Biochimica et Biophysica Acta, 687 (1982) 94–96
Elsevier Biomedical Press

BBA Report

BBA 70029

EVIDENCE FOR THE EXISTENCE OF A MONOVALENT CATION-STIMULATED, MAGNESIUM-DEPENDENT ADENOSINE TRIPHOSPHATASE ACTIVITY IN THE ISOLATED PLASMA MEMBRANES OF AMOEBAS OF THE SLIME MOLD *DICTYOSTELIUM DISCOIDEUM*

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(Received February 8th, 1982)

Key words: Mg^{2+} ATPase; Monovalent cation; Plasma membrane; (D. discoideum)

Evidence for a magnesium-dependent ATPase activity that can be stimulated by Na^+ and K^+, or equally by Na^+ or K^+ alone, has been found in the plasma membranes isolated from amoebas of the slime mold *Dictyostelium discoideum* when the membranes are isolated from cultures grown up to the stationary phase. This ATPase activity is scarcely inhibitable by ouabain or phlorizin, but is very sensitive to low concentrations of azide or thimerosal. When the plasma membranes are isolated from amoebas growing in logarithmic phase, this monovalent cation-stimulated Mg^{2+}-dependent activity is barely detectable.

Preliminary data, suggesting the existence of a magnesium-dependent ATPase activity which is Na^+- and K^+-activated in crude preparations of plasma membranes of the amoebas of the slime mold *Dictyostelium discoideum*, are presented here.

Cells from *Dictyostelium discoideum* strain AX-2, a strain adapted to growth axenically in artificial media devoid of bacteria [1], were cultivated at 23°C on a growth medium containing 1.8% maltose [2]. The cells required for obtaining the membrane fraction were grown up to cell densities of $(6–8) \cdot 10^6$ per ml, then were washed three times in cold Sørensen phosphate buffer (0.017 M, pH 6.0) and were resuspended in 0.6 mM $ZnCl_2$ adjusted to a cell concentration of about $6 \cdot 10^7$ cells per ml. Then the cells were incubated under agitation for 20 min at 23°C, and isolation of the membrane fraction was performed following the procedure of Brunette and Till [3], except that $ZnCl_2$ was not used in the two-phase system. After the membranes were collected, they were washed once with 5-times their volume in 10 μM Mg^{2+} EDTA. The membranes were sedimented from the suspension by a low-speed centrifguation (1000 rpm for 15 min) ($500 \times g$) and the pellet was washed once more with doubly-distilled water and finally resuspended in water. Na^+ or K^+ were not detectable in membranes prepared by this method as determined by flame photometry (García-Cañero, R., personal communication). All procedures for membrane isolation were carried out in plastic flasks and tubes.

The magnesium-dependent ATPase activity of the membranes was assayed in 1 ml reaction mixture containing 3 mM ATP-Tris, 3 mM $MgCl_2$, 30 mM imidazole buffer, pH 7.4, and variable concentrations of NaCl or KCl as indicated in Fig. 1. All samples were incubated for 30 min at 37°C. The reaction was initiated by introducing 50 to 80 μg of membrane protein per ml. The inorganic phosphate liberated was determined by the method of Fiske and SubbaRow [4]. Protein concentration was determined by the method of Lowry et al. [5]. All procedures for membrane assay of ATPase activity were carried out in plastic tubes.

Fig. 1 shows the action of Na^+ or K^+ or different inhibitors on the magnesium-dependent ATPase activity of the plasma membrane isolated for amoebas of *Dictyostelium discoideum* grown to stationary phase. Fig. 1A shows the ATPase activ-

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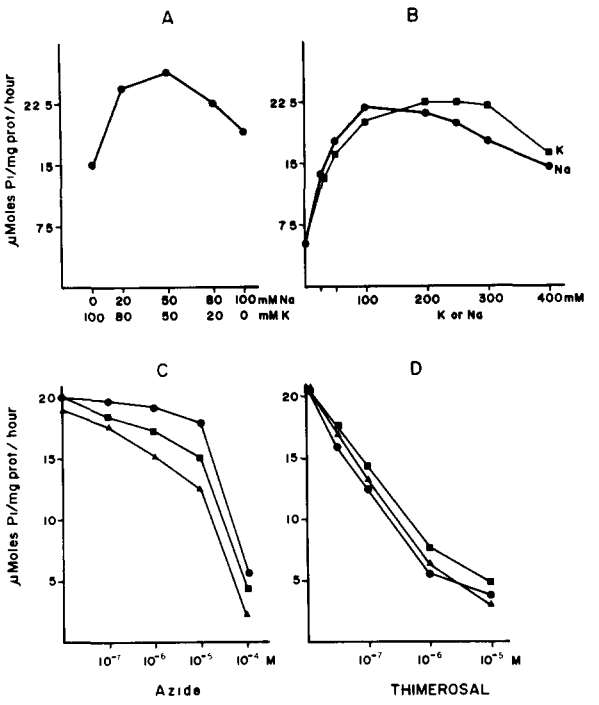

Fig 1 Monovalent cation-activated magnesium-dependent ATPase activity of plasma membrane of amoebas of *Dictyostelium discoideum* grown up to stationary phase (A) Effect of the combined addition of Na^+ and K^+ (B) Effect of the addition of Na^+ (●———●) or K^+ (■———■) alone in increasing concentrations (C) Effects of increasing concentrations of azide on the ATPase activity assayed in the presence of 150 mM Na^+ (●———●), 150 mM K^+ (■———■) or 50 mM Na^+ plus 50 mM K^+ (▲———▲) (D) Effects of increasing concentrations of thimerosal on the ATPase activity assayed in the presence of 150 mM Na^+ (●———●), 150 mM K^+ (■———■) or 50 mM Na^+ plus 50 mM K^+ (▲———▲)

ity as a function of different combinations of sodium plus potassium concentrations. In the absence of both sodium or potassium, the 3 mM magnesium-dependent ATPase activity was about 6 µmol phosphate/mg protein per hour. The combined concentration of sodium and potassium is represented in Fig. 1A as done by Skou in his initial reports of (Na^+-K^+)-dependent ATPase (for review, see Ref. 6). This experiment suggests that there is a monovalent cation-stimulated Mg^{2+}-dependent ATPase activity in these membrane preparations. Fig. 1B shows a significant stimulation of the magnesium-dependent ATPase activity of the membranes just by the presence of each of the cations alone in the test medium; 100 mM K^+ alone or 100 mM Na^+ alone increases the Mg^{2+}-dependent activity more than 3-times. In these experiments, we searched respectively after Na^+ or K^+ in the membrane preparation and they were not found. When the membranes were prepared from cell cultures in exponential growth phase (cell densities between $1 \cdot 10^6$ and $4 \cdot 10^6$ cells/ml), the monovalent cation-stimulated Mg^{2+}-ATPase activity were barely detectable in our experimental conditions.

The cation-activated Mg^{2+}-ATPase activity of the membranes isolated from cells in stationary phase was studied for sensitivity to inhibitors. Sensitivity to ouabain and phlorizin was found to be very low, less than 20% inhibition of the activity measured in the presence of Na^+, K^+ or Na^+ plus K^+, up to 2 mM concentration (not shown) of the inhibitors. However, azide (Fig. 1C) and thimerosal (Fig. 1D) were very effective inhibitors of the ATPase activity whether it was tested in the presence of Na^+, K^+ or Na^+ plus K^+. Thimerosal has been reported as an inhibitor of (Na^+-K^+)-dependent ATPase activity which acts without diminishing Na^+-dependent ATPase activity in mammalian preparations [7].

The results suggest at least three possibilities in describing the existence of an Na^+ and K^+-activated Mg^{2+}-ATPase activity in plasma membrane of amoebas of the slime mold *Dictyostelium discoideum*, grown up to stationary phase: (1) that the cation-stimulated Mg^{2+}-ATPase activity measured in our experiments corresponds to a single (Na^+-K^+)-dependent ATPase enzyme similar to the first described by Skou [6], but with two very dissociable Na^+-dependent and K^+-dependent stimulations which represent enzyme sites with similar affinities; this possibility is ruled out because the enzyme does not require Na^+ plus K^+ for maximal activity and is not inhibited by ouabain: (2) that the ATPase activity encountered corresponds mostly to the existence of an 'ionic strength-dependent ATPase'; it seems that this possibility can be ruled out if we relate the different enzyme activity obtained to the ionic strength of the assay conditions, as has been done in Table I: (3) that the activity encountered corresponds to the existence of a K^+-dependent ATPase similar, for instance, to those described in *Escherichia coli*

TABLE I

VARIATION OF Mg^{2+}-DEPENDENT ATPase ACTIVITY AS MEASURED WITH MEDIA OF INCREASING IONIC STRENGTH

Other components of the assay medium were imidazole, Tris-HCl or choline-HCl, depending on the experiment, and in all cases Mg^{2+} and ATP

Ionic strength	ATPase activity (nmol P_i per mg protein)	K^+ content (mM)	Na^+ content (mM)
17	7	–	–
25	8	–	–
37	12	25	–
37	14	–	25
50	15	50	–
50	16	–	50
67	11	50	50
75	22	50	50
75	17	100	–
75	20	–	100
75	28	50	50
75	23	80	20
75	22	20	80
80	16	50	100
100	20	50	100
125	22	200	–
125	20	–	200
150	22	250	–
150	18	–	250
175	21	300	–
175	16	–	300
225	17	400	–
225	14	–	400

[8] or soybean root [9]. In this case, the K^+ site would be of low affinity and specificity and would react almost equally with K^+ and with Na^+. The specificity is not so low, in our hands, that Na^+ could be substituted by Tris or choline, which again rules out possibility 2. At the present time, our experimental evidence decides us in favor of the third possibility. Thus the enzyme would have to be termed monovalent cation-stimulated Mg^{2+}-dependent.

This work was supported by grant 12-154-79 from the 'Instituto Nacional de la Salud' (Madrid). The author is grateful to Dr. Gunter Gerish for his sample of axenically grown Ax2 *Dictyostelium discoideum* amoebas to initiate the cultures

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