MOLECULAR PARAMETERS OF THE BEEF HEART SUCCINATE DEHYDROGENASE*

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Received 11 January 1971

1. Introduction

Succinate dehydrogenase (succinate:(acceptor) oxidoreductase, EC 1.3.99.1) is quite well characterized in its catalytic behaviour, but some of its fundamental parameters such as molecular weight, composition, subunit number etc. are still unsettled. So far the molecular weight has been estimated from the ratio of flavin to protein and values reported in the literature range from 220,000 [1] to 120,000 daltons [2].

The difficulty in obtaining homogeneous preparations of the enzyme has, until recently, delayed progress in this field. In our laboratory we obtained a highly purified preparation of the flavoprotein [3] and were thus able to investigate the molecular properties of the enzyme. We thus gained some new insight on its substructure and molecular weight. A report of these results is given here.

2. Materials and methods

Acrylamide and methylene-bisacrylamide were from Bio-Rad, *N, N, N′, N′*-tetramethylelethylenediamine and β-mercaptoethanol from Eastman, bromophenol blue, Coomassie brilliant blue-R-250 and urea from Mann, sodium dodecyl sulfate (SDS) (specially pure) from B.D.H.

Ribonuclease A, chymotrypsinogen A and ovalbumin were from Pharmacia, bovine serum albumin (BSA), cytochrome *c* and catalase from Sigma, aldolase was from Boehringer, human haemoglobin was a gift from Dr. Luigi Rossi Bernardi.

Succinate dehydrogenase was purified from beef heart as described elsewhere [3]. The preparation contains 7–8 nmoles of peptide bound flavin per mg protein and is reconstitutively active.

Iron was determined by the dipyridyl method [4] and labile sulfide was assayed by the procedure of Fogo and Popowsky [5] as modified by Brumby et al. [6].

Conditions for SDS electrophoresis were those of Dunker and Rueckert [7] with the following modifications [8]: the unknown and the standards were run side by side on a gel slab. At the end of the run the distance travelled by each polypeptide was measured and was plotted against the logarithm of the molecular weight [9].

Ultracentrifugation on a density gradient for approximate molecular weight determination was done according to Martin and Ames [10]. Succinate dehydrogenase and the standard proteins used catalase, aldolase and haemoglobin, (approximately 400 μg each, in 0.1 ml) were layered on the top of each centrifuge tube and spun in the conditions given in the legend to fig. 3. At the end of the run 48 fractions of 4 drops each were collected from each tube.

Succinate dehydrogenase was determined with the spectrophotometric phenazine methosulphate assay, as described earlier [3], catalase was monitored with an oxygen electrode [11], aldolase was assayed using the Boehringer UV test kit and haemoglobin was measured spectrophotometrically at 540 nm.

3. Results and discussion

A preparation of succinate dehydrogenase, homogeneous in the analytical ultracentrifuge, and which on gel electrofocusing was found free of any detect-

* Part IV.

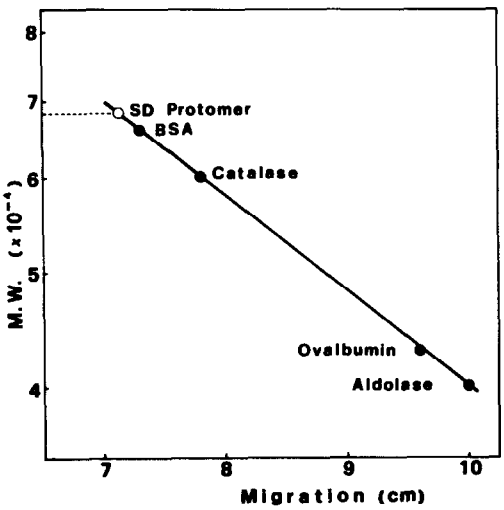

Fig. 1. Molecular weight determination of the heavier protomer of succinate dehydrogenase (SD). The enzyme and the standards were denatured in SDS and approximately 40 μg of each were layered in the pockets of a 5% polyacrylamide gel slab. The electrophoresis was run for 12 hr at 200 mA, at 10°, in a chamber from E-C Apparatus Corp. (Philadelphia). Staining and destaining were performed according to [7]. *Abscissa*: distance travelled from the origin.

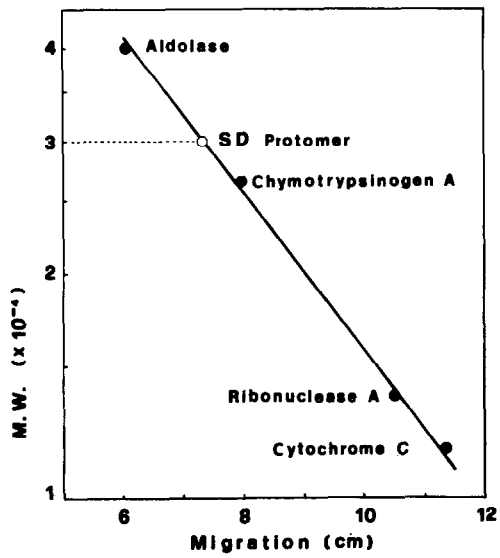

Fig. 2. Molecular weight determination of the lighter protomer of succinate dehydrogenase (SD). The electrophoresis was run in a 10% polyacrylamide gel slab using the procedure detailed in the legend to fig. 1.

able impurity, exhibited on SDS electrophoresis two bands corresponding to polypeptide chains of MW 68,500 and 30,000 daltons. This result was routinely confirmed. In order to get accurate estimates of MW, the samples were analysed on 5% polyacrylamide gels with standards ranging from 40,000 to 70,000 daltons and on 10% polyacrylamide gels with standards ranging from 10,000 to 40,000 daltons. The MW was found to be 68,500 ± 1,000 daltons for the heavier chain from succinate dehydrogenase (fig. 1) and 30,000 ± 500 daltons for the lighter one (fig. 2).

The polypeptides were eluted from the gel and SDS was removed from the protein moiety according to Pitt-Rivers and Impiombato [12]. Both subunits contain iron and labile sulfide, while peptide bound flavin is present only in the larger one. Flavin fluorescence is seen also before elution from the gel and is much increased by soaking the slab 1 hr in 1 M acetate buffer pH 3.0. This allows identification of the flavin containing protomer in preparations at earlier stages of purification. Before DEAE Sephadex chromatography six bands appear on SDS electrophoresis.

The MW of the undenatured flavoprotein determined by density gradient ultracentrifugation was 110,000 ± 10,000 daltons (fig. 3). This value does not unequivocally establish whether the heavier and the lighter protomer appear in the flavoprotein in a ratio 1:1 or 1:2. Therefore the gel slab with the two protomers separated by SDS electrophoresis and stained with Coomassie blue was scanned in a Joyce Loebl densitometer and the ratio between the absorbance areas corresponding to the heavier and to the lighter polypeptide was found to be 2:1. Since their MW's are approximately in the same ratio and we may assume that Coomassie blue reacts uniformly with homologous sites in either polypeptide, this shows that the two protomers exist in a 1:1 ratio in the flavoprotein.

When standards and unknown are run on separate columns errors arise which are related to asymmetries in the apparatus or to differences in polyacrylamide composition at the top of the columns. The work of Righetti and Drysdale [8] showed that using slabs for SDS electrophoresis these errors are minimized and accuracy in MW estimation is thus much improved.

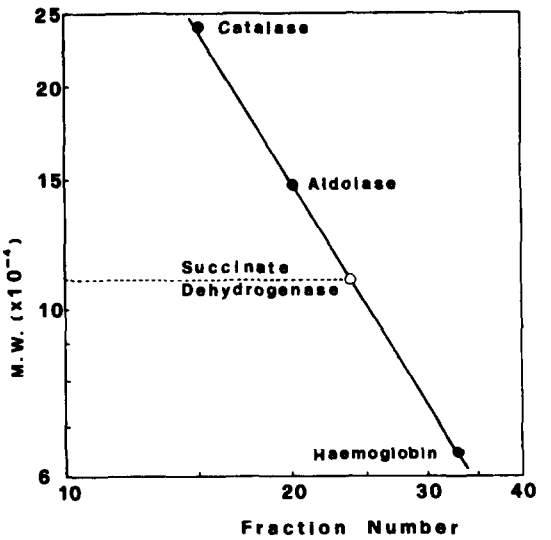

Fig. 3. Molecular weight determination of undenatured succinate dehydrogenase by ultracentrifugation in a 5 to 25% (v/v) linear glycerol gradient. The gradient was in 30 mM phosphate, pH 7.6 and 20 mM succinate; the two solutions for the gradient were degassed and flushed repeatedly with nitrogen. The tubes, each containing 5.1 ml, were spun for 14 hr at 45,000 rpm in a Spinco L2 65B ultracentrifuge, using a SW 65Ti rotor. At the end of the run, 48 fractions were collected from each tube. *Abscissa:* fraction number from the bottom of the tube.

The best estimate of the MW of the flavoprotein is obtained by summing up the MW of the two subunits and comes to 99,000 daltons. This agrees fairly well with the MW determined by density gradient ultracentrifugation in view of the approximation of the method of Martin and Ames.

Hatefi and coworkers [2] have recently separated their preparation of succinate dehydrogenase into two fractions which may correspond in composition the protomers we have isolated. We believe that the two polypeptides are subunits of the same protein. The idea that the smaller polypeptide accompanies the flavin containing polypeptide as an impurity seems untenable on the following basis: 1) drastic treatment is required to separate the preparation of succinate dehydrogenase into two polypeptide chains; 2) other components except for the two polypeptides are absent; 3) the two protomers are present in an integer ratio.

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