Connection Between Membrane Adenosinetriphosphatase Activity and Potassium Transport in Erythrocyte Ghosts

In our earlier experiments[1-3] we have stated that ATP produced by glycolysis serves as energy source for K^+ accumulation in human erythrocytes. In the present paper we report on the stoichiometric connection found between ATP-splitting and K^+-influx.

The preparation of ATP-rich ghosts was carried out by the method published in our earlier paper[2]. The ghosts contained 2–6 mg ATP/ml, i.e. 2–8 times the physiological ATP content of the erythrocytes at 0 min. As glycolytic substrate was not added to the system, glycolysis activity was very low. The cells were reverted with hypertonic NaCl; the ghosts thus contained more Na^+ than K^+. The ghosts were suspended in a medium containing 124 m*M* NaCl, 25 m*M* $KHCO_3$ and 4 m*M* $MgSO_4$. The 'haematocrite' value was 35–45%, the incubation time $1\frac{1}{2}$–4 h at 37°C.

We studied the relation between K^+-influx of the cells and ATP-splitting in ATP-rich ghosts. Figure 1 shows one of these experiments, in which we observed the kinetics of K^+ accumulation and ATP breakdown. The experiment points to a parallelism between the transport procedure and enzyme activity.

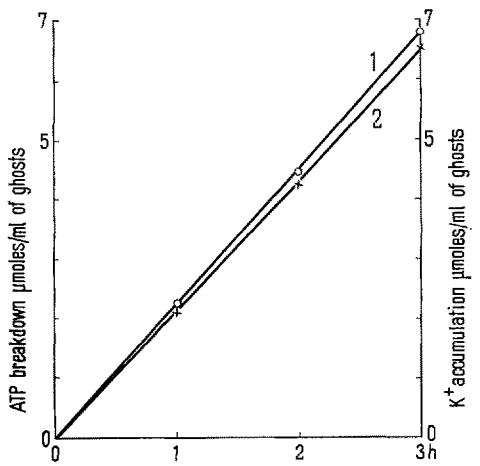

Fig. 1. Kinetics of ATPase activity and K^+ accumulation in ATP-rich erythrocyte ghosts. 1 = ATP breakdown, 2 = K^+ accumulation.

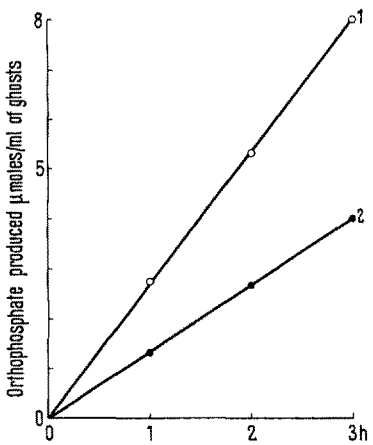

Fig. 2. ATPase activity in ATP-rich erythrocyte ghosts. 1 = Control, 2 = in the presence of 10^{-5} *M* ouabain.

As is known, hydrolysis of ATP supplies not only the energy need of the active cation transport but also ensures the presence of other factors required for the maintenance of physiological conditions in the red cells. The experiments of DUNHAM and GLYNN[4] also emphasized that only part of the total membrane-ATPase activity was 'transport ATPase'. This 'transport ATPase' can be specifically inhibited by cardiac glycosides, especially ouabain, and activated by monovalent cations, K^+ and Na^+[4,5]. In Figure 2 an experiment is presented in which we investigated what percentage of the total ATPase activity consisted of ouabain-sensitive ATPase. The results – supported by other authors[4,6] – show that under our experimental conditions 50% of the total ATPase activity was 'transport ATPase'.

We then examined whether there exists a stoichiometric connection between 'transport ATPase' activity and K^+ accumulation. The results of 10 experiments are summarized in the Table. This series of experiments points to a stoichiometric relation between ATP-splitting and ion movement: the hydrolysis of 1 ATP molecule is accompanied by the accumulation of 2 K^+ ions. This experimental result seems to support the hypothesis that membrane-ATPase is part of the carrier producing ion movement.

Stoichiometric connection between 'transport ATPase' activity and K^+ accumulation in ATP-rich erythrocyte ghosts

No.	Incubation time at 37° h	K^+ accumulation nmoles/l ghosts	'Transport ATPase' activity mmoles/l ghosts	K^+ accumulation / ATP split
1	1.5	8.00	3.88	2.06
2	1.5	5.45	2.50	2.18
3	2	2.20	1.16	1.90
4	2	5.50	2.65	2.08
5	3	7.06	4.00	1.77
6	3	6.10	3.49	1.75
7	3	5.34	2.38	2.24
8	3	6.40	3.50	1.83
9	4	9.15	3.94	2.32
10	4	7.80	3.23	2.42
		Average of 10 experiments		2.05 ± 0.24

Zusammenfassung. In ATP angereicherten menschlichen Erythrocytenstroma konnte zwischen Ouabain-empfindlicher Membran-ATPase-Aktivität und aktiver K^+-Akkumulation ein Zusammenhang festgestellt werden: die Hydrolyse eines ATP-Moleküls ist mit der Akkumulation von zwei K^+-Ionen verbunden.

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