RELEASE AND BINDING OF PROTEINS AND ENZYMES WITH ISOLATED INNER MITOCHONDRIAL MEMBRANES

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SUMMARY

Extramitochondrial substances trigger specific and reversible release of proteins and enzymes from inner membranal compartment towards intermembranal space. This shuttling phenomenon has been shown to occur with isolated inner membranes.

In previous experiments we were able to show that rat liver mitochondria respond to variations of the extramitochondrial environment by releasing proteins and enzymes from the inner membranal compartment towards the intermembranal space. This phenomenon shows some specificity as for the nature of the released proteins and is characterized by the capacity of the inner membrane to recognize various extramitochondrial "releasing effectors". Furthermore rebinding of released proteins is observed when the "releasing effectors" are withdrawn from the extramitochondrial medium[1,2].

The present paper compares results obtained with inner membranes prepared by two different methods[3,4]. They show good concordance in the effects produced by the "releasing effectors" on these two preparations. They suggest the equivalences of the inner membranes obtained by different methods as far as the release and binding phenomena are concerned. They exclude the possibility for digitonin to synergize the action of the "releasing effector" or to weaken the membrane as to provoke the release and binding process. They also suggest independance of the release phenomenon from ionic strength.

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EXPERIMENTAL METHODS

Rat liver mitochondria were prepared according to Harel *et al.*[6] as described by Levy *et al.*[5].Mitochondrial subfractions were prepared either by the digitonin method[3,5] or by the swelling-condensing-sonication method[4]. For the release experiments, inner membrane obtained with both methods and corresponding to 25 mg of initial mitochondrial preparation, were incubated for 5 min at 37 °C in presence of various"releasing effectors"at diverse concentrations. Final sucrose concentration was 0.25 M ; final volume was 1 ml. After incubation, the assay mixture was centrifuged for 15 min at 60,000 x g in a type 40 rotor at 4 °C, in a Spinco model L centrifuge. Supernatants were collected and pellets were resuspended and homogenized in 3 ml ice cold water and stored overnight at 4 °C.

Aspartate aminotransferase (AAT) activity was determined by measuring the rate of oxidation of NADH at 340 nm in the presence of exogenous malate dehydrogenase. Malate dehydrogenase (MDH) was assayed by measuring the rate of NADH oxidation in presence of oxalacetate at 340 nm[7].The spectrophotometric measurements were performed in a Beckman Acta III type spectrophotometer at 22 °C. The method of Lowry *et al.*[8] was used for protein determinations.

For binding experiments mitochondria corresponding to 100 mg of protein were suspended in sucrose containing 50 mM succinate, and incubated as mentionned above. They were then treated with digitonin according to Schnaitman *et al.*[3], and the pellet was separated from the supernatant under the already mentionned conditions.

The supernatant fraction was divided in two equal parts, one of which was dialyzed against 0.25 M sucrose, and the other one against 0.25 M sucrose containing 12.5 mM succinate. Dialysis was performed under constant stirring at 4 °C for 18 h, against five changes of 10 volumes of dialyzing fluid. The dialyzed supernatants were than incubated with freshly prepared inner membranes obtained from either digitonin treated, succinate exposed mitochon-

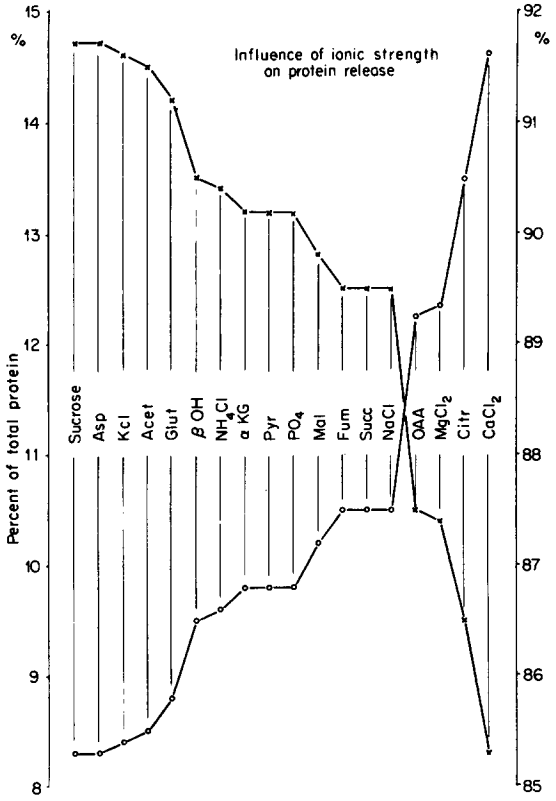

Fig. 1.

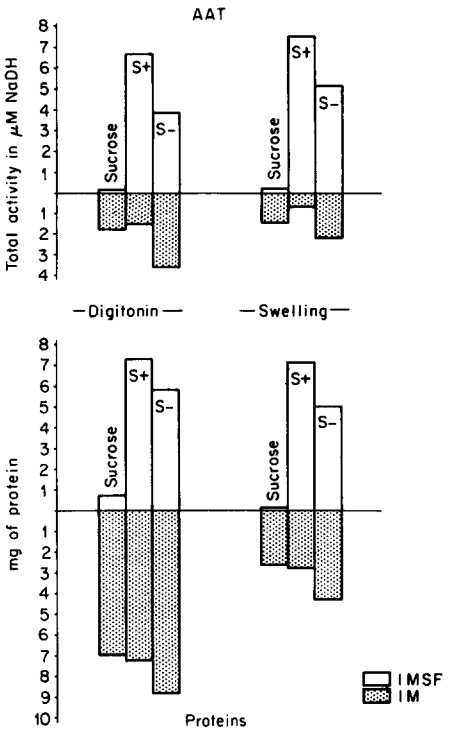

Fig. 2.

Fig. 1. Release of proteins by isolated inner membranes at equivalent ionic strength. Results are expressed as percent of total protein. Succinate 10 mM-acetate 30 mM-citrate 5 mM. IM : inner membrane. IMSF : intermembranal soluble fluid. Asp : aspartate ; Acet : acetate ; Glut : glutamate ; βOH : β-hydroxybutyrate ; Pyr : pyruvate ; αKG : α-ketoglutarate ; Succ : succinate ; Mal : malate ; Fum : fumarate; Citr : citrate ; OAA : oxalacetate.

Fig. 2. Release and binding of proteins and AAT by inner membranes isolated either by the digitonin or by the swelling-condensing-sonication method. Sucrose : in presence of sucrose alone (control). S+ : in presence of succinate containing intermembranal fluid. S- : in presence of sucrose dialyzed succinate containing intermembranal fluid. IM : inner membrane ; IMSF : intermembranal soluble fluid.

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dria (25 mg of protein), or from swelling-condensing-sonication, succinate exposed mitochondria (25 mg of protein). A control incubation in presence of 0.25 M sucrose was run simultaneously. Thereafter the preparations were centrifuged at 60,000 x g for 15 min at 4 °C in a Spinco model L centrifuge.

Table 1 - Release of Proteins and Enzymes : Comparison of the Digitonin and the Swelling Method

	Subfractions	Sucrose		Succinate	
		Digitonin	Swelling	Digitonin	Swelling
Protein	IM	88.6	73	83	65
	IMSF	11.4	27	17	35
AAT	IM	96.2	88.8	23.4	33.7
	IMSF	3.8	11.2	76.6	66.3
MDH	IM	90	50.5	51.3*	28
	IMSF	10	49.5	48.7	72

Results are expressed as percent of total activity for AAT and MDH, and as percent of total protein. IM : inner membrane. IMSF : inter-membranal soluble fluid. Columns compare the results obtained either by the digitonin method or by the swelling-condensing-sonication method.
*10 mM succinate.

The pellets were homogenized in 3 ml water. Suspended pellets and supernatants were allowed to stay overnight at 4 °C. AAT, MDH and protein determinations were made on the different fractions.

RESULTS AND DISCUSSION

Release of AAT, MDH and proteins from isolated inner membranes

Table 1 compares AAT, MDH and protein release from inner membranes obtained by either the digitonin or the swelling-condensing-sonication method. They show that both preparations are able to release AAT, MDH and proteins and that this release is of the same order of magnitude in both preparations.

These results exclude the possibility for digitonin to be the agent responsible for the release, and thus suggest that the inner mitochondrial membrane reacts in response to exogenous effector succinate by releasing proteins and enzymes.

Influence of ionic strength on protein release

Fig. 1 shows the differential effect of diverse releasing effectors at comparable ionic strength as far as protein release is concerned. The results, expressed in percent of total release, suggest that the observed phenomena is not due to changes in ionic strength only, for at equivalently ionic strength protein release is not equal in each assay.

Binding of released AAT, MDH and protein on isolated inner membranes

For these experiments inner mitochondrial membranes obtained by the above described methods were incubated either in sucrose, or in intermembranal fluid from succinate treated mitochondria dialyzed against sucrose or sucrose containing succinate. The results in Fig. 2 show that under our experimental conditions both types of inner membranes have still rebinding properties and that there is good agreement between the two preparations although the isolation procedures of the organels and their subfractions are quite different in both cases. Thus inner mitochondrial membranes obtained either by digitonin or sonication show releasing capacity and are characterized by rebinding potentiality. The observed phenomenon does not seem to be directly dependent upon the variation of ionic strength of the system.

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