# THE MOLECULAR WEIGHT AND SUBUNITS OF THE ISOZYMES OF GLUTAMIC ASPARTIC TRANSAMINASE.*

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SUMMARY: The molecular weight of the isozymes of glutamic aspartic transaminase has been measured by gel filtration on Sephadex G-200 and by membrane osmometry. The molecular weights were: 91,200 ± 2,700 for the mitochondrial enzyme and 93,000 ± 2,800 for the supernatant isozyme. Guanidine hydrochloride induces dissociation into two subunits of half the molecular weight. Gel filtration and sucrose density gradient centrifugation measurements failed to reveal dissociation of each isozyme's dimer in the protein concentration range of 2.5 to 1 x $10^{-5}$ mg/ml.

After purification of the pig heart supernatant glutamate aspartate transaminase (S-GAT), the molecular weight of the enzyme was reported as 110,000 ± 1,600 (1). Later chemical and gel filtration data of highly purified preparations of (S-GAT) gave values in the 90,000 to 100,000 molecular weight range (2,3). All reports agree on the enzyme being a dimer and on the basis of fluorescence depolarization measurements, it was even postulated that upon dilution it dissociates into catalytically active monomer subunits (4). This concept has recently been challenged by Banks et.al. (5,6) who by ultracentrifugation and gel filtration experiments reported a figure of 78,600 ± 2,400 as the molecular weight of (S-GAT) and the lack of a dilution induced dissociation.

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The above controversy is further complicated by the fact that the aspartate transaminase also exists as a mitochondrial isozyme (M-GAT) whose structural dissimilarities with S-GAT are well documented (7). Many conflicting values of molecular weights exist for the supernatant isozyme but only one value of 100,000 daltons has been reported for M-GAT using sedimentation techniques (8). Therefore, we investigated the effects of extreme dilutions upon the aggregation state of the subunits of each isozyme. We have also determined the molecular weight of both isozymes and their subunits by a non-transport method, membrane osmometry.

EXPERIMENTAL:

Sedimentation behavior was followed in sucrose density gradients by the method of Martin and Ames (9) with catalase as the reference marker. After collection of the samples the enzymes were located by their absorbance at 280 mμ or catalytic activity using previously described assays (2).

Molecular weight determinations by chromatography in Sephadex G-200 was performed by the procedure of Andrews (10). Blue dextran and tritiated water were used to measure the column's void (Vo) and intrinsic (Vi) volumes. Osmotic pressure values were determined in a Hewlett-Packard high speed membrane osmometer Model 503 with B-19 membranes. The purification of the α form of (S-GAT) and of (M-GAT) have appeared elsewhere (2,7).

RESULTS:

No change in the sedimentation coefficient was observed

TABLE I: Sedimentation Coefficient Values of the GAT Isozymes[a]

| ISOZYME | CONCENTRATION[b] (mg/ml) | S |
|---|---|---|
| S-GAT[c] | 2.5 | 5.95 |
| | $3.5 \times 10^{-1}$ | $5.75 \pm 0.1$ |
| | $5.0 \times 10^{-2}$ | $5.89 \pm 0.1$ |
| | $5.0 \times 10^{-3}$ | $5.85 \pm 0.1$ |
| | $3.0 \times 10^{-4}$ | $5.67 \pm 0.1$ |
| | $1.1 \times 10^{-5}$ | $5.75 \pm 0.1$ |
| M-GAT | $4.4 \times 10^{-1}$ | $5.78 \pm 0.1$ |
| | $4.4 \times 10^{-3}$ | $5.70 \pm 0.1$ |
| | $4.4 \times 10^{-5}$ | $5.84 \pm 0.1$ |
| APO-GAT | $5.1 \times 10^{-1}$ | $5.76 \pm 0.1$ |
| | $5.1 \times 10^{-3}$ | $5.68 \pm 0.1$ |
| | $5.1 \times 10^{-5}$ | $5.86 \pm 0.1$ |

[a]Sedimentation in a 5 to 20% sucrose gradient in 0.05 M Tris buffer, pH 7.5, at 4° and 39,000 rpm for 12 hours. Catalase was the internal standard.

[b]Enzyme was maintained at the proper dilution for 4 hours at room temperature prior to layering on the sucrose gradient.

[c]$\alpha$ form.

after dilution of either isozyme in the holoenzyme or apoenzyme forms to several orders of magnitude lower than those reported in the fluorescence polarization studies (Table I). The lowest dilutions are the limit of detection of enzymatic activity and were obtained using a 0 to 0.1 full scale deflection in a Gilford model 2000 spectrophotometer at 30°. Values in Table II show the complete recovery of the enzyme units placed in the sucrose gradient and the validity of the method to check en-

TABLE II: Enzymatic Recovery and Effect of pH on the Sedimentation Coefficients

| Isozyme | (mg/ml) | pH | S[a] | Units[b] Initially Present | Units Recovered |
|---|---|---|---|---|---|
| S-GAT | $3 \times 10^{-1}$ | 7.5 | $5.67 \pm 0.1$ | 12.70 | 12.00 |
| | $3 \times 10^{-3}$ | 7.5 | $5.80 \pm 0.1$ | 1.88 | 1.53 |
| | $3 \times 10^{-4}$ | 7.5 | $5.77 \pm 0.1$ | 0.29 | 0.25 |
| S-GAT[c] | $3 \times 10^{-1}$ | 2.8 | $2.93 \pm 0.3$ | 1.93 | 1.19 |
| S-GAT[c] | $3 \times 10^{-1}$ | 11.5 | $2.84 \pm 0.2$ | 1.84 | 1.52 |
| M-GAT[c] | $3 \times 10^{-1}$ | 11.5 | $2.76 \pm 0.2$ | 1.95 | 1.81 |

[a]Sedimentation conditions as in Table I, extreme pH buffers were 0.02M Glycine-NaOH, pH 11.5 and 0.02 M Glycine-HCl, pH 2.8.

[b]One unit is change of 0.01 optical density units per minute under the assay conditions employed (2).

[c]followed by the absorbance at 280 mμ of each fraction collected after puncturing of the centrifuge tube.

zymatic dissociation under conditions of extreme pH known to induce dissociation (11).

To substantiate the results obtained from sucrose density gradient centrifugation, a gel filtration method was employed. An approximate molecular weight was determined after standardization of the Sephadex G-200 gel (10). All experiments with concentrations of the two isozymes of GAT ranging from 1 mg/ml to 3 μg/ml gave no indication of a concentration dependent dissociation of the molecule. Gel chromatography gave a molecular range of 95-103,000 for both S and M-GAT.

TABLE III: The Molecular Weights of Native and Dissociated Proteins and the Number of Subunits as Determined by Osmometry

| Isozyme | Solvent Density | $\pi^a / C^b$ cm/g/l | M. W. | Subunits |
|---|---|---|---|---|
| S-GAT | 1.012 | 0.250 | 93,000 $\pm$2,800 | 2 |
| S-GAT | 1.150 | 0.460 | 44,500 $\pm$1,600 | |
| M-GAT | 1.012 | 0.255 | 91,200 $\pm$2,700 | 2 |
| M-GAT | 1.150 | 0.465 | 44,000 $\pm$1,700 | |

$^a\pi$, osmotic pressure; $^b$C, protein concentration

To determine the molecular weight by a method independent of the ultracentrifuge and where the presence of dissociating agents such as guanidine hydrochloride (Gu-HCl) would exert little effect in the values, we chose membrane osmometry. This method offers particular advantage to examine the true molecular weight of the enzyme subunits after dissociation by Gu-HCl, because unlike ultracentrifugation it does not depend on determinations of the partial specific volume $\bar{v}$ of the protein in the solvent used and, interactions between protein and solvent show little interference since the data is extrapolated to zero protein concentration (12).

The data obtained in the osmometer are plotted in Figure 1 for the two isozymes of GAT at neutral pH and in the presence

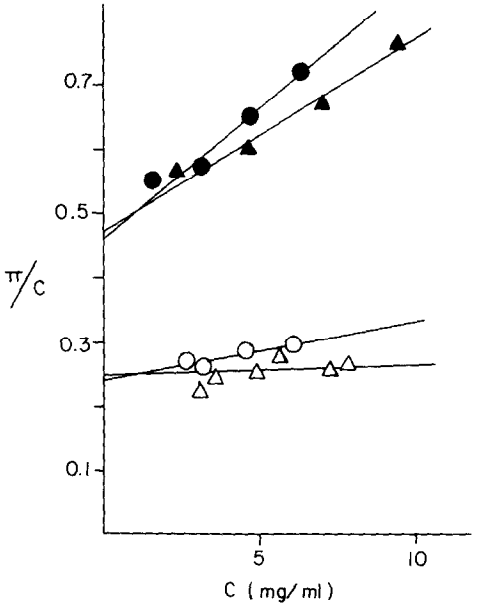


Figure 1 --- Plots of $\pi/C$ vs C for the isozymes of GAT in, 0.1 M KCl, 0.1 m potassium phosphate buffer, pH 6.8. O, or Δ and in 6 M guanidine hydrochloride with 0.5 M mercaptoethanol at 5°, ● or ▲. Circles, M-GAT, Triangles, S-GAT.

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of 6M Gu-HCl. The points shown are averages of three experiments and the calculated values are summarized in Table III.

DISCUSSION:

Two methods namely, sucrose density gradient centrifugation and gel filtration, failed to detect any dilution induced dissociation of either isozyme of aspartate amino-transferase. Even after the removal of the prosthetic group, pyridoxal phosphate, S-GAT maintained its dimeric structure at a concentration of $5.1 \times 10^{-5}$ mg/ml. Because our location of the enzyme at very high dilution was based on detection of enzymatic activity it could be argued that dissociation occurs but, contrary to

Polyanovskii and Sphikiter's hypothesis (11), the monomeric subunits are inactive. This possibility or their original proposal of dissociation into monomeric subunits with higher specific activities are eliminated on the basis of the quantitative recovery of enzymatic activity on Table II. Using gel filtration on Sephadex G-100 Banks et.al. (6) have also reported lack of dissociation within the range investigated by the Russian workers.

It must be concluded that dilution does not induce dissociation of either isozyme necessitating another explanation for the observed fluorescence polarization changes upon dilution of S-GAT. Our results of the osmotic pressure data indicate a molecular weight of 93,000 ± 2,800 for S-GAT and 91,200 ± 2,700 for M-GAT and establishes the molecular weights of the respective subunits at half these values. These results agree with those calculated for S-GAT in our gel filtration experiments, the N-terminal analysis and pyridoxal phosphate content (2), with the molecular weight determinations of the S-GAT apoenzyme through gel filtration (3) and with our previous chemical evidence of a dimeric structure for each isozyme (8). The low molecular weight obtained by Banks et.al. (5) will have to be considered a result of their choice of Sephadex G-100 as a fractionating gel or their reported lack of buffer in the eluant. Indeed, it has already been pointed out by Andrews (10) that Sephadex G-100 may give lower molecular weight estimates than Sephadex G-200. The low molecular weight on the ultracentrifuge (5) also disagrees with those already published using a similar approach (1,7). The later discrepancy is harder to explain but could be due to their uncertain diffusion coefficient obtained through centrifugation techniques. On the other hand Banks

et.al. (6) report on the lack of dissociation of S-GAT in 6M Gu-HCl. These results conflict with our osmotic pressure determinations in 6M Gu-HCl, 0.1M 2-mercaptoethanol which are the experimental conditions when proteins exist as random coils (12,13). No explanation for this discrepancy can be forwarded. However, Banks et.al. (16) calculations of a molecular weight in Gu-HCl are uncertain since they are based on sedimentation coefficient values using a partial specific volume identical to that of the protein in water and without the proper corrections for solvent-protein interactions. Thus, the magnitude of this effect cannot account for the disagreement in the values of the subunit molecular weight of S-GAT by the methods employed.

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