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PHOSPHOLIPID METABOLISM OF STIMULATED LYMPHOCYTES

COMPARISON OF THE ACTIVATION OF ACYL-CoA: LYSOLECITHIN ACYLTRANSFERASE WITH THE BINDING OF CONCANAVALIN A TO THYMOCYTES

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SUMMARY

Calf thymocytes were isolated and incubated with concanavalin A. The effect of the mitogen on the enzyme activity of membrane-bound lysolecithin acyltransferase (acyl-CoA: 1-acylglycero-3-phosphorylcholine-*O*-acyltransferase, EC 2.3.1.23) was determined as also the binding of ^{125}I-labelled concanavalin A to intact cells and isolated membranes.

The lysolecithin acyltransferase was found to be activated three times in microsomal membranes. The activation occurred directly after binding of concanavalin A and was temperature independent, since similar activities were found in cells treated with concanavalin A at 0 and 37 °C.

The acyltransferase activation using increasing concentrations of concanavalin A revealed a different behaviour, as compared to the binding of concanavalin A. While the binding of concanavalin A to intact cells expressed a normal hyperbolic saturation function the activation process of the acyltransferase described a sigmoidal relationship. Correspondingly, the interaction coefficients for both functions were different (Sips coefficient for binding = 1.0 and Hill coefficient of the enzyme activation = 1.8).

These results indicate that the acyltransferase activation is due to a cooperative interaction between the ligand-receptor complex and the enzyme.

INTRODUCTION

We previously reported that in lymphocytes the acyl-CoA: lysolecithin acyltransferase (EC 2.3.1.23) is predominantly located in the plasma membrane, and that

Abbreviations: Nbs_2, 5,5′-dithio-bis(2-nitrobenzoic acid); HEPES, 4-(hydroxyethyl)-1-piperazinyl-ethane-2-sulfonic acid.

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this enzyme is activated in cells stimulated with phytohemagglutinin. Since those transferases which transfer polyunsaturated fatty acids to membrane phospholipids exhibited the highest activities in stimulated cells, we suggested that this activated enzyme is responsible for the increased content of polyenoic fatty acids [1, 2] and the subsequent changes of membrane fluidity in activated lymphocytes [3]. However, the elucidation of the mechanism of the enzyme activation had not been undertaken.

In the following study we report experiments which throw light on this ligand-dependent enzyme activation process.

MATERIALS AND METHODS

(1) *Chemicals.* Concanavalin A was obtained from Pharmacia, Frankfurt, Germany, α-methyl-D-mannoside from Roth, Karlsruhe, Germany; agarose-bound concanavalin A (Glycosylex A) from Miles Laboratories, Frankfurt, Germany, Nbs_2 (5,5'-dithio-bis(2-nitrobenzoic acid)) from Boehringer, Mannheim, Germany, HEPES (4-(hydroxyethyl)-1-piperazinyl-ethane-2-sulfonic acid) from Serva, Heidelberg, Germany.

(2) *Substrates.* Unlabelled lysolecithin (1-acylglycero-3-phosphorylcholine) was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A. Labelled lysolecithin (1-[1'-^{14}C]palmitoylglycero-3-phosphorylcholine) was prepared by enzymatic acylation of unlabelled 2-acylglycero-3-phosphorylcholine with [1-^{14}C]-palmitic acid and subsequent cleavage of the 2-acyl group with phospholipase A_2 according to Ferber and Resch [1]. Oleoyl-CoA and arachidonoyl-CoA were prepared according to Reitz and Lands [4] and Okuyama et al. [5] with slight modifications: the starting compounds were the acid chlorides, obtained from Nu Chek Prep. (Bast, Copenhagen, Denmark) and instead of Santoquin as antioxidant, butylated hydroxytoluene was added directly after the termination of the reaction. The total amount of added butylated hydroxytoluene was 50 mg/120 μmol acyl-CoA in 20 ml phosphate buffer, pH 7.4. The purity of the compounds was checked by gas-liquid chromatography. The quantitative determination of acyl-CoAs was performed with the photometric acyl-CoA : lysolecithin acyltransferase assay according to Lands and Hart [6] using an excess of microsomal enzyme.

(3) *Suspensions of calf thymus cells.* Thymocytes were prepared from calf thymus as described previously for the preparation of lymph node cells [7, 8]. Incubation of cell suspensions was carried out with 5×10^9 cells/100 ml Eagle's medium (Dulbeccos modification, Grand Island Biological Company (GIBCO), Grand Island, N.Y. U.S.A.) containing 20 mM HEPES buffer, pH 7.2.

(4) *Isolation of subcellular components.* After incubation, the cells were washed, resuspended and disrupted using the nitrogen cavitation method. Mitochondria, microsomes, plasma membranes and endoplasmic reticulum were isolated as described previously [8].

Most experiments were carried out with microsomal membranes since we found that thymus microsomes consist to 60–80 % of outer cell membrane. The high proportion of plasma membrane in the microsomal fraction is documented by a similar composition (cholesterol, phospholipid) and specific activities of the alkaline phosphatase when compared to purified membranes (Table I).

TABLE I

COMPOSITION OF MICROSOMES AND PLASMA MEMBRANES FROM CALF THYMOCYTES

	Cholesterol (nmol/mg protein)	Phospholipid (nmol/mg protein)	Cholesterol/ phospholipid molar ratio	Alkaline phosphatase (nmol · mg^{-1} · min^{-1})
Microsomes	522	864	0.60	320
Plasma membranes	750	1002	0.75	410

(5) *Iodination of concanavalin A.* ^{125}I-labelled concanavalin A was prepared according to the method of McConahey and Dixon [9]. A solution of 40 mg concanavalin A/4 ml 1 M NaCl, 0.01 M phosphate, pH 7.0, was clarified by centrifugation (55 000 rev./min for 60 min, Spinco Rotor SW 56) and then reacted at 0 °C under stirring for 10 min with 3 mCi Na^{125}I and 0.4 mg (0.5 ml) chloramine T. Then, 0.4 mg (0.5 ml) sodium metabisulfite was added and the mixture dialyzed five times against 2 l of 1 M NaCl. After removal of aggregates by centrifugation, as above, the ^{125}I-labelled concanavalin A solution was stored at −20 °C. The characterization of the labelled compound showed, that it contained less than 2 % free ^{125}I and exhibited a high sugar binding capacity since 92 % could be precipitated with excess of glycogen. Furthermore, binding of ^{125}I-labelled concanavalin A to thymocytes was identical with unlabelled concanavalin A, since mixtures with different ratios of ^{125}I-labelled concanavalin A/unlabelled concanavalin A resulted in the same amount of total bound concanavalin A.

(6) *Binding of concanavalin A to thymocytes.* Binding of concanavalin A was carried out in Falcon plastic tubes (No. 2058, 12×75 mm) with 2 ml of a thymocyte suspension in phosphate-buffered saline ($5 \cdot 10^7$ cells/ml) and increasing amounts of ^{125}I-labelled concanavalin A as indicated.

Unspecific binding was calculated from samples containing 0.1 M α-methyl-D-mannoside, which was mixed with concanavalin A before addition of the cells. After incubation for 30 min at 37 °C, the cells were spun down and 1 ml supernatant was collected for radioactivity measurement. The sedimented cells were washed once with 1.0 ml phosphate-buffered saline, resuspended in 1 ml, and their radioactivity determined in a Packard gamma-spectrometer. Bound concanavalin A, as determined from measurements of the supernatant alone, was nearly identical to the calculation based on cell measurements. This shows that during the single washing step of cells at 4 °C, concanavalin A was not removed from the cells to a significant extent.

(7) *Binding of concanavalin A to thymocyte membranes.* Binding of concanavalin A to thymocyte membranes was carried out in polycarbonate ultracentrifuge tubes (5/8×3 inch, Beckman Instruments) in a total volume of 2.2 ml of 0.01 M HEPES buffer, pH 7.4, containing 1.0 mg bovine serum albumin, 160 μg membrane protein and the same amounts of ^{125}I-labelled concanavalin A as used for intact cells (3–60 μg/ml). Unspecific binding was calculated from samples containing 0.1 M α-methyl-D-mannoside, which was mixed with concanavalin A before addition of the membranes. After incubation for 60 min at 37 °C, the samples were centrifuged in a Beckman 50 Ti rotor at 40 000 rev./min for 90 min, and 1.0 ml supernatant was

collected for radioactivity measurement. Bound concanavalin A was calculated from the difference of total radioactive concanavalin A and free concanavalin A measured in the supernatant.

Measurement of supernatant radioactivity revealed more reliable results as compared to the determination of the radioactivity in pellets probably because of losses of material during washing the pellets. A further advantage of this method, avoiding washing procedures, is that it is carried out under real equilibrium conditions of free and bound concanavalin A.

(8) *Enzyme assays. Acyl-CoA : 1-acylglycero-3-phosphorylcholine-O-acyltransferase, EC 2.3.1.23.* The activity of the acyltransferase was determined by reaction of arachidonoyl-CoA or oleoyl-CoA with labelled lysolecithin (1-[1′-^{14}C]palmitoyl-glycero-3-phosphorylcholine) as described earlier [1]. The standard assay was performed with arachidonoyl-CoA as donor substrate.

From the percentage conversion of lysolecithin to phosphatidylcholine, the enzyme activities were calculated in terms of nmol · mg^{-1} · min^{-1}.

Lysophospholipase (1-acylglycero-3-phosphorylcholine acylhydrolase, EC 3.1.1.5 The lysophospholipase activity was followed using the same conditions as described for the determination of the acyltransferase, but without addition of acyl-CoA. The calculation of enzymic activities was based on the percentage of cleaved labelled fatty acid.

Acyl-CoA hydrolase: The acyl-CoA hydrolase activity was determined using arachidonoyl-CoA as substrate and assayed measuring liberated CoASH with Nbs_2 using the photometric assay according to Lands and Hart [6]. The reaction mixture contained, in a total volume of 1.0 ml, 0.1 M phosphate buffer, pH 7.4, 0.5 μmol Nbs_2, 50 nmol arachidonoyl-CoA and 120 μg membrane protein.

Alkaline phosphatase (EC 3.1.3.1): The activity of the alkaline phosphatase was followed with *p*-nitrophenylphosphate as substrate. The reaction mixture contained in a total volume of 1.0 ml, 100 μmol diethanolamine, pH 9.5, 1.0 μmol $MgCl_2$, 5 mg Triton X-100, 2.5 μmol *p*-nitrophenylphosphate and 3–16 μg membrane protein. After 10 min incubation at 37 °C the reaction was stopped by addition of 0.2 ml 5 M NaOH. Liberated nitrophenol in supernatants was measured at 405 nm and calculated using a molar extinction coefficient of 17 840 l · mol^{-1} · cm^{-1}.

(9) *Chemical determinations.* Cholesterol was determined in membrane lipid extracts, prepared as described previously [2], after saponification with ethanolic KOH according to Abell et al. [10] with $FeCl_3$ according to Zlatkis et al. [11]. The phospholipid content was measured by phosphorus determination according to the method of Lowry et al. [12]. Protein was determined by the ninhydrin method [13] with bovine serum albumin as standard.

RESULTS

Activation of the acyl-CoA : lysolecithin acyltransferase

The acyl-CoA : lysolecithin acyltransferase is activated in thymocytes by concanavalin A in a similar way as in lymphocytes by phytohemagglutinin [1]. However, the degree of activation is more pronounced in the thymocyte concanavalin A system. Table II demonstrates that membranes from cells stimulated at 37 °C exhibited a generally 3–4 times higher acyltransferase activity as compared to controls. Incubation

TABLE II

ACYL-CoA : 1-ACYLGLYCERO-3-PHOSPHORYLCHOLINE-*O*-ACYLTRANSFERASE IN MICROSOMES OF NORMAL AND CONCANAVALIN A-STIMULATED CALF THYMOCYTES

Values are given as means ±S.D. from three independent experiments with duplicate determinations of the enzyme activities. Enzyme activities are expressed as nmol/mg protein per min and as percentage of controls.

	Temperatures during incubation of cells			
	0 °C	%	37 °C	%
Control	15.7±1.6	100	9.47±0.58	100
Concanavalin A (20 μg/ml)	33.7±1.5	215	34.55±3.5	365

at 0 °C with concanavalin A also causes a significant degree of activation, which in turn indicates that metabolites or effectors are not responsible for the enzyme activation, thus being a direct process requiring no metabolic energy. In addition, the reproducibility of the determinations of enzyme activities was much higher than in the lymphnode-phytohemagglutinin system.

Table III shows that the activation of the enzyme is dependent on the specific interaction of sugar moieties of the cell surface, as demonstrated by the ability of α-methyl-D-mannoside to interrupt the process of activation. In these experiments α-methyl-D-mannoside was administered at different points of time (before and after the onset of the concanavalin A stimulation). α-Methyl-D-mannoside blocks completely the process of activation if added prior to the 60 min incubation with concanavalin A, and terminates the activation at distinct levels if added later. However, α-methyl-D-mannoside did not reverse the raised activities to control levels. It appears from these experiments, that the interruption of binding of concanavalin A by α-methyl-D-mannoside also immediately terminates the process of enzyme activation.

TABLE III

TERMINATION OF CONCANAVALIN A ACTIVATION OF ACYL-CoA : 1-ACYLGLYCERO-3-PHOSPHORYLCHOLINE-*O*-ACYLTRANSFERASE BY α-METHYL-D-MANNOSIDE

Concentration of concanavalin A 20 μg/ml; concentration of α-methyl-D-mannoside, 4 mg/ml. Controls were also incubated 60 min at 37 °C. Enzyme activities are expressed as nmol/mg protein per min and as percentage of controls.

Incubation period with concanavalin A (min)	Incubation period with α-methyl-D-mannoside (min)	Acyl-CoA : lysolecithin acyltransferase	%
None (control)	None (control)	9.47	100
0–60	(−10)–60*	12.74	135
0–60	0–60	12.97	136
0–15	15–60	17.29	182
0–30	30–60	20.23	214
0–45	45–60	31.26	330
0–60	60–90	35.15	371
0–60	None	37.48	396

* In this experiment α-methyl-D-mannoside was added 10 min before the addition of concanavalin A.

Dependence of lysolecithin acyltransferase activation on concanavalin A concentration. In order to analyze the effect of increasing concanavalin A concentrations on the enzyme activation process, we incubated calf thymocytes at 37 °C for 60 min with 0.8–10 μg concanavalin A per ml. This response describes a sigmoidal relationship between concanavalin A concentration and enzyme activation (Fig. 1A). Correspondingly, the reciprocal plot is parabolic (Fig. 1B) which is proved by the linear relationship between the reciprocal of the square of the concanavalin A concentration and the reciprocal of the enzyme activation (Fig. 1C). The degree of cooperativity (interaction coefficient) was calculated from the Hill plot (Fig. 2). The slope of $N = 1.8$ expresses a relatively high cooperativity of concanavalin A with the enzyme.

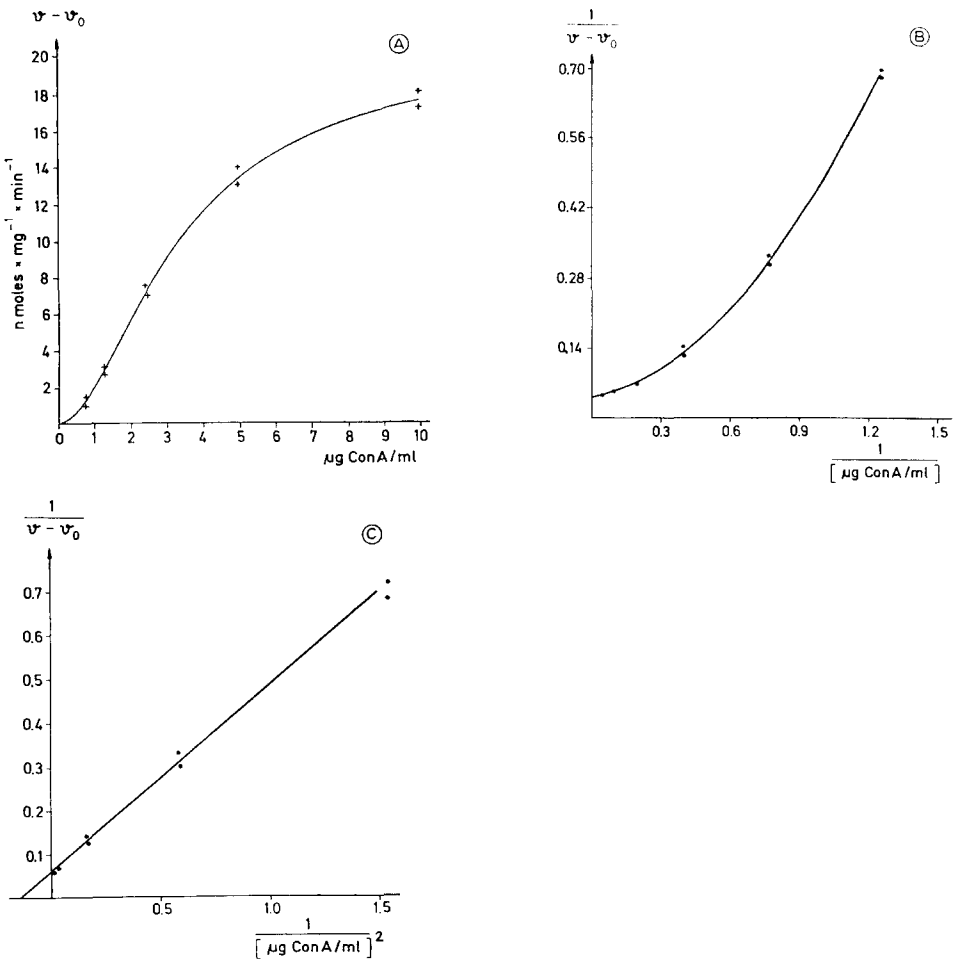

Fig. 1. Dependence of the concanavalin A concentration on the activation of the acyl-CoA : 1-acylglycero-3-phosphorylcholine-*O*-acyltransferase. (A) Specific activities with (v) and without (v_0) added concanavalin A (con A). (B) A plot of the reciprocal of the velocity against the reciprocal of the concanavalin A concentration. (C) A plot of the reciprocal of the velocity against the reciprocal of the square of the concanavalin A concentration.

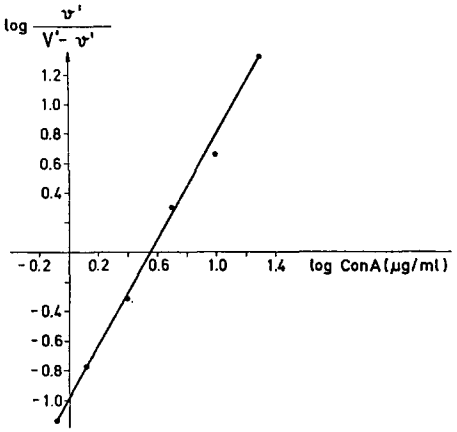

Fig. 2. Dependence of the activation of the acyl-CoA : 1-acylglycero-3-phosphorylcholine-*O*-acyltransferase on the concanavalin A (con A) concentration. Hill plot. Mean values from Fig. 1 were plotted using the Hill equation [28, 29]: $\log v/V-v = N \cdot \log [S] - N \cdot \log K$ with $v' = v - v_0$; $V' = 21$. The slope N (Hill coefficient) = 1.8.

Binding of ^{125}I-labelled concanavalin A to intact calf thymocytes and to thymocyte membranes

In order to compare binding of cancanavalin A to the cell surface with the enzyme activation process, we incubated calf thymus cells with ^{125}I-labelled concanavalin A at conditions analogous to the procedure used for activating the acyltransferase. In contrast to the enzyme activation kinetics, the binding of concanavalin A demonstrates an usual hyperbolic saturation function (Fig. 3A). The Sips plot of these data (Fig. 3C), which is mathematically analogous to the Hill plot, has a slope of $a = 1.00$ thus indicating the lack of cooperativity or heterogeneity of the binding sites. In addition to these results, previous experiments showed that the time required for optimal binding of concanavalin A at 37 °C is about 45 min. Table IV shows that the binding parameters obtained with thymocyte membranes compare well with those from intact cells, since a normal hyperbolic saturation function (Sips coefficient = 1.096), and a nearly identical association constant was also observed with isolated membranes.

TABLE IV

BINDING OF CONCANAVALIN A TO INTACT CALF THYMOCYTES AND TO THYMOCYTE MEMBRANES AT 37 °C

	Intact cells	Microsomal membranes
Maximal number of binding sites*	$7.95 \cdot 10^5$ molecules/cell	$6.1 \cdot 10^{14}$ molecules/mg protein
Association constant*	$31.6 \cdot 10^6$ l/mol	$37.2 \cdot 10^6$ l/mol
Sips coefficient *a*	1.000	1.096

* Using a molecular weight of 106 000.

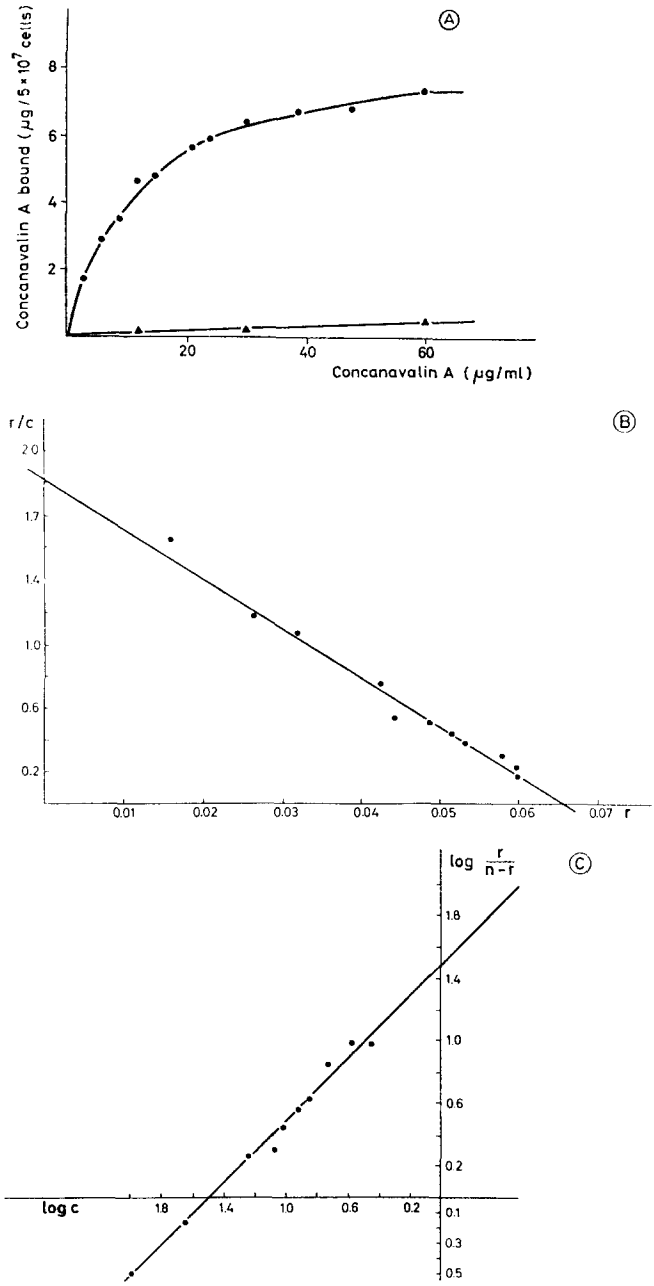

Fig. 3. Binding of concanavalin A to calf thymocytes. (A) ● -- ●, total binding; ▲ · · ▲, unspecific binding in the presence of 0.1 M α-methyl-D-mannoside. (B) Scatchard [30] plot and (C) Sips [31, 32] plot of specific binding. Sips equation: $\log r/(n-r) = a \log K_{ass} + a \log c$. In the Scatchard and Sips plot r (bound concanavalin A) and c (free concanavalin A) are expressed as nmol/ml of samples containing $5 \cdot 10^7$ cells/ml using a molecular weight of 106 000. From these plots the following parameters were calculated: maximal number of binding sites $n = 0.066$ nmol/$5 \cdot 10^7$ cells corresponding to $7.95 \cdot 10^5$ molecules/cell; association constant $K_{ass} = 31.6 \cdot 10^6$ l/mol; Sips coefficient $a = 1.000$.

Substrate kinetics of the acyl-CoA : lysolecithin acyltransferase

We carried out detailed studies in order to determine whether the enzyme activation is due to changes in the affinity of substrates or maximal velocities. These substrate kinetics were followed using microsomal membranes from normal and stimulated thymocytes. The kinetics for all substrates revealed common Michaelis-Menten saturation functions. The characteristic parameters (V and K_m) are summarized in Table V. It is noteworthy that the enzyme activation results exclusively in an increase of the maximal velocity (V). The substrate specificity is underlined by the fact that long chain polyenoic fatty acids (arachidonoyl-CoA) exhibited a 15 times higher affinity to the enzyme with a correspondingly higher maximal velocity (V), as compared to oleoyl-CoA. In addition, the ratio of the maximal velocities of normal and stimulated cells using arachidonoyl-CoA is twice as high as in experiments with oleoyl-CoA.

TABLE V

MAXIMAL VELOCITIES AND MICHAELIS CONSTANTS OF ACYL-CoA : 1-ACYL-GLYCERO-3-PHOSPHORYLCHOLINE-*O*-ACYLTRANSFERASE IN MICROSOMES FROM NORMAL AND CONCANAVALIN A-STIMULATED CALF THYMOCYTES

Enzyme activities are expressed as nmol/mg protein per min with (a) 1-acylglycero-3-phosphorylcholine as acceptor substrate and with (b) arachidonoyl-CoA as donor substrate.

	Oleoyl-CoA (a)		Arachidonoyl-CoA (a)		1-Acylglycero-3-phosphorylcholine (b)	
	V	K_m (M)	V	K_m (M)	V	K_m(M)
Control	3.9	$1.0 \cdot 10^{-5}$	9.5	$6.4 \cdot 10^{-7}$	9.8	$8.0 \cdot 10^{-6}$
Concanavalin A	7.9	$1.2 \cdot 10^{-5}$	34.6	$8.5 \cdot 10^{-7}$	36.8	$8.2 \cdot 10^{-6}$

Stimulation of thymocytes by concanavalin A bound to Sepharose

In a previous study we reported that the acyl-CoA : lysolecithin acyltransferase in lymphocytes predominantly is located in the plasma membrane. Consequently, the acyltransferase should also be activated by insoluble matrix-bound concanavalin A. In experiments using concanavalin A bound to Sepharose we found similar levels of enzyme activation.

Effects of colchicine

In order to determine whether actin-like proteins are involved in the acyltransferase activation, we studied the effect of colchicine on the process of activation. The conditions were identical to those used by Edelman et al. [14], however, colchicine had no effect on the acyltransferase, neither as activator nor as inhibitor.

DISCUSSION

Concanavalin A-stimulated lymphocytes represent an adequate model of antigen-stimulated cells. In addition, since concanavalin A binds preferentially to the outer cell surface, it allows the investigation of mitogen-induced biochemical

processes occurring within the plasma membrane. Our previous studies [1–3, 7, 8] focussed on changes of lipid-metabolizing enzymes and their possible role during the stimulation of lymphocytes.

The present study concentrates on the substrate kinetics and on the kinetics of activation of the acyl-CoA : lysolecithin acyltransferase by concanavalin A. This process of activation appears to be independent of metabolic effectors and protein synthesis, since the activation occurs equally at 0 and 37 °C. The assumption that the enzyme activation is a primary event, is further supported by time course studies. The period of 45 min was optimal for both binding of concanavalin A and enzyme activation.

While the binding of concanavalin A to intact cells expressed a normal hyperbolic saturation function, the activation process of the acyltransferase describes a sigmoidal function against increasing concentrations of concanavalin A. This function has a Hill coefficient of $N = 1.8$, thus indicating a relatively high degree of cooperativity.

Due to the complexity of the system studied our results do not allow final conclusions concerning the nature of the cooperating assembly, however, the following modes of interaction may be discussed. It is well known that optimal lymphocyte stimulation occurs when only 5 % [15–17] to 25 % [18] of the total binding sites are occupied by stimulating lectins. A similar behaviour we observed for the activation of the acyltransferase since under the conditions we used saturation of all binding sites was obtained when 60–70 μg concanavalin A/ml were added whereas optimal enzyme activation was obtained with concentrations between 10 and 15 μg/ml. Therefore, it is likely that only a small fraction of the total number of binding sites is in close topological relationship with the acyltransferase and thus their cooperative interaction cannot be detected in binding assays.

Furthermore, the existence of a desensitization phenomena, loss of regulatory activity but continued catalytic activity, in isolated membranes thus can be explained by a loss of interaction between discrete receptors and the enzyme due to the fragmentation of the membrane. It is improbable that the activation is caused by metabolic events occurring exclusively in the intact cell (second messengers) since the enzyme activation occurred equally well at 0 °C.

We were able to exclude the possibility that lipid-metabolizing enzymes play a part in the expression of sigmoidicity of the acyltransferase activation. The activities

TABLE VI

ACYL-CoA : 1-ACYLGLYCERO-3-PHOSPHORYLCHOLINE-*O*-ACYLTRANSFERASE LYSOPHOSPHOLIPASE AND ACYL-CoA HYDROLASE IN MEMBRANES OF NORMAL AND CONCANAVALIN A-STIMULATED CALF THYMOCYTES

Enzyme activities are expressed as nmol/mg protein per min.

	Control	Concanavalin A (20 μg/ml)
Acyltransferase	9.47	34.55
Lysophospholipase	0.42	0.48
Acyl-CoA hydrolase	1.12	1.20

of the other lysolecithin- and acyl-CoA-converting enzymes (lysophospholipase and acyl-CoA hydrolase) are so low and unchanged in stimulated cells (Table VI), that artifactual sigmoidal kinetics due to the complex participation of these reactions could be excluded.

The time course of the activation of the acyltransferase paralleled the time course of binding of concanavalin A to thymocytes, both reaching their maximum after 30–45 min at 37 °C. This suggests that the activation of the acyltransferase occurs immediately upon the binding of concanavalin A to appropriate receptors. However, α-methyl-D-mannoside did not reverse previously activated acyltransferase to control levels, although we have evidence* that concanavalin A is almost completely removed from the cell surface. Similar irreversibly activated functions of the outer cell membrane were reported for amino acid transport in stimulated lymphocytes [19] and were discussed in terms of a membrane memory phenomena [20].

The model presented by Edelman et al. [14] and Cunningham et al. [21] that lymphocyte surface receptors are connected to a colchicine-binding protein system which in turn regulates not only the mobility of the receptors but also lymphocyte stimulation, does not, however, appear to be involved in the concanavalin A-receptor-enzyme system we studied, since colchicine had no effect on the acyltransferase activation process. This demonstrates that processes of completely different nature can simultaneously occur within the membrane.

It is now becoming clear that membrane-bound enzymes such as $(Na^+ + K^+)$-ATPase, Ca^{2+}-ATPase or acetylcholine esterase show cooperativity towards various allosteric effectors [22–26]. It is especially interesting that the degree of cooperativity of these enzymes (as expressed by the Hill coefficient) is a function of the ratio of unsaturated/saturated fatty acids of membrane phospholipids. Farias et al. [27] discuss the influence of lipid fluidity on these cooperative membrane-bound enzymes as an important form of regulation of the metabolism. Since in lymphocytes, changes of phospholipid fatty acids are mainly due to the acyl-CoA : lysolecithin acyltransferase, it is tempting to speculate that in these cells many key membrane-bound enzymes are in turn regulated by the activity of the acyltransferase.

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* $1 \cdot 10^8$ calf thymocytes were incubated with 5.8–58 μg ^{125}I-labelled concanavalin A in 2.0 ml phosphate-buffered saline 30 min at 37 °C and then treated with 0.1 M α-methyl-D-mannoside for another 30 min period at 37 °C. Under these conditions 85 % (for the lowest concanavalin A concentration) up to 95 % (for the highest concanavalin A concentration) of cell-bound concanavalin A could be liberated with α-methyl-D-mannoside.

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