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Molecular Weight of Rat Liver Ornithine-Ketoacid Aminotransferase[1] (38422)

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(Introduced by J. F. Thomson)

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Molecular properties of rat liver ornithine-ketoacid aminotransferase [L-ornithine: 2-oxoacid aminotransferase (EC 2.6.1.13)] have been studied extensively (1–6). The estimations of molecular weight from three different laboratories (3–5) are not in good agreement, however; they range from 132,000 to 180,000 daltons. To understand the basis for the observed discrepancies, we have studied the effects of purification and concentration on the molecular weight of the enzyme.

Methods. Ornithine-ketoacid aminotransferase was purified from rat liver as previously described (4), except that the mitochondria were disrupted by freezing at −130° prior to sonication and the sonication time was reduced to 1 hr. The purified enzyme, three times crystallized, was dissolved in buffer (0.01 *M* tris, 10^{-3} *M* dithiothreitol, and 10^{-4} *M* pyridoxal phosphate, pH 8.0) to a concentration of approximately 10 mg/ml and frozen in small aliquots at −130°.

Crude extracts of ornithine-ketoacid aminotransferase were prepared from individual rat livers. The liver plus 1.5 vol of ice-cold buffer (0.1 *M* tris, 10^{-3} *M* EDTA, 10^{-3} *M* dithiothreitol, and 10^{-4} *M* pyridoxal phosphate, pH 8.0) was homogenized in a Potter–Elvehjem homogenizer with Teflon pestle. After sonication the homogenate was centrifuged at 226,000*g* for 30 min. The supernatant fraction was then heated to 55° for 1 min by swirling in a hot water bath, cooled quickly in ice water, and centrifuged at 226,000*g* for 15 min. The clear supernatant fraction was used without further treatment.

Ornithine-ketoacid aminotransferase activity was assayed as previously reported (6). The method is based on the formation of a yellow adduct (m*M* $\epsilon_{440} = 2.71$, 1-cm light path) between the reaction product, Δ^1-pyrroline-5-carboxylate, and *o*-aminobenzaldehyde, which is added to the reaction mixture. One unit of activity is defined as 1 μmole of product formed per minute under the conditions of the assay.

Protein concentrations were determined according to the procedure of Lowry *et al.* (7).

The molecular weight of the undissociated enzyme was estimated by gel filtration chromatography (8). Columns, 2.5 × 85 cm, prepared from Sephadex G-200 which had been equilibrated with buffer (0.05 *M* tris, 0.1 *M* NaCl, and 0.1% NaN_3, pH 7.0) were standardized with the following proteins: apoferritin and glutamate dehydrogenase (Calbiochem), aldolase, ovalbumin, chymotrypsinogen A, and ribonuclease A (Pharmacia). Similar results were obtained whether the columns were run at 25 or 4°. When high concentrations of purified enzyme were used, its emergence from the column was detected either as an A_{280} peak or by measurement of enzyme activity. In the chromatography of dilute purified enzyme, or enzyme in crude liver extracts, activity measurements were the sole means of detection. When detection was by activity measurement, pyridoxal phosphate (10^{-4} *M*) was added to the column buffer.

The molecular weight of the ornithine-ketoacid aminotransferase subunit was estimated by

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electrophoresis of the pure enzyme [in polyacrylamide gel slabs (18 cm long, 3 mm thick) containing 0.1% sodium dodecyl sulfate] in a vertical gel electrophoresis cell (E. C. Apparatus Corp., St. Petersburg, FL). Gels were prepared by dissolving 8.0 g Cyanogum in 150 ml of buffer (0.08 *M* tris, 3.4 m*M* EDTA, 6.2 m*M* boric acid, and 0.1% sodium dodecyl sulfate, pH 9.2); to the filtered solution were added 0.2 ml *N,N,N',N'*-tetramethylethylenediamine and 0.2 g ammonium persulfate. Gel slabs were preelectrophoresed for 30 min prior to applying samples. Proteins at a concentration of 10 mg/ml were preincubated in the gel preparation buffer plus 1% dithiothreitol for 2 hr at room temperature. Approximately 50 μl of each preincubated protein solution was applied to the gel and electrophoresed for 1.5 hr at 200 V. Gel slabs were then fixed in 10% trichloroacetic acid, sliced longitudinally, and scanned at 280 nm. Protein standards used in the sodium dodecyl sulfate-polyacrylamide gel electrophoresis experiments were those listed above plus catalase (Mann Research Laboratories) and bovine serum albumin (Sigma).

Results and Discussion. When 160 units of ornithine-ketoacid aminotransferase (specific activity, 16 units/mg; concentration, 10 mg/ml) were chromatographed on a standardized Sephadex G-200 column (Fig. 1A), a molecular weight of 202,000 daltons was obtained. This value was an average of seven separate determinations; the standard deviation was 14,000 daltons.

This enzyme preparation was diluted 100-fold in column buffer and allowed to stand in an ice bath for 15 days, at which time the specific activity of the preparation was 14 units/mg. After applying 2.8 units to the column the molecular weight was estimated at 116,000 daltons.

These results show the molecular weight of the enzyme is concentration dependent; *i.e.*, aggregation of the enzyme occurs with increasing concentration. The similarity in specific activity of the different aggregate forms suggests that the interaction of enzyme molecules during aggregation does not block catalytic sites.

The molecular weight of the enzyme in a freshly prepared crude liver extract was then determined. Two milliliters of the extract, containing 0.8 units of activity, were applied to the column. The average molecular weight from three separate determinations was 116,000 daltons, with a standard deviation of 14,000 daltons.

Analysis of the molecular weight of the orni-

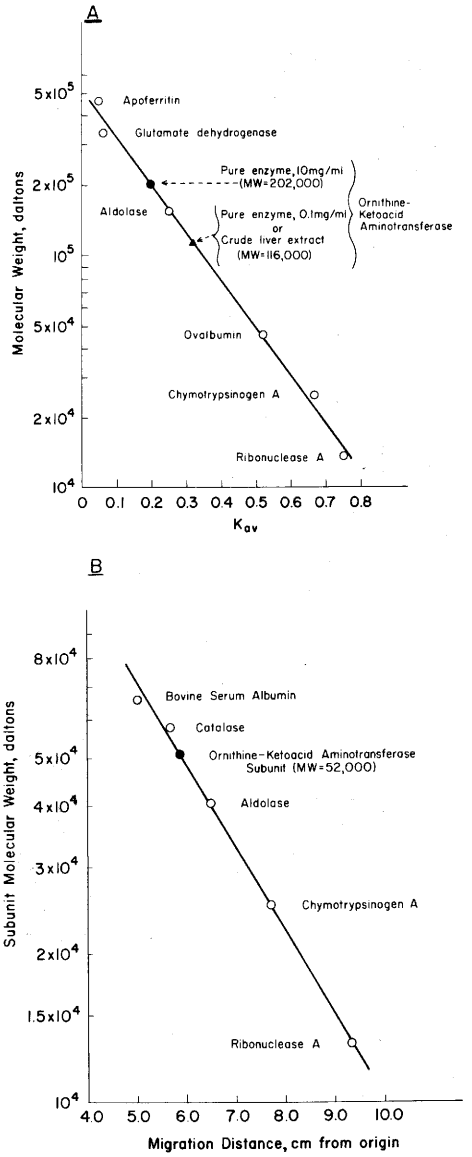

FIG. 1. Molecular weight of ornithine-ketoacid aminotransferase based on (A) gel filtration chromatography on Sephadex G-200. K_{av} (abscissa) = V_e-V_0/V_t-V_0, where V_e = elution volume of a given protein, V_0 = elution of blue dextran 2000 (MW = 2,000,000), and V_t = total bed volume of column. (B) Electrophoretic migration in polyacrylamide gel in the presence of sodium dodecyl sulfate (see "Methods" for details of both procedures). In both analyses the lines were fitted to the points by the method of least squares.

thine-ketoacid aminotransferase subunit under conditions which dissociated the subunits (Fig. 1B) yielded a value of 52,000 daltons, with a standard deviation of 2300 daltons (11 separate determinations). In these analyses ornithine-ketoacid aminotransferase migrated as a single band in the electrophoresis gels; this indicates that the enzyme contains identical subunits.

The pyridoxal phosphate content of the enzyme was previously estimated at 18 μmole/g

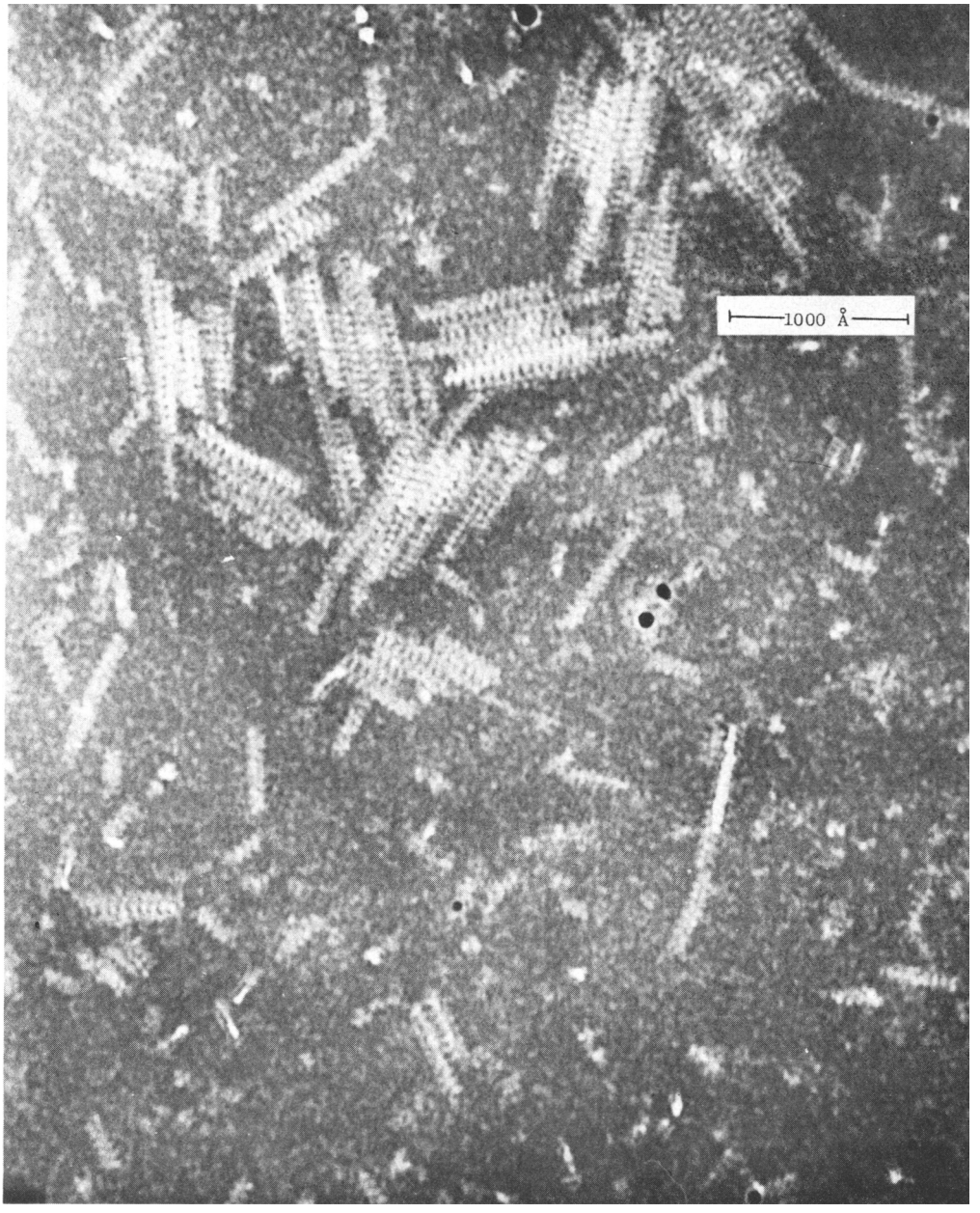

FIG. 2. Aggregates of ornithine-ketoacid aminotransferase subunits, formed when a drop of the purified but not previously crystallized enzyme (10 mg/ml in 0.01 *M* tris buffer, pH 8.0) was allowed to evaporate to dryness on a carbon-coated grid. The buffer residue was washed away, and the preparation was negatively stained with sodium silicotungstate and viewed in the JEM 7A electron microscope.

enzyme (6). This corresponds to 0.94 mole of pyridoxal phosphate per 52,000 daltons, indicating that the pure enzyme binds one molecule of pyridoxal phosphate per subunit.

Figure 2 is an electron micrograph showing aggregates containing large numbers of subunits (oblong white particles, approximately 25 × 40 Å, Ref. 4). These aggregates formed as a droplet of solution containing 10 mg enzyme/ml was allowed to evaporate on a carbon grid. Although this enzyme preparation had not previously been crystallized, the aggregates resembled the electron micrographs of crystal fragments described earlier (4).

Taken together, the present results suggest that ornithine-ketoacid aminotransferase exists as a dimer in dilute solutions (0.1 mg/ml) of the pure enzyme and may also exist as a dimer *in vivo*. At higher concentrations the pure enzyme associates into aggregates with the tetrameric form predominating at enzyme concentrations of 10 mg/ml. Larger aggregates are produced as the concentration is increased further (Fig. 2). This tendency to aggregate is probably responsible for the variations in the earlier estimates of the molecular weight of ornithine-ketoacid aminotransferase (3–5).

Summary. The molecular weights of ornithine-ketoacid aminotransferase in pure form and in crude extracts of rat liver were compared. The molecular weight of the pure enzyme was concentration dependent. At concentrations of 10 mg/ml its molecular weight was 202,000 daltons, but this value was reduced to 116,000 daltons when the enzyme was diluted and allowed to equilibrate at a concentration of 0.1 mg/ml. The crude enzyme preparation also had a molecular weight of 116,000 daltons. The molecular weight of the enzyme subunit was 52,000 daltons and the pure enzyme contained one pyridoxal phosphate moiety per subunit. The data suggest that the enzyme may exist as a dimer *in vivo*.

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