concentrations of T = 3.081, 4.108, 5.135, 6.162, and 7.189. The buffer concentration in the gels was 0.1 M sodium phosphate buffer pH 7.0 containing 1% SDS. 50 mM sodium phosphate buffer pH 7.0 with 0.1% SDS was used as electrophoresis buffer.

To all protein samples, myoglobin was added as front marker. Aliquots of the samples containing 6 µg protein were applied per well. Electrophoresis runs were performed at 4°C and 50 mA per slab gel (1.5 x 145 mm across), until the bromophenolblue marker had migrated about 10 cm towards the cathode. The mobilities of the polypeptide chains were expressed relative to myoglobin.

RESULTS AND DISCUSSION

Our earlier molecular weight determinations of the monomers of the components of the PDHC were based on calibration curves with marker proteins of known molecular weights which show a linear relationship between free electrophoretic mobility (M_o) and retardation coefficient (K_R) (8). To determine these parameters for the three components of the PDHC, their mobility in comparison with marker proteins (Table 1) was examined for different gel concentrations ranging from T = 3.081 to T = 7.189 (see methods). Fig. 1 shows the R_F values for the protein-SDS complexes from these experiments plotted against increasing gel concentrations. The relationship is linear for all proteins within the range of chosen gel concentrations.

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[1] C = 100 x g bisacrylamide/T; T = g acrylamide + g of N,N'-methylene-bisacrylamide per 100 ml solution, employing the terminology of Hjerten (11) as proposed by Banker & Cotman (8).

TABLE 1. List of proteins and their molecular weights used in this study.

No.	Protein	mol.weight	Ref.
1	myoglobin	17 800	a
2	lactate dehydrogenase	36 000	cf 2
3	ovalbumin	45 000	a
4	bovine serum albumin	67 000	a
5	phosphorylase a	95 000	a
6	ß-galactosidase	130 000	cf 2
7	dihydrolipoamide dehydrogenase	56 000	5
8	dihydrolipoamide transacetylase	80 000	4
9	pyruvate dehydrogenase	100 000	3

a) Values given by the supplier, determined by gel filtration.

The free electrophoretic mobilities (intercept at T = 0) and the corresponding retardation coefficients (slopes of the curves) for the different proteins can be taken from Fig. 1. The relationship of both parameters, as shown in Fig. 2, is linear for all tested proteins i.e. the components of the PDHC form "normal" SDS-complexes with respect to their molecular weights.

The linear relationship between molecular weight, free electrophoretic mobility and retardation coefficient (8) implies that the apparent molecular weights at different gel concentrations are constant. This is shown for the three components of the PDHC in Fig. 3. The average molecular weights from these plots are

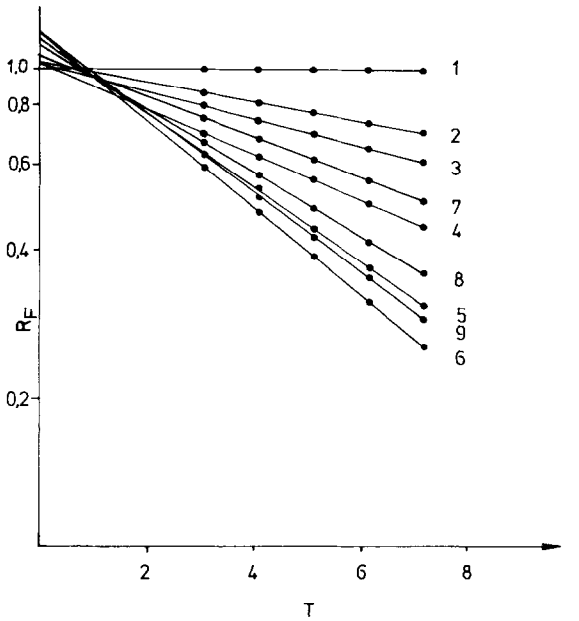

Fig. 1 Ferguson plot of marker proteins and the components of the PDHC as listed in Table 1. Within the chosen gel concentrations the relationship is linear in all cases. Each point represents the mean of four R_F values.

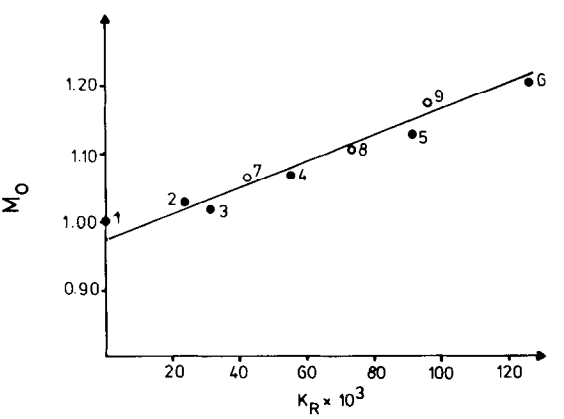

Fig. 2 Relationship between free electrophoretic mobility (M_O) and retardation coefficient (K_R) of the polypeptide chains listed in Table 1. The M_O and K_R values are calculated from the corresponding points in Fig. 1 by the least square method.

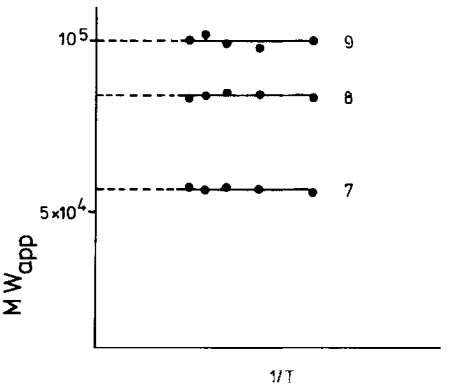

Fig. 3 Dependence of the apparent molecular weights on the gel concentration for the components of the PDHC. The MW_o (intercept at 1/T=o) for the pyruvate dehydrogenase is 100 000 daltons, for the transacetylase 83 000 daltons, and for the flavoprotein 56 500 daltons.

100 000 daltons for the pyruvate dehydrogenase, 83 000 for the transacetylase and 56 500 for the flavoprotein.

The data presented here prove the validity of the molecular weights of the PDHC components as determined by us and others (3-6) on SDS-polyacrylamide gels because all components behave normal as compared to standard proteins. Eley et al. (7) have found a somewhat lower molecular weight for the transacetylase by sedimentation equilibrium studies in 5 M guanidinium chloride. This lower value might reflect a partial heterogeneity of their transacetylase preparations caused by a small percentage of fragmented material. Furthermore, as also pointed out by these authors, the $\bar{v}$ value, which is difficult to approximate in 5 M guanidinium chloride, may be a source of error.

We know that the ratio of the polypeptide chains in the "core" complex (12) is unity. Although this composition can be found

"in vivo", too (1), a varying excess of pyruvate dehydrogenase seems more usual "in vivo" (12,13). Studies are in progress to eludicate the conditions which might influence the "in vivo" composition of the complex.

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