INTRAMITOCHONDRIAL RELEASE AND BINDING OF MITOCHONDRIAL ASPARTATE AMINOTRANSFERASE AND MALATE DEHYDROGENASE IN THE PRESENCE AND ABSENCE OF EXOGENOUS SUCCINATE

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SUMMARY

Differential shuttling of aspartate aminotransferase and malate dehydrogenase between submitochondrial fractions was shown to occur. This phenomenon is dependent upon the nature of the extramitochondrial compartment.

It has been shown previously (1,2) that under defined experimental conditions, aspartate aminotransferase (AAT) (EC 2.6.1.1) is released from the inner mitochondrial membrane. If this phenomenon was found to be reversible, this would suggest that some enzymes may belong to the mitochondrial membrane system only temporarily, rather than to constitute permanent elements of the integrated structural and functional mitochondrial membrane system.

The present study shows unequivocally that released intramitochondrial AAT and malate dehydrogenase (MDH) (EC 1.1.1.37) may rebind to the mitochondrial membrane, depending upon the environmental conditions of the organelle (1).

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EXPERIMENTAL METHODS

Rat liver mitochondria were prepared by the method of Harel et al. (3), as described by Levy et al. (4). When examined under the electron microscope, these mitochondria were found to be in the condensed state as described by Hackenbrock (5).

Aliquots of mitochondria suspended in 0.25 M sucrose were incubated for 5 min at 37°C in the presence or absence of succinate (2 mM to 50 mM). In rebinding experiments, the mitochondria were incubated consecutively in sucrose, sucrose containing succinate, and sucrose, for 5 min periods each at 37°C. In parallel 15 min incubations were run in which the mitochondria were incubated in either sucrose alone or in sucrose-containing succinate.

For submitochondrial fractionation, the mitochondria were treated with digitonin, according to the method of Levy et al. (4) as modified by Schnaitman et al. (6). AAT activity was determined by measuring the rate of oxidation of NADH at 340 mμ in the presence of exogenous MDH (1). MDH was assayed by measuring the rate of oxidation of NADH in the presence of oxaloacetate, at 340 mμ. The spectrophotometric measurements were performed in a Cary type 14 spectrophotometer at 27°C. The method of Lowry et al. (7) was used for protein determinations.

RESULTS

Localization of AAT and MDH activities after successive incubation of mitochondria in sucrose, succinate, and sucrose.

The results of Fig. 1 clearly show that in mitochondria incubated in a 0.25 M sucrose medium, 86 % of the AAT activity is localized in the inner membrane-enriched fraction. When these mitochondria were transferred to a succinate medium (50

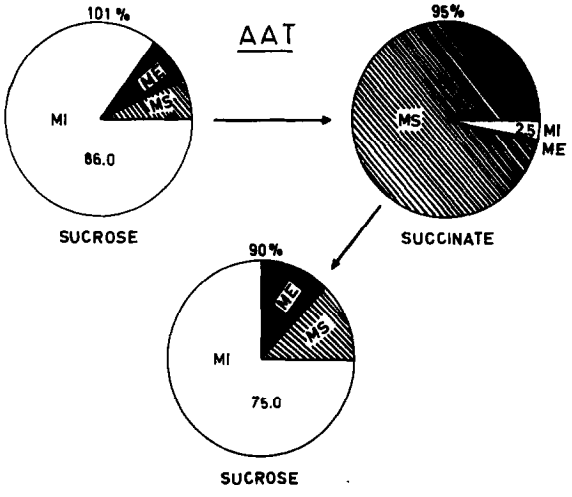

Figure 1. - Effect of consecutive incubation of mitochondria in sucrose-succinate-sucrose on AAT localization. MI : enriched internal membrane fraction ; ME : enriched external membrane fraction ; MS : soluble matrix. Figures on top of the circles indicate the per cent recovery of AAT activity. Figures in the circles indicate the per cent AAT activity bound to MI.

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mM), about 96 % of the total bound AAT left the membrane compartment and passed into the soluble matrix. Reincubation of the succinate-incubated mitochondria in a sucrose medium resulted in the rebinding of 75 % of the solubilized AAT to the inner membrane-enriched fraction. A similar cycle of solubilization-binding was also observed for MDH, but at lower concentrations of succinate (0.2 µmoles/mg mitochondrial protein) than the solubilization of AAT (0.8 µmoles/mg mitochondrial protein). This difference in the critical substrate level for the release of the enzymes suggests that the mitochondrial membranes may react specifically towards compounds, which give rise to differential release-binding cycles of certain transitorily bound proteins. As may be seen in Fig. 2, the concentrations of succinate at which these enzymes were released are of the same order of magnitude as that required to produce a

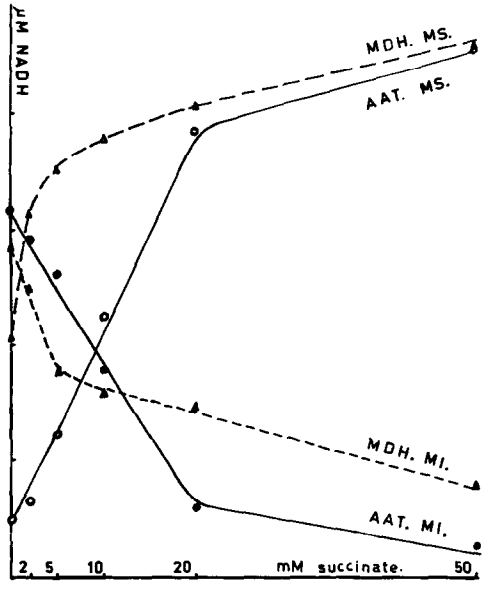

Figure 2. - AAT and MDH activity in MI and MS as a function of succinate concentration. Ordinate : µM NADH oxidized/min.

morphological change of the mitochondria detectable by electron microscopy (5).

This preliminary demonstration of a differential release of membrane-bound mitochondrial enzymes is confirmed in Fig. 3. This figure illustrates the variation in the specific activity of AAT in the different submitochondrial fractions. Indeed,the initial increase in the specific activity of AAT in the inner membrane indicates a differential solubilization of non-AAT protein.

It is conceivable that during the period of transient release from the membrane, some of the solubilized enzymes come into contact with the new and complex environment of the intermembranal space. They may, in these conditions, interact with molecules of both extra and intramitochondrial origin (8).Thus an interesting possibility is that the shuttling of enzymes between membrane and soluble fraction might play a role in the

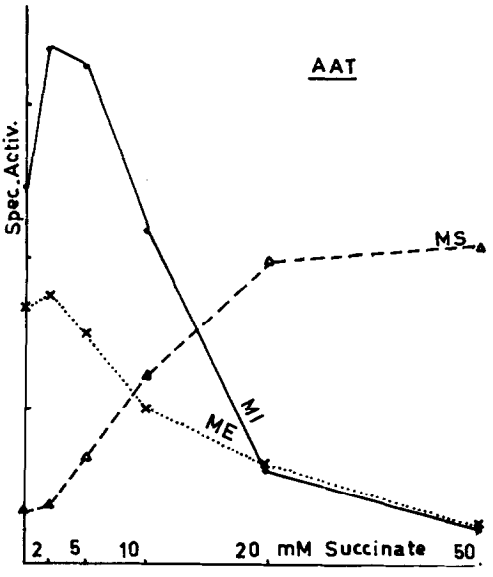

Figure 3. - Specific activity of AAT in different submitochondrial fractions as a function of succinate concentration.

transport of constituents of the intermembranal space towards the inner membranal comportment.

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