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EFFECT OF CONCANAVALIN A ON MEMBRANE-BOUND ENZYMES FROM MOUSE LYMPHOCYTES

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SUMMARY

The ionic influence and ouabain sensitivity of lymphocyte Mg^{2+}-ATPase and Mg^{2+}-$(Na^+ + K^+)$-activated ATPase were studied in intact cells, microsomal fraction and isolated plasma membranes. The active site of 5′-nucleotidase and Mg^{2+}-ATPase seemed to be localized on the external side of the plasma membrane whereas the ATP binding site of $(Na^+ + K^+)$-ATPase was located inside the membrane.

Concanavalin A induced an early stimulation of Mg^{2+}-ATPase and $(Na^+ + K^+)$-ATPase both on intact cells and purified plasma membranes. In contrast, 5′-nucleotidase activity was not affected by the mitogen. Although the thymocyte Mg^{2+}-ATPase activity was 3–5 times lower than in spleen lymphocytes, it was much more stimulated in the former cells (about 40 versus 20 %). $(Na^+ + K^+)$-ATPase activity was undetectable in thymocytes. However, in spleen lymphocytes $(Na^+ + K^+)$-ATPase activity can be detected and was 30 % increased by concanavalin A. Several aspects of this enzymic stimulation had also characteristic features of blast transformation induced by concanavalin A, suggesting a possible role of these enzymes, especially Mg^{2+}-ATPase, in lymphocyte stimulation.

INTRODUCTION

Concanavalin A is a potent stimulant of in vitro metabolic and morphologic changes by mouse lymphocytes [1–3]. The process also resembles immunological stimulation by specific antigens. On the other hand, it has been found previously that while lymphocyte stimulation by mitogenic agents requires an initial interaction with the cell surface, a more complex process is required for activation. This latter process could be related to an early critical event which induces molecular alterations in the plasma membrane [4–7]. Among these early events are increased ion [8, 9] and amino acid [10] transport, activation of phospholipid metabolism [11, 12] and glycoprotein synthesis [13], alterations in the activity of enzymes which are engaged in cyclic AMP synthesis [14, 15] and ATP degradation [16].

In order to be able to gain insight into the mechanism by which lymphocytes are stimulated to become active blast cells, we have chosen syngeneic mouse lymphocytes as a starting material because they have been widely studied for immunological purposes. The present communication reports a comparative study of concanavalin A-induced changes in membrane-bound enzymes from such fundamentally different lymphoid cell populations as spleen lymphocytes and thymocytes. In addition, this study was carried out on both intact cells and isolated membrane fractions.

MATERIALS AND METHODS

Animals and cell suspensions

Balb/c mice, 4–5 weeks old, were killed by sulfuric ether. All following steps were carried out at 0 °C. The spleens and thymuses were immediately removed and put directly either in Hanks solution or in 10 mM Tris · HCl buffer, pH 7.5, 130 mM NaCl, 0.5 mM $MgSO_4$. The lymphoid organs were carefully cleaned of adherent connective and fat tissues, cut into small pieces and gently pressed to yield single cell suspensions. Clumps and debris were removed by filtering through nylon tissue (125 μm pore size). At this stage, more than 90 % of the cells were viable as judged by trypan blue exclusion.

Preparation of lymphocyte suspensions

The cell suspension was overlaid on a discontinuous gradient of a 9 % Ficoll/40 % sodium lotalamate (Isopaque) mixture (24 : 10, v/v) at a final density of 1.076 and run for 20 min at $400 \times g_{av}$. The cell population which remained at the interface consisting of over 95 % lymphocytes was removed and washed once with 10 mM Tris · HCl buffer, pH 7.5, 130 mM NaCl, 0.5 mM $MgSO_4$ containing 0.2 % methylcellulose and once with the same buffer without methylcellulose. The cell viability was never less than 85 %.

Cell disruption

The cell suspension was centrifuged (10 min at $300 \times g_{av}$) and the pellet was resuspended in 10 mM Tris · HCl buffer, pH 8.0, 130 mM NaCl, 0.5 mM $MgSO_4$, diluted 5 times with 10 mM Tris buffer, pH 8.0, and kept at 0 °C for 3 min under rapid magnetic stirring. The iso-osmolarity was restored by progressively adding 2 M sucrose solution to give a final concentration of 0.25 M. The cells were then centrifuged for 10 min at $300 \times g_{av}$ and the pellet resuspended at a concentration of $1 \cdot 10^8$–$5 \cdot 10^8$ cells/ml in 20 mM Tris · HCl buffer, pH 7.5, 1 mM $MgSO_4$. An equal volume of 0.5 M sucrose was then added with constant gentle stirring to avoid cell aggregates [17]. This hypo-osmotic treatment was used as the first stage of cell disruption. It was also found to be a suitable lymphocyte purification procedure since the only cells lysed were erythrocytes. Microscopic observation of cell preparations submitted to osmotic forces showed that about 90 % of the cells were lymphocytes. However, the cell viability was relatively low (70 %) showing that the lymphocytes were slightly damaged. On the other hand, the yield of lymphocytes is large. This purification procedure was, therefore, alternatively used for the preparation of plasma membranes but never for studies on whole cells.

The breakage of the cells was then achieved by 10–15 strokes of a Dounce homogenizer. All stages of the procedure were examined under the light microscope.

Subcellular fractionation

Subcellular fractionation was performed at 0–4 °C. The procedure adopted was derived from the one described by Wallach and Kamat [18]. The homogenate was centrifuged first at $1500 \times g_{av}$ for 10 min to remove nuclei. The supernatant was then centrifuged at $12\,000 \times g_{av}$ for 10 min (Sorvall SS-34 rotor): this step was repeated once for the best elimination of mitochondria and lysosomes. The "microsomal fraction" was then pelleted at $220\,000 \times g_{av}$ for 90 min (Spinco 50 Ti rotor), carefully resuspended in a large volume of 10 mM Tris · HCl buffer, pH 7.5, and 250 mM sucrose by means of a Potter-Elvehjem homogenizer, and pelleted at $220\,000 \times g_{av}$ for 90 min and then the washed microsomes were identically resuspensed but in 4 ml of the same buffer ($d = 1.041$).

By this treatment, we removed most of the entrapped material within the membrane vesicles as judged by the appreciable *p*-nitrophenylphosphate-hydrolysing activity recovered in the wash of the $220\,000 \times g_{av}$ pellet. The microsomal material was then layered onto a discontinuous sucrose gradient of density 1.085–1.236. After centrifugation at $55\,000 \times g_{av}$ for 150 min (Spinco SW-25 rotor), fractions of 1 ml were removed from the upper portion of the tube with a Buchler Auto-Densiflow and assayed for their protein and enzymic content. 5'-Nucleotidase [19, 20], Mg^{2+}-ATPase [21] and $(Na^+ + K^+)$-ATPase were used as reliable plasma membrane markers. NADH cytochrome *c* reductase, antimycin A insensitive, was used as a marker for endoplasmic reticulum [22]. The presence of mitochondria was detected by assaying for cytochrome *c* oxidase [23].

Fraction 1 (interface 1.041/1.085) contained mainly soluble material which exhibited trace amounts of the marker enzyme activities. Fraction 2 and Fraction 3 (interfaces 1.085/1.132 and 1.132/1.182) consisted essentially of plasma membranes, PM_1 and PM_2, as judged by the highest specific activities of plasma membrane markers (enrichment of about 7–12-fold with respect to the homogenate and recovery of about 40–60 % with respect to the microsomal fraction). NADH cytochrome *c* reductase was found with an appreciable activity in Fraction 4 (interface 1.182/1.236) but with the highest specific activity in Fraction 3. This surprising repartition of this endoplasmic reticulum marker was previously found in lymphocyte membranes by several authors [17, 24, 25] and shows that a more accurate characterization of endoplasmic reticulum by other criteria is necessary. Fraction 4 was the only one which exhibited a cytochrome *c* oxidase activity and so contained some membranes resulting from the breakage of mitochondria during the cell disruption. The enzymic properties of our membrane fractions are essentially comparable with those reported in previous studies of lymphocytes plasma membranes [17, 21, 26–30]. (For more details see Tables II and III).

Incubation of lymphocytes with concanavalin A

Spleen lymphocytes or thymocytes were incubated at a density of $4 \cdot 10^6$–$5 \cdot 10^6$ cells/tube for 2 h at 37 °C in 10 mM Tris · HCl buffer, pH 7.5, 130 mM NaCl, 0.5 mM $MgSO_4$ with various concentrations of concanavalin A. Cells were harvested at the end of the incubation period and resuspended in the appropriate buffer for

enzyme assays or subcellular fractionation as described above.

Analytical methods

Chemical determinations. Protein was determined by the method of Lowry et al. [31] with bovine serum albumin (Fraction V) as standard. Intact cells were solubilized by the addition of an equal volume of 5 % sodium deoxycholate before the protein assay.

Assay of enzymic activities. Mg^{2+}-ATPase (EC 3.6.1.3) and ouabain-sensitive ($Na^+ + K^+$)-activated ATPase activities were measured at pH 7.5 for 10 min at 37 °C by a modification of the procedure used by Bakkeren and Bonting [32]. The standard medium containing 97 mM Tris · HCl, pH 7.5, 3 mM $MgCl_2$, 50 mM NaCl, 9 mM KCl, 0.1 mM EDTA and 2 mM Tris · ATP gave the total ATPase activity. The same medium plus 10^{-3} M ouabain gave the Mg^{2+}-ATPase activity. The difference between these two enzymic activities represents the ($Na^+ + K^+$)-activated ATPase activity. 5'-Nucleotidase (EC 3.1.3.5) was measured as described by Michell and Hawthorne [33]. The P_i liberated by these enzymes was measured by the method of Martin and Doty [34] in the case of subcellular fractions and according to Chen et al. [35] in the case of intact cells. In all cases, the blank values were subtracted. NADH cytochrome *c* reductase (EC 1.6.2.1), antimycin A insensitive, was assayed in a 0.05 M Tris · HCl buffer, pH 8.5, with about 100–200 μg of protein incubated with antimycin A (8 μg/ml) for 10 min at 30 °C. Then 3 mg of cytochrome *c* was added. After a slight increase in absorbance due to the NADH present in the extract, 0.3 ml of NADH (1 mM) was added and the reaction was followed by measuring the increase in absorbance at 550 nm with the help of a PMQ_2 Zeiss recording spectrophotometer. Cytochrome *c* oxidase (EC 1.9.3.1) activity was determined according to the method of Azoulay et al. [36]. *p*-Nitrophenylphosphate-hydrolysing activity was assayed as described by Schlesinger [37].

Balb/c mice were obtained from the Centre de sélection et d'élevage d'animaux de laboratoire, CNRS, France. Tris · ATP, AMP, NADH, antimycin A, cytochrome *c* and bovine serum albumin (Fraction V) were purchased from Sigma chemical Co., U.S.A., concanavalin A was obtained from Calbiochem Inc., U.S.A., *p*-nitrophenylphosphate from Merck, Germany, Isopaque (Angio Contrix 48) from Lab. Guerbet, France, and Ficoll from Pharmacia Fine Chemicals, Sweden. All other chemicals were reagent grade.

RESULTS AND DISCUSSION

Ionic influence and ouabain action on Mg^{2+}-ATPase and ($Na^+ + K^+$)-ATPase

This study was carried out on the microsomal fraction obtained from spleen lymphocytes. Mg^{2+}-ATPase as well as ($Na^+ + K^+$)-ATPase activities were linear only during the first 10 min of the enzymic reaction at pH 7.5 and 37 °C. Ionic influence on enzymic activities was investigated at constant ionic strength (by adjusting the Tris molarity, pH 7.5) and ATP molar concentration (2 mM).

Without adding any Mg^{2+} there were trace amounts of ATPase activities. This result is normal since the ATPase substrate is the Mg^{2+}-ATP complex and the assay performed in the presence of EDTA. Optimal Mg^{2+}-ATPase activity was reached at an Mg^{2+}/ATP ratio of 1. The 3 mM Mg^{2+} concentration used in our

standard conditions (see Materials and Methods) gave about 98 % suboptimal activity. The $(Na^+ + K^+)$-activated ATPase activity was maximum at 3 mM Mg^{2+} (Mg^{2+}/ATP ratio of 1.5), 50 mM Na^+ and 9 mM K^+. Higher ionic concentrations had a strong inhibitory effect on this enzymic activity. The glycoside ouabain is a well known specific inhibitor of $(Na^+ + K^+)$-ATPase activity [38, 39]. The mouse lymphocyte $(Na^+ + K^+)$-ATPase was completely inhibited by 10^{-2} M ouabain but it had no effect on Mg^{2+}-ATPase or 5′-nucleotidase activities. The half maximal inhibition was obtained with about $0.5 \cdot 10^{-4}$ M ouabain showing a relatively low sensibility to this inhibitor (Fig. 1). The 10^{-3} M ouabain concentration used in our assays gave 85 % inhibition. In addition, $(Na^+ + K^+)$-ATPase represents a relatively low amount of the total ATPase activity (10–15 %).

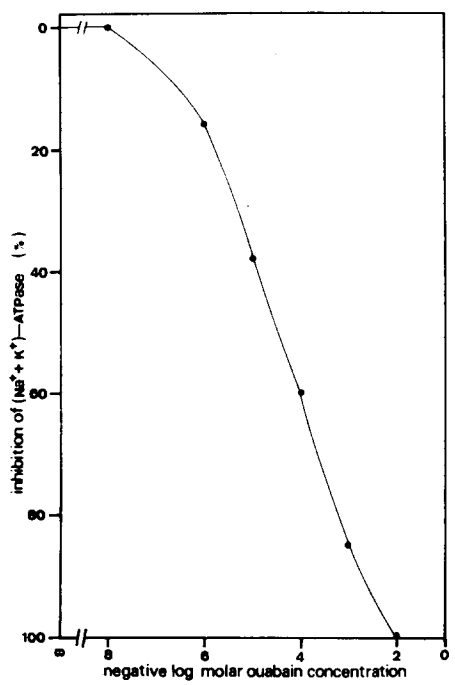

Fig. 1. Effect of ouabain on $(Na^+ + K^+)$-ATPase activity in spleen lymphocyte microsomal fraction. The enzyme activity was assayed as described in Materials and Methods. The medium contained 147 μg microsomal protein and various ouabain concentrations.

Sidedness of mouse lymphocyte plasma membrane enzymes

Our experimental approach to the problem of marker enzyme sidedness was to compare the plasma membrane enzyme activity of suspensions of whole isolated lymphocytes with that of cell homogenates (disrupted cells) [40].

When isolated intact cells were incubated in an isotonic medium containing the enzyme substrate, an assay for metabolic products should then reflect only the activity of an ecto-enzyme, i.e. an enzyme whose active site(s) faces the external medium. If the cells were disrupted the substrate would be ideally exposed to the action of the enzyme on both sides of the plasma membrane. Assuming that the total

TABLE I
PLASMA MEMBRANE ENZYME ACTIVITY IN ISOLATED INTACT AND DISRUPTED SPLEEN LYMPHOCYTES AND THYMOCYTES

Cell isolation and enzyme assays were done as described in Materials and Methods. The values represent the average of two separate determinations performed on 3 preparations. N.D., not determined.

Enzyme	Specific activity in nmol P_i liberated/mg protein per min			
	Spleen lymphocytes		Thymocytes	
	Intact cells	Disrupted cells	Intact cells	Disrupted cells
$(Na^+ + K^+)$-ATPase	0	17.6	N.D.	N.D.
Mg^{2+}-ATPase	121	124	27.2	33.7
5′-Nucleotidase	9.5	8.7	7.1	9.0

measured activity presented the sum of the catalytic activities of both sides, no increase in activity should be observed for an ecto-enzyme when the intact cells are compared with the disrupted cells.

As shown in Table I, no inhibition of ATPase activity was found in the presence of 10^{-3} M ouabain for both intact spleen and thymus lymphocytes. This lack of inhibition proves that this ATPase activity was not related to the $(Na^+ + K^+)$-ATPase. The latter enzyme activity was detected only in the disrupted cells. On the other hand, nearly the same level of Mg^{2+}-ATPase and 5′-nucleotidase specific activities was observed in intact cells and homogenate of both spleen lymphocytes and thymocytes. These results might suggest that Mg^{2+}-ATPase and 5′-nucleotidase exhibited an activity which appeared to be associated with the external aspect of the plasma membrane. The substrate binding site of the $(Na^+ + K^+)$-ATPase was found to be associated only with the inside of the plasma membrane. Such a situation is not particular for mouse lymphocytes but appears to be a rather general feature of most eucaryotic cells [19, 40]. However, this comparison between intact and disrupted cells requires two major reservations. (1) The permeability of whole cells to substrate might be a possible source of error. (2) Upon disruption plasma membranes might form sealed vesicles. Moreover, the normal membrane situation of outside-out would be reversed. This could lead to erroneous conclusions regarding the activity of membrane enzymes which are localized on the interior aspect of these sealed vesicles. Our data on the sidedness of $(Na^+ + K^+)$-ATPase whose substrate binding site localization on the inside of the plasma membrane is well known [41, 42] indirectly suggest that substrate permeation into intact cells and exclusion of substrate by vesicle formation are minor factors. However, our data are insufficient for definitive evaluation of the amount of Mg^{2+}-ATPase and 5′-nucleotidase activity which occurred on the exterior face of the lymphocyte membrane. Therefore, we have concluded, with these reservations in mind, that at least a significant part of these latter enzymes are ecto-enzymes.

Concanavalin A stimulation of spleen and thymus intact lymphocytes

Concanavalin A is a potent inducer of DNA synthesis and blast transformation in spleen and thumus lymphocytes. A characteristic feature of concanavalin A stimulation is the existence of an optimal concentration which has a maximal stimulatory effect. This optimal dose was about 1–2 μg/ml [2, 5, 43]. Specific inhibition of $(Na^+ -$

K^+)-ATPase and cation transport by ouabain leads to a suppression of lymphocyte transformation [8, 9]. Moreover, Mg^{2+}-ATPase was found to be concanavalin A stimulated with intact rat lymphocytes [16]. So we tested the effect of a short incubation (2 h) of intact lymphocytes with various concanavalin A concentrations on some membrane-bound enzymes. No significant stimulatory effect of concanavalin A on 5'-nucleotidase was observed. On the contrary a slight inhibition was suspected but it was difficult to evaluate its significance, since the values determined on intact cells were low and, therefore, difficult to measure (specific activities were, respectively, 9.5 and 7.1 nmol P_i liberated/mg protein per min for spleen lymphocytes and thymocytes). On the other hand, concanavalin A stimulated a Mg^{2+}-ATPase activity catalyzed by intact cells (Fig. 2). Maximal stimulatory effect was obtained with different concanavalin A concentrations for spleen lymphocytes (3 μg/ml) and thymocytes (4–5 μg/ml). Although the absolute magnitude of Mg^{2+}-ATPase specific activity was 4.5 times lower for thymocytes (27.2 nmol P_i/mg protein per min) as compared to spleen lymphocytes (121 nmol P_i/mg protein per min), the former cells were more stimulated by concanavalin A (49.7 %) than the latter cells (24.8 %).

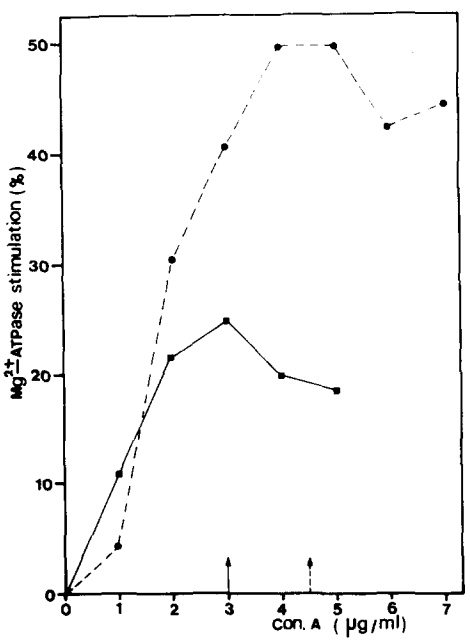

Fig. 2. Effect of concanavalin A concentration on Mg^{2+}-ATPase specific activity of intact thymocytes and spleen lymphocytes. Intact spleen lymphocytes (■—■) and thymocytes (●- - -●) were incubated with various concentrations of concanavalin A for 120 min at 37 °C and assayed for Mg^{2+}-ATPase activity as described in Materials and Methods. The specific activities of unstimulated cells were respectively 121 and 27.2 nmol P_i/mg protein per min for spleen lymphocytes and thymocytes.

Modifications of enzyme activities on isolated membrane fractions from concanavalin A-stimulated spleen and thymus lymphocytes.

Single cell suspensions of spleen lymphocytes and thymocytes were separated into two equal parts. One part was submitted to subcellular fractionation, whereas

TABLE II

MODIFICATIONS OF ENZYME SPECIFIC ACTIVITIES ON SUBCELLULAR FRACTIONS FROM CONCANAVALIN A-STIMULATED SPLEEN LYMPHOCYTES

Spleen lymphocytes were incubated with concanavalin A (Con A) (3 μg/ml) and submitted to subcellular fractionation as described in Materials and Methods. Specific activities are expressed as nmol P_i liberated /mg protein per min. The values represent the average of two separate determinations performed on 3 preparations.

Subcellular fraction	5'-Nucleotidase			Mg^{2+}-ATPase			$(Na^+ + K^+)$-ATPase		
	−Con A	+Con A	Stimulation (%)	−Con A	+Con A	Stimulation (%)	−Con A	+Con A	Stimulation (%)
Homogenate	8.7	8.2	None	124	163	31.4	17.6	22.8	29.5
Microsomal fraction	26.2	26.6	None	466	618	32.6	76.1	115.1	51.4
Fraction 1	10.8	8.5	None	104	131	26.0	0	0	0
Fraction 2 (PM 1)	68.8	67.7	None	1566	1905	21.6	175.0	224.2	28.1
Fraction 3 (PM 2)	50.5	41.1	None	850	980	15.3	78.2	101.4	29.6
Fraction 4	18.2	19.0	None	374	413	10.4	0	0	0

TABLE III

MODIFICATIONS OF ENZYME SPECIFIC ACTIVITIES ON SUBCELLULAR FRACTIONS FROM CONCANAVALIN A-STIMULATED THYMOCYTES

Thymocytes were incubated with concanavalin A (Con A) (4 μg/ml) and submitted to subcellular fractionation as described in Materials and Methods. Specific activities are expressed as nmol P_i liberated/mg protein per min. The values represent the average of two separate determinations performed on 3 preparations.

Subcellular fraction	5′-Nucleotidase			Mg^{2+}-ATPase		
	−Con A	+Con A	Stimulation (%)	−Con A	+Con A	Stimulation (%)
Homogenate	9.0	6.5	None	33.7	46.2	37.2
Microsomal fraction	60.5	55.8	None	188.4	281.8	49.5
Fraction 1	19.5	23.4	None	15.2	15.8	4.0
Fraction 2 (PM 1)	96.2	111.3	None	275.7	375.3	36.2
Fraction 3 (PM 2)	306.0	254.6	None	496.3	710.0	43.0
Fraction 4	39.5	34.9	None	0	0	0

the other was incubated in the presence of the optimal concanavalin A concentration, i.e. 3 μg/ml for spleen lymphocytes and 4 μg/ml for thymocytes, and submitted to the same fractionation procedure as described in Materials and Methods. Thus we could compare the enzymic results obtained from subcellular fractions derived from the same lymphocyte population, stimulated or not by concanavalin A. Concanavalin A had no stimulatory effect on NADH cytochrome *c* reductase, antimycin A insensitive, and cytochrome *c* oxidase. As shown in Table II for spleen lymphocytes and Table III for thymocytes, no stimulation of 5′-nucleotidase was found. In contrast, $(Na^+ + K^+)$-activated ATPase of spleen lymphocytes exhibited a significant concanavalin A stimulation. This enzymic activity was only present in fractions containing plasma membranes (Fraction 2 and Fraction 3). It was at an undetectable level in thymocyte subcellular fractions even after stimulation. This increment of spleen $(Na^+ + K^+)$-ATPase activity induced by concanavalin A was not found by Novogrodsky [16] but is in agreement with previous studies of Kaplan and co-workers [8, 9]. Mg^{2+}-ATPase was also found to be stimulated by concanavalin A in subcellular fractions derived from either spleen or thymus lymphocytes. A nearly identical percentage of Mg^{2+}-ATPase stimulation was found in intact cells and isolated plasma membranes (PM 1 and PM 2). This result clearly shows that the isolated membrane material was not damaged by our isolation procedure. Almost no stimulation was observed in Fraction 1 and Fraction 4, i.e. fractions containing soluble material or contaminants, except spleen lymphocyte Fraction 1. Although this latter result remains unexplained it might suggest that within an immunologically mature population, such as spleen lymphocytes, there is an early activation of a soluble ATPase beyond the plasma membrane.

CONCLUSION

Our study of ATPases activities in microsomal fraction, isolated plasma membranes and intact cells has provided accurate information about the existence of two

lymphocyte plasma membrane ATPases, one Mg^{2+}-dependent and the other Mg^{2+}-dependent and $(Na^+ + K^+)$-activated. The physiologic significance of the Mg^{2+} $(Na^+ + K^+)$-ATPase is now well defined. This enzyme is directly involved in active cation transport across the cell membrane [38, 39]. Our results confirm the localization of the ATP-binding site at the inside of the membrane. The level of this enzymic activity is low in mouse spleen lymphocytes and undetectable in mouse thymocytes. Similar observations were made by others [21, 25]. Moreover, its ouabain sensitivity is low as compared to human lymphocytes [9] but is in agreement with recent findings showing that mouse antibody-forming cells are poorly inhibited by ouabain as compared to other species [44].

Some speculations about Mg^{2+}-ATPase function have been made by others. Thus, alteration of the cell surface mediated by ATP [45, 46] seems to be an essential ecto-ATPase function. Likewise, Mg^{2+}-ATPase may play a role in cell adhesion [47], translocation of molecules which participate in metabolic processes requiring ATP [48–50] or production of extracellular ATP by cells in culture [51]. Our results show that this enzyme is notably enriched in isolated plasma membranes (about 13-fold as compared to homogenate). Moreover, its active site seems to be localized on the external aspect of the plasma membrane. It might be of interest to point out that Mg^{2+}-ATPase specific activity measured on intact cells and subcellular fractions was always found about to be 3–5 times lower in thymocytes than in spleen lymphocytes. This marked activity difference of an enzyme involved in some energy-dependent processes in the lymphocyte membrane is at the present time difficult to explain but might be related to a different behaviour of T (thymus derived) and B (bursa-equivalent derived) lymphocytes or to the immunological competence of the lymphocyte populations studied.

Mg^{2+}-ATPase stimulation and lymphocyte transformation induced by concanavalin A are characterized by similar features suggesting a possible correlation between these two phenomena. (1) Enzyme stimulation reaches a maximum for an optimal concentration of concanavalin A (Fig. 2). For higher doses, a decrease of the stimulatory effect is observed. (2) The same optimal dose of concanavalin A produces a maximal stimulatory effect both for Mg^{2+}-ATPase and blast transformation. (3) Although in absolute terms the thymocyte Mg^{2+}-ATPase activity is lower than in spleen lymphocytes, thymocytes display a greater percentage of Mg^{2+}-ATPase stimulation at the optimum concanavalin A-concentration than do spleen lymphocytes (Fig. 2). The same observation was made by Stobo et al. [5] by measuring DNA synthesis. Our results indicate that Mg^{2+}-ATPase might be involved in the initial biochemical events of lymphocyte stimulation at the plasma membrane level. The stimulation of the plasma membrane Mg^{2+}-ATPase might be a critical event by which concanavalin A initiates blast transformation or a consequence of other cellular changes induced by concanavalin A. We also found that concanavalin A stimulates the $(Na^+ + K^+)$-ATPase activity present in isolated plasma membranes. This result in accord with previous findings [8, 9] confirms the prominent part of the sodium pump in the early events of lymphocyte stimulation. The hypothesis of a concanavalin A-stimulated multienzyme sequence which would trigger lymphocyte transformation is especially attractive. Within this sequence, plasma membrane Mg^{2+}-ATPase would act as a vectorial enzyme which would work in reverse. Such a mechanism was proposed for mitochondrial ATP synthesis [52]. If this assumption is verified, Mg^{2+}-

ATPase would be necessary for ATP production, ATP becoming the substrate for $(Na^+ + K^+)$-ATPase. More work on the elucidation of this hypothesis is in progress.

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