ENZYME–ENZYME COMPLEXES BETWEEN ASPARTATE AMINOTRANSFERASE AND MALATE DEHYDROGENASE FROM PIG HEART MUSCLE

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1. Introduction

Several functional complexes of enzymes that catalyze a sequence of reactions are known, for example, the α-keto acid dehydrogenase complexes [1]. Such multienzyme complexes probably have several advantages for the living cell as compared to non-interacting enzymes. By channelling the substrate of the first enzyme through the chain to the final product, time consuming free diffusion is avoided, the intermediates in the sequence are withheld from other reactions and should the concentration of initial substrate change abruptly steady state is quickly re-established. Though the multienzyme complexes studied thus far have been relatively strong complexes, systems involving weakly-bound enzymes could also be of considerable importance. However, the determination of weak interactions between proteins encounters methodological problems.

A simple method to detect protein–protein interactions is to study the mutual influence of the proteins on their respective partitions in liquid–liquid biphasic systems, suitable ones being the so-called aqueous biphasic systems developed by Albertsson [2]. Proteins have relatively high solubilities in both phases of these systems and the partition can be adjusted by addition of electrolytes [3].

In the present work interaction between malate dehydrogenase, MDH (EC 1.1.1.37) and aspartate aminotransferase, AAT (EC 2.6.1.1) has been studied by partition using a counter-current distribution technique.

2. Materials and methods

2.1. *Preparation*

All enzymes were prepared from fresh pig heart muscle. Mitochondrial (m-form) and cytoplasmic (s-form) MDH [4] as well as mitochondrial and cytoplasmic AAT [5,6] were prepared as described previously.

2.2. *Aqueous biphasic systems*

The biphasic systems were made up of water, dextran, $M_w = 5 \times 10^5$ (Pharmacia Fine Chemicals, Uppsala, Sweden) and trimethylaminopoly(ethylene glycol), TMA-PEG [7] or carboxymethyl-poly-(ethylene glycol), CM-PEG [8], both with $M_n = 6000$.

The systems contained 6.4% (w/w) dextran, 6.6% (w/w) TMA-PEG or CM-PEG and 2.5 mmol/kg potassium phosphate buffer, pH 6.0, 7.4 or 7.5.

2.3. *Counter-current distribution, CCD*

CCD with 60 transfers was carried out in the thin-layer CCD machine described elsewhere [2]. The volume ratio between the upper and lower phase was 1, except for the experiments with m-AAT and m-MDH where it was 2. A phase system containing protein(s) was introduced into chambers No. 0 and 1. The time for separation was 5 min and the shaking time 30 s. All experiments were carried out at 21 ± 1°C. After the experiment was completed 1.5 ml of water (1.2 ml in the m-AAT and m-MDH experiments) was added to each chamber to obtain homogenous solutions. The contents of each chamber were collected and analysed for enzymatic activity.

2.4. *Assay of enzymatic activity*

The activity of s-AAT was measured by a direct method [6] and the activity of m-AAT by a coupled method [9], because of product inhibition [10]. m-MDH and s-MDH were analysed for activity by following the reduction of NAD^+ [4].

3. Results

The counter-current distributions of s-AAT and s-MDH, each distributed separately, are shown in fig.1a. When the enzymes are distributed together, with an activity ratio of 12, fig.1b, and 24, fig.1c, between AAT and MDH, the s-AAT curves are not affected, but the shapes of the s-MDH curves change substantially. The material spreads out and the MDH activity splits into two peaks, one in the same position as the peak of s-MDH distributed separately and one very close to the peak of s-AAT. Furthermore, when the ratio of s-AAT to s-MDH becomes larger, more s-MDH is found close to the s-AAT peak.

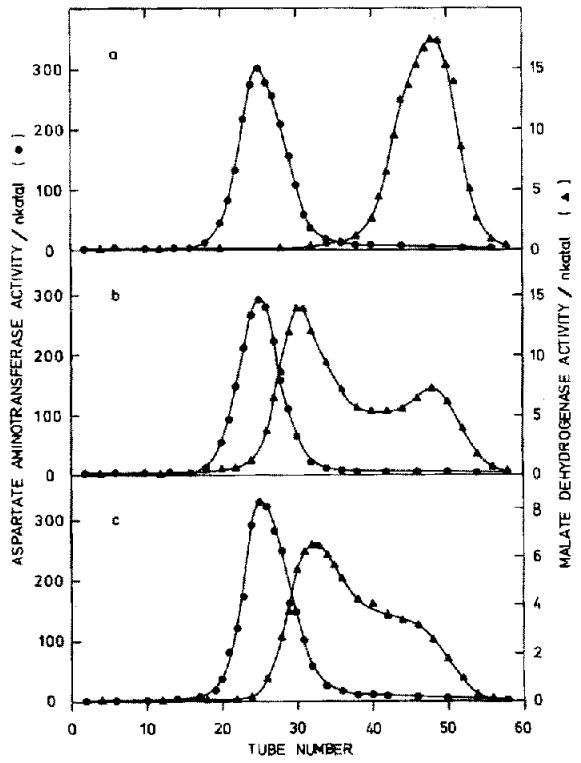

Fig.1. Counter-current distribution curves of s-AAT and s-MDH. (a) The curves of s-AAT respectively s-MDH distributed separately. (b) s-AAT and s-MDH distributed together with an activity ratio of 12 between s-AAT and s-MDH. (c) Same as in (b) but with an activity ratio of 24. The aqueous biphasic systems contained 6.4% (w/w) dextran, 6.6% (w/w) TMA-PEG and 2.5 mmol/kg potassium phosphate buffer, pH 6.0.

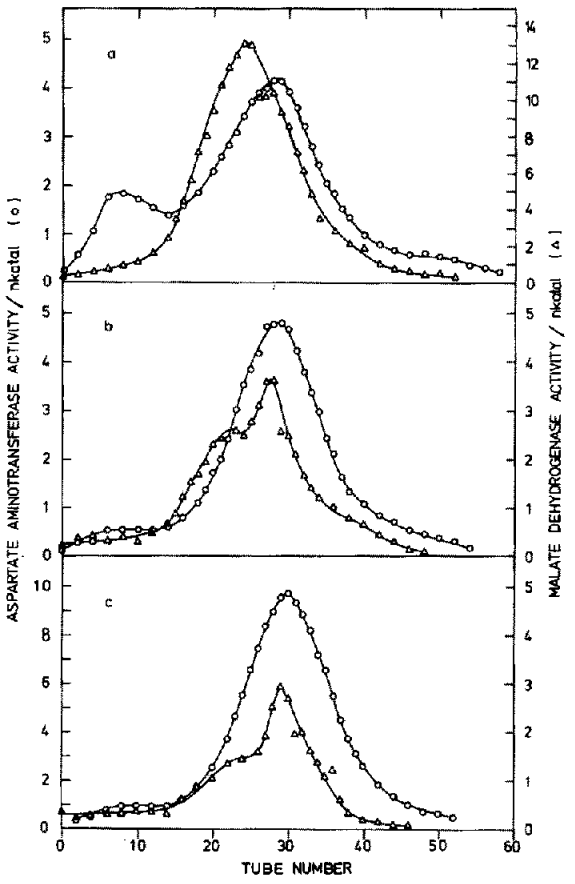

Fig.2. Counter-current distribution curves of m-AAT and m-MDH. (a) The curves of m-AAT respectively m-MDH distributed separately. (b) m-AAT and m-MDH distributed together with an activity ratio of 2 between m-AAT and m-MDH. (c) Same as in (b) but with an activity ratio of 4. The aqueous biphasic systems contained 6.4% (w/w) dextran, 6.6% (w/w) CM-PEG and 2.5 mmol/kg potassium phosphate buffer, pH 7.5.

The mitochondrial forms of the enzymes, m-AAT and m-MDH, behave similarly, fig.2a shows the counter-current distribution curves for the enzymes distributed separately and fig.2b and 2c the curves

when the enzymes are distributed together. The activity ratio of m-AAT to m-MDH was 2 (fig.2b) and 4 (fig.2c). The curves for both m-AAT and m-MDH change their shapes when the enzymes are distributed together. When distributed separately, m-AAT activity occurs in two peaks but in the presence of m-MDH, the m-AAT peak around tube No 7 is strongly reduced. The peak obtained when m-MDH is distributed singly begins to split into two peaks when m-AAT is present, with the main peak close to the main peak of m-AAT. In the same way as with the cytoplasmic enzymes, more m-MDH is found under the m-AAT peak when the ratio of the concentrations of m-AAT and m-MDH is increased.

The distributions of the single enzymes s-AAT and m-MDH are shown in fig.3a and those of m-AAT and s-MDH in fig.4a. The distributions of these enzymic activities in a mixture of s-AAT and m-MDH are shown in fig.3b, and of m-AAT and s-MDH in fig.4b. They do not differ from the distributions of the single enzymes.

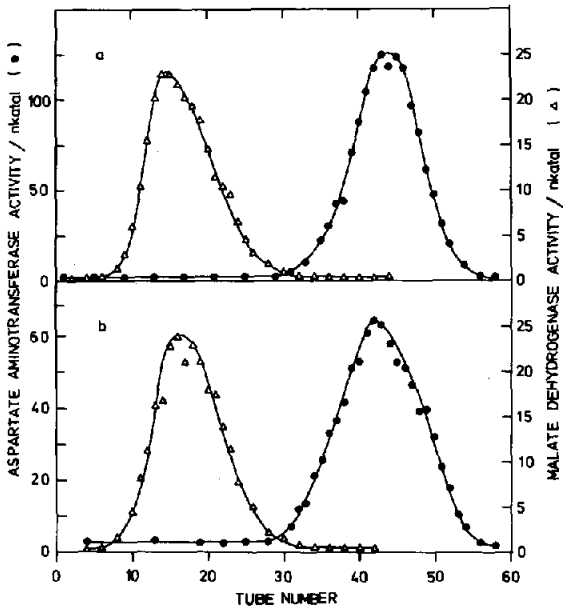

Fig.3. Counter-current distribution curves of s-AAT and m-MDH. (a) The curves of s-AAT respectively m-MDH distributed separately. (b) s-AAT and m-MDH distributed together. The aqueous biphasic systems contained 6.4% (w/w) dextran, 6.6% (w/w) TMA-PEG and 2.5 mmol/kg potassium phosphate buffer, pH 7.4.

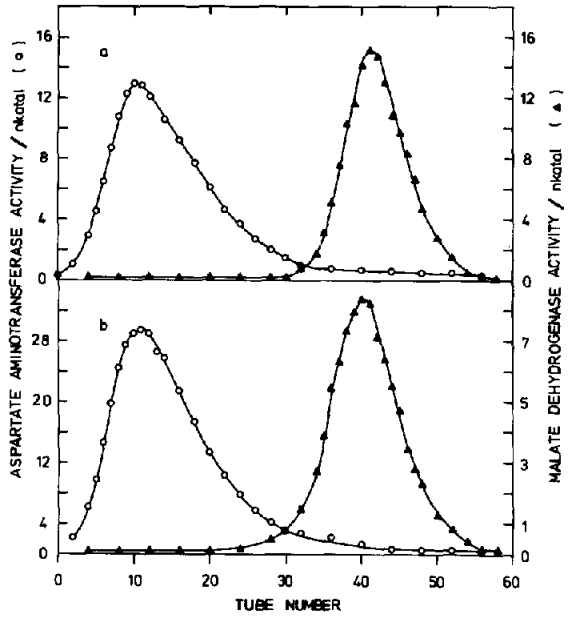

Fig.4. Counter-current distribution curves of m-AAT and s-MDH. (a) The curves of m-AAT respectively s-MDH distributed separately. (b) m-AAT and s-MDH distributed together. The preparation of s-MDH contained a little impurity in form of s-AAT which has been withdrawn from the curve of m-AAT. The aqueous biphasic systems contained 6.4% (w/w) dextran, 6.6% (w/w) TMA-PEG and 2.5 mmol/kg potassium phosphate buffer, pH 7.5.

4. Discussion

It is well known that both AAT and MDH consist of at least one cytoplasmic and one mitochondrial form. The differences in the distribution behaviour of the enzymes between when they are mixed and when they are separate show that an interaction exists between AAT and MDH. This interaction is specific in that the cytoplasmic forms of the enzymes, s-AAT and s-MDH, interact with each other as do the mitochondrial forms, m-AAT and m-MDH. No interaction between a cytoplasmic enzyme and a mitochondrial one could be detected.

4.1. *Enzyme–enzyme complexes*

Because of the demonstrated affinity, the two enzymes may, in vivo, be present partly as enzyme–enzyme complexes, one in the cytoplasm (s-AAT–s-MDH complex) and one in the mitochondria (m-AAT-

m-MDH complex). No complex will arise between s-AAT and m-MDH, or between m-AAT and s-MDH. The specificity in affinity between the two isoenzymic forms of the two enzymes, indicates that the proposed complexes are of fundamental importance for the metabolic function of the enzymes.

To our knowledge such complexes between AAT and MDH have not been reported previously. The interaction between the enzymes must be relatively weak otherwise the distribution patterns would have been different [11]. If the interaction had been strong, it would be possible to isolate these complexes.

The water content of the aqueous biphasic systems is similar to that in the living cell. These systems provide a milieu which may be more favourable for complex formation than pure water is. Furthermore, the concentrations of the enzymes in the CCD experiments did not exceed the concentrations prevailing in the cell.

An enzyme–enzyme complex between AAT and MDH should have many biological advantages for the living cell compared with free enzymes. The complex should prevent the intermediate metabolite, oxaloacetate, from diffusing into the surrounding solution where it might undergo enzymatic as well as non-enzymatic reactions, e.g. decarboxylation. It should also increase the efficiency of the overall process, as in the case of strong complexes, e.g. α-ketoglutarate dehydrogenase complex and fatty acid synthetase of yeast [1]. An intermediate metabolite bound to the complex would most likely continue through the complete reaction sequence. Malate is in this way channelled through to aspartate. Further, the velocity of the overall reaction will not depend on the rate of diffusion of oxaloacetate.

4.2. *Role in regulation*

The assemblage of enzymes of a particular metabolic sequence into a multienzyme complex could be involved in the regulation of metabolism. If the enzymes of the complex could associate or dissociate when necessary, their overall activity could be controlled. Another possibility is that complex formation changes the conformational state of the enzymes and thereby influences their activities. The formation of AAT–MDH complexes could therefore play a role in metabolic regulation in the following ways:

(1) Oxaloacetate is a substrate for a number of enzymes. When confined to the complex it is only available to the enzymes of the complex. The ratio between the free and associated enzymes can therefore regulate this metabolic crossroad, this ratio could in turn be influenced by other factors, e.g. metabolites or metallic ions.

(2) The association of AAT and MDH may also give rise to an enzyme conglomerate which might be allosteric. Both reactions could then be regulated by one effector.

(3) It has been proposed that reduced units can be transferred through the mitochondrial membrane by the malate shuttle [12]. This shuttle requires the presence of AAT and MDH on both sides of the membrane and the permeability of aspartate and malate. The AAT–MDH complexes might be involved in the regulation of the $NAD^+/NADH$ ratio inside, as well as outside the mitochondria, via this shuttle.

The counter-current distribution technique used here should prove valuable in determining whether interactions occur between enzymes of other metabolic sequences.

Acknowledgements

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