CONVERSION OF THE Na^+ AND K^+ INDEPENDENT PART OF THE BRAIN MICROSOMAL ATPASE TO A FORM REQUIRING ADDED Na^+ AND K^+

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The Na^+ and K^+ activated, Mg^{++}-dependent ATPase is found in almost all tissues and especially in subcellular fractions containing cell membrane fragments. It has been observed by many workers that disruption of the membrane fragments, beyond that obtained during the homogenization, by procedures such as freezing and thawing (Deul and McIlwain, 1961), or inclusion of detergents in the homogenization medium (Skou 1962, Schwartz 1963) produces a higher yield of the Na^+ and K^+ dependent activity.

It is the purpose of this communication to demonstrate, that the ATPase of the brain microsomal fraction can be converted from a largely Na^+ and K^+ independent form, to a form which is almost absolutely dependent on added Na^+ and K^+.

Preparation of enzyme. Brain microsomes were isolated from 0.25M sucrose homogenates of rat brains as described previously (Järnefelt, 1962), and suspended in 0.25M sucrose to a final concentration of 7.5 mg protein/ml. This preparation, is used either as such for assays, or is treated further in the following manner. To the microsomal suspension is added an equal volume of 1M NaCl or KCl containing a desired amount of Na-deoxycholate (DOC) (or the

equivalent amount of a tris salt of deoxycholic acid). The concentrations of DOC used were: 0, 0.15, 0.45, 0.75 and 1.5 mg/ml; the final concentration of salt was always 0.5M. The particle-salt-DOC mixture was immediately centrifuged at 100000 g for 60 minutes, the sediment was suspended in 0.25M sucrose or 0.02 M tris-Cl buffer, pH 7.5, and made up to a volume equal to twelve times the original volume of the microsomal suspension, and centrifuged again at 100000 g for 60 minutes The sediment was now suspended in sucrose or tris-Cl, and adjusted to a volume equal to the original.

Assays. The ATPase activity of the preparations obtained was measured in the usual manner (Järnefelt, 1962), both in the absence and in the presence of added Na^+ and K^+ . Mg^{++} was used throughout the assays at a concentration of 4 mM, and Na^+, when added, was always 0.1 M, K^+, 5mM. The Na^+ and K^+ content of the microsomal pellets were determined in a Baird-Atomic DB-5 flame photometer after resuspension in distilled water, using Li_2SO_4 as internal standard.

Results and Discussion. Table 1 shows the ATPase activity of the various preparations. The activities are expressed as total activities of the enzyme preparation studied, and therefore indicate the total enzymic activity recovered. It is immediately obvious that the rather high ATPase activity in absence of added Na^+ and K^+ found in the original microsomes disappears rapidly on exposure to increasing concentrations of DOC. However, this does not indicate a true loss of enzyme, since full activity can be restored by the addition of Na^+ and K^+ to the medium. Up to a DOC concentration of 0.75 mg/ml the ATPase activity in presence of Na^+ and K^+ hardly

Table 1. Effect of treatment of rat brain microsomes with deoxycholate and salt (NaCl or KCl) on the ATPase activity and on the Na and K content.

Concentration of DOC used (in mg/ml) in treatment of microsomes:	0	0.15	0.45	0.75	1.5
ATPase activity, μmoles, P_i liberated/10 min:					
Na and K not added	18.5	15.8	8.5	6.4	2.0
Na and K added (*)	28.8	25.6	23.6	25.6	2.5
The pellet from one ml of microsomes contains:					
K, mμmoles	103	49	26	24	13
Na, mμmoles	262	77	60	52	65

*Na = 0.1M, K = 5 mM.

decreases at all. Thus, it has been possible to convert, through treatment with deoxycholate and salt, the brain microsomal ATPase from a form that to a large extent (70%) does not require added Na^+ and K^+ to a form that shows an almost absolute (80%) requirement on added Na^+ and K^+, without any significant loss in total activity.

It is tempting to assume that the original particles contain enough Na^+ and K^+ to partially activate the enzyme, and that DOC treatment removes one or both of these ions from the enzyme. Determination of K^+ and Na^+ in the microsomal pellet bears this assumption out, as shown in Table 1. Both K^+ and Na^+ are removed by DOC, and there is a reasonably close parallelism, especially between the amount of K^+ and the activity of the ATPase when assayed without added Na^+ and K^+.

Since the amounts of Na^+ and K^+ originally present are very much smaller than those required for the activation of the enzyme, it can be assumed that the bound Na^+ and K^+ occur at specific sites, where they act. Support for this view is given by the inhibiting effect of ouabain on the two preparations, the untreated microsomes, and the

microsomes treated with 0.75 mg DOC/ml. Table 2 shows an experiment, where the ATPase was assayed in absence of ouabain and in the presence of two concentrations of ouabain (10^{-5}M and 5 x 10^{-5}M). In both types of microsomes, ouabain is unable to inhibit significantly the ATPase activity in absence of added Na^+ and K^+. The additional

Table 2. Effect of ouabain at two concentrations on the ATPase of untreated brain microsomes and microsomes treated with 0.75 mg DOC/ml and 0.5 M NaCl.

	ATPase μmoles P_i liberated/10 min:			
	treated		untreated	
	-Na -K	+Na*+K*	-Na -K	+Na*+K*
No ouabain	4.2	25.7	14.5	22.8
10^{-5} ouabain	3.6	10.3	15.5	19.9
5 x 10^{-5} ouabain	2.8	6.9	14.5	17.0

*Na = 0.1M; K = 5mM; all assays contain Mg = 4 mM.

activity found in the presence of Na^+ and K^+ is, on the other hand, strongly inhibited by ouabain (70-80%). The activity of the treated enzyme in presence of added Na^+ and K^+ is depressed by ouabain considerably below the level of the activity of the untreated enzyme in absence of added Na^+ and K^+.

Under conditions, where the conversion of the activity pattern takes place, Na^+ and K^+ are removed from the particles, and it is therefore postulated that the loss of endogenous Na^+ and K^+ is responsible for the emerging requirement for added Na^+ and K^+. The functional differences between the endogenous and added Na^+ and K^+ are emphasized by the effect of ouabain: it can not reach the activity supported by endogenous Na^+ and K^+, whereas it powerfully inhibits the activity restored by the addition of these ions, irrespective of whether this constitutes 30% of the total activity, as

in the untreated microsomes, or 80%, as in the DOC-salt treated microsomes. A similar pattern with respect to kidney microsomes was observed by Landon and Norris (1963). Through dialysis, the ATPase, which originally was not Na^+ and K^+ dependent, was converted into a Na^+ and K^+ dependent form, which was also ouabain sensitive. K^+, and possibly Na^+, were lost from the preparation during the dialysis.

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