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MODULATION OF THE ORGANIZATION OF ERYTHROCYTE MEMBRANE PHOSPHOLIPIDS BY CYTOPLASMIC ATP

THE SUSCEPTIBILITY OF ISOIONIC HUMAN ERYTHROCYTE GHOSTS TO ATTACK BY DETERGENTS AND PHOSPHOLIPASE C

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Summary

Human erythrocyte ghosts were prepared in media of physiological ionic composition, and these "isoionic" ghosts were then lysed and resealed in media of varying Ca^{2+}, Mg^{2+} and ATP concentrations. The susceptibilities of these ghosts to limited attack by various detergents and by phospholipases C were then compared with the susceptibilities of intact cells to similar attack: attack was assessed by measurements of lysis and of phospholipid hydrolysis. Ghosts were more readily attacked than cells by anionic detergents (cholate, glycocholate, dodecyl sulphate) and by phospholipases C, but Triton X-100 and cetyltrimethylammonium attacked cells and ghosts to the same extent. $Mg \cdot ATP^{2-}$ partially protected ghosts from attack by the anionic detergents and by the phospholipases C of *Bacillus cereus* and of *Clostridium perfringens*. Protection by $Mg \cdot ATP^{2-}$ occurred only if $Mg \cdot ATP^{2-}$ had access to the cytoplasmic surface of the membrane. Adenylyl(β-γ-methylene)diphosphonate, a non-hydrolysable ATP analogue, protected as effectively as did $Mg \cdot ATP^{2-}$. Internal $Mg \cdot ATP^{2-}$ caused a marked reduction in the hydrolysis by phospholipases of phosphatidylethanolamine and sphingomyelin, but had no appreciable effect upon the simultaneous hydrolysis of phosphatidylcholine. It therefore seems that interaction of ATP with sites on the cytoplasmic surface of the erythrocyte membrane can, without ATP hydrolysis, cause changes in the organization of the outer surface of the membrane that specifically render phosphatidylethanolamine and sphingomyelin less accessible to attack by extracellular phospholipases.

Abbreviations: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; $AppCH_2p$, adenylyl(β-γ-methylene)diphosphate; EGTA, ethyleneglycol bis(α-aminoethylether)-*N,N'*-tetraacetic acid.

Introduction

Erythrocytes which have been depleted of intracellular ATP show changes in various properties, including shape [1,2], deformability [3] and susceptibility to chemical and enzymic modification of their membranes [4—6]. However, it has been diffucult to determine from such studies with whole cells whether any of these changes are direct results of depleting the erythrocyte membrane of ATP (see for example, ref. 7). Attempts have therefore been made to extend such studies to erythrocyte ghosts, in which ATP levels can be adjusted directly during lysis. Some effects of ATP upon the shape of such ghosts have been reported [8,9].

When erythrocytes are lysed to produce erythrocyte ghosts their membranes can undergo substantial changes in shape, stability, composition and permeability [10]. These changes are most marked in ghosts prepared and maintained in media of low ionic strength, and some of the changes can be reversed by restoration of approximately physiological ionic conditions, by incubation at 37°C and by control of factors such as the internal concentrations of divalent cations and ATP. Such restoration of relatively normal physiological parameters in "resealed" ghosts is usually assessed from the extent of recovery of impermeability of the ghosts to small molecules or ions. However, it is not known to what degree this is an appropriate way of determining whether the membrane organization has been returned to a state close to that in the intact, healthy cell.

We recently described a method for obtaining haemoglobin-free erythrocyte ghosts under conditions in which an approximately physiological ionic environment is maintained throughout the isolation procedure [11]. These isoionic ghosts can be prepared in a form in which they are substantially impermeable to a variety of large and small molecules [12] and changes in their internal medium can easily be made by controlled lysis in appropriate media. We have now made comparisons of the susceptibilities of these sealed isoionic ghosts and of intact erythrocytes to limited external attack by detergents and by phospholipases. It was hoped that such techniques would provide a sensitive way of detecting any subtle perturbations to the native organization of the membrane which might have occurred during the preparation of the ghosts and which persisted even in the resealed ghosts.

In particular, we were interested in determining whether changes in the internal enviroment of the ghosts (e.g. changed ATP, Ca^{2+} or Mg^{2+} concentrations) would be able to influence the accessibility to detergents or phospholipases of membrane components exposed at the outer surface of the membrane.

Materials and Methods

Preparation of ghosts

Human blood was used within 4—5 days of donation as it has been observed that susceptibility of intact cells to phospholipase C increases after this period (Shukla, S.D., unpublished results). Pig blood was used fresh.

The isoionic media for different ghosts preparations contained 130 mM KCl/

10 mM NaCl/ 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid) pH 7.0, and, in addition, varying amounts of Ca^{2+}, Mg^{2+}, $Mg \cdot ATP^{2-}$ etc. as specified in the text. In some experiments the $Mg \cdot ATP^{2-}$ was supplemented with 0.5 mM cyclic AMP (Sigma, London) and in others it was replaced by an ATP analogue (adenylyl(β-γ-methylene)diphosphonate, $AppCH_2p$, obtained from Boehringer, Mannheim). The general procedure for the preparation was similar to the earlier report from this laboratory [11] except that whenever glycol-loaded cells were lysed they were allowed to stand for 5 min at 0° C before centrifugation. Ghosts were resealed by incubating for 1 h at 37° C [12]. Ghosts obtained after one lysis were used for the haemolysis studies. Resealed one-lysis and haemoglobin-free ghosts were used for experiments in which phospholipid hydrolysis by phospholipase C was studied.

Treatment of ghosts with detergents

The procedure followed was essentially as described by Coleman and Holdsworth [13]. The erythrocyte or resealed ghost concentrations were adjusted to approximately 2 μmol membrane phospholipid per ml. 1 vol. of this suspension was incubated at 37° C for 10 min with 3 vols. isoionic medium (130 mM KCl/10 mM NaCl/10 mM HEPES, pH 7.0) in the presence of various concentrations of detergents. Supernatants were obtained by centrifuging at 14 000 × *g* for 3—4 min and were then appropriately diluted for estimation of haemoglobin at either 418 or 525 nm. The extent of lysis was determined by comparing the absorbance of each supernatant with that of an incentrifuged control completely lysed by 0.1% Triton X-100 (v/v).

Treatment of ghosts with phospholipases C

Phospholipase C from *Clostridium perfringens* was purified on an immunosorbent column by the method of Bird et al. [14]. One unit of activity is the amount of enzyme which releases 1 μmol of water-soluble organic phosphate from soyabean lipid in 30 min at 37° C under conditions specified by Low and Finean [15]. Purified phospholipase C from *Bacillus cereus* was a gift from Dr. C. Little and had been prepared by the procedure of Little et al. [16]. Due to the difference in susceptibility of cells and ghosts to *B. cereus* and *C. perfringens* enzymes, the number of units of enzyme required for comparable effects were much greater for the *B. cereus* than from the *C. perfringens.*

Resealed haemoglobin-free ghosts were treated with enzyme by incubating them in a mixture which contained ghosts (200—300 nmol phospholipid) and enzyme in a final volume of 0.5 ml of HEPES saline solution (130 mM KCl/ 10 mM NaCl/10 mM HEPES, pH 7.0). In the case of *C. perfringens*, $CaCl_2$ was added to a final concentration of 1 mM. Incubation was at 37° C for 30 min and the reaction was stopped by the addition of 2 mM EDTA and 0.5 mM *o*-phenanthroline in 0.5 ml of HEPES saline solution, pH 7.0.

Extraction and analysis of lipids

Lipids were extracted by the method of Lapetina and Michell [17] except that the washing stage was omitted. Lipids were analysed on activated silica gel H thin layer plates in a solvent system of chloroform/methanol/acetic acid/ water (75 : 45 : 12 : 3, v/v) [18]. Organic phosphorus was determined by the method of Bartlett [19].

Results

Susceptibility of ghosts to attack by detergents and phospholipase C

Treatment of one-lysis isoionic ghosts with various concentrations of glycocholate and phospholipase C (*B. cereus*) in the presence of 2 mM Mg^{2+} and 10 μM Ca^{2+} caused a graded attack upon ghosts. Lysis by glycocholate (Fig. 1a) or phospholipid hydrolysis by phospholipase C (Fig. 1b) occurred at much lower concentrations than with the intact cells. The inclusion of 1 mM $Mg \cdot ATP^{2-}$ during the preparation of ghosts reduced the susceptibility of the ghosts to attack (Fig. 1, a and b). Further experiments using cholate, glycocholate, dodecyl sulphate and phospholipase C at low levels showed that the effect of $Mg \cdot ATP^{2-}$ was a relatively common phenomenon (Table I). 1 mM ATP or 2 mM EDTA did not bring about this protection showing that the effect was due to $Mg \cdot ATP^{2-}$ rather than an unspecific effect; e.g. chelation of divalent cations (Table I). The possibility that ATP might act through such a chelating effect appears to have been further eliminated by the information in Fig. 2, where it can be seen that partial protection against lysis (e.g. by glycocholate, Fig. 2a) or phospholipid hydrolysis (e.g. by phospholipase C (*B. cereus*), Fig. 2b) was afforded by Ca^{2+} (0.1—1.0 μM) and by Mg^{2+} ($\leqslant$0.1 mM), but much the greatest protective effect was that of $Mg \cdot ATP^{2-}$, suggesting that there might be some specificity to this effect of the nucleotide.

ATP was less effective in the absence of added Mg^{2+} (Table I); presumably in these circumstances its chelation effects tended to counteract its protective effects. Other nucleoside triphosphates (UTP, GTP, CTP) and other adenosine nucleotides (AMP, ADP, cyclic-AMP) conferred neither substantial nor consistent protection (results not shown). Studies with Triton X-100 and cetyltri-

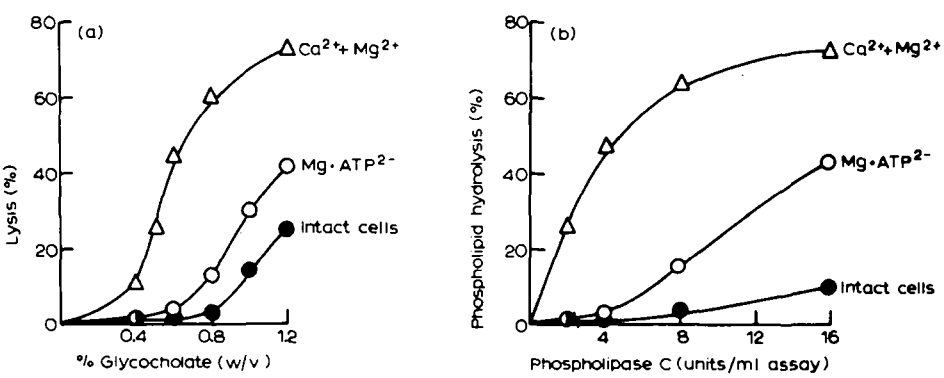

Fig. 1. Susceptibility of erythrocytes and ghosts to glycocholate and phospholipase C (*B. cereus*) Resealed, one-lysis, human erythrocyte ghosts were prepared in isoionic media containing 1 mM $Mg \cdot ATP^{2-}$ or 10 μM Ca^{2+} + 2 mM Mg^{2+} as described in Materials and Methods. At various concentrations of glycocholate or phospholipase C, lysis or phospholipid hydrolysis, respectively, of the ghosts or intact erythrocytes were determined (see Materials and Methods). Lysis was monitored by the release of haemoglobin at either 418 nm or 525 nm. Phospholipid hydrolysis was determined from the organic phosphorus content of the extracted lipid. Values are represented as percentage lysis or phospholipid hydrolysis compared with ghosts or cells as appropriate. (a) Experiments with glycocholate; (b) experiments with phospholipase C (*B. cereus*). △———△, Ca^{2+} + Mg^{2+}; ○———○, $Mg \cdot ATP^{2-}$; ●———●, intact cells.

TABLE I

EFFECTS OF THE PREPARATIVE MEDIUM ON THE LYSIS OF CELLS AND GHOSTS BY VARIOUS DETERGENTS

A variety of resealed one-lysis ghosts was prepared (see Materials and Methods) in the presence of different ions, chelators and nucleotides. They were then treated with a concentration of each detergent at which the attack was limited, and percentage lysis or phospholipid hydrolysis was determined as in Fig. 1. The values represent mean ± S.D. with the number of observations in parenthesis. The results were assessed using a *t* test and significant differences against the control are designated * ($P < 0.001$).

Additions to isoionic medium (HEPES/NaCl/KCl)	Percentage lysis			Phospholipid hydrolysis (%)
	Cholate (0.3%, w/v)	Glycocholate (0.8%, w/v)	Dodecyl sulphate (0.0028%, w/v)	Phospholipase C (*B. cereus*) (8 units/ml assay)
Intact cells	4 ± 1 (4)	3 ± 1 (5)	3 ± 1 (4)	3 ± 1 (5)
Ghosts				
10 μM Ca^{2+}, 2 mM Mg^{2+} (control)	42 ± 9 (5)	53 ± 14 (5)	31 ± 3 (4)	60 ± 2 (5)
1 mM ATP	34 ± 11 (4)	24 ± 9 (4)	27 ± 3 (4)	51 ± 2 (5)
1 mM Mg · ATP^{2-}	9 ± 3 (5) *	10 ± 6 (5) *	4 ± 1 (4) *	17 ± 1 (5) *
2 mM EDTA	69 ± 15 (5)	46 ± 5 (5)	46 ± 3 (4)	63 ± 2 (5)

methylammonium presented a different picture. In these two cases there was no clear difference in susceptibility to detergent attack between intact cells and Mg · ATP^{2-} ghosts (results not shown).

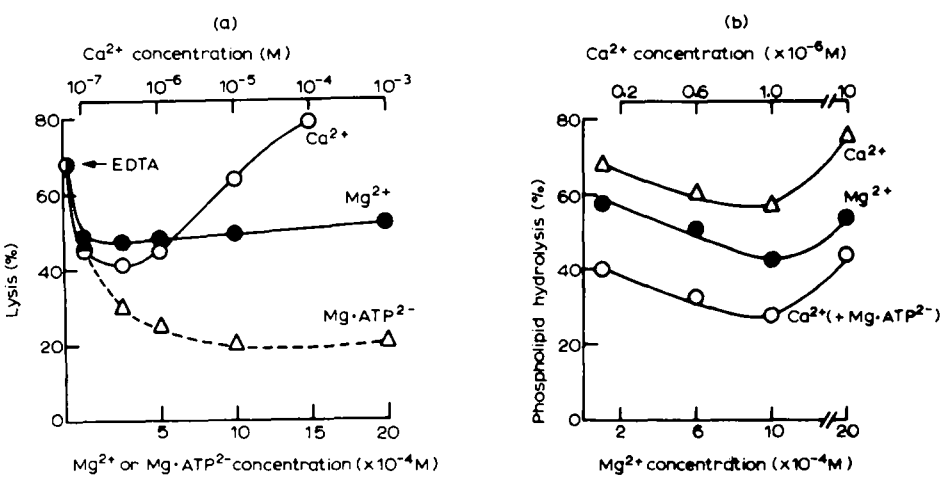

Fig. 2. Attack of glycocholate or phospholipase C (*B. cereus*) on ghosts prepared in different concentrations of Ca^{2+}, Mg^{2+} and Mg · ATP^{2-}. Various resealed, one-lysis human isoionic ghosts for glycocholate experiments were prepared in different concentrations of free Ca^{2+} (established using Ca^{2+}-ethyleneglycol bis(α-aminoethylether)-*N*,*N*′-tetraacetic acid (EGTA), see ref. 23) or of Mg^{2+} or of Mg · ATP^{2-} (with 2 mM EGTA throughout) (a). For phospholipase C experiments (b), resealed haemoglobin-free ghosts were prepared in different concentrations of free Ca^{2+} (as above), of Ca^{2+} (using EGTA buffer) with the inclusion of 1 mM Mg · ATP^{2-}, or of Mg^{2+} (with 0.1 μM Ca^{2+} as Ca^{2+}-EGTA buffer). Resealed ghosts were washed twice with isoionic medium and then treated with 1% glycocholate, (a) or phospholipase C (*B. cereus*), (b), 1 unit/ml assay under conditions described in Materials and Methods. The percentage lysis or phospholipid hydrolysis was determined as in Fig. 1.

Attack by phospholipases C on haemoglobin-free isoionic ghosts

The membrane of the intact erythrocyte is attacked readily by purified phospholipase C of *C. perfringens* but to a negligible extent by the phospholipase C of *B. cereus*. This is confirmed in Fig. 3. Both enzymes, however, attacked resealed haemoglobin-free erythrocyte ghosts prepared under isoionic conditions. Such ghosts are impermeable to large molecules such as enzyme proteins [12] and it must therefore be concluded that the organization of their external surface is subtly modified in some way which allows greater access to the phospholipases than exists in the native membrane. When 1 mM $Mg \cdot ATP^{2-}$ was included in the media throughout the preparation of ghosts attack by low concentrations of either of the phospholipases was substantially reduced (Fig. 3). A similar protective effect of $Mg \cdot ATP^{2-}$ was also observed if the nucleotide was absent throughout the preparation of the ghosts but was added to the interior of the haemoglobin-free ghosts during an additional glycol-induced lysis after their preparation. If $Mg \cdot ATP^{2-}$ was added to the final resealed haemoglobin-free ghost preparation, without lysis being induced (i.e. the ATP was available only at the external surface of the membrane), then it had no protective effect (results not shown), indicating that protection by the $Mg \cdot ATP^{2-}$ requires its interaction with the cytoplasmic surface of the membrane. Experiments with pig isoionic ghosts gave essentially identical results showing protection by $Mg \cdot ATP^{2-}$ against both types of phospholipase C.

Possible explanations of the protective effect of $Mg \cdot ATP^{2-}$ could include the phosphorylation of either membrane proteins or lipid. Erythrocytes also possess kinases for membrane lipids, and diacylglycerol (the substrate for one of these kinases) is a major product of phospholipase C action: in intact erythrocytes attacked by phospholipase C diacylglycerol is rapidly phosphorylated [20,21]. Analyses of the lipids of ghosts made in the presence and absence of $Mg \cdot ATP^{2-}$ were therefore undertaken to determine whether they differed in their contents of phosphatidate produced by phosphorylation of diacylglycerol, and of phosphatidylinositol phosphate and diphosphate: no

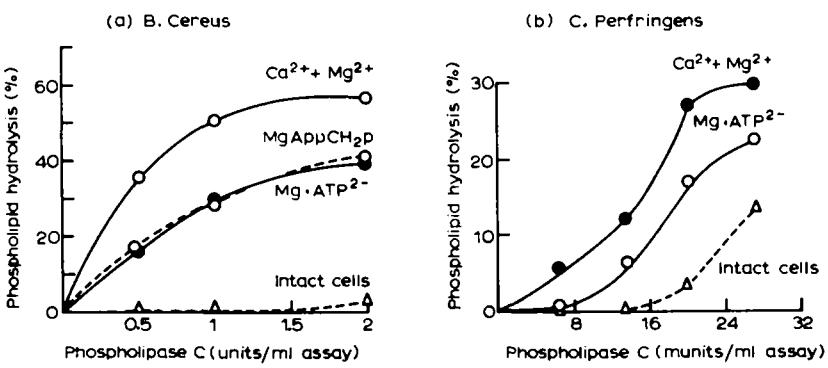

Fig. 3. Hydrolysis of lipids of human isoionic ghosts by phospholipase C of (a) *B. cereus* and (b) *C. perfringens*. Resealed haemoglobin-free ghosts were prepared in isoionic media containing 1 mM $Mg \cdot ATP^{2-}$ or 1 mM $MgAppCH_2p$ or 10 μM Ca^{2+} + 2 mM Mg^{2+} and treated with purified phospholipases C as described in Materials and Methods. The percentage hydrolysis of total phospholipid at various phospholipase C concentrations are represented.

differences were found (results not shown). We therefore tested the effect of $AppCH_2p$, an ATP analogue which does not possess a hydrolysable β-γ bond and which is therefore not a substrate for kinases or ATPases [22]. This compound was as effective as ATP at protecting (Fig. 3a) ghosts from attack. Thus, is seems likely that the protective effect of internal $Mg \cdot ATP^{2-}$ does not arise from its utilisation either as a phosphate donor for a kinase reaction or as an energy source by an ATPase, but through its binding to some site at the interior of the plasma membrane. Essentially similar results were obtained when pig isoionic ghosts were used.

Effects of $Mg \cdot ATP^{2-}$ upon the phospholipase C susceptibility of individual phospholipids in the ghost membranes

In order to determine whether $Mg \cdot ATP^{2-}$ was having an unspecific effect upon the characteristics of the entire lipid phase of the membrane, erythrocyte ghosts made with and without $Mg \cdot ATP^{2-}$ were subjected to limited attack by the two phospholipases C. Under the conditions chosen, the rate of hydrolysis by the *B. cereus* enzyme was somewhat higher than that by the *C. perfringens* enzyme, both with and without $Mg \cdot ATP^{2-}$, and attack by both enzymes was substantially decreased in the ghosts which contained $Mg \cdot ATP^{2-}$ (Fig. 4).

At the concentrations employed, both phospholipases C brought about

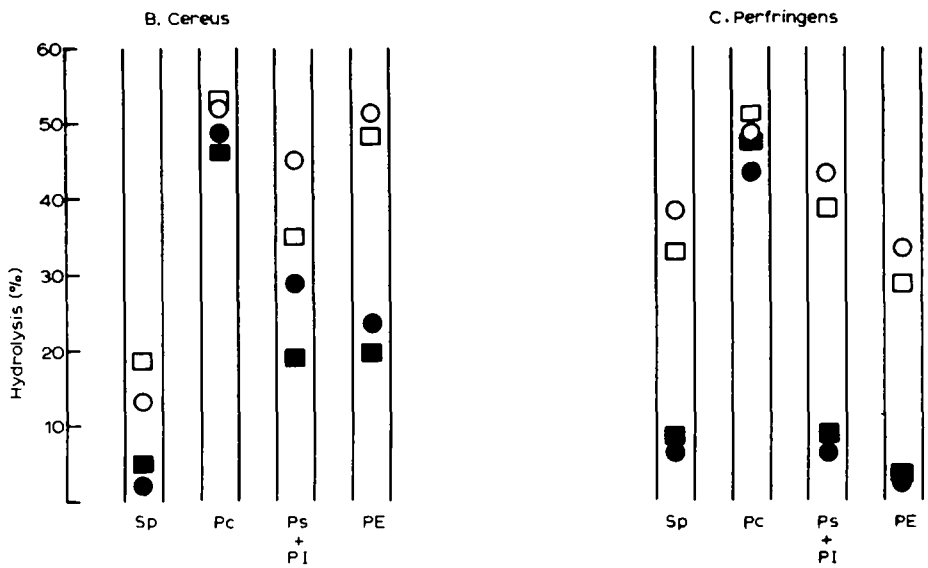

Fig. 4. Analysis of phospholipids of human isoionic ghosts treated with phospholipases C. Resealed haemoglobin-free ghosts were prepared from fresh human blood and treated with purified *B. cereus* (1 unit/ml assay) or *C. prefringens* (20 munits/ml assay) phospholipase C as described in Materials and Methods. The reaction was stopped and the ghosts were sedimented at 20 000 × *g* for 15 min at 4°C. A control incubation of ghosts without enzyme was run in each case. Lipids were extracted from the pellet and analysed on silica gel H thin layer chromatography plates in a solvent system of chloroform/methanol/acetic acid/water (75 : 45 : 12 : 2, v/v). The spots were visualised by iodine vapour and their organic phosphorus determined. Values of two experiments are represented as percentage hydrolysis of individual phospholipids. Experiment I: ○, control ghosts; ●, internal $Mg \cdot ATP^{2-}$ ghosts. Experiment II: □, control ghosts; ■, internal $Mg \cdot ATP^{2-}$ ghosts. PC, phosphatidylcholine; PS + PI, phosphatidylserine + phosphatidylinositol; PE, phosphatidylethanolamine; Sp, sphingomyelin.

substantial phospholipid hydrolysis in the control ghosts. When ghosts were made with internal $Mg \cdot ATP^{2-}$, however, the hydrolysis was considerably reduced. This effect was seen in the reduction of the hydrolysis of phosphatidylethanolamine, sphingomyelin and phosphatidylserine plus phosphatidylinositol, whereas the hydrolysis of phosphatidylcholine was only slightly affected by inclusion of $Mg \cdot ATP^{2-}$ during ghost preparation. Essentially similar observations were made on the effects of internal $Mg \cdot ATP^{2-}$ on phospholipid hydrolysis in pig erythrocyte ghosts.

Discussion

From the results with phospholipase C and with anionic detergents, ghosts which were resealed to many molecules and showed substantial resealing to K^+ [12] are seen to have suffered some subtle, but significant, change in their surface organization during isolation. The recognition of these structural modifications is important in view of the assumption frequently made (e.g. Bretscher, ref. 24) that the molecular organization of the erythrocyte membrane does not change significantly during ghost preparation.

It seems that this surface modification seen in ghosts can be substantially corrected if ATP (as $Mg \cdot ATP^{2-}$) is present within the ghosts during lysis. The effect is mimicked by $AppCH_2p$, an ATP analogue which is not hydrolysable and which is not a phosphate donor for phosphokinases, and the protective effect of ATP or $AppCH_2p$ occurs only if the nucleotide has access to the inner, cytoplasmic face of the membrane. Thus it seems that ATP, through its binding to some site(s) on the cytoplasmic surface of the membrane, must be able to perturb the organization of the lipids exposed at the outer surface of the membrane. Furthermore, this change in organization appears to have much more effect on the disposition of some lipids than of others. There was no evidence that internal ATP had any consistent effect upon the accessibility of phosphatidylcholine, whereas the availabilities of both sphingomyelin and phosphatidylethanolamine to phospholipases C were drastically reduced in ghosts containing ATP.

Although Zwaal et al. [25] have reported in some detail on the specificities of attack of *B. cereus* phospholipase C and *C. perfringens* phospholipase C, they only did so in approximate terms (0 or 100% hydrolysis) and more recent studies have revealed substantial discrepancies in the substrate specificities of these enzymes when purified in different laboratories or allowed to attack different lipid and membrane preparations. For example, all reports agree that the enzyme from *B. cereus* attacks phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine readily, but there is substantial disagreement as to whether phosphatidylinositol [25—30] and sphingomyelin [25—27,31] are substrates for this enzyme. Similarly, it is agreed that phosphatidylcholine, phosphatidylethanolamine and sphingomyelin are substrates of the *C. perfringens* enzymes, but there is disagreement as to whether phosphatidylserine is a substrate [25,26,28,32]. In our experiments we found a broad spectrum of activity with both purified enzymes.

One of the most important points to emerge from our studies is probably that the presence of an evironmental modification at the inner surface of the

plasma membrane can change the apparent specificity of both of these phospholipases. This is not due to an effect of ATP on the enzyme itself, since ATP does not change the rates of attack upon the lipids in a mixed lipid emulsion (Shukla, S.D., unpublished). Although we do not yet understand the process by which intracellular ATP affects the susceptibility to attack of the individual phospholipids, it emphasises that caution must be applied when attempting to distinguish between the specificity of an enzyme for substrates of particular chemical structures and the apparent specificity which can be caused by changes in the mode of presentation of a lipid substrate to an enzyme.

Specific effects of the ATP levels upon the susceptibility to phospholipase attack of erythrocyte ghost lipids have not been reported previously, but what may be a similar effect, again involving phosphatidylethanolamine and sphingomyelin, has been reported in intact erythrocytes. In one study it was found that when cells were depleted of ATP by inhibition of glycolysis their phosphatidylethanolamine became more susceptible to attack by a phospholipase A_2 [33] and also more available for reaction with 2,4,5-trinitrobenzene sulphonic acid [6]. A suggested interpretation of these data was that phosphatidylethanolamine underwent inversion from the inner to the outer leaflet of the membrane when cells were depleted of ATP [33]. In the other study [5] it was found that ATP depletion of cells increased the susceptibility of sphingomyelin to hydrolysis by extracellular sphingomyelinase. It is thought that most of the sphingomyelin of the membrane faces outwards [34], so in this case the proffered explanation emphasised some reorganisation of the outer leaflet of the membrane caused by depletion of intracellular ATP. If one assumes that the effects we have seen in ghosts arise from the same structural perturbation as is responsible for the effects seen in intact cells then it seems unlikely that the explanation will lie in the movement of lipid molecules between the two faces of the membrane. It is difficult to envisage a redistribution of phosphatidylethanolamine from interior to exterior leaflet during ghost preparation that could later be reversed simply by the binding of ATP to the inner aspect of the membrane. It seems far more likely that an interaction of cytoplasmic ATP with some membrane-spanning (glyco)protein, as suggested by Haest and Deuticke from experiments with thiol reagents [33], modulates the local lipid environment surrounding the portion of the molecule that reaches through to the outer half of the membrane. The nature of the ATP binding site is unknown.

One problem posed by these results relates to the interpretation of experiments on phospholipase hydrolysis of membrane lipids in terms of the asymmetric distribution of lipids in the lipid bilayer of membranes. It would appear from our experiments that three quite different assessments of the amount of phosphatidylethanolamine in the outer leaflet of the erythrocyte membrane could be obtained simply by choosing to experiment on intact cells, sealed ghosts containing ATP or sealed ghosts with no ATP. Obviously this casts some doubt on the assumption, implicit in many experiments on lipid asymmetry, that the membrane structure in ghosts is unchanged relative to the membranes of intact cells.

The only previous example of well defined effects of ATP on red cell ghosts are those involving ATP-induced vesiculation and shape changes [8–10, 35,36].

Recent studies by Singer and his colleagues [8,9] have shown that an important component of these effects is the phosphorylation by ATP of spectrin. It is therefore interesting to note that the protective effects of ATP binding that are reported here presumably depend upon quite different interactions between ATP and the cytoplasmic surface of the erythrocyte membrane. One effect of ATP is to phosphorylate spectrin and thus modulate cell shape, whilst the other is to bind to the membrane, presumably at specific sites, and thus modulate the organization of the membrane lipid phase.

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