BIKEN JOURNAL Vol. 12, 141-143, 1969

SHORT COMMUNICATION

INHIBITION OF SODIUM AND POTASSIUM INDEPENDENT ADENOSINE TRIPHOSPHATASE BY URANYL ION

AKIO MIYAMA, JUNKO KOGAMI and SHUZO KASHIBA
Department of Bacteriology, Nara Medical University, Kashihara, Nara
(Received February 28, 1969)

The adenosine triphosphatase (ATP phosphohydrolase EC 3.6.1.3) of stromata prepared from red cells has recently been investigated by many workers. It has been demonstrated that there are two fractions of this ATPase, one being activated by Mg^{2+} (Mg^{2+} ATPase) and the other, by Mg^{2+}, Na^+, and K^+ (Na^+-K^+ ATPase). The part activated by Na^+ and K^+ is further characterized by its specific inhibition by cardiac glycosides and it participates in the active transport of Na^+ and K^+ through the cell membrane. While the cellular functions of Mg^{2+} ATPase are at present unknown, there is some evidence (Marchesi et al., 1967) that Mg^{2+} ATPase may be structurally associated with the cell membrane, like actomyosine of muscle. In studies on the mechanisms of destruction of the red cell membrane during immune hemolysis, it was demonstrated (Miyama et al., 1968) that a low concentration of uranyl ion inhibits the lysis of sheep red cells which had reacted with antibody and all components of complement (E*), and that Mg^{2+} ATPase associated with stromata was selectively activated during immune hemolysis (Miyama et al., 1968). The precise mechanisms of inhibition by uranyl ion of E* transformation are not understood. However, the level of membrane ATPase of E* is higher than that of the intermediate $EAC_{1,4,2}$ (Miyama et al., 1968), so that the inhibition of E* transformation by uranyl ion may be closely related to modification of Mg^{2+} ATPase.

This communication reports the inhibitory effects of uranyl ion on Mg^{2+} ATPase associated with human red cell ghosts. In these experiments red cell ghosts, prepared from fresh human blood by a slight modification of the method of Post et al. (1960), were used as a source of ATPase. They were suspended in 40 mM Tris buffer (pH 7.7) at a concentration of 1×10^9 ghosts per ml. The activity of ATPase was assayed by the following methods. Two ml of reaction mixture containing 1.0 ml of ghost suspension, 0.2 ml of ATP (12 mM) and 0.8 ml of 40 mM Tris buffer (pH 7.7) were incubated at 37°C for 60 min. The reaction was stopped by addition of 0.5 ml of 25% $HClO_4$ to the mixture and the orthophosphate contents of the deproteinized sample was determined by the method of Fiske and Subbarow (1925). Total ATPase activity of the red cell ghosts was determined in 40 mM Tris buffer (pH 7.7) containing 140 mM NaCl, 14 mM KCl and 5 mM $MgCl_2$. Mg^{2+} ATPase activity was determined in the same buffer but without NaCl and KCl or in the presence of 5×10^{-5} M g-strophanthin (ouabain). The activity of the fraction sensitive to Na^+ and

K^+ (Na^+ -K^+ ATPase) was then estimated as the difference between the activities of total ATPase and Mg^{2+} ATPase, i.e., ouabain insensitive ATPase.

The effect of various concentrations of uranyl ion on the ATPase activity of human red cell ghosts is shown in Table 1. As seen in the table, the activity of Mg^{2+} ATPase was markedly inhibited by uranyl ion, whereas the activity of Na^+-K^+ ATPase was unaffected by less than 20 μM of the ion. Next, the activities of 3 human red cell ghost preparations with different ratios of ouabain sensitive to insensitive ATPase activity, were tested in the presence of 12.5 μM uranyl ion. As shown in Table 2, ouabain insensitive ATPase can be selectively inhibited by uranyl ion in all cases, although, with sample No. 3 which contains relatively large amount of ouabain sensitive ATPase, uranyl ion also inhibited the ouabain sensitive ATPase slightly. It has been demonstrated that inhibition of E* transforma-

TABLE 1. *Effect of uranyl ion on stromal ATPase*

Final concentration of uranyl ion (μM)	ATPase activity (μM Pi/10^9 ghosts/hr) Total ATPase	Mg^{2+} ATPase	Na^+-K^+ ATPase
20	0.172	0.055	0.117
10	0.270	0.070	0.200
5	0.300	0.119	0.181
2.5	0.347	0.114	0.233
1.25	0.341	0.119	0.222
—	0.408	0.220	0.188

TABLE 2. *Effect of uranyl ion on ouabain insensitive ATPase*

Blood No.	Treatment with uranyl ion	ATPase activity (μM Pi/10^9 ghosts/hr) Total	Ouabain insensitive	Ouabain sensitive
1	12.5 μM	0.480	0.336	0.144
	none	0.700	0.583	0.117
2	12.5 μM	0.502	0.255	0.247
	none	0.803	0.595	0.208
3	12.5 μM	0.261	0.000	0.261
	none	0.541	0.206	0.335

TABLE 3. *Effect of washing red cell ghosts treated with uranyl ion*

Preincubation at 37°C for 10 min	Washing fluid	Mg^{2+} ATPase (μM Pi/10^9 ghosts/hr)
12.5 μM uranyl ion	buffer	0.160
12.5 μM uranyl ion	EDTA	0.391
Tris buffer	buffer	0.330
Tris buffer	EDTA	0.450
12.5 μM uranyl ion	no washing	0.115
Tris buffer	no washing	0.325

tion by uranyl ion is completely reversed by the addition of EDTA (Miyama et al., 1968), so the possibility that EDTA could restore the ATPase activity of red cell ghosts treated with uranyl ion was tested. Ghost suspensions were treated with 12.5 μM uranyl ion at 37°C for 10 min, washed once with either 40mM Tris buffer (pH 7.7) or the same buffer containing 0.01 M EDTA and then twice with 40 mM Tris buffer (pH 7.7). Table 3 shows that washing with Tris buffer did not completely restore the activity of Mg^{2+} ATPase but washing with EDTA decreased the effect of uranyl ion. However, a high concentration of uranyl ion (100 μM) was found to inhibit both ouabain sensitive and insensitive ATPase activity irreversibly. This table also shows that the ATPase activity of EDTA-control was higher than that of the buffer control. This may be because treatment of red cell ghosts with EDTA elevates the stromal ATPase activity as reported by Schwartz (1963) for rat liver microsomal ATPase.

These findings clearly show that, at an appropriate concentration, uranyl ion inhibits stromal Mg^{2+} ATPase selectively and reversibly. Many divalent cations are known to inhibit Na^+ -K^+ ATPase (Toda et al., 1967, Marchesi et al., 1967), but no inhibitors to Mg^{2+} ATPase are known except rose bengal which inhibits Mg^{2+} ATPase irreversibly (Dastoli et al., 1965). Thus use of uranyl ion as an inhibitor may be useful in clarifying the physiological role of Mg^{2+} ATPase in the red cell membrane and also the mechanism of cell damage during immune hemolysis.

REFERENCES

Dastoli, F. R. and J. W. Green. 1965. A study of rose bengal and sulfhydryl reagents on stromal fragments of rabbit and human erythrocytes. J. Cell and Comp. Physiol. 65 : 313–318.

Fiske, C. H. and Y. Subbarow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66 : 375–400.

Marchesi, V. T. and G. E. Palade. 1967. Inactivation of adenosine triphosphatase and disruption of red cell membranes by trypsin : protective effect of adenosine triphosphatase. Proc. Nat. Acad. Sci. 58 : 991–995.

Marchesi, V. T. and G. E. Palade. 1967. The localization of $Mg^{2+}-Na^+-K^+$ activated adenosine triphosphatase on red cell ghost membranes. J. Cell Biol. 35 : 385–404.

Miyama, A., J. Kogami, S. Yamada, S. Yamada and S. Kashiba. 1968. Terminal step of immune hemolysis. I. Inhibition of E* transformation by uranyl ion. Biken J. 11 : 101–110.

Miyama, A., J. Kogami, S. Kashiba and T. Amano. 1968. The effect of ATP content of human erythrocytes on immune hemolysis. US-Japan Complement Seminar. Tokyo.

Post, R. L., C. R. Meritt, C. R. Kinsolving and C. D. Albright. 1960. Membrane adenosine triphosphatase as a participant in active transport of sodium and potassium in human erythrocytes. J. Biol. Chem. 235 : 1796–1802.

Schwartz, A. 1963. A $Na^+ + K^+$ — stimulated adenosine triphosphatase in " microsomal " fractions from rat liver. Biochim. Biophys. Acta 67 : 329–331.

Toda, G., T. Hashimoto, T. Asakura and S. Minakami. 1967. The inhibition of $(Na^+ - K^+)$ — activated ATPase by beryllium. Biochim. Biophys. Acta 135 : 570–572.