REASSESSMENT OF THE MOLECULAR WEIGHT OF MITOCHONDRIAL ATPase FROM BEEF HEART*

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1. Introduction

A recent investigation by Lambeth and Lardy [1] of purified rat liver mitochondrial ATPase indicated a molecular weight of 360,000 ± 10,000, and a $S^{\circ}_{20,w}$ of 12.9S. Values for the molecular weight obtained by the high speed sedimentation equilibrium method of Yphantis [2] and by gel filtration were in excellent agreement. As the above values were significantly higher than the values of 280–284,000 reported for beef heart ATPase [3, 4], we decided to reinvestigate the molecular weight of that enzyme using the above techniques. Our results indicate the molecular weight of beef heart ATPase is also near 360,000. However, although rat liver ATPase gives linear plots of log J vs. r^2 in sedimentation equilibrium runs [1], the corresponding plots for beef heart ATPase show considerable curvature due to significant dissociation of the enzyme. This dissociation appears to be responsible for previous underestimation of the molecular weight.

2. Materials and methods

The procedures used for preparing rat liver ATPase [1] and beef heart ATPase ([5], purification taken through step 5) have been reported previously. The estimation of molecular weight by gel filtration of ATPase with marker proteins (phosphofructokinase, pyruvate kinase and yeast alcohol dehydrogenase) on Bio-Gel P-300, the method of estimating the enzymatic activity in each fraction, and the results obtained for rat liver ATPase have been reported in detail [1].

High-speed sedimentation equilibrium by the method of Yphantis [2] was carried out at 25°, using a Beckman Model E ultracentrifuge equipped with Rayleigh interference optical system and a RTIC temperature control. ATPase enzyme was prepared for ultracentrifugation studies by equilibrating the enzyme with Buffer I (0.1 M KCl, 20 mM potassium phosphate, pH 7.3, 4 mM ATP, 2 mM EDTA) on a Sephadex G-50 column, following closely the procedure of Penefsky and Warner [4].

3. Results and discussion

The dependence of log J on r^2 for a high-speed sedimentation equilibrium run of beef heart ATPase at 25° and 10,589 rpm is shown in fig. 1. The most significant feature is the upward curvature of the plot which indicates that the molecular weight increases as the bottom of the cell is approached. This variation could result from size heterogeneity due to partial dissociation of the enzyme into subunits. As discussed in the following communication [10] and elsewhere [1], electrophoresis in polyacrylamide gels in the presence of sodium dodecyl sulfate indicated that rat liver and beef heart ATPases are identical with respect to the molecular weights and relative proportions of subunits. However, rat liver ATPase does not give a curved plot when run under the same conditions as in fig. 1. We believe this is due to much more complete association at 25° of the rat liver enzyme subunits.

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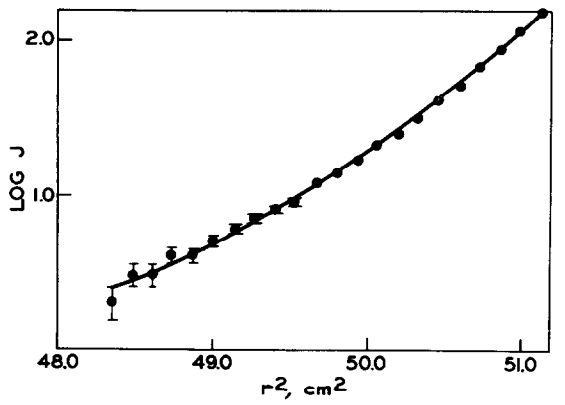

Fig. 1. Dependence of log c on r^2 for high speed sedimentation equilibrium of beef heart ATPase equilibrated with buffer I [4]. J is the fringe displacement which is proportional to c, the concentration. The rotor speed was 10,589 rpm, the temperature was 25°, the initial protein concentration was 0.70 mg/ml, and r is the distance from the axis of rotation. Photographs were taken after 20 hr, using Rayleigh interference optics, and were read on a Gaertner two-dimensional microcomparator.

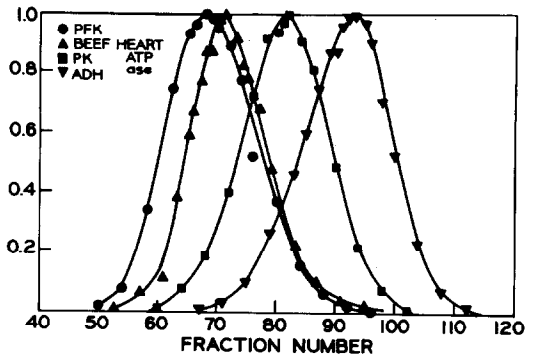

Fig. 2. Chromatography at 25° of beef heart ATPase and marker proteins on a 0.9 × 23 cm column of Bio-Gel P-300. A mixture containing 0.7 mg of beef heart ATPase, 2.5 mg of phosphofructokinase (PFK), 0.4 mg of pyruvate kinase (PK) and 0.7 mg of yeast alcohol dehydrogenase (ADH) was dissolved in 0.2 ml of Buffer I and equilibrated with the buffer by dialysis before applying to the column. Five drop fractions (about 0.1 ml) were collected and the flow rate was 1.0 ml per 35 min. The distribution of enzymatic activity in the collected fractions was determined by spectrophotometric assays as described previously [1] The activity of each enzyme in each fraction is expressed as the ratio of the observed activity to the maximum activity observed for that enzyme. The molecular weights of the marker proteins are PFK, 380–385,000; PK, 237,000; ADH, 150,000.

Forrest and Edelstein [3] also clearly showed that beef heart ATPase is significantly dissociated at 25° and that the degree of dissociation increased as the temperature was lowered to 5°. They attempted to determine the molecular weight of the heaviest component present in a sedimentation equilibrium mixture by correcting for the lightest component present. Although we believe their method is theoretically valid, they reported molecular weight values ranging from 240–320,000, the variation probably being due to technical difficulties in estimating the slopes at the top and bottom of the cell. Furthermore, the number of species present may be greater than they assumed.

From the limiting slope of fig. 1 which corresponds to the bottom of the cell, a molecular weight of 310,000 is calculated, assuming the partial specific volume of 0.74 found by Penefsky and Warner [4]. This value represents a weight-average molecular weight of the species present in the bottom of the cell and, therefore, underestimates the weight of the heaviest component. However, this value is somewhat higher than 284,000 reported by Penefsky and Warner [4], using the Archibald method. The latter method also provides a weight average molecular weight if the system is polydisperse, and measurements made at the meniscus should give a lower weight average molecular weight than measurements made at the bottom of the cell. However, no data were given by the authors with respect to these values.

Since both gel filtration and high-speed sedimentation equilibrium gave values of 360,000 for rat liver ATPase, we decided to use the former technique to study the molecular weight of beef heart ATPase. Gel filtration has the advantages that the ATPase can be chromatographed simultaneously with marker proteins of known molecular weight and that only enzymatically active (fully associated) ATPase is detected in the collected fractions when enzymatic assays are used to determine protein distribution. Fig. 2 shows the result of chromatographing ATPase with three marker proteins whose molecular weights are well established and which had been used earlier to determine the molecular weight of rat liver ATPase [1]. As reported elsewhere, a plot of the log of molecular weight of each marker protein versus the fraction number of the peak gives a straight line [1], and the mobility of beef heart ATPase in this run indicated its molecular weight was

345,000. Two other determinations gave values of 360,000 and 370,000. The mobility of beef heart ATPase is not affected by the presence or absence of phosphofructokinase. Since the mobility of beef heart and rat liver ATPases were the same within experimental error, we have concluded that the molecular weight of beef heart ATPase is also 360,000. It is worth noting that the buffer and temperature used in gel chromatography are the same used for the ultracentrifuge experiments.

We have tested a suggestion by Racker [6] that a lower molecular weight for ATPase is obtained if the enzyme is heat-treated during purification. However, heating beef heart ATPase at 65° for 2 min as described by Senior and Brooks [5] did not alter the molecular weight obtained by gel filtration. Actually, examination of a sample of the heated enzyme by high speed sedimentation equilibrium showed no curvature in the log J versus r^2 plot and gave a molecular weight consistent with gel filtration data. Therefore, the history of the sample may affect the pattern observed in the ultracentrifuge but it does not affect the molecular weight of the active enzyme. Finally, as shown in the following communication [10], a calculated molecular weight based on the molecular weights of subunits obtained by polyacrylamide gel electrophoresis is in excellent agreement with a molecular weight of 360,000.

Yeast mitochondrial ATPase [7] and spinach chloroplast ATPase [8] have reported molecular weights of 340,000 and 325–358,000, respectively, and a membrane-bound energy-linked microbial ATPase [9] has a reported molecular weight of 385,000. It is therefore possible that these membrane-bound, energy-linked ATPases have a similar molecular architecture and are derived from a common ancestral protein.

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