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ACETOLACTATE SYNTHASE OF *PSEUDOMONAS AERUGINOSA*

II. EVIDENCE FOR THE PRESENCE OF TWO NONIDENTICAL SUBUNITS

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SUMMARY

Homogeneous preparations of acetolactate synthase (acetolactate pyruvate-lyase (carboxylating), EC 4.1.3.18, formerly also known as acetohydroxy acid synthetase) from *Pseudomonas aeruginosa* gave rise to two protein peaks when subjected to gel filtration in the presence of 6 M guanidine·HCl, indicating the presence of two nonidentical subunits. The ratio of protein in the two peaks was 4.6 to 1. Acrylamide gel electrophoresis in the presence of sodium dodecyl sulphate confirmed this finding. Estimates of the molecular weights of the subunits yielded values of 60 000 and 15 000. Summation of these two molecular weight values yields a value which is about one-eighth the molecular weight of the native enzyme.

INTRODUCTION

The accompanying paper[1] describes the purification and some of the allosteric properties of acetolactate synthase (acetolactate pyruvate-lyase (carboxylating), EC 4.1.3.18, formerly also known as acetohydroxy acid synthetase) of *Pseudomonas aeruginosa*. The large s value observed in sedimentation velocity experiments with the pure protein suggested that the acetolactate synthase is a large molecular weight enzyme and prompted a more detailed study of the size and subunit structure of the enzyme. In this paper evidence is presented showing that acetolactate synthase is composed of two nonidentical subunits with approximate molecular weights of 60 000 and 15 000, and that the native enzyme probably contains eight subunits of each type.

MATERIALS AND METHODS

Chemicals

Reagents for polyacrylamide gel electrophoresis were obtained from Eastman.

Sodium dodecyl sulphate and the proteins used for molecular weight standards were obtained from Schwarz/Mann. Guanidine·HCl was purchased from Heico Chemical Co. The other chemicals used have been described elsewhere[1].

Enzyme preparation

Acetolactate synthase was isolated from *P. aeruginosa* (ATCC 7700) as described previously[1].

Acrylamide gel electrophoresis in the presence of sodium dodecyl sulphate

Acrylamide gel electrophoresis in sodium dodecyl sulphate was performed essentially as described by Weber and Osborn[2]. The proteins were incubated at 37 °C for 2 h in 0.01 M sodium phosphate buffer (pH 7.0) containing 1% sodium dodecyl sulphate and 1% β-mercaptoethanol and then dialyzed overnight at room temperature against 0.01 M sodium phosphate buffer (pH 7.0) containing 0.1% sodium dodecyl sulphate and 0.1% β-mercaptoethanol. Electrophoresis was carried out at room temperature with a constant current of 8 mA per tube. The gels were stained with Coomassie brilliant blue and destained electrophoretically.

Gel filtration in the presence of guanidine·HCl

The procedures employed for gel filtration in the presence of guanidine·HCl were essentially the same as those described by Davison[3] and Fish *et al.*[4]. The solution used for equilibrating and eluting the columns of Bio-Gel A-0.5 m contained 6 M guanidine·HCl–0.1 M β-mercaptoethanol (pH 7.0). Protein was dialyzed against this same solution for 24 h prior to application to the column. Protein in the fractions eluted from the column was determined by the turbidimetric method reported by Rosenbloom *et al.*[5].

Ultracentrifuge studies

Sedimentation equilibrium experiments were carried out with a Spinco Model E ultracentrifuge equipped with interference optics and a rotor temperature indicator control unit. Standard double sector cells were used. Measurements of fringe displacement against radial distance were made with a Gaertner comparator. Fringe displacements were corrected for window distortion with water blanks.

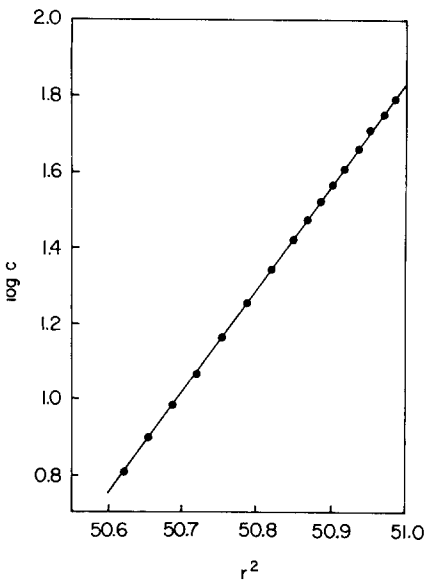
RESULTS AND DISCUSSION

Sedimentation equilibrium studies

The enzyme, at several concentrations, was subjected to equilibrium centrifugation by the meniscus depletion method of Yphantis[6]. Fig. 1 illustrates a plot of the log of the fringe displacement against the distance from the center of the rotor for one such experiment. Such plots produced a straight line in all experiments indicating the homogeneity of the enzyme preparation with respect to molecular weight. Assuming a partial specific volume of 0.740, the apparent weight average molecular weight of the acetolactate synthase was found to be approx. 600 000.

Gel filtration in the presence of guanidine·HCl

In the presence of 6 M solutions of guanidine·HCl containing reducing agents



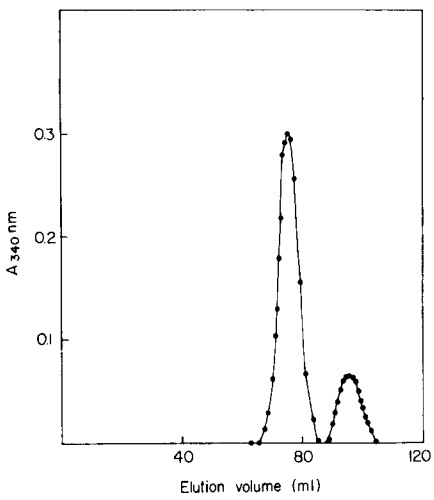


Fig. 1. Sedimentation equilibrium data for the purified α-acetolactate synthase at an initial protein concentration of 0.015%. The buffer was 0.04 M potassium phosphate (pH 7.0). Centrifugation was at 14 000 rev./min and 20 °C. Equilibrium was attained after 36 h. Log c represents the log of the fringe displacement in arbitrary units and r represents the distance, in centimeters, from the center of rotation.

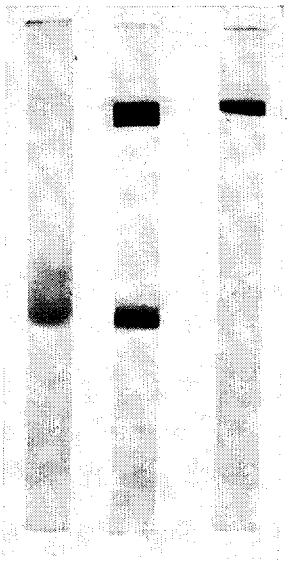
Fig. 2. Elution profile of α-acetolactate synthase chromatographed on Bio-Gel A-0.5 m in the presence of 6 M guanidine·HCl. A 0.5-ml sample (approx. 6 mg of protein) was applied to a 1.5 cm × 65 cm column. Fractions of 0.9 ml were collected.

most proteins are dissociated to their constituent polypeptide chains and these chains adopt a conformation close to a random coil[7]. The elution volume of the polypeptide chains from columns of molecular-sieving media such as Bio-Gel A-0.5 m is a function of their molecular weight. When a homogeneous preparation of acetolactate synthase is subjected to gel filtration on Bio-Gel A-0.5 m in the presence of 6 M guanidine·HCl, the elution profile shown in Fig. 2 is obtained. Assuming that under these conditions the enzyme is fully denatured the presence of at least two subunits of different molecular weights is indicated. Estimation of the total protein in each of the two peaks yields a ratio of 4.6.

Acrylamide gel electrophoresis in the presence of sodium dodecyl sulphate

Fig. 3 shows the results obtained by electrophoresing acetolactate synthase in the presence of sodium dodecyl sulphate. The enzyme gives rise to two components, again indicating the existence of at least two subunits of different molecular weights. The results in Fig. 3 also show that the two subunits separated by gel filtration in the presence of guanidine·HCl as described above correspond to the two subunits identified by acrylamide gel electrophoresis in the presence of sodium dodecyl sulphate.

The molecular weights of each subunit were estimated by comparing their relative migration distances to those of marker proteins of known molecular weight.



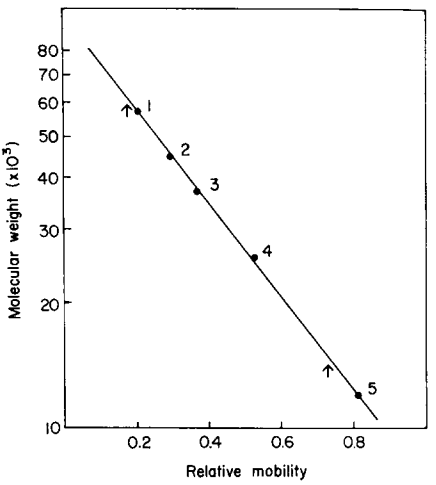


Fig. 3. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate of α-acetolactate synthase and the subunits obtained from chromatography in the presence of guanidine·HCl. Conditions for electrophoresis and staining are described in Materials and Methods. From left to right are small subunit, native enzyme and large subunit.

Fig. 4. Determination of the molecular weights of the subunits of α-acetolactate synthase by polyacrylamide gel electrophoresis in sodium dodecyl sulphate buffer. The samples were prepared and electrophoresis carried out as described in Materials and Methods. 1, pyruvate kinase; 2, ovalbumin; 3, alcohol dehydrogenase; 4, chymotrypsinogen; 5, cytochrome *c*. The arrows indicate the mobility of the α-acetolactate synthase subunits.

The results are shown in Fig. 4. Molecular weights of the subunits may be estimated at 60 000 and 15 000.

The data obtained from both gel filtration in the presence of guanidine·HCl and acrylamide gel electrophoresis in the presence of sodium dodecyl sulphate support the conclusion that acetolactate synthase is composed of at least two nonidentical subunits. If it is assumed that there are only two subunits of molecular weights 60 000 and 15 000 and that these are present in equimolar amounts a ratio of 4.0 for the total protein in the larger molecular weight peak to that in the smaller molecular weight peak is expected. This is in good agreement with the ratio of 4.6 obtained in the experiment illustrated in Fig. 2. Since the sedimentation equilibrium experiments show that the native enzyme has a molecular weight of about 600 000 it seems reasonable to suggest that there may be eight of each type of subunit present in the native enzyme.

As yet we have been unable to successfully separate the two subunits under non-denaturing conditions. Thus a functional role cannot yet be assigned to the two subunits. Experiments designed to elucidate the functional role of the subunits are in progress.

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