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CONCANAVALIN A AUGMENTS THE TURNOVER OF ELECTROPHORETICALLY DEFINED THYMOCYTE PLASMA MEMBRANE PROTEINS

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SUMMARY

1. Rabbit thymocytes were labelled in vitro with ^{14}C-labelled amino acids either before or during stimulation with concanavalin A.

2. Plasma membranes were isolated from control and stimulated thymocytes and their proteins fractionated by sodium dodecylsulfate–polyacrylamide gel electrophoresis.

3. No qualitative and quantitative differences between the sodium dodecylsulfate–polyacrylamide gel electrophoresis patterns of membrane proteins from normal and concanavalin A-treated cells could be detected.

4. Plasma membrane protein turnover is generally augmented by concanavalin A stimulation.

5. The turnover of a sodium dodecylsulfate–polyacrylamide gel electrophoresis component with an apparent molecular weight of 55 000 is particularly enhanced. Moreover, concanavalin A induces excretion of this component into the cultivation medium.

6. The data are discussed in terms of possible modes of interaction between concanavalin A and thymocyte plasma membranes.

INTRODUCTION

Activation of thymocytes and other lymphoid cells by immunoglobulins and lectins, such as concanavalin A and kidney bean phytohemagglutinin, leads to diverse functional plasma membrane alterations, including redistribution of surface receptors [1, 2], activation of ion transport [3, 4] and enhanced aminoacid transport [5]. Moreover, the plasma membrane lipid composition of thymocytes changes dramatically within 30 min after addition of concanavalin A [6], an effect attributable to an activation of the membrane-bound lysolecithin-acyltransferase [7].

Abbreviation: HEPES, 4-(hydroxymethyl)-1-piperazinylethane-2-sulfate.

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Here we initiate an exploration of possible membrane protein changes subsequent to concanavalin A stimulation of thymocytes. For this we compare the protein compositions as defined by sodium dodecylsulfate–polyacrylamide gel electrophoresis of plasma membranes derived as in refs 8 and 9 from control and stimulated cells.

We also evaluate the effect of concanavalin A on the turnover of the various sodium dodecylsulfate–polyacrylamide gel electrophoresis components from the incorporation and loss of [^{14}C] aminoacid labelled protein entities. Some kinetic aspects of these experiments have been reported previously [10].

MATERIALS AND METHODS

Chemicals

Unless stated otherwise, all chemicals were of highest purity available. We obtained 4-(hydroxymethyl)-1-piperazinylethane-2-sulfate (HEPES), Coomassie Brilliant Blue, sodium dodecylsulfate, acrylamide, *N,N'*-diallyltartrate diamide, dithiothreitol, and pyronin G from Serva (Heidelberg, Germany), Dextran-150 from Pharmacia Inc. (Frankfurt, Germany), Ficoll from Pharmacia (Sweden), Isopaque (sodium metrizoate, 75 %) from Nyegaard and Co. (Oslo, Norway) and nylon wool (LP-1-Leukopak) from Fenwal Laboratories (Ill.). We purchased a ^{14}C-labelled amino acid mixture (high specific activity) 250 μCi per 5 ml in 2 % aqueous ethanol and [5-^{3}H]uridine (TRK 178) 23 Ci/mmole from Buchler Amersham. The latter was diluted with unlabelled uridine to a concentration of 200 nmoles/ml.

To obtain thymocytes for cultivation, we proceed sterilely. We first exsanguinate rabbits (strain: silver champagne; 2–3 months old) by heart puncture, then isolate their thymuses, remove blood vessels and connective tissue, mince, suspend these fragments in phosphate-buffered saline (Gibco), pH 7.2, liberate the cells with a Tenbroeck homogenizer (Bellco; clearance 0.10–0.18 mm) and pass the cell suspension over a short column of nylon wool to remove cell aggregates and debris. When a thymocyte suspension contain more than 2–3 % erythrocytes, we lyse these by addition of 0.017 M Tris–HCl, 0.14 M NH_4Cl, pH 7.4 [11], and when the proportion of Trypan blue (dead) cells exceeded 10 %, these are eliminated by means of Ficoll-isopaque gradient principally as in ref. 12.

We wash the isolated thymocytes twice with phosphate-buffered saline ($800 \times g_{av}$, 5 min, 20 °C) and resuspend to a concentration of $5 \cdot 10^7$ cells/ml in Eagle's minimal essential medium (Gibco), 10 % in fetal calf serum, inactivated for 30 min at 56 °C. For cultivation, we use 250-ml Petri dishes kept in 5 % CO_2, 95 % air, 37 °C using CO_2 incubators (Heraeus, Hanau KB 600).

To obtain good stimulation with concanavalin A, we precultivate the thymocytes for 20–24 h, resuspend in fresh minimal essential medium containing 5 μg concanavalin A per ml, and, after 30 min, make the medium 10 % in fetal calf serum and cultivate for 4–24 h.

To monitor the stimulating effect of concanavalin A by [^{3}H]uridine incorporation, we add 50 μl [1-^{3}H]uridine (0.5 μCi) to 10^8 cells in 2 ml minimal essential medium after a given period of stimulation, cultivate for another 4 h, chill, make the suspension 10 % in trichloroacetic acid, wash the precipitates twice with ice-cold trichloroacetic acid ($1600 \times g$, 10 min), solubilize them with 1 ml Soluene (Hewlett-

Packard), take up in 10 ml Aquasol (Hewlett Packard) and count in a scintillation spectrometer Hewlett-Packard (Tricarb Model 3820). The ratio (^{3}H incorporation per 10^8 stimulated cells) to (^{3}H-incorporation per 10^8 unstimulated cells) (in percent) is taken as stimulation index.

To establish turnover rates for membrane proteins we measure the incorporation and elimination of amino acid ^{14}C during cultivation. For incorporation, we precultivate thymocytes ($5 \cdot 10^7$ cells/ml minimal essential medium 10 % fetal calf serum) for 20 h, then wash twice in minimal essential medium resuspend in medium containing only 20 % of the amino acid concentration of minimal essential medium, add 0.7 ml per 100 ml (35 μCi) of a ^{14}C-labelled amino acid mixture and 5 μg concanavalin A per ml medium, and cultivate as before for various time periods.

To evaluate amino acid ^{14}C loss, we first precultivate thymocytes in amino acid-depleted minimal essential medium, 10 % fetal calf serum, in the presence of the ^{14}C-labelled amino acid mixture for 20–24 h, then wash twice in minimal essential medium and continue cultivation with concanavalin A as described (stimulation) and without concanavalin A (control).

To isolate the cells' plasma membranes after completion of cultivation, we first wash the cells twice with minimal essential medium, resuspend at a concentration of $5 \cdot 10^7$ cells/ml in 0.075 M KCl, 0.065 M NaCl, 0.25 mM $MgCl_2$, 0.01 M HEPES, pH 7.4, disrupt the cells by N_2 cavitation as in ref. 8, sediment nuclei, mitochondria and lysosomes together at $6 \cdot 10^5 \times g \cdot$ min (Beckman centrifuge J 21) and pellet small particles at $10^7 \times g \cdot$ min (Beckman Spinco ultracentrifuge L2 65, Rotor 50.1). We then wash the particles first with 0.01 M HEPES, pH 7.5, and then with 0.001 M HEPES, pH 7.5 ($10^7 \times g \cdot$ min), to eliminate trapped cytoplasmic proteins [8], suspend in 0.001 M HEPES, pH 7.5, dialyze for 90 min against 0.001 HEPES, 0.001 M $MgCl_2$, pH 8.2, then apply the particles to the top of a dextran gradient (maximum density 1.09) as in ref. 8, and centrifuged for $10^8 \times g \cdot$ min (Beckman Spinco L2 65, Rotor SW 56) to obtain two membrane fractions, plasma membrane fractions 1 and 2 and an endoplasmic reticulum pellet, as in [8] and [9].

We fractionate membrane proteins of the microsomes and plasma membrane fraction 1 [9], by sodium dodecylsulfate–polyacrylamide gel electrophoresis. For this we make membrane fractions 1 % in sodium dodecylsulfate $\pm$40 mM dithiothreitol and heat about 2 min at 100 °C. We also examine the proteins in fetal calf serum-free culture media by sodium dodecylsulfate–polyacrylamide gel electrophoresis after ultracentrifugation at $10^8 \times g \cdot$ min (Beckman Spinco L2 65, Rotor SW 56). For this we concentrate by ultrafiltration (Diaflo membrane PM 10, Amicon) before sodium dodecylsulfate addition. Sodium dodecylsulfate–polyacrylamide gel electrophoresis is on 6-mm gels, 7 % polyacrylamide cross-linked with 3.75 % *N,N'*-diallyltartrate diamide, containing 1 % sodium dodecylsulfate, using a Tris–acetate–EDTA (40 mM : 20 mM : 2 mM) buffer, pH 7.4 [13], applying exactly 60 μg protein per gel (determined as in refs 14 and 15) and using pyronin G as tracking dye and electrophorese for 240 min at 2 mA/gel. We stain for protein with Coomassie Brilliant Blue [13], and for carbohydrate with HIO_4 Schiff reagent [13], scanning the gels at 620 and 560 nm respectively, using a Gilford scanner (Model 240). We quantify staining by means of an integrator (Hewlett-Packard Model 3370B). For ^{14}C analysis we prestain membrane proteins with *O*-phthalaldehyde [16]. By this, the various components can be detected under ultraviolet light in intact gels and the gels sliced

accordingly. For counting, we dissolve each gel slice in 0.5 ml 2 % HIO_4, add 1 ml Soluene and, after 12 h, 10 ml Aquasol (vigorous shaking).

We express the ^{14}C specific activity of various sodium dodecylsulfate–polyacrylamide gel electrophoresis components in terms of Coomassie blue staining. Assuming constant staining for each sodium dodecylsulfate–polyacrylamide gel electrophoresis component and knowing the amount of protein applied per gel, the stain distribution can be taken as a relative measure.

RESULTS

Thymocyte survival

With $5 \cdot 10^7$ thymocytes per ml we usually obtain a 70 % survival after 44–48 h cultivation time in presence of 10 % fetal calf serum and at least 60 % without fetal calf serum was added. We observed no cell division during cultivation, even during the first 24 h of stimulation.

Activating effect of concanavalin A

To obtain satisfactory concanavalin A stimulation, we find it necessary to precultivate fresh thymocytes for 20–24 h ($5 \cdot 10^7$ cells/ml, 10 % fetal calf serum). Then 5 μg/ml concanavalin A gives a [^{3}H]uridine incorporation after 24 h of about 30 times that of controls (Table I). Concurrently, protein synthesis, as monitored by ^{14}C-labelled amino acid incorporation into proteins, increases nearly 3-fold, producing an increase of 120 % in the specific ^{14}C activity of the isolated microsomes and of about 100 % in the plasma membrane fraction (Table I).

Quantitative changes in plasma membrane protein composition during cultivation

To evaluate the influence of cultivation on the thymocyte plasma membranes, those obtained from freshly prepared cells are compared to membranes isolated after 48 h cultivation (Table II). In both instances, ten sodium dodecylsulfate–polyacrylamide gel electrophoresis components comprise 94 and 95 % of Coomassie staining. Cultivation induces quantitative differences in protein composition (Table II, Fig. 1). These do not depend on the presence of 10 % fetal calf serum. Specifically, proteins 3.2 (apparent molecular weight 105 000) and protein 6 (apparent molecular weight 44 000) increase by about 40 and 15 %, respectively, while protein 4 (apparent molecular weight 80 000) and protein 7 (apparent molecular weight 36 000) both decrease by 10 %.

TABLE I

CONCANAVALIN A STIMULATION OF RABBIT THYMUS LYMPHOCYTES

RNA synthesis ([^{3}H]uridine uptake) and protein synthesis (^{14}C-labelled amino acid uptake). Cells precultivated for 20 h and then stimulated with concanavalin A (5 μg/ml) for 24 h using a cell concentration of $5 \cdot 10^7$ per ml. Values from 6 experiments.

	Concanavalin A	Control
[^{3}H]Uridine (10^8 cells)	45 800 (± 3700)	1480 (± 155)
^{14}C activity (10^8 cells)	18 500 (± 1650)	7600 (± 310)
^{14}C activity (microsomes, 10^8 cells)	2 800 (± 310)	1300 (± 122)
^{14}C activity (plasma membrane, $2 \cdot 10^8$ cells)	900 (± 105)	460 (± 40)

TABLE II

PROTEIN COMPOSITION OF RABBIT THYMOCYTE PLASMA MEMBRANES (PM_1) OBTAINED FROM FRESH AND CULTIVATED CELLS

Values are given for the major components only. Composition according to Coomassie blue staining. Fresh cells were fractionated immediately after isolation, cultivated cells after 48 h.

Peptide	Apparent mol. wt	% Coomassie blue staining	
		0 h	48 h
1.1–1.2	≈230 000	6.5	5.8
2.1–2.3	≈160 000	9.8	9.5
3	120 000	6.2	5.9
3.2	105 000	4.8 (±0.2)	7.3 (±0.3)
4	80 000	15.9 (±0.8)	14.2 (±0.6)
5, 5.1	≈ 55 000	15.0 (±0.8)	15.7 (±0.9)
6	44 000	8.3 (±0.4)	10.5 (±0.6)
7	36 000	10.4 (±0.5)	9.0 (±0.4)
8	27 000	6.5	6.2
9	21 000	6.8	6.7
10	16 500	3.2	3.8
		93.4	94.6

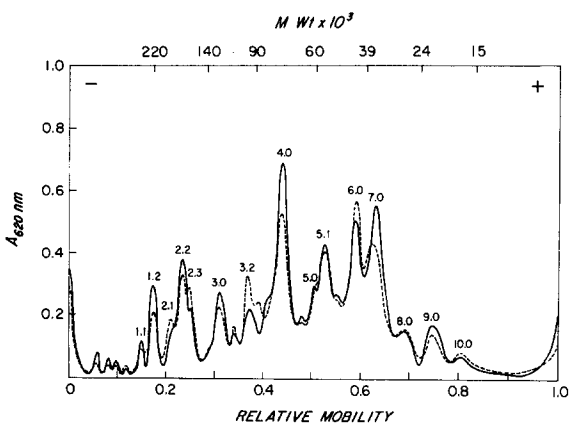

Fig. 1. Sodium dodecylsulfate–polyacrylamide gel electropherogram of proteins in rabbit thymocyte plasma membranes. Abscissa. relative mobility: apparent molecular weight. Ordinate. absorbance at 620 nm for Coomassie blue staining: —, fresh thymocytes; - - -, after 24 h culture in minimal essential medium (Eagle's).

Influence of stimulation on thymocyte membrane protein composition

Concanavalin A stimulation induces no differences in qualitative and quantitative protein composition in thymocyte microsomes (Fig. 2) or plasma membranes isolated therefrom. No concanavalin A can be detected in either fraction upon sodium dodecylsulfate–polyacrylamide gel electrophoresis (Coomassie blue staining).

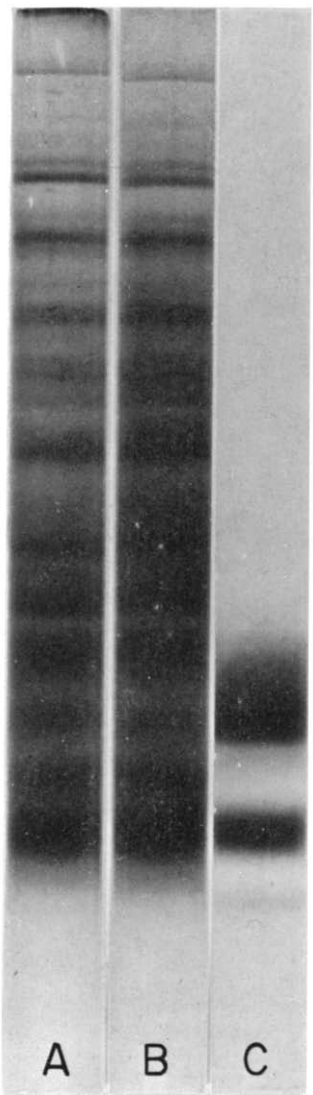

Fig. 2. Sodium dodecylsulfate–polyacrylamide gel electropherogram of microsomal proteins from unstimulated (A) and concanavalin A stimulated (B) thymocytes. (C) Sodium dodecylsulfate–polyacrylamide gel electrophoresis of concanavalin A.

Concanavalin A-induced changes in plasma membrane protein kinetics

By comparison of membrane protein labelling in cells after stimulation in the presence of ^{14}C-labelled amino acids with that of pre-labelled cells, stimulated in the presence of unlabelled amino acids yields a measure of protein turnover. (After fractionating the plasma membrane proteins by sodium dodecylsulfate–polyacrylamide gel electrophoresis, we obtain the specific ^{14}C activity of each sodium dodecylsulfate–polyacrylamide gel electrophoresis protein component). As shown in the Table III, stimulation after precultivation augments the turnover of components

TABLE III

SPECIFIC ^{14}C ACTIVITY IN PLASMA MEMBRANE PROTEINS OF CONCANAVALIN A-STIMULATED RABBIT THYMOCYTES IN COMPARISON TO UNSTIMULATED CELLS

After precultivation of the cells for 20 h ^{14}C-labelled amino acid uptake within 24 h is determined. Plasma membrane proteins separated by sodium dodecylsulfate–polyacrylamide electrophoresis. Values are given from 4 separate determinations. Increase in specific activity is referred to the control as 1.0. Average deviation for spec. act. $\leqq 14\,\%$.

Peptide	Coomassie blue staining (%)	Protein (μg)	Control		Concanavalin A		Spec. act. concanavalin A/spec. act. control
			^{14}C (cpm)	(Spec. act. (cpm/μg protein)	^{14}C (cpm)	Spec. act. (cpm/μg protein)	
1.1–1.2	5.8	3.8	150	39.5	280	73.7	1.87
2.1–2.3	9.5	6.2	260	41.9	590	95.2	2.27
3	5.9	3.8	160	42.1	275	72.4	1.72
3.2	7.3	4.3	230	48.9	510	108.5	2.22
4	14.2	9.2	280	30.4	580	63.0	2.07
5.1	10.3	6.7	400	59.7	1220	182.1	3.05
6	10.5	6.8	220	32.4	360	52.9	1.63
7	9.0	5.8	190	32.8	330	56.9	1.74
8	6.2	4.0	130	32.5	190	47.5	1.46
9	6.7	4.3	140	32.6	200	46.5	1.43
10	3.8	2.5	130	52.0	130	52.0	0
	89.2	57.8	2290		4665		

TABLE IV

LOSS OF ^{14}C LABEL FROM PLASMA MEMBRANE PROTEINS AS DETERMINED BY MEANS OF DECREASE IN SPECIFIC ^{14}C ACTIVITY OF EACH PROTEIN

Influence of concanavalin A stimulation in comparison to unstimulated cells (control). Values are given from 3 separate determinations. Decrease in specific activity is referred to the control as 1.0. Plasma membrane proteins separated by sodium dodecylsulfate–polyacrylamide electrophoresis. Average deviation for spec. act. $\leqq 16\,\%$.

Peptide	Coomassie blue staining (%)	μg protein	*Precultivation* (^{14}C aminoacid uptake)		Control		Concanavalin A		Spec. act. concanavalin A/ spec. act. control
			^{14}C (cpm)	Spec. act. (cpm/μg protein)	^{14}C (cpm)	Spec. act. (cpm/μg protein)	^{14}C (cpm)	Spec. act. (cpm/μg protein)	
1.1–1.2	6.0	4.0	175	43.8	115	28.8	90	22.5	0.782
2.1–2.3	9.6	6.4	320	50.0	230	36.0	135	21.1	0.586
3	5.7	3.8	105	54.0	135	35.6	100	26.3	0.740
3.2	7.0	4.7	285	60.7	210	44.7	150	22.3	0.499
4	13.9	9.3	380	40.8	290	31.2	160	17.2	0.550
5.1	11.2	7.5	515	68.7	375	50.1	155	20.7	0.413
6	10.5	7.0	290	41.5	195	27.9	180	25.7	0.921
7	8.6	5.7	225	39.5	150	26.4	125	21.9	0.830
8	5.9	3.9	160	41.0	105	26.9	95	24.4	0.907
9	6.6	4.4	175	39.8	115	26.2	110	25.0	0.955
10	3.9	2.6	165	63.5	110	42.3	120	46.2	0.943
	89.9	59.3	2795		2030		1420		

TABLE V

HALF LIFE TIME ($T_{\frac{1}{2}}$) OF PLASMA MEMBRANE PROTEINS DERIVED FROM CONCANAVALIN A-STIMULATED RABBIT THYMOCYTES

The $T_{\frac{1}{2}}$ values of all molecular weight classes exceed 24 h in the case of unstimulated cells.

Peptide	$T_{1/2}$ (h)
1.1–1.2	24 (±4)
2.1–2.3	18 (±2)
3	>24
3.2	20 (±2.5)
4	18 (±2.3)
5.1	10 (±1.8)
6	>24
7	>24
8	>24
9	>24
10	>24

1.1–1.2, 2.1–2.3, 3.2, 4 and 5.1 more than components 3 and 6–10. Also, when prelabelled cells are cultivated with or without concanavalin A, the specific activities of high-molecular weight components drop more rapidly than most of other sodium dodecylsulfate–polyacrylamide gel electrophoresis entities (Table IV). When unstimulated cells are labelled during precultivation (20–24 h) we obtain higher specific activities than when labelling is after precultivation (Tables III and IV). Also the concanavalin A augmented ^{14}C incorporation without prelabelling exceeds the concanavalin A-induced ^{14}C release after prelabelling. The data show that membrane protein turnover is generally higher in stimulated cells than in unstimulated ones. The specific ^{14}C activities of the various sodium dodecylsulfate–polyacrylamide gel electrophoresis components in Tables III and IV, relative to each other, are rather similar with exception of 5.1 (apparent molecular weight 55 000), whose apparent turnover is significantly greater than that of other proteins. The apparent low turnover of the low molecular weight components 8–10 may relate to the possibility that these proteins are cytoplasmic contaminants [9].

From the kinetics of ^{14}C incorporation and release into membrane proteins, one can calculate a half life ($T_{\frac{1}{2}}$) for each plasma membrane protein. The $T_{\frac{1}{2}}$ values for concanavalin A stimulated thymocytes are listed in Table V. The very low half life of protein 5.1 of about 10 h stands out. In contrast, the $T_{\frac{1}{2}}$ values of the higher-molecular weight components lie between 18 and 24 h, and those for the lower-molecular weight components exceed 24 h.

Release of membrane proteins during stimulation and cultivation

We observe differences in the protein composition in the culture media when we cultivate unstimulated and stimulated thymocytes in minimal essential medium without fetal calf serum. These differences derive not only from the presence of concanavalin A in the medium. The supernatant of non-stimulated cells contains more of the higher molecular weight components (molecular weights 140 000–100 000), particularly component 3.2 (molecular weight 105 000). Also, release of sodium do-

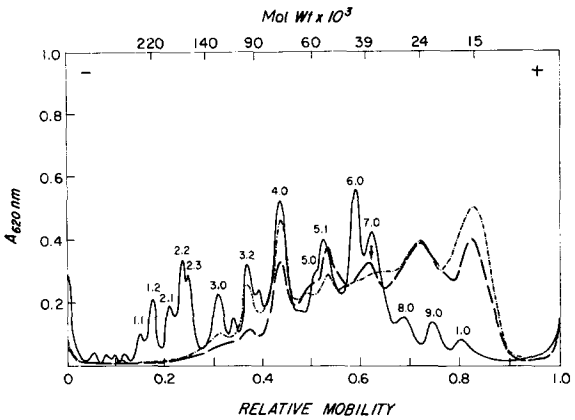

Fig. 3. Sodium dodecylsulfate–polyacrylamide gel electrophoresis of plasma membrane proteins of rabbit thymocytes (—) and of cultivation medium: - - -, stimulated cells; - · - · -, controls. Arrow indicates concanavalin A.

decylsulfate–polyacrylamide gel electrophoresis component 5.1, which we have shown to exhibit high membrane turnover, rises 50–80 % during 24-h stimulation period (Fig. 3).

DISCUSSION

Freshly prepared thymocytes exhibit a different plasma membrane protein composition than cultivated cells (Table II). This cannot be explained by cell death during cultivation, since independent tests show more than 90 % viable cells in both cases. Protease activity can also be excluded since we find no systematic decreases in high-molecular weight components and increase in low-molecular weight entities (as found with peptidolysis). Finally, one can eliminate selection of a special cell population, since there is essentially no cell division during cultivation. The observed differences must, thus, arise in response to the cultivation process per se.

We detect neither qualitative nor quantitative changes in the plasma membrane proteins after concanavalin A stimulation of rabbit thymocytes (Fig. 2). Studies with ^{125}I-labelled concanavalin A show that about 10^6 molecules of lectin bind per mouse spleen cell [17]. Resch (personal communication) observes similar values for intact rabbit thymocytes. Our independent experiments show that this quantity should be revealed by sodium dodecylsulfate–polyacrylamide gel electrophoresis followed by staining with Coomassie Brilliant Blue. Since we cannot detect concanavalin A in the electropherograms of washed thymocyte microsomes, we suspect that the lectin is eluted during preparation and/or redistributed to other subcellular fractions.

While the proportions of the various plasma membrane sodium dodecyl-proteins do not change significantly after concanavalin A, this lectin induces appreciable alterations in membrane protein turnover (Tables III–V). When we precultivate cells for 24 h and then measure incorporation of ^{14}C-labelled amino acids for 24 h after concanavalin A stimulation, we find this uptake to exceed the loss of ^{14}C, after thymocytes, labelled during 24 h precultivation, are stimulated with concanavalin A.

This suggests that concanavalin A produces a net increase in the amount of plasma membrane per cell. In this, the relative selective functional alteration, in the membranes do not relate to any detectable enrichment of specific sodium dodecylsulfate–polyacrylamide gel electrophoresis components.

High-molecular weight components turn over more rapidly than low-molecular weight proteins (Tables III–V). Although concanavalin A causes proportionally identical changes of ^{14}C-labelled specific activity for most membrane proteins (Tables III–IV), component 5.1 (apparent molecular weight 55 000), corresponding to a HIO_4 Schiff reagent positive electrophoresis zone, behaves in a unique fashion. From the incorporation of amino acid ^{14}C into and loss of label from this component, we compute a half life of 10 h, much below that of all other proteins.

We know that component 5.1 of isolated plasma membrane represents a protein exposed at the external surface of intact thymocytes, because it is strongly labelled with ^{125}I after radioiodination of the cells with lactoperoxidase [9]. Unfortunately, lactoperoxidase radioiodinated cells survive less well in culture than untreated thymocytes (unpublished observation). Therefore, we could not measure turnover of ^{125}I-labelled membrane proteins. However, we find a selective increase of 5.1 as defined by sodium dodecylsulfate–polyacrylamide gel electrophoresis, in serum-free culture medium after concanavalin A stimulation. Our data, thus, indicate that concanavalin A apart from increasing the turnover of all high-molecular weight membrane proteins, causes preferential extrusion of component 5.1. However, this is replaced biosynthetically, and the combination of effects appears as increased turnover.

The behavior of 5.1 after thymocyte stimulation with concanavalin A suggests two major, possible mechanisms: First, stimulation induces a generalized membrane restructuring and/or expansion, which involves preferential extrusion and replacement of 5.1. Second, 5.1 is a concanavalin A receptor, whose steady state association with the membrane becomes perturbed upon reaction with concanavalin A, leading to its extrusion into the medium and subsequent biosynthetic replacement. These possibilities are not mutually exclusive. New data show that 5.1 binds concanavalin A (in preparation).

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