The Molecular Weight of the Polypeptide Chains of
L-Glutamate Dehydrogenase*

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L-Glutamate dehydrogenase, originally reported as having a molecular weight of about 1,000,000 (1), is known to be readily dissociated into subunits of smaller size (1–3). The size of the ultimate subunits, the individual polypeptide chains, has, however, not been definitely established. Jirgensons (4) reported finding about 20 moles of NH₂-terminal acid per 10⁶ g of enzyme, suggesting a molecular weight of about 50,000 per polypeptide chain. He also estimated an approximate molecular weight of 43,000 from sedimentation velocity and viscosity measurements carried out in the presence of detergent. Frieden (5) has given a possible range of 30,000 to 60,000 on the basis of sedimentation velocity data in several solvents.

Work carried out in this laboratory during the last few years has suggested that guanidine hydrochloride is probably the best reagent for breaking noncovalent interactions which are responsible for the folding and aggregation of proteins. The method most likely to dissociate an aggregated protein entirely into its constituent polypeptide chains, therefore, is to dissolve it in concentrated guanidine hydrochloride in the presence of a reducing agent. The latter will rupture disulfide bonds when they are present, and will prevent exposed sulfhydryl groups in the unfolded protein from forming new disulfide bonds by oxidation. Molecular weight determination by sedimentation equilibrium can be carried out directly on the protein dissolved in this medium. If the procedure recommended by Yphantis (6) is used, it can be established in a single experiment whether the dissociated protein exists as polypeptide chains which have substantially identical molecular weights, or whether chains of different size are present. We have applied this technique to the determination of the molecular weight of the polypeptide chains of γ-globulin (7) and fumarase (8). In γ-globulin the constituent chains appear to be of two kinds with different molecular weights, while in fumarase they are identical.

This paper reports application of the same method to glutamate dehydrogenase.

EXPERIMENTAL PROCEDURE

L-Glutamate dehydrogenase was a crystalline beef liver product, obtained from the California Corporation for Biochemical Research (Lot 36145). It was dissolved in 5.8 M guanidine hydrochloride containing 0.01 M 2-mercaptoethanol, and adjusted to pH 8.0. The guanidine hydrochloride was prepared from guanidine carbonate by the method of Anson (9).

The protein solution was dialyzed against solvent. The solution subjected to sedimentation equilibrium had a concentration of 0.12 g per liter, as determined from the absorbance at 280 mp, with the extinction coefficient reported by Olson and Anfinsen (1). This low protein concentration permits neglect of the effect of thermodynamic nonideality on the equilibration of the protein in the sedimentation experiment. It also is a factor which favors complete dissociation, although we believe that the protein would remain completely dissociated to substantially higher concentrations in this solvent system.

Sedimentation equilibrium was carried out in a Spinco model E ultracentrifuge, with the interferometric method described by Yphantis (6). The rotor speed was 23,150 r.p.m. A solution column 5.4 mm long was used. Double sector cells with 12-mm centerpieces were employed. The temperature was maintained constant at 25.0°. Fringe displacements were measured as a function of the radial position in the cell (r) by means of a Gaertner two-dimensional comparator. They were corrected for cell distortion, as described by Yphantis (6).

The solvent density was measured in a modified Lipkin pycnometer (10), at 25.0°, and was found to be 1.1434 g per cc.

RESULTS AND DISCUSSION

As a test for the establishment of equilibrium, fringe displacements at a fixed position near the bottom of the solution column were recorded at intervals, until no further change occurred. These data are shown in Fig. 1, and it is seen that equilibrium was reached after about 80 hours.

Fig. 2 shows the logarithm of the fringe displacement at equilibrium, plotted against r². Under the conditions of the experiment, the upper meniscus of the solution column is essentially depleted of protein. The fringe displacement is therefore directly proportional to protein concentration, and the linearity of the plot indicates that the polypeptide chains of the protein are essentially homogeneous with respect to molecular weight. The molecular weight itself can be obtained from the slope of Fig. 2, but knowledge of the partial specific volume (δ) of the protein is required. Olson and Anfinsen (1) determined a value of 0.75 cc per g in 0.1 ionic strength phosphate buffer. Dr. G. M. Tomkins has provided us with a somewhat lower figure (0.737 cc per g), calculated from the amino acid composition and applicable to dilute salt solutions. Since δ in concentrated guanidine hydrochloride solution is likely to be between 0.01 and 0.02 cc per g lower than in dilute salt or buffer solutions (7, 11), these figures lead to an estimate of δ = 0.72 to 0.73 cc per g for the...
conditions used in the sedimentation experiments. A direct determination of \( \bar{v} \) in guanidine hydrochloride has been reported by Reithel and Sakura (12), but they consider their result (0.682 to 0.725 cc per g at 25\(^{\circ}\)) unreliable. A direct determination has been attempted in our laboratory also, but the result (0.69 to 0.72 cc per g at 25\(^{\circ}\)) is uncertain because we were unable to measure the concentration of protein in our solutions with sufficient precision. The most probable value of \( \bar{v} \) which emerges from these data in 0.72 cc per g, and the corresponding molecular weight is 50,000. The uncertainty is however large. With \( \bar{v} = 0.73 \) cc per g, the molecular weight becomes 53,000. With \( \bar{v} = 0.70 \) cc per g, it becomes 44,000.

We have also made separate determinations of number, weight, and \( z \)-average molecular weights from the same data, using the numerical procedures described by Yphantis (6). All three averages agreed within less than 1% with each other, and with the value obtained from the slope of Fig. 2.

The molecular weight of the smallest, active molecular unit of glutamate dehydrogenase lies in the range of 250,000 to 350,000 (2, 3, 5, 13). The molecular weight given here for the individual polypeptide chains thus suggests that the smallest active molecule may contain five or six polypeptide chains.

**SUMMARY**

The mixture of polypeptide chains of glutamate dehydrogenase, obtained by solution of the protein in concentrated guanidine hydrochloride, has been examined by sedimentation equilibrium. The chains all have the same, or nearly the same, molecular weight. The actual value is approximately 50,000, but an exact molecular weight cannot be assigned, despite the good precision of our data, because of uncertainty in the partial specific volume. Within the limits of uncertainty, our result agrees well with previous determinations, by other methods, reported by Jirgensons (4).

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**REFERENCES**

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