ATP-dependent translocation of amino phospholipids across the human erythrocyte membrane

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Trace amounts of radiolabeled phospholipids were inserted into the outer membrane leaflet of intact human erythrocytes, using a non-specific lipid transfer protein. Phosphatidylcholine, phosphatidylserine and phosphatidylethanolamine were transferred from the donor lipid vesicles to the membrane of the intact red cell with equal ease, whilst sphingomyelin was transferred 6-times less efficiently. The transbilayer mobility and equilibrium distribution of the labeled phospholipids were assessed by treatment of the intact cells with phospholipases. In fresh erythrocytes, the labeled amino phospholipids appeared to move rapidly towards the inner leaflet. The choline phospholipids, on the other hand, approached an equilibrium distribution which strongly favoured the outer leaflet. In ATP-depleted erythrocytes, the relocation of the amino phospholipids was markedly retarded.

Erythrocyte membrane *Phospholipid distribution* *Transbilayer mobility* *Lipid transfer protein* *Phospholipase* *ATP depletion*

1. INTRODUCTION

An asymmetric distribution of phospholipids has been demonstrated in many biological membranes, but most notably in that of the erythrocyte [1–3]. The choline phospholipids are localized mainly in the outer leaflet of the erythrocyte membrane, whilst the amino phospholipids are confined almost exclusively to the inner leaflet of the bilayer [4,5].

The transbilayer mobility of phosphatidylcholine (PC) across the red cell membrane has been extensively studied (see [3] for review). Whilst the translocation rate of PC has been found to be quite slow ($t_{1/2}$ = 13–24 h), this is insufficient to explain the maintenance of phospholipid asymmetry over the 120-day life span of the cell.

Changes in erythrocyte shape, deformability, and membrane stability have long been known to be consequent upon depletion of the intracellular level of ATP [6,7]. Recently, however, metabolic factors have also been implicated in the maintenance of phospholipid asymmetry [8]. Seigneuret and Devaux have studied the uptake and transbilayer mobility of spin-labeled phospholipid analogues. When added to a suspension of cells, these probes partition into the outer leaflet of the membrane bilayer of erythrocytes, thereby causing them to adopt a crenated morphology. The spin-labeled amino phospholipids, phosphatidylethanolamine (PE) and phosphatidylserine (PS), were found to be quite rapidly translocated to the inner leaflet – a rearrangement

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which was concomitant with the conversion of the echinocytes to discocytes and/or stomatocytes. PC translocation was shown to be significantly slower. Furthermore, the translocation rate for the amino phospholipids was found to be energy dependent. When erythrocytes were deprived of ATP, the translocation of the amino phospholipids was severely inhibited.

In the present study, a non-specific lipid transfer protein has been used to mediate the transfer of trace amounts of radioactively labeled long-chain diacyl phospholipids and sphingomyelin to the erythrocyte membrane. Under the conditions applied, the incorporation of these probes does not alter the cell shape. The distribution adopted by the exogenously added probes was assessed by phospholipase degradation of the outer-leaflet phospholipids. It appears that the establishment of asymmetric distribution of the exogenously added amino phospholipid is indeed under metabolic control. However, once asymmetry is established, the non-random localization of the phospholipids is not immediately disrupted by ATP depletion.

2. MATERIALS AND METHODS

1,2-[^{14}C]Dioleoylphosphatidylcholine, 1,2-dioleoylphosphatidyl[^{14}C]serine, 1,2-dioleoylphosphatidyl[^{14}C]ethanolamine and [*N-methyl*-^{14}C]sphingomyelin (bovine) were obtained from Amersham International, England. Egg PC, egg phosphatidic acid, bovine brain phosphatidylserine, bee venom phospholipase A_2 and *Naja naja* phospholipase A_2 were obtained from Sigma (St. Louis, MO), and cholesterol and iodoacetic acid from Merck (Darmstadt). All other chemicals were of reagent grade or higher. Sphingomyelinase C was purified from *S. aureus* cultures by the method described by Zwaal et al. [9].

2.1. *Erythrocytes*

After obtaining informed consent, fresh blood from healthy donors was collected into acid/citrate/dextrose buffer and used immediately. The cells were washed 3 times with a buffer composed of 10 mM Tris-HCl, pH 7.4, 280 mM sucrose, 10 mM NaCl, 1 mM EDTA, 20 mM glucose (sucrose buffer) [10]. The buffy coat was aspirated at each step.

2.2. *Preparation of vesicles*

Vesicles were prepared from an equimolar mixture of cholesterol and phospholipid. The phospholipid was either egg PC/phosphatidic acid (10:1) or bovine brain PS. Trace amounts of [^{14}C]PC, [^{14}C]PE and [^{14}C]PS were included in the lipid mixture. The lipid was dried from chloroform/methanol (1:1, v/v). Vesicles were prepared by dispersing the dried lipid in the above sucrose buffer at a phospholipid concentration of about 0.15 mM. The lipid dispersion was sonicated under N_2 at 65 W, using a Branson sonifier, and then centrifuged at 19000 × *g* for 10 min to remove any titanium particles.

2.3. *Preparation of the transfer protein*

A non-specific lipid transfer protein was partially purified from bovine liver according to the method of Bloj and Zilversmit [11], as adapted by Crain and Zilversmit [12]. The two peaks eluting from the carboxymethylcellulose column were pooled and used without further purification. The final preparation contained 260 units of activity/mg protein. Protein concentrations were determined using the method of Ross and Schatz [13]. Transfer activity is defined as the ability of the preparation to transfer [^{14}C]PE from PE/PC (0.8:1.2) vesicles to bovine heart mitochondria [12].

2.4. *Phospholipid incorporation conditions*

Incubations were carried out at room temperature, in plastic tubes, in a clinical blood rotator operated at 4 rpm. Erythrocytes were incubated for 30 min as a 30–40% suspension (approx. 1.5 mM erythrocyte phospholipid) in sucrose buffer containing 1 mg/ml of the non-specific lipid transfer protein preparation and about 0.035 mM vesicle phospholipid. The percentage incorporation of exogenous phospholipid into the erythrocyte membrane was determined by measuring radioactivity/mol phosphorous in extracts of ghosts prepared from the labeled erythrocytes. Lipid extractions were performed using the method of Rose and Oklander [14]. Phosphorus was assayed according to Rouser et al. [15].

Vesicle phospholipid, containing trace amounts of [^{14}C]PC, [^{14}C]PE, and [^{14}C]PS, was incorporated into intact erythrocytes to a final level of about 1 mol exogenous phospholipid per 100 mol erythrocyte phospholipid.

2.5. *Incubation of labeled cells and determination of the distribution of incorporated labels*

After washing the labeled cells at 4°C, with a buffer composed of 20 mM Tris-HCl, 90 mM KCl, 45 mM NaCl, 44 mM sucrose and 10 mM glucose, pH 7.4, at 37°C, they were suspended at 10% haematocrit in the same buffer and incubated at 37°C. Samples were removed at timed intervals and washed with a buffer composed of 10 mM Tris-HCl, 90 mM KCl, 45 mM NaCl, 10 mM $CaCl_2$, 0.25 mM $MgCl_2$, 22 mM sucrose, pH 7.4. To discriminate between radioactive phospholipids in the two halves of the bilayer, intact cells were incubated, at 20% haematocrit, at 37°C for 1 h, with a combination of two phospholipases A_2 (bee venom and *N. naja*, 15 IU/ml each) [16]. In some cases, the above incubation was continued for a further hour in the presence of sphingomyelinase C (3.8 IU/ml) [9].

2.6. *ATP depletion of erythrocytes*

Erythrocytes were either starved of their energy source by incubation for 22 h in a buffer composed of 50 mM Tris-HCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 90 mM NaCl, 0.2 mg/ml streptomycin and 200 IU/ml penicillin, pH 7.4, at 37°C or, alternatively, depleted of ATP by incubation for 1 h in the presence of 1 mM iodoacetate as described by Mohandas et al. [17]. ATP levels in fresh and treated erythrocyte suspensions were determined using the hexokinase/glucose-6-phosphate dehydrogenase assay of Lamprecht and Trautschold [18].

3. RESULTS

Fig.1 shows the transfer of radioactively labeled phospholipids from unilamellar vesicles to human erythrocytes in the presence and absence of the transfer protein. The incubation mixture contained 1.6 mM erythrocyte phospholipid and 0.03 mM vesicle phospholipid. The results indicate that, under the conditions of the experiment, about 35% of the donor vesicle phospholipid label is transferred to the erythrocyte acceptor. Assuming that the bulk lipid is transferred to the acceptor with the same efficiency as the labeled phospholipid, this is equivalent to the incorporation of exogenous lipid to a level of about 1% of the total erythrocyte membrane phospholipid. It can be seen that the

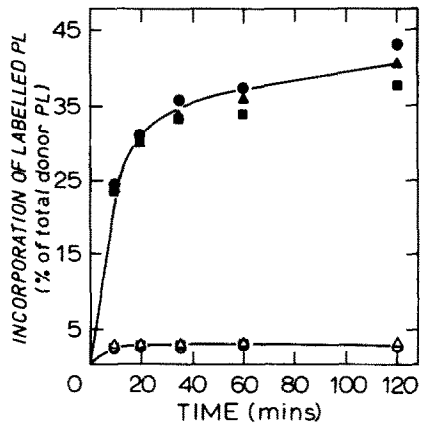

Fig.1. Transfer of radioactively labeled phospholipids from unilamellar vesicles to human erythrocytes. Incorporation of radiolabeled PC (■, □), PE (●, ○) and PS (▲, △) was followed in the presence (closed symbols) and absence (open symbols) of the protein which mediated non-specific lipid transfer. The data are expressed as percentage of the initial radioactivity present in the donor system as [^{14}C]PC, [^{14}C]PE and [^{14}C]PS, respectively. Each point represents the average of duplicate determinations.

labeled PC, PE, and PS are transferred with approximately equal efficiency. In the absence of the protein, incorporation of lipid still occurs to a small extent during the 30 min incubation period (about 0.1% of the total erythrocyte phospholipid). PC/PA/cholesterol (1:0.1:1, mol/mol) was normally used as the carrier lipid in the preparation of vesicles. This composition was chosen to cause minimum disturbance of the normal erythrocyte lipid composition. The incorporation of exogenous lipid did not change the shape of the erythrocytes to any appreciable extent, nor did it affect the osmotic fragility of the cells.

After the incorporation of labeled phospholipid, the cells were washed and then incubated at 37°C. Samples were taken at timed intervals and then subjected to phospholipase A_2 digestion. Fig.2 shows the percentage of labeled phospholipid which remains susceptible to phospholipase digestion at various time intervals after the incorporation and washing steps. Most of the PS appears to very quickly adopt a location in which it is inaccessible to hydrolysis by phospholipase A_2 – presumably this represents flip to the inner leaflet of the bilayer. The apparent rate of PE flip is

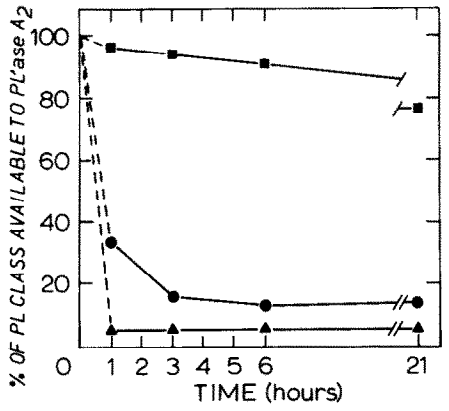

Fig.2. Transbilayer migration of exogenously added diacyl glycerophospholipids. Erythrocytes labeled in vitro with [^{14}C]PC, [^{14}C]PE and [^{14}C]PS were subsequently incubated at 37°C under the conditions described in section 2. The percentage of the radioactive PC (■), PE (●) and PS (▲) remaining in the outer leaflet of the bilayer, was assessed by determining accessibility to phospholipase A_2. Each point represents the average of duplicate determinations. The results are typical of those observed in three different experiments. The time scale represents the time of incubation of cells at 37°C (including the period of incubation with phospholipase A_2) after the introduction of the labeled phospholipids.

somewhat slower and a stationary transbilayer distribution appears to be reached in which 15% of the PE remains accessible to phospholipase A_2. PC flip appears to be significantly slower.

The data presented in fig.2 refer to digestion of the radiolabeled phospholipids by treatment of the intact erythrocytes with a combination of two phospholipases A_2 (bee venom and *N. naja*). In some experiments, the incubation mixture was supplemented (after 1 h) with sphingomyelinase C and the incubation was continued for a further hour [9]. This did not significantly affect the level of phospholipase A_2 digestion of the labeled compounds.

[*N-methyl*-^{14}C]Sphingomyelin (SM) was also transferred to erythrocytes using the non-specific transfer protein, albeit with a 6-fold lower efficiency than the glycerophospholipids. The exogenously incorporated sphingomyelin, however, showed only very limited transbilayer mobility. Even after 22 h at 37°C, 90% of the incorporated sphingomyelin label remained accessible to sphingomyelinase digestion (not shown).

Similar results to those presented in fig.2, were also obtained when [^{14}C]PS was carried in vesicles which were comprised of an equimolar ratio of cold PS/cholesterol. [^{14}C]PS was still found to be 95% inaccessible to the phospholipases A_2 at the initial time point.

An attempt was also made to examine the redistribution of exogenously added phospholipids using erythrocytes which had been starved of glucose for 22 h. These energy-depleted cells were a uniform population of echinocytes and contained no ATP, as assessed by the hexokinase/glucose-6-phosphate dehydrogenase assay [18]. Exogenous trace-labeled phospholipid was incorporated into these cells under the same conditions as were described for normal cells. The distribution of the label was then assessed by subjecting the erythrocytes to phospholipase A_2 attack.

The first two rows of table 1 show the data obtained for control and glucose-starved erythrocytes, respectively. It can be seen that the flip of the incorporated [^{14}C]PS and [^{14}C]PE was significantly inhibited in the ATP-depleted cells, compared with the control, since a much larger proportion of the populations of PS and PE remained accessible to the phospholipase. To test whether this difference might be due simply to an altered reactivity of these phospholipids with the phospholipase A_2 in echinocytic cells, a further sample was examined in which the radioactive phospholipids were incorporated into the erythrocyte membrane prior to the ATP-depletion period. The results of this experiment are given in the third row of table 1. The [^{14}C]PS and [^{14}C]PE distributions in these cells more closely resemble the control sample. It appears that the echinocytic shape is not per se responsible for the altered accessibility of the amino phospholipids to the phospholipase. In this third case, the PC distribution can be seen to resemble more closely the expected equilibrium distribution [4,9]. This is because the cells have been incubated for 22 h prior to analysis.

ATP depletion by glucose starvation is quite a lengthy process and involves irreversible rearrangements within the membrane skeleton [6]. A more rapid method for depleting the intracellular

Table 1

Transbilayer migration of diacyl glycerophospholipids in energy-starved erythrocytes

Sample	% non-hydrolyzable labeled phospholipid		
	PC	PE	PS
Control	4	75	95
Glucose-starved	4	7	36[a]
Labeled, then glucose-starved	28	65	89

[a] The non-hydrolyzable [^{14}C]PS, in glucose-starved cells, may well be over-estimated. Lyso-PS is quite water-soluble and, for this sample, a proportion (nearly 40%) of the PS radioactivity was lost during the phospholipase treatment and subsequent preparation of ghosts. The recovery of radioactivity after phospholipase treatment was >90% for all other erythrocyte incubations

The accessibility to phospholipase A_2 of exogenously added [^{14}C]PC, [^{14}C]PE and [^{14}C]PS was examined in erythrocytes which had been deprived of glucose for 22 h. Control cells were kept at 4°C in acid/citrate/dextrose buffer during this incubation time. Erythrocytes, into which exogenous phospholipid was first incorporated and which were subsequently depleted of ATP, were also analyzed for accessibility of the incorporated label to phospholipase A_2. The non-hydrolyzable phospholipid, found after treatment of intact cells with phospholipase A_2, is assumed to represent that fraction of the labeled diacyl phospholipid that has been translocated to the inner leaflet. Each value represents the average of duplicate determinations in a typical experiment

ATP level has been described [17]. Cells depleted of ATP by this method, involving iodoacetate, are not a uniform population of echinocytes but are mostly discocytes with occasional stomatocytes. The level of ATP in these treated cells was about 5% of the control as determined by the hexokinase/glucose-6-phosphate dehydrogenase assay [18]. Table 2 shows the results which were obtained for control cells, for cells which were treated with 1 mM iodoacetate prior to the incorporation of labeled exogenous phospholipid and for cells which were first labeled and then subsequently depleted of ATP. The results are similar to those for the glucose starvation experiment, although the

Table 2

Transbilayer migration of diacyl glycerophospholipids in iodoacetate-treated erythrocytes

Sample	% non-hydrolyzable labeled phospholipid		
	PC	PE	PS
Control	4	64	95
Iodoacetate-treated	3	16	82
Labeled, then iodoacetate-treated	4	80	93

The accessibility to phospholipase A_2 of exogenously added [^{14}C]PC, [^{14}C]PE and [^{14}C]PS was examined in erythrocytes which had been incubated at 37°C for 1 h in the presence of 1 mM iodoacetate. Control cells were incubated under the same conditions but without iodoacetate. Erythrocytes, into which exogenous phospholipid was first incorporated and which were subsequently depleted of ATP, were also analyzed for accessibility of the incorporated label to phospholipase A_2. The non-hydrolyzable phospholipid, found after treatment of intact cells with phospholipase A_2, is assumed to represent that fraction of the labeled diacyl phospholipid that had been translocated to the inner leaflet. The recovery of radioactivity after the phospholipase incubation was >90% for all samples. Each value represents the average of duplicate determinations in a typical experiment

inhibition of amino phospholipid relocation in iodoacetate-treated cells was less dramatic in the case of PS.

The percentage of cells which was haemolyzed during the phospholipase A_2 incubations was determined for each sample in the above experiments and was less than 3% for samples taken early in the equilibration incubations. Cells incubated overnight, with or without an energy source, showed increased fragility but percentage haemolysis was always less than 10%.

In preliminary experiments with erythrocyte ghosts, which had been resealed in the presence or absence of ATP [8], it was found that exogenous trace-labeled phospholipid could be incorporated under the same conditions as described for intact cells. The redistribution of the labeled phospholipid was assessed by subjecting the ghosts to bee venom phospholipase A_2 attack using buffer conditions (0.25 mM $CaCl_2$) designed to limit the level

of haemolysis of the resealed ghosts to less than 10%. In the presence of ATP, in the concentration range 0.3–2 mM, the exogenously incorporated PS was largely inaccessible to the phospholipase, whereas about 80% of the radiolabeled PC was hydrolyzed under these conditions. In the absence of ATP, transmigration of the incorporated labeled PS was markedly inhibited.

4. DISCUSSION

Under the conditions of these experiments, the non-specific lipid transfer protein mediates the transfer of PC, PE, and PS with equal efficiency, and can be used to incorporate trace amounts of exogenous phospholipid into human erythrocytes.

It is difficult to exclude the possible occurrence of some fusion between donor (vesicle) and acceptor (erythrocyte) membranes during the incorporation step. It has been suggested that the non-specific transfer protein may induce fusion under conditions similar to those used for these experiments [10]. However, the fact that the [^{14}C]PC is still 97% accessible to the phospholipase at the initial time point indicates that fusion is not, in any case, the major mechanism for phospholipid transfer under these conditions. Similarly, we cannot exclude the possibility that the composition of the erythrocyte membrane may be changed slightly during the incorporation step. In the worst case, assuming that all the incorporation of exogenous phospholipid occurs by net transfer rather than by exchange, then the total amount of phospholipid in the erythrocyte would be increased by about 1% and the amount in the outer monolayer, therefore, by 2%. This would have caused the cells to adopt a crenated morphology [19] which was not observed. To minimize the disturbance of the phospholipid composition of the outer leaflet of the erythrocyte, PC was chosen as the bulk phospholipid for most experiments. It was found, however, that the accessibility of [^{14}C]PS to phospholipase A_2 was very small, even when this probe molecule had been introduced into the red cell membrane using PS as the bulk phospholipid for the donor vesicles.

Exogenously added amino phospholipids were found to move very quickly into a pool which was inaccessible to phospholipase A_2, i.e., presumably, into the inner leaflet of the bilayer. Various techniques have previously been used to demonstrate that the phospholipids in the human erythrocyte membrane are distributed over the two halves of the bilayer in an asymmetric fashion [1,4]. After 22 h of incubation in a buffer which contained glucose, the four exogenously added phospholipid classes appear to have adopted this same asymmetric distribution.

The apparent flip rates for the labeled phospholipids decreased in the order PS > PE ≫ PC > SM. Seigneuret and Devaux have previously reported flip rates for spin-labeled analogues of PS, PE, PC, and SM in the order found in this study [8,20]. These authors also reported that the rapid inward translocation of the amino phospholipids could be inhibited by depleting the ATP content of the erythrocytes or by preparing pink resealed ghosts in the absence of ATP [8]. Their findings are supported by the present study. We found that the inward migration of exogenously incorporated PS was markedly inhibited in ghosts resealed in the absence of ATP. Furthermore, depletion of intracellular ATP levels of intact erythrocytes, either by starvation or by treatment of the cells by iodoacetate, inhibited the inward movement of amino phospholipids, which had been incorporated into the outer leaflet of the bilayer. The effect is less dramatic for iodoacetate-treated cells than for glucose-starved cells. This may indicate that time-dependent protein rearrangements are responsible for the altered translocation rates, or may simply reflect the fact that the ATP level is not completely reduced to zero by the 1 h incubation with the oxidizing agent.

It has previously been reported that the asymmetry of the erythrocyte phospholipids is maintained in ATP-depleted cells [21], which is consistent with the present study. We found that, once the labeled amino phospholipids have adopted their inner leaflet positions, subsequent depletion of the cellular ATP level does not result in an immediate loss of asymmetry. The backward diffusion rates for these amino phospholipids are obviously very slow. This observation, in combination with reports that PS interacts with both spectrin and band 4.1 in reconstituted systems [22], may also lend support to the idea [3,21] of binding sites for the amino phospholipids at the inner surface of the erythrocyte membrane.

Seigneuret and Devaux have suggested that there is an energy-dependent, specific transfer of the amino phospholipids to the inner leaflet [8,20]. Facilitated translocation of phosphatidylcholine was, in fact, proposed by Bretscher [23] more than a decade ago, as a possible mechanism for generating phospholipid asymmetry. The present studies support the idea of facilitated translocation of the amino phospholipids, but to assign definitively the mechanism for the observed phenomena, further studies are necessary.

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REFERENCES

[1] Op den Kamp, J.A.F. (1979) Annu. Rev. Biochem. 48, 47–71.
[2] Etemadi, A.-H. (1980) Biochim. Biophys. Acta 604, 423–475.
[3] Van Deenen, L.L.M. (1981) FEBS Lett. 123, 3–15.
[4] Zwaal, R.F.A., Roelofsen, B. and Colley, C.M. (1973) Biochim. Biophys. Acta 300, 159–182.
[5] Roelofsen, B. (1982) J. Toxicol. Toxin Rev. 1, 87–197.
[6] Palek, J. and Liu, S.C. (1979) J. Supramol. Struct. 10, 79–96.
[7] Sheetz, M.P., Sawyer, D. and Jackowski, S. (1978) in: The Red Cell (Brewer, G.J. ed.) pp.431–450, Alan R. Liss, New York.
[8] Seigneuret, M. and Devaux, P.E. (1984) Proc. Natl. Acad. Sci. USA 81, 3751–3755.
[9] Zwaal, R.F.A., Roelofsen, B., Comfurius, P. and Van Deenen, L.L.M. (1975) Biochim. Biophys. Acta 406, 83–96.
[10] Franck, P.F.H., De Ree, J.M., Roelofsen, B. and Op den Kamp, J.A.F. (1984) Biochim. Biophys. Acta 778, 405–411.
[11] Bloj, B. and Zilversmit, D.B. (1977) J. Biol. Chem. 252, 1613–1619.
[12] Crain, R.C. and Zilversmit, D.B. (1980) Biochem. 19, 1433–1439.
[13] Ross, E. and Schatz, G. (1973) Anal. Biochem. 54, 304–306.
[14] Rose, H.G. and Oklander, M. (1965) J. Lipid Res. 6, 428–431.
[15] Rouser, G., Fleischer, S. and Yamamoto, A. (1970) Lipids 5, 494–496.
[16] Franck, P.F.H., Bevers, E.M., Lubin, B.H., Comfurius, P., Chiu, D.T.-Y., Op den Kamp, J.A.F., Zwaal, R.F.A., Van Deenen, L.L.M. and Roelofsen, B. (1985) J. Clin. Invest. 75, 183–190.
[17] Mohandas, N., Wyatt, J., Mel, S.F., Rossi, M.E. and Shohet, S.B. (1982) J. Biol. Chem. 257, 6537–6543.
[18] Lamprecht, W. and Trautschold, I. (1974) in: Methods of Enzymatic Analysis (Bergmeyer, H.U. ed.) vol.4, pp.2101–2110, Academic Press, New York.
[19] Ferrell, J.E. jr, Lee, K.-Y. and Huestis, W.H. (1985) Biochem. 24, 2849–2857.
[20] Zachowski, A., Fellman, P. and Devaux, P.F. (1985) Biochim. Biophys. Acta 815, 510–514.
[21] Haest, C.W.M. (1982) Biochim. Biophys. Acta 694, 331–352.
[22] Wagner, C.M., Schwartz, R.S., Chiu, D.T.-Y. and Lubin, B.H. (1985) Clinics Haematol. 14, 183–200.
[23] Bretscher, M.S. (1973) Science 181, 622–629.