

Title	Inhibition of Sodium and Potassium Independent Adenosine Triphosphatase by Uranyl Ion
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Citation	Biken journal : journal of Research Institute for Microbial Diseases. 1969, 12(2), p. 141-143
Version Type	VoR
URL	<a href="https://doi.org/10.18910/82842">https://doi.org/10.18910/82842</a>
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## SHORT COMMUNICATION

## INHIBITION OF SODIUM AND POTASSIUM INDEPENDENT ADENOSINE TRIPHOSPHATASE BY URANYL ION

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(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  $1 \times 10^9$  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  $5 \times 10^{-5}$  M g-strophanthin (ouabain). The activity of the fraction sensitive to  $Na^+$  and

K<sup>+</sup> (Na<sup>+</sup>-K<sup>+</sup> ATPase) was then estimated as the difference between the activities of total ATPase and Mg<sup>2+</sup> 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<sup>2+</sup> ATPase was markedly inhibited by uranyl ion, whereas the activity of Na<sup>+</sup>-K<sup>+</sup> ATPase was unaffected by less than 20 μM of the ion. Next, the ac-

tivities 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)	Total ATPase	ATPase activity (μM Pi/10 <sup>9</sup> ghosts/hr)	
		Mg <sup>2+</sup> ATPase	Na <sup>+</sup> -K <sup>+</sup> 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	Total	ATPase activity (μM Pi/10 <sup>9</sup> ghosts/hr)	
			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 <sup>2+</sup> ATPase (μM Pi/10 <sup>9</sup> 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  $\mu$ M 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<sup>2+</sup> ATPase but washing with EDTA decreased the effect of uranyl ion. However, a high concentration of uranyl ion (100  $\mu$ M) 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<sup>2+</sup> ATPase selectively and reversibly. Many divalent cations are known to inhibit Na<sup>+</sup>-K<sup>+</sup> ATPase (Toda et al., 1967, Marchesi et al., 1967), but no inhibitors to Mg<sup>2+</sup> ATPase are known except rose bengal which inhibits Mg<sup>2+</sup> ATPase irreversibly (Dastoli et al., 1965). Thus use of uranyl ion as an inhibitor may be useful in clarifying the physiological role of Mg<sup>2+</sup> ATPase in the red cell membrane and also the mechanism of cell damage during immune hemolysis.

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