# The Release of Aspartate Transaminase from Mitochondria by Dilute Ionic Solutions

by

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## SUMMARY

Aspartate transaminase is readily extracted from mitochondria into dilute salt solutions. The effect is probably attributable to a combination of osmotic rupture and adequate ionic strength for release of the enzyme from its binding sites.

## INTRODUCTION

The mitochondrial isoenzyme of aspartate transaminase (EC 2.6.1.1) was reported as being relatively difficult to elute into ionic solutions, requiring extensive mechanical degradation of the particles before complete extraction into 0.1M phosphate buffer could be achieved (1). We found, on the contrary, that the enzyme was extraordinarily easy to extract, provided that the correct salt concentration was used (2, 3). A number of recent reports suggest that

the enzyme is released (4 - 6) on the basis of specific energy-related interactions of the organelles with ATP, $Mg^{2+}$, or ionic "oxidisable substrates". More recently still it has been claimed that translocation of the enzyme between intra mitochondrial compartments occurs as a result of exposure to succinate (7) or other ionic solutions (8). All these effects may be related to the phenomenon described here.

## METHODS

Rat liver mitochondria were prepared by the method of Hogeboom (9). For solubilization experiments, twice sedimented mitochondria (i.e. after one sedimentation at 24,000 g) were used, since a second sedimentation at 24,000 g produced no improvement in the purity of the preparation of particles (see below). The mitochondrial pellet corresponding to 0.5 g of tissue was resuspended in 5.0 ml of 0.25 M sucrose, using a very loosely fitting glass pestle, rotated mechanically at 500 rev./min for 30 sec. 0.1 ml lots of suspension were added to 4.9 ml lots of the solution being tested, which were then capped and stored at $4^{\circ}C$. After periods of 0.5, 2, 4, and 24 h single tubes were centrifuged at 80,000 g for 60 min. 3.0 ml of the supernatant was then removed by pipette and the deposit was resuspended in the remaining supernatant using the pestle as described above. Aspartate transaminase activity was estimated in 0.2 - 0.5 ml of the supernatant and in 0.1 ml of the resuspended deposit, and was calculated as total supernatant activity and total deposit activity (corrected for that present in the 2.0 ml

of supernatant used for resuspension). Results were finally expressed in terms of supernatant activity as a proportion of the sum of supernatant and deposit activities. Aspartate transaminase was estimated by the method of Karmen (10), but at 25°C and using 33 mM L-aspartate and 33 mM phosphate buffer (final concentrations).

Twice sedimented mitochondria were found to contain 71% of the succinate dehydrogenase activity of the supernatant recovered after the first (600 g) centrifugation. Corresponding figures for acid phosphatase and glucose-6-phosphatase were 22% and 11%. For three times sedimented mitochondria the corresponding figures were 58%, 19% and 10%.

## RESULTS

Table I is a summary of several hundred experiments. The salts indicated were tested over a range of concentration of 1 - 200 mM and the following pattern was found.

i) Generally, there was a marked optimum concentration for the release of aspartate transaminase, varying only between ionic strength (I) = 10 mM and I = 30 mM for different salts. Sodium phosphate buffer gave a broad region of fairly similar behaviour between 7.5 mM and 50 mM concentration. With tris-acetate buffer, all concentrations tested above 10 mM (25 - 200 mM) showed a consistent pattern.

ii) By 30 minutes, the proportion of activity brought into solution was more than half the maximum figures, except in the case of tris-acetate. Despite some fluctuations, there was then usually a gradual increase with time.

TABLE I

Selected values of the percentage of total enzyme found in the supernatant (see text). Optimum ionic strength values are those giving, experimentally, the greatest solubilization - i.e. no interpolation was attempted. $MnSO_4$ and $ZnSO_4$ behaved much like $MgSO_4$. Except where indicated, pH was not adjusted.

| SALT | OPTIMUM IONIC STRENGTH (mM) | Concn. (mM) | EXPERIMENTAL RESULTS SUPERNATANT ACTIVITY % 30min | 24 h. |
|---|---|---|---|---|
| NaCl | 20 | 10 | 21 | 25 |
| | | 20 | 54 | 67 |
| | | 50 | 38 | 42 |
| | | 200 | 33 | 33 |
| $MgCl_2$ | 30 | 10 | 49 | 64 |
| $MgSO_4$ | 12 | 3 | 26 | 48 |
| Tris-acetate buffer pH 7.4 (concn. as tris) | | 10 | 53 | 84 |
| | | 25 | 25 | 97 |
| | | 200 | 33 | 98 |
| Sodium citrate pH 7.4 | 18 | 3 | 58 | 84 |
| Sodium phosphate buffer pH 7.4 (concn. as phosphate) | | 3 | 10 | 46 |
| | | 7.5 | 75 | 90 |
| | | 10 | 73 | 97 |
| | | 20 | 73 | 96 |
| | | 50 | 60 | 90 |
| | | 75 | 54 | 42 |
| EDTA disodium salt | 30 | 10 | 70 | 82 |

iii) In sodium phosphate buffers of constant ionic strength (10 mM) the proportion of activity brought into

solution increased generally with pH (range 6.0 - 8.0 pH), but minima were observed at pH 7.2 for tests at 2 h, pH 6.8 (4 h), and pH 6.6 (24 h).

iv) Neither water nor sucrose (0.25 M) yielded any activity in the supernatant at any time.

## DISCUSSION

Rat liver mitochondria swell in hypotonic solutions, but microscopically detectable loss of matrix material by extrusion occurs only below 15 mosmols/l (11). The present observations indicate that hypo-osmotic treatment alone does not suffice to solubilize aspartate transaminase, but the existence of an optimum salt concentration may be used to argue that osmotic swelling is part of the mechanism. Where release occurs at substantially more than 15 mosmols/l we are constrained to postulate either damage to some or all mitochondria during isolation, or that ions can penetrate or partially disrupt the previously intact mitochondrion to allow release from the inner compartment. Phosphate and tris are presumably exceptionally effective in this regard.

Below the optimum, extraction increases with salt concentration. Expressed in molar terms, the optimum is much lower for salts with polyvalent ions - which give solutions combining low osmolarity with relatively high ionic strength. This argues for a combined effect, low osmolarity improving access of ions to the binding sites and high ionic strength increasing release. In view of the influence of osmolarity one would not expect all salts to show the same optimum even if expressed in terms of ionic

strength, but it is noteworthy how small the range proves to be.

Possibly, the cationic transaminase occurs normally in association with proteins of opposite charge : the coulombic interaction is eliminated in the presence of sufficiently high ionic strength. An extremely sharp critical concentration would be expected only if access to the binding sites was always perfect.

There is some apparent conflict with the results of Rendon and Waksman (7, 8) who found only a progressive release of enzyme with salt concentration, and no maximum. It should be recalled, however, that their experiments involve a treatment with digitonin to prepare submitochondrial fractions.

There would appear to be no evidence yet of any special effect of ionic solutions beyond simple physical interactions with the mitochondria. The present results cast doubt on the validity of mitochondrial (matrix) enzyme assays carried out in buffer solutions and demand re-evaluation of the state of the mitochondria within the cell - since some enzyme release occurs in isotonic salt solutions.

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