Hypothesis

# A FORM OF $(Ca^{2+} + Mg^{2+})$-ATPase OF HUMAN RED CELL MEMBRANES WITH LOW AFFINITY FOR Mg-ATP: A HYPOTHESIS FOR ITS FUNCTION

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We have shown before that the cell membrane is responsible for the entire true ATPase activity of human red cells and that in the absence of membranes a steady state of glycolysis cannot exist [1]. Thus the ATP consumption by membrane ATPases is the main controlling factor of red cell glycolysis and is equal to the ATP production which amounts to ~2 mmol ATP per liter cells and hour at pH 7.2 and 37°C.

The known ion transport ATPase activities amount to ≲25% of the total ATP consumption. Most of it is due to $(Na^+ + K^+ + Mg^{2+})$-ATPase activity, the share of 20% of which is indicated by the effect of ouabain on red cell glycolysis [2] and on the total ATP-breakdown of glucose-free cells (fig.1). It is even smaller at low pH-values and temperatures [3].

The extent of $Ca^{2+}$ transport under physiological conditions is very small indeed, owing to the low passive permeability of the mature human red cell for $Ca^{2+}$. Only ~1 μmol calcium per liter cells and hour enters the cells at 37°C [4]. The low permeability of the red cell membrane to $Ca^{2+}$ is changed little during cellular ATP depletion [5]. Thus <1% of the ATP production is used for $Ca^{2+}$ transport in intact red cells.

The correctness of this assessment is borne out by the negligible effect of lanthanum ions, the most effective inhibitor of active $Ca^{2+}$-transport in human red cells [6], on the ATP-breakdown of glucose-free cells (fig.1).

Investigations on the ATP depletion of glucose-free cells with and without ouabain demonstrated that the rate of ATP-breakdown declined with lower ATP concentrations ([3] and fig.1). These results indicated the presence of an ATPase with low affinity for Mg-ATP in intact red cells (~1 mM Mg-ATP). Therefore we began a search for an ATPase with such characteristics. Fig.2 demonstrates that the $(Ca^{2+} + Mg^{2+})$-ATPase activity of 'isotonic' membranes has $K_m$ ~0.76 mM Mg-ATP, in contrast to a 'hypotonic' membrane preparation, which had a 20-fold higher affinity ($K_m$ 0.035 mM). Washing of 'isotonic' membranes with a hypotonic solution resulted in a 5-fold increase of affinity for Mg-ATP.

The $K_m$-value of Mg-ATP of the 'hypotonic' preparation corresponds to that of the 'B-state' of the $(Ca^{2+} + Mg^{2+})$-ATPase as in [7]. The data presented indicate that there exists a factor in red cells in addi-

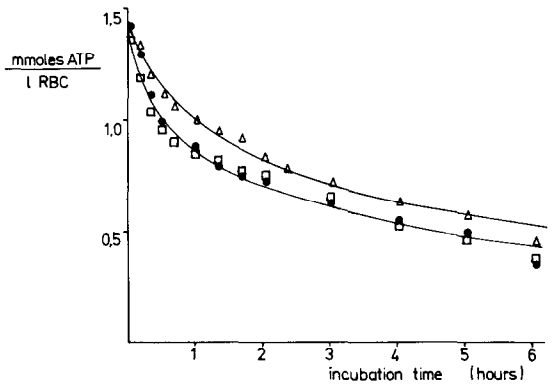


Fig.1. ATP-breakdown in intact glucose-free human red blood cells at 37°C incubated in a medium containing 145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $Na_2HPO_4$, 1.5 mM $CaCl_2$ and 10 mM imidazole at pH 7.4. Red cells from fresh, heparinized blood were washed twice with glucose (5 mM) containing solution (as above) at 37°C to maintain the steady state concentrations of metabolites. The red cells were depleted of glucose by two further washing steps without glucose at 37°C and incubated at hematocrit values of 42%. (●---●) Control; (△---△) addition of 0.2 mM ouabain or 0.2 mM ouabain + 0.1 mM $La^{3+}$; (□---□) addition of 0.1 mM $La^{3+}$.

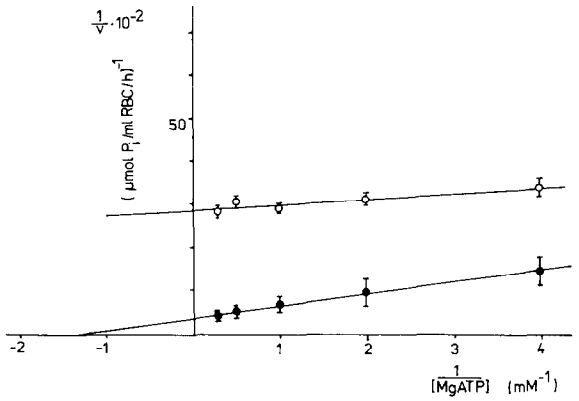


Fig.2. Double reciprocal plot of ($Ca^{2+}$ + $Mg^{2+}$)-ATPase activity as a function of Mg-ATP concentration in media with 1 mM $Mg^{2+}$. The reaction mixture contained 120 mM KCl, 20 mM NaCl, 1 mM dithioerythritol, 0.2 mM ouabain and 10 mM imidazole (pH 7.2). Membranes were assayed for ATPase activity by incubation at 37°C for 15 min and the $P_i$ liberated was determined by the method in [12]. The ATPase activities of the membranes were normalized to 1 ml red blood cells in relation to the recovery of acetylcholinesterase activity [13] of the whole hemolysate. (o---o) 'Hypotonic' membranes, prepared under hypotonic conditions in 10 mM imidazole (pH 7.2) and freeze–thawed for ATPase determination. (•---•) 'Isotonic' membranes; prepared in a solution containing 120 mM KCl, 4 mM 2-mercaptoethanol, 10 mM imidazole (pH 7.2), 1 mM $MgEt_2$, 20 mM NaCl. Hemolysis was carried out with a 10% cell suspension by a freeze–thaw procedure or by addition of 0.02% saponin.

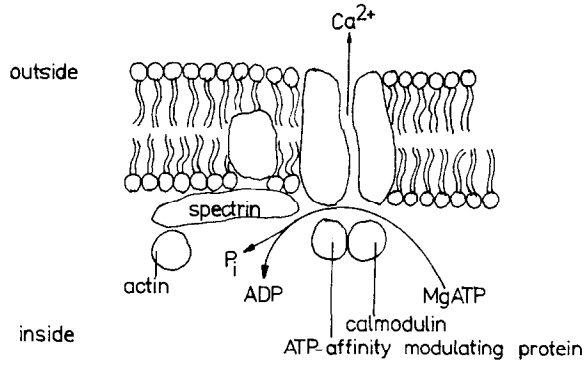


Fig.3. Hypothetical model for the action of red blood cell membrane ($Ca^{2+}$ + $Mg^{2+}$)-ATPase at the intracellular [$Ca^{2+}$] (probably 1 µM). A Mg-ATP affinity modulating protein is assumed to form a complex with Ca-ATPase and calmodulin which are bound to the membrane. The following properties are postulated: (1) At low [$Ca^{2+}$] the complex is assumed to have both Mg-ATP-dependent kinase activity and $Ca^{2+}$-dependent phosphatase activity with low Mg-ATP affinity which phosphorylate and dephosphorylate so far unspecified membrane proteins; (2) Increase of intracellular [$Ca^{2+}$] dissociates the modulating protein from the complex so that ($Ca^{2+}$ + $Mg^{2+}$)-ATPase + calmodulin acts as a $Ca^{2+}$-transporting system with high ATP affinity.

tion to calmodulin, which modulates the properties of ($Ca^{2+}$ + $Mg^{2+}$)-ATPase in red cells.

Calmodulin is known to increase the affinity for $Ca^{2+}$ and the maximal activity of the enzyme [8], while the affinity for Mg-ATP may not be changed greatly [9]. The additional factor apparently affects primarily the affinity for Mg-ATP and maintains the high ($Ca^{2+}$ + $Mg^{2+}$)-ATPase activity of isotonically isolated red blood cell membranes. It is possible that this postulated factor is identical with the modulator-binding protein found in brain, which is reported to antagonize the ($Ca^{2+}$ + $Mg^{2+}$)-ATPase activation of red blood cell membranes by calmodulin [10,11].

We assume that ($Ca^{2+}$ + $Mg^{2+}$)-ATPase, calmodulin and the modulator-binding protein constitute a system by which the affinity and activity of this ATPase is adjusted over a wide range. Presumably the modulation of the affinity for Mg-ATP is of greater importance than that for $Ca^{2+}$ in intact red cells. Furthermore we should like to propose that this system has functions which are not limited to $Ca^{2+}$ transport through the cell membrane.

We suggest that its function is a combination of a kinase reaction dependent on Mg-ATP with a phosphatase reaction dependent on $Ca^{2+}$, which might result in an apparent ($Ca^{2+}$ + $Mg^{2+}$)-ATPase activity separate from the $Ca^{2+}$-pump and which affects the structure and dynamics of the cell membrane. We assume that this system is responsible for the bulk of ATP consumption of the red cell membrane. A scheme of our hypothesis is shown in fig.3.

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