INTRAMITOCHONDRIAL RELEASE AND BINDING OF MITOCHONDRIAL ASPARTATE AMINOTRANSFERASE AND MALATE DEHYDROGENASE IN THE PRESENCE AND ABSENCE OF MONOVALENT AND BIVALENT CATIONS

A. RENDON* and A. WAKSMAN**

Centre de Neurochimie du CNRS, Faculté de Médecine, 67-Strasbourg (France)

Received February 5, 1971

SUMMARY

Intramitochondrial release and binding of mitochondrial aspartate aminotransferase and malate dehydrogenase was shown to be controlled by a sucrose-cation-sucrose cycle *in vitro*.

The effect of ion concentration on the aspartate aminotransferase release suggests distinct modes of action for bivalent and monovalent cations.

Differential shuttling of intramitochondrial aspartate amino transferase (AAT) (EC 2.6.1.1) and malate dehydrogenase (MDH) (EC 1.1.1.37), between submitochondrial fractions, has been shown to occur in the presence and absence of succinate. The connection of these release-binding cycles and the sucrose-succinate-sucrose cycle was established (1), suggesting that some enzymes may only temporarily belong to the mitochondrial membrane system, rather then constituting permanent elements of the integrated structural and functional mitochondrial membrane.

As ions play a fundamental role in the regulation of the functional physiological and morphological state of the mitochondrion (2, 3, 4), the possibility of a relation between extra- intramitochondrial ion gradients and enzyme movements was

---

* Attaché de recherche au CNRS.
**Chargé de recherche au CNRS.

investigated.

EXPERIMENTAL METHODS

Rat liver mitochondria were prepared as described by Harel et al. (5) and Levy et al. (6). Enzyme assays were as reported by Waksman and Rendon (1). Assays in the presence of cations were performed at ion concentrations varying from 2 mM to 100 mM. Incubation cycles in the presence and absence of cations were carried out as described for succinate cycles by Waksman and Rendon (1).

Submitochondrial fractions were obtained after action of digitonin according to Levy et al. (6) as modified by Schnaitman et al. (7). The method of Lowry et al. (8) was used for protein determination.

RESULTS

Effect of ionic concentration on intramitochondrial release of mitochondrial "bound" AAT and MDH

Fig.1 shows the effects of increasing concentration (2 mM to 100 mM) of NaCl, KCl, $CaCl_2$ and $MgCl_2$ on the release of bound AAT. Two modes of interactions were observed. With Ca^{++} and Mg^{++} AAT release is directly proportional to the ion concentration, and has the same shape as with sodium succinate (broken line). The only difference is that with Mg^{++} the curve reaches a plateau at lower levels of released enzyme. With Na^{+} and K^{+} however, the release curve has a sigmoidal shape with a very marked "latency".

These results suggest that two distinct mechanisms of release are involved. The first one is presumably the result of a direct action of the cation, affecting the enzyme-membrane interacting site. The second one is either a release through an al-

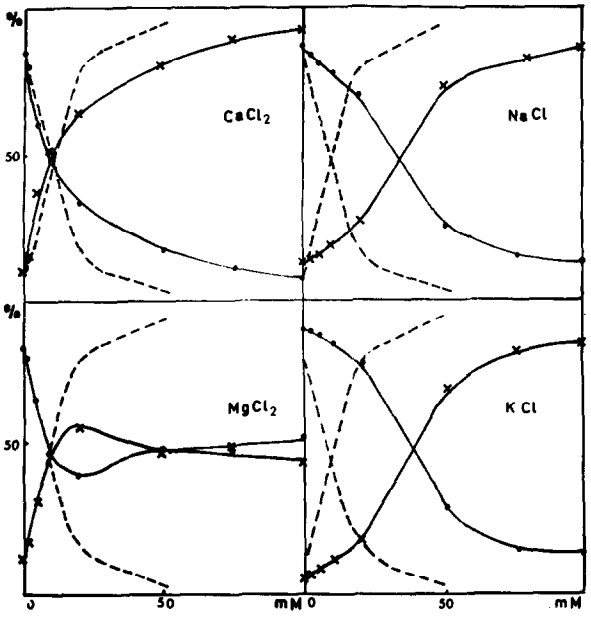

Figure 1. - Effect of ions concentration on AAT release. x——x activity in soluble matrix ; ●——● activity in internal membrane enriched fraction ; ------ succinate effect.

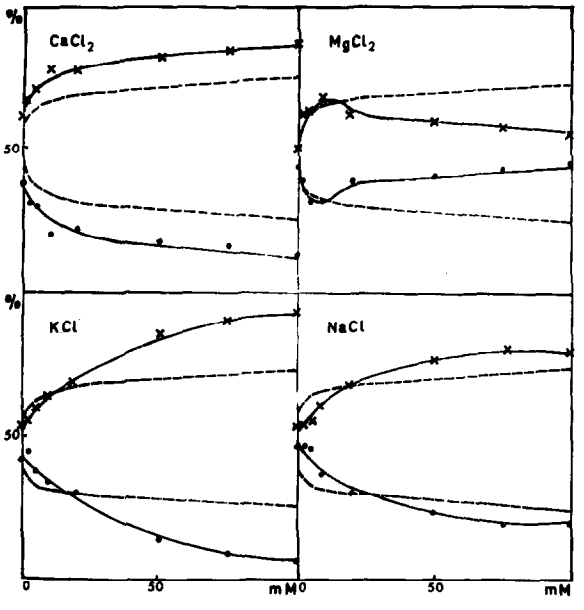

Figure 2. - Effect of ion concentration on MDH release. x——x activity in soluble matrix ; ●——● activity in internal membrane enriched fraction ; ------ succinate effect.

Table 1

Localization of AAT in digitonine treated mitochondria as a function of exogenous conditions

Cation	Sample	AAT as % of total recovery		
		Sucrose*	Cation*	Sucrose*
Na^+, 50 mM	Outer membrane	4.4	1.0	8.5
	Inner membrane	92.0	36.0	87.0
	Soluble matrix	3.6	63.0	4.5
K^+, 50 mM	Outer membrane	3.0	1.0	6.5
	Inner membrane	88.0	49.0	85.0
	Soluble matrix	9.0	50.0	8.5
Ca^{++}, 10 mM	Outer membrane	7.0	1.0	3.0
	Inner membrane	87.0	22.0	86.0
	Soluble matrix	6.0	77.0	11.0
Mg^{++}, 10 mM	Outer membrane	4.0	1.0	2.0
	Inner membrane	89.0	39.0	87.0
	Soluble matrix	7.0	60.0	11.0

*The mitochondria were incubated consecutively in sucrose, sucrose containing cation at indicated concentration, and sucrose for 5 min periods each at 37°C. Thereafter submitochondrial fractionation was performed and AAT activity was measured in each fraction.

losteric effect on the mitochondrial inner membrane, or the result of a diffusion phenomenon through a membrane, mediated by the monovalent cations.

In the case of MDH (Fig.2) all ions have a similar effect.A progressive release of the enzyme was observed as a function of the concentration of the monovalent and divalent ions tested.The only pecularity observed is with Mg^{++} for which the curve plateaus at lower levels of released MDH, than for the three other ions tested. No clear correlation can be drawn, as yet, between the nature of the ion and the intramitochondrial release of mitochondrial MDH.

Generation of intramitochondrial release-binding cycles of mitochondrial AAT and MDH in presence and absence of monovalent and bivalent cations

Under the specified conditions released AAT and MDH can rebind with the mitochondrial membrane fraction. The release-binding cycles are comparable to those obtained with succinate (1). Table 1 summarizes the results obtained with AAT and shows clearly the relationship between the shuttling of the enzymes from membrane to intramitochondrial soluble compartments and the presence of exogenous ions. Similar results were obtained for MDH.

A challenging hypothesis would be to relate the functional significance of these phenomena to the transport of ions and small molecules from the intramitochondrial soluble compartment to the membrane.

ACKNOWLEDGEMENTS

We are grateful to Dr. H. Hadjilazaro for her valuable contribution to this paper. We wish to acknowledge technical assistance of Misses E. Bouvart and J. Valora.

REFERENCES

1. Waksman, A. and Rendon, A. Biochem. Biophys. Res. Commun.,in press.
2. Lehninger, A.L., in "The mitochondria", ed.A.L. Lehninger, W.A. Benjamin Inc. New-York-Amsterdam, p.157 (1965).
3. Chappell, J.B. and Haarhoff, K.P., in "Biochemistry of mitochondria", ed. E.C. Slater, Z. Kaminga and L. Wojtezac, Academic Press, London, p.75 (1967).
4. Hackenbrock, C. and Caplan, H.L., J. Cell Biol. 42, 221 (1969).
5. Harel, L., Jacob, A. and Moulé, Y. Bull. Soc. Chim. Biol.,39, 819 (1957).
6. Levy, M., Toury, R. and André, J. Biochim. Biophys. Acta, 135, 599 (1967).
7. Schnaitman, C., Erwin, V.G. and Greenawalt, J.W., J. Cell Biol., 32, 719 (1967).
8. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. J. Biol. Chem., 193, 265 (1951).