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CONCANAVALIN A STIMULATION OF RAT LYMPHOCYTE ATPase

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SUMMARY

1. Concanavalin A, a lectin which induces transformation of lymphocytes was tested for its effect on lymphocyte ATPase.

2. Concanavalin A stimulates ATPase activity catalyzed by intact rat lymphocytes and by lymphocyte microsomal fraction.

3. Concanavalin A stimulates selectively (Na^+–K^+)-independent ATPase of lymphocyte microsomal fraction.

4. The stimulatory effect of concanavalin A on lymphocyte ATPase can be reversed by methyl-α-D-mannopyranoside which binds specifically to concanavalin A.

5. Concanavalin A also stimulates ATPase of a brain microsomal fraction and has no effect on ATPase of a liver microsomal preparation.

6. It is suggested that concanavalin A-stimulated lymphocyte ATPase might be involved in an energy-dependent process in the lymphocyte membrane, induced by the lectin.

INTRODUCTION

Lymphocytes undergo metabolic and morphological changes, referred to as transformation or blastogenesis, upon interaction with a variety of agents which affect the cell membrane[1]. The binding and the mitogenic activity of the lectins phytohemagglutinin and concanavalin A can be reversed by specific saccharides[2–4], suggesting that a saccharide-containing site in the cell membrane is involved in lymphocyte transformation. The lymphocyte membrane itself undergoes biochemical changes upon interaction with blastogenic agents. Fisher and Mueller[5] reported a stimulatory effect of phytohemagglutinin on the incorporation of $^{32}P_i$ into a phospholipid fraction of the lymphocyte membrane. Smith and Hollers[6] found that fluorescein-labelled concanavalin A, shortly after its binding to the lymphocyte membrane, is pinocytized into the cytoplasm by an energy-dependent process. Recently, Taylor *et al.*[7] reported redistribution and pinocytosis of lymphocyte immunoglobulin molecules on the surface of the lymphocyte induced by anti-immunoglobulin antibody, an agent which induces lymphocyte transformation.

In a study of the biochemical alterations in the lymphocyte membrane induced by phytomitogens we tested the effect of concanavalin A on some enzymic properties of the cell membrane. We have found that concanavalin A stimulates

ATPase activity catalyzed by rat lymphocytes and by lymphocyte membrane preparation (microsomal fraction). Some of the characteristics of this phenomenon are described in the present communication.

MATERIALS AND METHODS

Materials

[γ-^{32}P]ATP was synthesized according to the method of Jagendorf and Avron[8]. Concanavalin A, twice crystallized, was obtained from Miles–Yeda Ltd, Rehovot, Israel. Methyl-α-D-mannopyranoside and D-galactose were obtained from Pfanstiehl Laboratories, Inc., Illinois. Methyl-α-D-galactopyranoside was a gift from Dr. E. Harel and was synthesized according to the method of Frahn and Mills[9].

Animals

Male Wistar rats weighing 200–240 g were used after being killed by ether.

Preparation of lymphocytes and lymphocyte microsomal fraction

Rat peripheral and mesenteric lymph nodes were removed and minced in phosphate-buffered saline[10]. The large pieces of connective tissue were allowed to settle and the supernatant suspension was harvested. Lymphocytes were suspended ($80 \cdot 10^6$ per ml) in a solution containing: 0.25 M D-mannitol, 0.001 M EDTA, 0.02 M Tris–HCl (pH 7.4) and broken in the Yeda-Press[11] at 600 lb/inch^2 under argon, at 0 °C. The suspension was centrifuged at 6000 $\times$ g for 10 min, the pellet discarded and the supernatant centrifuged at 105000 $\times$ g for 60 min. The pellet was suspended in the above-mentioned D-mannitol-containing solution (one-fifth of the original volume), and was used as the lymphocyte microsomal fraction. Small aliquots were frozen at —20 °C. Each aliquot was thawed, as needed, only once.

Preparation of liver and brain microsomal fractions

Rat brain or liver tissue was homogenized with 9 vol. of 0.25 M D-mannitol, 0.001 M EDTA, 0.02 M Tris–HCl (pH 7.4) at 0 °C using a motor-driven teflon pestle. The homogenate was treated in the Yeda-Press at 600 lb/inch^2 and the microsomal fraction was isolated by the procedure described above for the preparation of lymphocyte microsomal fraction.

Protein determinations

Protein determinations were carried out by the method of Lowry *et al.*[12] with the use of bovine plasma albumin as a standard.

ATPase assay

Assay conditions are outlined in the figure legends and tables. Incubation mixtures were shaken in a Dubnoff bath at 37 °C for 20 min. The reaction was terminated by the addition of 0.1 vol. of 50% trichloroacetic acid. $^{32}P_i$ formed was determined as the phosphomolybdate complex according to the method of Conway and Lipmann[13]. ATPase assays were performed in triplicate and the results are expressed as the mean value of the three determinations. The range of deviation from the mean value was not more than 5%.

5'-Nucleotidase assay

Lymphocyte microsomal fractions (0.92 mg/ml) in a solution of 0.25 M D-mannitol, 0.001 M EDTA, 0.02 M Tris–HCl (pH 7.4) were incubated (where indicated) with concanavalin A (40 μg/ml) for 20 min at 23 °C. The incubation mixture (0.1 ml) was then mixed with 0.1 ml of a solution of 0.1 M Tris–HCl (pH 7.4), 0.02 M KCL 0.02 M $MgCl_2$, 0.01 M AMP, and incubation continued for 60 min at 37 °C. 0.1 vol. of a solution of 50% trichloroacetic acid was then added and P_i was determined according to the method of Chen *et al.*[14].

RESULTS

Concanavalin A stimulates ATPase activity catalyzed by intact lymphocytes (Table I) and by lymphocyte microsomal fraction (Table II). A characteristic feature of concanavalin A stimulation of lymphocyte ATPase is an optimal concentration of concanavalin A which has maximal stimulatory effect. Higher concentrations of concanavalin A are less stimulatory. A similar dose response curve was recorded for concanavalin A-stimulated RNA and DNA synthesis in rat lymphocytes[4].

TABLE I

EFFECT OF CONCANAVALIN A AT DIFFERENT CONCENTRATIONS ON ATPase ACTIVITY OF INTACT RAT LYMPHOCYTES

Rat lymph node lymphocyte suspensions ($5 \cdot 10^6$/ml) in a solution of 0.02 M Tris–HCl (pH 7.4) 0.15 M NaCl, 0.004 M $MgCl_2$ were incubated with concanavalin A at different concentrations for 30 min at 23 °C. Aliquots of 0.15 ml were added to 0.05 ml of a solution of 0.014 M [γ-^{32}P]ATP (specific activity 500 cpm/nmole). Incubation was continued for 20 min at 37 °C and $^{32}P_i$ formed was determined.

Concanavalin A (*μg/ml*)	*ATPase activity* (*nmoles $^{32}P_i$ formed per 10^6 lymphocytes per 20 min*)
—	3.45
6	4.10
15	6.03
30	5.52

TABLE II

EFFECT OF CONCANAVALIN A AT DIFFERENT CONCENTRATIONS ON ATPase ACTIVITY OF LYMPHOCYTE MICROSOMAL FRACTION

Lymphocyte microsomal fractions (0.96 mg protein per ml) in a solution of 0.25 M D-mannitol, 0.001 M EDTA 0.02 M Tris–HCl (pH 7.4) were incubated with concanavalin A at different concentrations for 30 min at 23 °C. 20-μl aliquots were added to 0.18 ml of a solution of 0.022 M Tris–HCl (pH 7.4), 0.0039 M $MgCl_2$, 0.0039 M [γ-^{32}P]ATP (specific activity 220 cpm/nmole). Incubation was continued for 20 min at 37 °C and $^{32}P_i$ formed was determined.

Concanavalin A (*μg/ml*)	*ATPase activity* (*μmoles $^{32}P_i$ formed per mg protein per h*)
—	3.08
2	4.13
8	4.54
32	5.13
160	4.24

As is shown in Fig. 1, the activity of concanavalin A-induced stimulated ATPase is linear during an incubation period of 60 min.

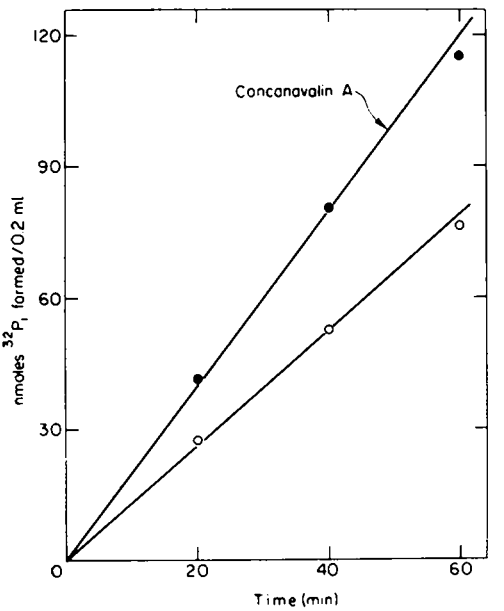

Fig. 1. Kinetics of concanavalin A-stimulated ATPase of lymphocyte microsomal fraction. Lymphocyte microsomal fraction (0.87 mg protein per ml) in a solution of 0.25 M D-mannitol, 0.001 M EDTA, 0.02 M Tris–HCl (pH 7.4) was incubated (where indicated) with concanavalin A (33 μg/ml) for 30 min at 23 °C. The incubation mixture (0.24 ml) was then mixed with 2.2 ml of a solution of 0.022 M Tris–HCl (pH 7.4), 0.133 M NaCl, 0.0055 M KCl, 0.0039 M $MgCl_2$, 0.0039 M [γ-^{32}P]ATP (specific activity 286 cpm/nmole). Incubation was continued at 37 °C and 0.2-ml aliquots were withdrawn at 20-min intervals and $^{32}P_i$ formed was determined. Concanavalin A-treated microsomal fraction (●—●); untreated microsomal fraction (○—○).

The effect of several cations (Mg^{2+}, Na^+, K^+) on ATPase of lymphocyte microsomal fraction treated with concanavalin A was investigated. As is shown in Table III, (Na^+–K^+)-independent ATPase is stimulated by concanavalin A, whereas (Na^+–K^+)-dependent ATPase is slightly inhibited by the lectin.

The effect of several saccharides on concanavalin A stimulation of lymphocyte microsomal fraction ATPase is shown in Table IV. Methyl-α-D-mannopyranoside, a saccharide which binds specifically to concanavalin A[15], markedly reduced the stimulatory effect of the lectin, whereas methyl-α-D-galactopyranoside and D-galactose, saccharides which are not bound by concanavalin A[16], have much less inhibitory effects. The above finding suggests that methyl-α-D-mannopyranoside abolishes the stimulatory effect of concanavalin A on lymphocyte microsomal fraction ATPase as a result of a competition reaction in which methyl-α-D-mannopyranoside and the concanavalin A-binding sites on the lymphocyte membrane preparation, compete for the specific bindings sites of concanavalin A.

Concanavalin A was tested for its effect on the ATPase activity of microsomal fractions from non-lymphoid tissues. As is shown in Table V, concanavalin A also stimulates ATPase of a brain microsomal fraction. In contrast concanavalin A has no effect on the ATPase activity of a liver microsomal preparation.

TABLE III

EFFECT OF CATIONS ON ATPase OF LYMPHOCYTE MICROSOMAL FRACTION TREATED WITH CONCANAVALIN A

Lymphocyte microsomal fractions (0.87 mg protein per ml) in a solution of 0.25 M D-mannitol, 0.001 M EDTA, 0.02 M Tris–HCl (pH 7.4) were incubated (where indicated) with concanavalin A (33 μg/ml) for 30 min at 23 °C. 20-μl aliquots were added to 0.18 ml of a solution containing 0.022 M Tris–HCl (pH 7.4), 0.0039 M [γ-^{32}P]ATP (Tris salt) (specific activity 258 cpm/nmole) and where indicated 0.133 M NaCl, 0.0055 M KCl, 0.0039 M $MgCl_2$. Incubation was continued for 20 min at 37 °C and $^{32}P_i$ formed was determined.

Additions	*ATPase fraction*	*ATPase activity (μmoles $^{32}P_i$ formed per mg protein per h)*	
		– Concanavalin A	*+ Concanavalin A*
—		0.20	0.13
Mg^{2+}, Na^+, K^+	Total	3.96	5.99
Mg^{2+}	$(Na^+–K^+)$-independent	2.55	5.04
(Calculated)	$(Na^+–K^+)$-dependent	1.41	0.95

TABLE IV

REVERSION OF CONCANAVALIN A STIMULATION OF LYMPHOCYTE MICROSOMAL FRACTION ATPase BY METHYL-α-D-MANNOPYRANOSIDE

Expt A. Lymphocyte microsomal fractions (0.75 mg protein per ml) in a solution of 0.25 M D-mannitol, 0.001 M EDTA, 0.02 M Tris–HCl (pH 7.4) were incubated (where indicated) with concanavalin A (30 μg/ml) and saccharides (20 mg/ml) for 30 min at 23 °C. 20-μl aliquots were added to 0.18 ml of a solution of 0.022 M Tris–HCl (pH 7.4), 0.0039 M $MgCl_2$ and 0.0039 M [γ-^{32}P]ATP (specific activity 240 cpm/nmole). Incubation was continued for 20 min at 37 °C and $^{32}P_i$ formed was determined. *Expt B*. Experimental conditions were similar to those outlined in the legend to Expt A, except that a different lymphocyte microsomal preparation was used and that the saccharides tested (at the concentrations outlined in the Table) were included also in the [γ-^{32}P]ATP-containing solution in order to maintain constant the saccharide concentration throughout the experiment.

Saccharide	*Concn (mg/ml)*	*ATPase activity (μmoles $^{32}P_i$ formed per mg protein per h)*		*Concanavalin A stimulation (%)*
		– Concanavalin A	*+ Concanavalin A*	
Expt A				
None	—	2.63	5.06	92
Methyl-α-D-mannopyranoside	20	2.91	3.59	23
Methyl-α-D-galactopyranoside	20	2.91	4.83	66
Expt B				
None	—	2.61	4.39	68
Methyl-α-D-mannopyranoside	15	3.03	2.94	−3
Methyl-α-D-mannopyranoside	1.5	3.07	3.40	11
D-Galactose	15	2.56	3.68	44

TABLE V

EFFECT OF CONCANAVALIN A ON ATPase ACTIVITY OF MICROSOMAL FRACTIONS FROM RAT LYMPHOCYTES, BRAIN AND LIVER

Microsomal fractions from lymphocytes (1.5 mg protein/ml), brain (0.67 mg protein per ml) or liver (1.0 mg protein per ml) in a solution of 0.25 M D-mannitol, 0.001 M EDTA, 0.02 M Tris–HCl (pH 7.4) were incubated (where indicated) with concanavalin A (33 μg/ml) for 30 min at 23 °C. 20-μl aliquots were added to 0.18 ml of a solution of 0.022 M Tris–HCl (pH 7.4), 0.133 M NaCl, 0.0055 M KCl, 0.0039 M $MgCl_2$ and 0.0039 M [γ-^{32}P]ATP (specific activity 416 cpm/nmole). Incubation was continued for 20 min at 37 °C and $^{32}P_i$ formed was determined.

Source of microsomal fraction	*ATPase activity (μmoles $^{32}P_i$ formed per mg protein per h)*	
	– Concanavalin A	*+ Concanavalin A*
Lymphocytes	3.71	5.44
Brain	12.06	17.06
Liver	5.22	5.33

We also investigated the effect of concanavalin A on 5′-nucleotidase of lymphocyte microsomal fraction, an enzyme which is used as a putative marker for the plasma membrane[17]. No stimulatory effect of concanavalin A was observed. The specific activity of 5′-nucleotidase (μmoles P_i formed per mg protein per h) of lymphocyte microsomal fraction treated with concanavalin A (under the experimental conditions outlined in Materials and Methods) was 0.33, whereas the control value was 0.37.

DISCUSSION

The mechanism by which concanavalin A stimulates lymphocyte ATPase is not known. It is possible that concanavalin A induces, upon interaction with a specific saccharide-containing site in the cell membrane, a conformational change which results in enhanced ATPase. However, a more indirect effect of the lectin on ATPase should obviously be considered. For example, if concanavalin A binding were to change the ionic permeability of the membrane, this could lead to a change in ATPase activity observed. Quastel and Kaplan[18] reported recently on phytohemagglutinin-induced stimulation of K^+ uptake into human lymphocytes. However, as was shown above, concanavalin A does not stimulate (Na^+–K^+)-dependent ATPase from rat lymphocytes.

Concanavalin A-stimulated ATPase might be involved in the active uptake of a substance from the culture medium which triggers lymphocyte transformation. The possible role of concanavalin A-activated ATPase in the pinocytosis reaction which occurred shortly after concanavalin A binding to lymphocytes[6] should also be mentioned. Further studies are required for the elucidation of the relationship between the stimulatory effect of concanavalin A on lymphocyte ATPase and its blastogenic activity.

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