Biochimica et Biophysica Acta, 1028 (1990) 1–8
Elsevier

BBAMEM 74982

Alterations in red blood cell sugar transport by nanomolar concentrations of alkyl lysophospholipid

Donald L. Melchior [1], Anthony Carruthers [1], Alexandros Makriyannis [2], Richard I. Duclos, Jr. [2] and O.H. Abdel-Mageed [2]

[1] *Department of Biochemistry, University of Massachusetts Medical School, Worcester, MA and* [2] *Department of Medicinal Chemistry, University of Connecticut, Storrs, CT (U.S.A.)*

(Received 28 March 1990)

Key words: Lysolipid; Sugar transport; Alkyl lysolipid; Red blood cell; Lipid–protein interaction; Antineoplastic immunomodulatory agent

Acyl lysolipids presented in vitro to red blood cells in amounts comparable to blood serum levels inhibit protein-mediated glucose transport (Naderi, A., Carruthers, A. and Melchior, D.L. (1989) Biochim. Biophys. Acta 985, 173–181). In this study, an alkyl lysolipid (2-*O*-methyl-1-*O*-octadecyl-*sn*-glycero-3-phosphocholine; ALP), was found to be an order of magnitude more effective in inhibiting sugar transport than the most potent acyl lysolipid. Bilayer concentrations of ALP as low as 5 ALP molecules per transporter (0.1 mol% of total membrane lipid) result in a 50% inhibition of transport activity. ALP acts as a competitive inhibitor of exchange L-glucose transport, of CCB binding to the glucose transporter and of D-glucose inhibition of CCB binding to the transporter. Inhibition of zero-trans sugar uptake by ALP is noncompetitive. The two enantiomers of ALP show a different ability to inhibit sugar transport. The action of ALP is consistent with a mechanism in which ALP interacts with a transmembrane portion of the sugar transport molecule resulting in a competitive displacement of D-glucose or cytochalasin B from the cytosolic facing side of the transport molecule. The simplest explanation of our findings is a direct interaction of the ALP molecule with the transport protein.

Introduction

Lysolipids have been identified in small but significant amounts in all biological membranes studied [1]. Although most membrane lysolipids are considered to serve as metabolic intermediates [2], specific lysolipid species participate in other membrane processes. For example, lysolipids are involved in cellular signalling [3,4] and have been shown to alter specific membrane functions in normal and pathological situations [5–10].

As part of an extended study on how membrane lipid composition governs a membrane transport process (for review, see Ref. 11), it was found that entry into the human erythrocyte membrane of small amounts of specific exogenous acyl lysolipids (1-acyl-n-lyso-*sn*-glycero-3-phospholipids) *, can significantly alter the activity of the glucose transport protein [12]. Since acyl lysolipids are substrates in membrane lipid metabolism, after their entry into the membrane, they can be altered by normal membrane metabolism. It was of interest, therefore, to investigate the action of a non-metabolizable lysolipid analogue. An alkyl lysophospholipid was chosen for this purpose [13]. Alkyl lysolipids are unable to enter the deacylation-acylation cycle of cellular phospholipids [14]. Specifically, we have chosen to work with *rac*-2-*O*-methyl-1-*O*-octadecylglycero-3-phosphocholine (*rac*-ALP, *rac*-ET-18-OCH_3) [15] as well as with both the individual (*R*)-ALP and (*S*)-ALP enantiomers (Fig. 1). ALP was also of interest due to its antimicrobial [15], antineoplastic [16–18] and immunomodulatory [19,20] activities which have been reviewed by Andreesen [21] and Baumann [22]. An additional advantage of this lipid is that we were able to synthesize both the (*R*)-ALP and the (*S*)-ALP as optically active enanti-

Abbreviations: *rac*-ALP, *rac*-2-*O*-methyl-1-*O*-octadecylglycero-3-phosphocholine; (*R*)-ALP, (*R*)-2-*O*-methyl-1-*O*-octadecyl-*sn*-glycero-3-phosphocholine; (*S*)-ALP, (*S*)-2-*O*-methyl-3-*O*-octadecyl-*sn*-glycero-1-phosphocholine; CCB, cytochalasin B; CCD, cytochalasin D; 3OMG, 3-*O*-methylglucose.

Correspondence: D.L. Melchior, Department of Biochemistry, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, U.S.A.

* The Nomenclature of Lipids (1978) Chem. Phys. Lipids 21, 159–173.

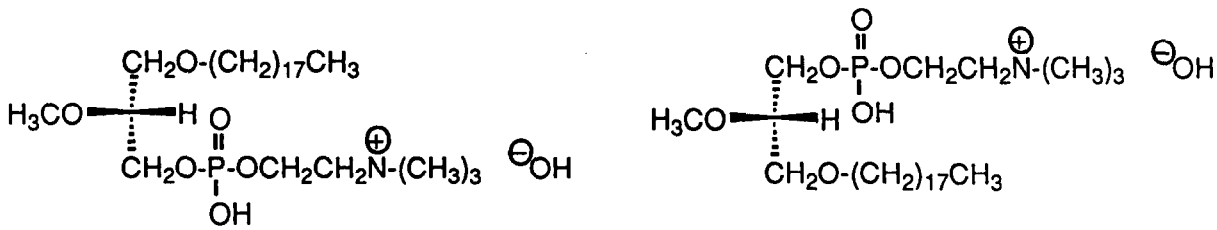

Fig. 1. Structures of (*R*)-ALP and (*S*)-ALP.

omers to investigate its effect on the activity of the sugar transport protein.

In this study, we find that ALP inhibits human red blood cell sugar transport at substantially lower concentrations than acyl lysophosphocholine. Bilayer concentrations of ALP as low as 5 ALP molecules per membrane transporter give 50% inhibition of transport activity. The low amounts of ether lipid required for inhibition, together with a differential potency between the two enantiomers suggest a direct interaction of the lipid with the transport protein. Competition experiments with cytochalasin B suggest that ALP interferes with sugar binding to the efflux site of the glucose transporter.

Materials and Methods

Solutions

The following solutions were used: (1) saline solution, containing 150 mm NaCl, 5 mM Tris-HCl, and 2 mM EDTA (pH 7.4); (2) Tris buffer, containing 50 mM Tris-HCl (pH 7.4); (3) lysis buffer, containing 10 mM Tris-HCl, 2 mM EDTA (pH 7.4); (4) efflux stopping buffer, containing saline solution, 50 μM CCB and 0.5 mM $HgCl_2$ brought up to 200 mM in sucrose; (5) influx stopping buffer, containing saline solution and 10 μM cytochalasin B (CCB), 2 μM $HgCl_2$ and 1.5 mM KI (pH 7.4). The pH of all solutions was adjusted using 1 M Tris-base.

Erythrocytes

Erythrocytes were collected from outdated blood by centrifugation and then washed three times in saline solution [23].

ALP synthesis

(*S*)-ALP was synthesized from 1,2-*O*-isopropylidene-*sn*-glycerol (Sigma) in seven steps in an overall yield of 34%. The 1,2-*O*-isopropylidene-*sn*-glycerol was first alkylated with 1-bromooctadecane (Aldrich) as reported [24] to give 1,2-*O*-isopropylidene-3-*O*-octadecyl-*sn*-glycerol. Transacetylation with benzaldehyde in the presence of catalytic *p*-toluenesulfonic acid gave the reported [25] benzylidene epimers of 1,2-*O*-benzylidene-3-*O*-octadecyl-*sn*-glycerol. Reduction with diisobutylaluminum hydride as described [25] or with 1 : 1 lithium aluminum hydride/aluminum chloride gave 1-*O*-benzyl-3-*O*-octadecyl-*sn*-glycerol as the major product. The 1-*O*-benzyl-3-*O*-octadecyl-*sn*-glycerol was then converted to (*S*)-ALP by the reported method [26]. The optical purity of 1-*O*-benzyl-3-*O*-octadecyl-*sn*-glycerol, and therefore of (*S*)-ALP, was determined to be 99.5 $\pm$ 0.5% by ^{1}H-NMR of the corresponding Mosher's ester, which was prepared by the reported method [27]. The (*R*)-ALP was prepared from 2,3-*O*-isopropylidene-*sn*-glycerol (Sigma) by the same synthetic sequence in 49% overall yield and was determined to be 99.0 $\pm$ 0.5% ee. The *rac*-ALP was prepared from *rac*-1,2-*O*-isopropylideneglycerol by the same synthetic sequence in 36% overall yield. Details of the synthesis will be published elsewhere.

Equilibrium exchange efflux

ALP dose response

Equilibrium exchange studies were carried out in a manner similar to that described by Naderi et al. [12]. To prepare cells for equilibrium exchange efflux studies, washed erythrocytes were loaded with glucose by suspending 10 ml of pelleted erythrocytes in 200 ml saline solution containing 200 mM unlabelled D-glucose and incubating at 37°C for 1 h. The glucose-loaded cells were then collected by centrifugation. 1ml aliquots of the pelleted cells were distributed into individual glass test tubes and each pellet brought up to 5 ml total volume with saline solution containing 200 mM D-glucose. Appropriate volumes of concentrated ethanolic solutions of ALP (volumes < 12.5 μl) were added to each tube to give ALP concentrations of 2, 20, 200, 1000 nM (CMC $\approx 10^{-5}$–10^{-6} M; Ref. 28). The maximum ethanol concentration was 0.25%. For control measurements, certain tubes had no added ALP and other tubes had no ALP and were made 0.5 mM in $HgCl_2$ and 50 μM in cytochalasin B. Control measurements were made with appropriate amounts of ethanol. Ethanol had no measurable effect on transport rates. The tubes were incubated at 37°C for 15 min to allow equilibration of the ALP with the erythrocytes. The cells were then pelleted by centrifugation, 1 ml of supernatant from each tube collected and the remaining supernatant discarded. 10 μl of D-[^{14}C]glucose (1 μCi at 265 mCi/mM) was then added to each tube, the respec-

tive supernatants added back to each tube and the mixtures gently mixed. The samples were then incubated at 37°C for 30 min, then pelleted and the supernatants removed.

Efflux experiments were carried out on ice. Efflux was initiated in each sample by the rapid addition of 10 ml of ice cold efflux buffer to 150 μl of the corresponding packed D-[^{14}C]glucose-loaded cells. Each efflux buffer was identical to those buffers employed for each sample, except that for the efflux buffers the D-glucose was unlabelled. For each time point, 1 ml samples were removed from each tube and added to tubes containing 50 μl of 100 mM $HgCl_2$. $HgCl_2$ is an inhibitor of protein-mediated sugar transport. The cells were pelleted, washed in 1 ml stopping buffer, pelleted and disrupted with 1 ml 3% perchloric acid. The extract was centrifuged and the activity in the clear supernatant counted by liquid scintillation spectrophotometry. To correct for non-transporter facilitated membrane leakage, control efflux experiments were made in the presence of $HgCl_2$. Protein-mediated equilibrium exchange sugar transport was calculated as the difference between corresponding uninhibited and inhibited ($HgCl_2$) time points and in all cases accounted for more than 90% of total transport. Each dose response study was carried out five to eight times.

Sugar concentration dependency of transport in ALP-treated cells

Aliquots of cells were loaded with D-glucose in the manner described above at sugar concentrations of 10, 25, 50, 75, 100 and 200 mM. All samples were incubated at 37°C for 15 min with 1 μM ALP, then loaded with labelled D-[^{14}C]glucose (1 μCi at 265 mCi/mM) and pelleted. Efflux experiments were carried out in a manner corresponding to that previously described and the rate constant for exit at any given sugar concentration was calculated from semi-log plots of exit versus time as in Ref. 12.

Zero-trans influx

Two 1 ml aliquots of pelleted erythrocytes were each brought up to 5 ml with saline solution. To one of these aliquots, 5 μl of a 0.4 mM ethanolic solution of ALP was added to give a final concentration of 0.4 μM ALP. The cells were incubated at 37°C for 15 min, pelleted in their respective tubes after which the supernatant was removed.

Uptake experiments were carried out on ice. Six influx buffers were made from Tris buffer and D-glucose at concentrations of 0.05, 0.1, 0.25, 0.5, 1 and 5 mM. The buffers were divided into two portions, ALP was added to one portion of each of the buffers and 3-*O*-[^{14}C]methylglucose (1 μCi/ml at 265 mCi/mM) was then added to each of the resulting 12 buffers.

For the uptake measurements, time points were taken at 0 s, 15 s and 30 s at ice temperature and 30 min at 37°C. For each condition (glucose concentration, ALP, time) 15 μl of packed erythrocytes were placed in a glass test tube. For 0 s time points, 1 ml ice cold influx buffer and 1 ml ice cold stopping buffer were simultaneously added to the cells, the cells immediately pelleted, washed in 1 ml stopping buffer, pelleted and disrupted with 1 ml 3% perchloric acid. The extract was centrifuged and the aliquots of the clear supernatant counted by liquid scintillation spectrometry. This process was repeated for the 15 s and 30 s time points with the appropriate influx buffer added at 0 time and the stopping buffer added after a 15 or 30 s interval. These samples were processed in the same manner as the 0 s time points. For the 30 min time point (infinity) the influx buffer was added at 0 time and the samples placed in a water bath at 37°C for 30 min. 1 ml stopping buffer was added at this time and the samples treated in the same manner as the other time points.

Uptake (mol/l cell water/min) was calculated as

$$v = \frac{\mathrm{cpm}_t - \mathrm{cpm}_0}{\mathrm{cpm}_\infty - \mathrm{cpm}_0} \cdot \frac{[3\mathrm{OMG}]}{t}$$

where cpm_0, cpm_t, cpm_∞ are counts associated with processed cells at time zero, time t and at equilibrium. $K_{m(app)}$ and V_{max} for 3-*O*-methylglucose uptake in control cells are estimated to be 0.28 mM and 400 μmol/l cell water per min, respectively. The corresponding parameters for uptake in ALP-treated cells are 0.29 mM and 110 μmol/l cell water per min. This corresponds to maximum rate constants ($V_{max}/K_{m(app)}$) for uptake by control and treated cells of 1.43 and 0.38 min^{-1}, respectively, or half-times for uptake at limitingly low [3OMG] of 29 and 109 s, respectively. Thus, estimates of initial rates of 3OMG uptake by ALP-treated cells at 15 and 30 s are considered to be accurate while estimates in control cells at low [3OMG] are somewhat underestimated even at 15-s intervals. Assuming monoexponential uptake of sugar, we calculate that our measurements of initial rates of uptake by control cells are underestimated by 14% at 0.05 mM 3OMG and by 1% at 5 mM 3OMG. Using these corrected values, we obtain $K_{m(app)}$ and V_{max} for corrected zero-trans, control 3OMG uptake of 0.24 mM and 401 μmol/l cell water per min, respectively. These considerations, while constrained by the arbitrary assumption of exponential sugar uptake, suggest that the use of a 15 s uptake interval in control cells results in overestimation of $K_{m(app)}$ for uptake by 17% without affecting the reliability of estimates of V_{max}. As net sugar transport by red cells displays a 10-fold asymmetry at 0°C (K_m and V_m for exit are 10-fold greater than K_m and V_{max} for entry [29]) and $K_{m(app)}$ for infinite-cis entry is reported to be some 40-fold greater than K_m for zero-trans uptake

[30], our assumption of exponential sugar uptake at both low and high [3OMG] is not unreasonable.

Cytochalasin B binding studies

Preparation of ghosts

Ghosts were prepared in the following manner. Red cells were washed three times in Tris buffer. The cells were then lysed by dispersing 1 volume of packed cells into 40 volumes of ice-cold lysis buffer. The suspension was gently stirred at 4°C for 10 min, centrifuged and the supernatant removed. The pellet was washed in lysis buffer several times to remove residual hemoglobin. The resulting ghosts were then suspended in Tris buffer, centrifuged and kept on ice as a pellet until use.

Effect of ALP on cytochalasin B binding

Six solutions were prepared using Tris buffer made 5 μM in cytochalasin D (CCD) (to inhibit binding of CCB to proteins other than the glucose transporter: Refs. 31, 32) and CCB in the following concentrations; 100 nM, 250 nM, 500 nM, 1 nM, 1 μM, 2.5 μM and 5 μM. 1 μl/ml of ^{3}H-CCB (18.5 Ci/mM) was added to each buffer. Each of the six solutions were divided into two portions, one portion of each solution was then made 5 μM in ALP.

Binding studies were carried out at room temperature. To determine CCB binding for each condition, 100 μl of ghosts (4 mg membrane protein/ml) were placed in a centrifuge tube, 100 μl of the appropriate solution added and the tube gently vortexed. The tube was allowed to sit for 1 min, after which 10-μl samples of the suspension were taken. The tubes were centrifuged to pellet the ghosts and 10-μl samples of the supernatant taken. Samples were counted by liquid scintillation spectrophotometry. Bound CCB was taken as the counts in the suspension minus the counts in the supernatant.

Effect of ALP on D-glucose inhibition of cytochalasin B binding

Tris buffer was made 100 μM in CCB, 5 μM in CCD with 1 μl/ml ^{3}H-CCB (18.5 Ci/mM). Six solutions were made from this buffer with D-glucose/mannitol added to give concentrations of (mM/mM); 0:100, 5:95, 10:90, 25:75, 50:50, 100:0. A portion of each of these buffers was made 5 μM in ALP. Binding measurements were made as described above.

Conversion values

1 μl of packed cells is equivalent to 6 μg membrane protein. For calculation purposes, a single human red blood cell membrane is considered to contain $7.2 \cdot 10^8$ lipid molecules, 0.6 pg membrane protein [33,34] and $1.5 \cdot 10^5$ sugar transport molecules [32].

Results

The ability of (*S*)-ALP, (*R*)-ALP, and *rac*-ALP to inhibit protein mediated D-glucose equilibrium exchange efflux is presented in Fig. 2. Concentrations of ALP as low as 2 nM gave 30% inhibition of transport. Inhibition increased with increasing ALP concentrations, inhibition being as high as 85% with 1 μM (*S*)-ALP. Above ALP concentrations of 5–10 μM, cell lysis was found to occur. It seems unlikely that 1 μM ALP caused significant disruption of the red cell bilayer for two reasons: (1) Cytochalasin B and $HgCl_2$-sensitive transport (transbilayer simple diffusion) was unaffected by 1 μM ALP; (2) we were unable to detect a significant release of Hb from those cells exposed to 1 μM ALP. The (*S*)-enantiomer was more potent in inhibiting D-glucose transport than the (*R*)-enantiomer. Interestingly, the racemic mixture was found less effective in inhibiting D-glucose exchange than either of its two component enantiomers. This was also the case for a 1:1 mixture of the (*S*)-ALP/(*R*)-ALP.

The concentration dependence of equilibrium-exchange D-glucose exit in the absence and presence of 1 μM *rac*-ALP is shown as a Hanes-Woolf plot (Fig. 3). In the absence of ALP, V_{max} for equilibrium exchange is 25 mmol/l per min and in the presence of ALP is 19.5

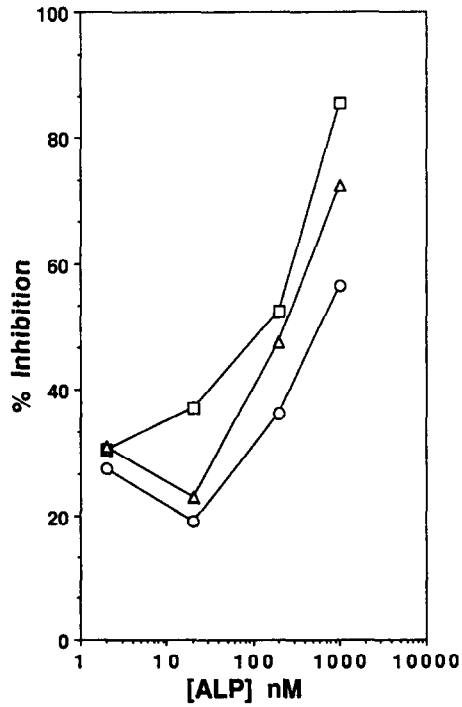

Fig. 2. Percentage inhibition of D-glucose equilibrium exchange efflux in human erythrocytes exposed to varying concentrations of different enantiomeric combinations of ALP: □, (*S*)-ALP; △, (*R*)-ALP; ○, *rac*-ALP. Abscissa: ALP concentration in nM. Ordinate: Percentage inhibition of equilibrium exchange efflux (200 mM D-glucose) relative to that of untreated red cell blood cells. Number of determinations per point, four or more; variation in data points, ±5%.

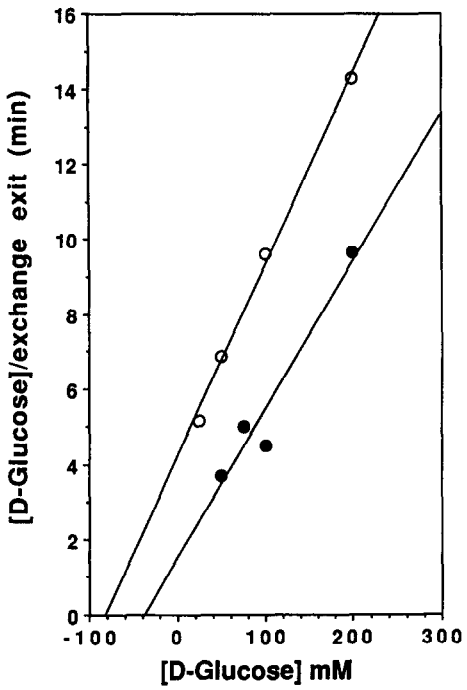

Fig. 3. Hanes-Woolf plot of the rate of equilibrium exchange D-glucose efflux from human erythrocytes in the absence (●) and presence (○) of *rac*-ALP (1 μM). Abscissa: D-glucose concentration in mM. Ordinate: D-glucose concentration (mM) divided by rate of efflux exit (mM/min), units in min. Number of determinations per point, four or more; variation in data points, ±5%. The straight lines drawn through the points were calculated by the method of least squares.

mmol/l per min. In the absence of ALP, $K_{m(app)}$ for exchange exit is 38.9 mM and in the presence of ALP is 81 mM.

ALP was found to reduce the affinity of the transporter for CCB. This is seen in Fig. 4 where a Scatchard plot is presented of CCD insensitive cytochalasin B binding to red cell membranes in the presence and absence of 5 μM *rac*-ALP. The K_d for CCB binding to the membranes is increased from a control value of 208 nM in the absence of ALP to a value of 389 nM in the presence of ALP.

The ability of D-glucose to inhibit CCD insensitive CCB binding to red cell membranes was found to be reduced in the presence of 5 μM ALP (Fig. 5). The presence of 5 μM *rac*-ALP increases $K_{i(app)}$ for D-glucose competitive inhibition of CCB binding to the glucose transporter from a control value of 35 mM to 108 mM. ALP (0.4 μM) reduces V_{max} for zero-trans 3OMG uptake at 4°C from 400 μmol/l cell water per min to 110 μmol/l cell water per min (Fig. 6). $K_{m(app)}$ for zero-trans 3OMG uptake (0.28 mM) was not significantly affected by ALP.

Discussion

In an earlier study by this laboratory on the modulation of red blood cell sugar transport by acyl lysolipid [12], several mechanisms were proposed that could account for the inhibition of glucose transport by lysolipid. These included: (1) nonspecific, detergent-like or membrane bilayer perturbing or distorting (e.g., red cell shape changes, 'invaginations' or 'crenations') action of lysolipid on red-cell membranes; (2) alteration in the packing of the lipids surrounding the transporter; and (3) the direct interaction of lysolipids (or a metabolite of lysolipid) with the glucose transport protein. The third explanation was considered the most likely.

2-*O*-Methyl-1-*O*-octadecyl-*sn*-glycero-3-phosphocholine (ALP) was found to be an order of magnitude more effective in inhibiting sugar transport than the most potent of the acyl lysolipids investigated ((*R*)-1-hexadecanol-*sn*-glycero-3-phosphatidylcholine) [12]. Like acyl lysolipids [33,12], ALP partitions from aqueous suspension into native membrane bilayers and artificial bilayers. NMR and X-ray diffraction studies demonstrate that it rapidly intercalates in the bilayer with its headgroup in the bilayer headgroup region and its alkyl chain parallel with the bilayer lipid chains (unpublished results, Makryannis, A., Xiu, X. and Yang, D.P.). The presence in the membrane bilayer of as little as 5 ALP molecules per transport molecule (0.1 mol% of membrane lipid) results in 50% inhibition of transport. Since

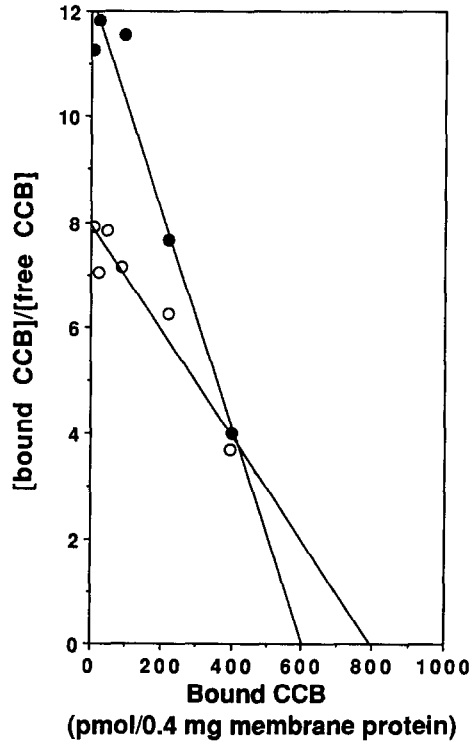

Fig. 4. Scatchard plot of cytochalasin B binding to the human erythrocyte sugar transport protein in the absence (●) and presence (○) of 5 μM (*rac*-ALP. Abscissa: bound cytochalasin B (pmols per 0.4 mg ghost membrane protein). Ordinate: [bound cytochalasin B] divided by [free cytochalasin B]. Number of determinants per point, four or more; variation in data points, ±5%. The straight lines drawn through the points were calculated by the method of least squares.

much of the ALP that enters the membrane bilayer might be expected to partition into regions of the membrane lacking the sugar transporter or else associate with membrane proteins other than the transporter, a 1 : 1 functional stoichiometry of ALP with the glucose transport molecule is not unreasonable.

The investigation of the action on sugar transport of an alkyl lysolipid in place of an acyl lysolipid eliminates the possibility that a metabolic derivative of the lysolipid is responsible for transport inhibition. Accordingly, the greater potency of alkyl lysolipid over acyl lysolipid may reflect a reduction of the membrane pool of endogenously added acyl lysolipid resulting from their metabolic conversion to other products. Another possibility for the greater potency of ALP over acyl lysolipids may lie in the major structural difference between the two lysolipid types. While the acyl lysolipids have a 2-OH, the ALP has a 2-OCH_3. This structural difference could result in differences in lipid–protein interactions or differential partitioning of lysolipid within regions of the membrane bilayer itself.

ALP has been reported to inhibit the activities of membrane proteins in addition to the red blood cell sugar transporter. For example *rac*-ALP has been demonstrated to inhibit membrane bound acyltransferase [34,35] as well as protein kinase activity [36]. Selective membrane effects have also been suggested by other investigators [39–45].

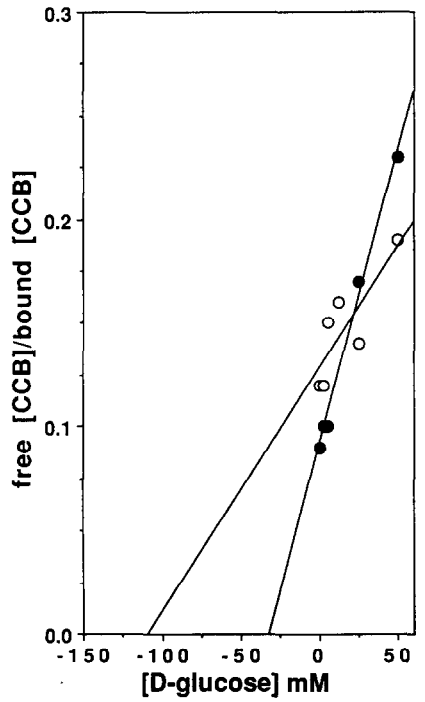

Fig. 5. Ability of D-glucose to inhibit cytochalasin B binding to the human erythrocyte sugar transport molecule in the absence (●) and presence (○) of 5 μM (*rac*-ALP. Abscissa: D-glucose concentration in mM. Ordinate: free [cytochalasin B] divided by [bound cytochalasin B]. Number of determinants per point, four or more; variation in data points, ±5%. The straight lines drawn through the points were calculated by the method of least squared.

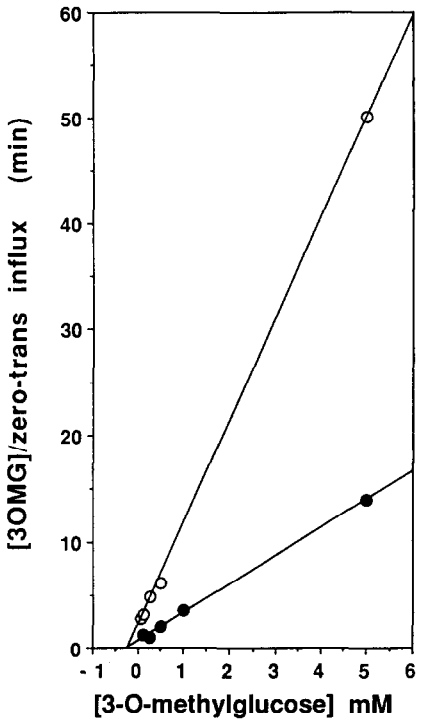

Fig. 6. Zero-trans influx of 3-*O*-methylglucose by red cells in the absence (●) and presence (○) of 0.4 μM *rac*-ALP. Abscissa: 3-*O*-methylglucose concentration in mM. Ordinate: 3-*O*-methylglucose concentration (mM) divided by zero-trans influx (mM/min), units in min. Number of determinations per point, four or more; variation in data points, ±5%. The straight lines drawn through the points were calculated by the method of least squares.

The two enantiomers, (*R*)-ALP and (*S*)-ALP, showed differential efficacy in inhibition of sugar transport, the (*S*)-enantiomer being more potent than the (*R*)-enantiomer. Surprisingly, the natural racemic mixture was less potent than either of the two enantiomers alone. When the two enantiomers were mixed in a 1 : 1 ratio, red cells treated with this mixture and sugar transport measured, the behavior of the natural racemic mixture was reproduced.

The relative potency of the (*R*)- and (*S*)-enantiomers of ALP on cellular functions appear to vary from system to system. In the in vitro activation of guinea pig peritoneal macrophages (*R*)-ALP had greater activity than (*S*)-ALP while the racemic mixture had intermediate activity [43]. The same was true for suppression of intraperitoneally transplanted sarcoma 180 tumors in mice when ALP was administered intravenously [39]. However, against intraperitoneally transplanted sarcoma 180 tumors in mice when administered intravenously, the (*R*)-ALP had comparable activity to the (*S*)-ALP. Comparable activity for the two enantiomers was also found against human promyelocytic leukemia HL-60 in vitro [39]. (*S*)-ALP was more effective than (*R*)-ALP [39] against mouse mammary tumor MM 46 propagated

intraperitoneally. Peritoneal exudate cells harvested from mice treated with (*R*)-ALP as opposed to (*S*)-ALP showed greater cytostasis against mouse leukemic EL-4 cells in vitro, although the racemic mixture was more effective than either of the optically active enantiomeric forms [39].

Our findings on the inhibition of human erythrocyte sugar transport by ALP are consistent with a mechanism in which an ALP molecule(s) present in the membrane bilayer interacts with a transmembrane portion of the sugar transporter in such a way as to cause a competitive displacement of D-glucose or cytochalasin B from the cytosolic facing side of the transport molecule. This hypothesis is suggested by the following observations: (1) Competitive inhibition of equilibrium exchange sugar transport (Fig. 3); (2) Non-competitive inhibition of sugar uptake by sugar-free cells (Fig. 6); (3) Competitive inhibition of cytochalasin B binding to the glucose transporter (Fig. 4); (4) increased $K_{i(app)}$ for D-glucose inhibition of cytochalasin B binding to the transport molecule (Fig. 5). It should be noted that red cell membranes are highly permeable to CCB which interacts with the sugar transporter at the cytosolic side of the membrane [46]. It is interesting to note that cellular uptake of *rac*-ALP was demonstrated to be inhibited in a variety of neoplastic cells by cytochalasin B [47].

A lyso-PC (monopalmitoyl lecithin, MPL) has previously been shown to inhibit equilibrium exchange D-glucose transport in human red cells [12]. Unlike ALP (a competitive inhibitor of exchange transport), MPL reduces both V_m and $K_{m(app)}$ for exchange transport and reduces $K_{i(app)}$ for D-glucose inhibition of cytochalasin B binding to the glucose carrier. The reasons for this quite disparate behaviour of two closely related compounds are unclear at this time. It is interesting, that in studies on glucose-6-phosphatase reconstituted into phosphatidylcholine bilayers, Chauhan and colleagues [8] found that while lysophosphatidylcholine acted as an inhibitor of enzymatic activity, 1-ether-deoxy lysophosphatidylcholine acted as a mild activator.

Acknowledgements

This work was funded by the National Science Foundation, Grant DMB-8416219 (D.L.M.), the National Institutes of Health, Grant DK 36081 (A.C.), the National Institute on Drug Abuse, Grant DA 3801 and Glaxo (A.M.). We gratefully acknowledge this support. The authors wish to thank Miss Annette Dion for her assistance in the preparation of this manuscript.

References

1 Ansell, G.B., Dawson, R.M.C. and Hawthorne, J.N. (1973) Form and Function of Phospholipids, Elsevier, Amsterdam.
2 Van den Bosch, H. (1974) Annu. Rev. Biochem. 43, 243–271.
3 Rittenhouse-Simmons, S. and Deykin, D. (1986) in Platelets in Biology and Pathology, 2nd Edn. (Gordon, J.L., ed.), pp. 349–371, Elsevier, Amsterdam.
4 Burgoyne, R.D., Cheek, T.R. and O'Sullivan, A.J. (1987) Trends Biochem. Sci. 12, 332–333.
5 Tamura, M., Harris, T.M., Higashirmori, K., Sweetman, B.J., Blair, I.A. and Inabami, T. (1987) Biochemistry 26, 2797–2806.
6 Kelly, R.A., O'Hara, D.S., Mitch, W.E. and Smith, T.W. (1986) J. Biol. Chem. 261, 1704–1711.
7 Abita, J.-P., Parniak, M. and Kaufman, S. (1984) J. Biol. Chem. 259, 14560–14666.
8 Chauhan, V.P.S., Ransammy, L.S. and Brockerhoff, H. (1984) Biochim. Biophys. Acta 772, 239–243.
9 Corr, P.B., Snyder, D.W., Lee, B.I., Gross, R.W., Keim, C.R. and Sobel, B.E. (1982) Am. J. Physiol. 243, H187–H195.
10 Sobel, B.E., Corr, P.B., Robinson, A.K., Goldstein, Witkowski, F.K. and Klein, M.S. (1978) J. Clin. Invest. 62, 546–553.
11 Carruthers, A. and Melchior, D.L. (1988) Annu. Rev. Physiol. 50, 257–271.
12 Naderi, S., Carruthers, A. and Melchior, D.L. (1989) Biochim. Biophys. Acta 985, 173–181.
13 Mangold, H.K. and Paltauf, F. (eds.) (1983) Ether Lipids, Academic Press, New York.
14 Hill, E.E. and Lands, W.E.M. (1970) in Lipid Metabolism (Wakil, S.J., ed.), pp. 185–277, Academic Press, New York.
15 Tsushima, S., Yoshioka, Y., Tanida, S., Nomura, H., Nojima, S. and Hozumi, M. (1982) Chem. Pharm. Bull. 30, 3260–3270.
16 Berdel, W.E., Andreesen, R. and Munder, P.G. (1985) in Phospholipids and Cellular Regulation, Vol. 2 (Kuo, J.F., ed.), pp. 41–73, CRC Press, Boca Raton.
17 Berdel, W.E., Fink, V. and Rastetter, J. (1987) Lipids 22, 967–969.
18 Andreesen, R., Osterhoz, J., Luckenbach, G.A., Costabel, U., Schulz, A., Speth, V., Munder, P.G. and Lohr, G.W. (1984) J. Natl. Cancer Inst. 72, 53–59.
19 Andreesen, R. and Giese, V. (1987) Lipids 22, 836–841.
20 Yamamoto, N. and Ngwenya, B.Z. (1987) Cancer Res. 47, 2008–2013.
21 Andreesen, R. (1988) Prog. Biochem. Pharmacol. 22, 118–131.
22 Baumann, W.J. (1987) Lipids 22, 787–974.
23 Carruthers, A. and Melchior, D.L. (1983) Biochim. Biophys. Acta 728, 254–266.
24 Hirth, G. and Barner, R. (1982) Helv. Chim. Acta 65, 1059–1084.
25 Takano, S., Akiyama, M. and Ogasawara, K. (1984) Chem. Pharm. Bull. 32, 791–794.
26 Berchtold, R. (1982) Chem. Phys. Lipids 30, 389–392.
27 Dale, J.A., Dull, D.L. and Mosher, H.S. (1969) J. Org. Chem. 34, 2543–2549.
28 Weltzien, H.U. and Munder, P.G. (1983) in Ether Lipids (Mangold, H.K. and Paultauf, F., eds.), pp. 277–308, Academic Press, New York.
29 Lowe, A.G. and Walmsley, A.R. (1986) Biochim. Biophys. Acta 857, 146–154.
30 Wheeler, T.J. (1986) Biochim. Biophys. Acta 862, 387–398.
31 Jung, C.Y. and Rampal, A.L. (1977) J. Biol. Chem. 252, 5456–5463.
32 Helgerson, A.L. and Carruthers, A. (1987) J. Biol. Chem. 262, 5464–5475.
33 Carruthers, A. and Melchior, D.L. (1984) Biochemistry 23, 6901–6911.
34 Carruthers, A. and Melchior, D.L. (1983b) Biochemistry 22, 5797–5807.
35 Weltzien, H.U. (1979) Biochim. Biophys. Acta 559, 259–287.
36 Herrmann, D.B.J. and Neumann, H.A. (1986) J. Biol. Chem. 261, 7742–7747.
37 Herrmann, D.B.J., Ferber, E. and Munder, P.G. (1986) Biochim. Biophys. 876, 28–35.

38 Helfman, D.M., Barnes, K.C., Kinkade, J.M., Jr., Vogler, W.R., Shoji, M. and Kuo, J.F. (1983) Cancer Res. 43, 2955–2961.
39 Kudo, I., Nojima, S., Chang, H.W., Yanoshita, R., Hayashi, H., Kondo, E., Nomura, H. and Inoue, K. (1987) Lipids 22, 862–867.
40 Snyder, F., Reard, M., Smith, Z., Blank, M.L. anf Hoffman, D.R. (1987) Aktuel Onkol. 34, 19–26.
41 Record, M., Hoffman, D. and Snyder, F. (1986) Proc. Am. Assoc. Cancer Res. 27, 14.
42 Hoffman, D.R., Hoffman, L.H. and Snyder, F. (1986) Cancer Res. 46, 5803–5809.
43 Hayashi, H., Kudo, I., Inoue, K., Onozaki, K., Tushima, S., Nomura, H. and Nojima, S. (1985) J. Biochem. 97, 1737–1745.
44 Andreesen, R., Modolell, M., Oepke, G.F., Common, H., Lohr, G.W. and Munder, P.G. (1982) Anticancer Res. 2, 95–100.
45 Hanahan, D.J., Munder, P.G., Satouchi, K., McManus, L. and Pinckard, R.N. (1981) Biochem. Biophys. Res. Commun. 99, 183–188.
46 Basketer, D.A. and Widdas, W.F. (1978) J. Physiol. 278, 389–401.
47 Storch, J. and Munder, P.G. (1987) Lipids 22, 813–819.