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METABOLICALLY CONTROLLED HEMOLYSIS OF CHICKEN ERYTHROCYTES

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SUMMARY

Commercial preparations of phospholipase C (*Clostridium perfringens*) contain a hemolysin which hemolyses red blood cells in the absence of bivalent cations and a phospholipase C whose activity is dependent on bivalent metals.

The hemolysin readily lysed fresh chicken erythrocytes but failed to act on ATP-depleted cells.

Heating of the commercial preparation of phospholipase C caused inactivation of the hemolytic activity while the hydrolytic activity remained relatively intact. A heated preparation failed to affect fresh erythrocytes but readily hemolysed ATP-depleted cells. Hemolysis of ATP-depleted cells took place only in the presence of bivalent cations such as Ca^{2+} or Mn^{2+}. ATP-depleted cells became sensitive to the hemolysin and resistant to the heated preparation again after restoration of the internal pool of ATP. It is suggested that ATP dependent changes of the erythrocyte membrane are responsible for these changes in sensitivity.

INTRODUCTION

Recently we have shown that commercial preparations of phospholipase C from *Clostridium perfringens* contain, besides the well-known hydrolytic activity, a hemolytic activity which hemolyses chicken red blood cells without hydrolysing their membrane phospholipids[1]. Hemolysis promoted by this hemolytic activity occurrs in the absence of bivalent cations whereas hydrolysis of phospholipids, by this phospholipase C is known to be absolutely dependent on their presence[2]. Phospholipids of intact red blood cells have been shown to be masked and insusceptible to hydrolysis by phospholipase C[3,4].

Roelofsen *et al.*[3] have recently shown that pure phospholipase C from *Bacillus cereus* is unable to hydrolyse the phospholipids of intact human erythrocytes unless the cells are previously treated with detergents. In addition, we have shown that phospholipids of human erythrocyte membrane might be exposed by incubating the cells in hypotonic solutions. The extent of hydrolysis of membrane phospholipids by phospholipase C of *B. cereus* increased as the hypotonicity of the incubation medium increased[4]. However, it has been shown that phospholipids of chicken erythrocyte membrane can be hydrolysed by phospholipase C from *Cl. perfringens*

in the presence of bivalent cations[1]. This is probably due to the fact that the hemolytic activity of these preparations exposes the phospholipids and paves the way for the hydrolytic activity. The enzyme devoid of hemolytic activity failed to hydrolyse the phospholipids of intact chicken erythrocytes whereas it readily hydrolysed them in ghosts prepared from the same cells[1].

From the above-mentioned works it seems that the phospholipids of either human or chicken erythrocytes are buried in the interior structure of the membrane and may be exposed in several ways.

Similarly to the phospholipids, some membrane carbohydrates of mammalian cells are also masked. Burger[5] and Inbar and Sachs[6] have shown that transformed cells agglutinate in the presence of certain glycoproteins whereas normal cells do not. Agglutination by these agents can be induced in normal cells by light trypsinization which probably exposes cryptic sites which are responsible for the process. Fox *et al.*[7] has shown that agglutination sites of normal cells are naturally accessible within a small part of the cell cycle, namely during mitosis. In addition, Inbar *et al.*[8] have shown that transformed cells can be agglutinated by concanavalin A only at 24 °C and not at 4 °C. These structural changes of the surface membrane were proposed as being associated with a specific metabolic activity[8].

Since red blood cells are virtually membrane sacs containing hemoglobin, they can serve as an excellent tool for studying metabolic dependent changes of biological membranes. In the present work, the effects of the hemolytic and hydrolytic activities of *Cl. perfringens* preparations have been studied on fresh and ATP-depleted chicken erythrocytes.

MATERIALS AND METHODS

Cells

Blood was collected from the necks of decapitated chickens in an erlenmeyer flask containing heparin (100 units/ml blood). The blood was washed and suspended in high-KCl medium as previously described[9].

Media

The following media were used:

(A) Solution K contained: 135 mM KCl, 5.4 mM NaCl, 0.8 mM $MgSO_4$ buffered with 20 mM Tricine–NaOH (pH 7.4).

(B) ATP depleting medium: if not otherwise stated the ATP depletion medium contained 1 mM KCN and 20 mM NaF dissolved in Solution K.

(C) ATP restoring medium contained: 2 mM adenine, 10 mM inosine, 5 mM glucose and 5 mM sodium phosphate buffered at pH 7.4, all dissolved in Solution K. Penicillin (100 units/ml) and streptomycin (10 μg/ml) were added to prevent growth of micro-organisms.

Hemagglutinin and hemolysis determinations

These were performed as previously described[9].

Phospholipids extraction

Phospholipids were extracted from chicken erythrocytes essentially according

to Burger *et al.*[10] with the modification previously described.[1] Total phosphate was determined according to Ames[11]

Heating of phospholipase C

Heating of phospholipase C was carried out essentially as described previously[1]; 500 μg/ml of phospholipase C in Solution K, which contained 5 mM Ca^{2+}, was heated for 10 min at 56 °C.

ATP determination

The method is based on that of Beutler and Baluda[12] which makes use of the bioluminescence of luciferin in the presence of luciferase and ATP. 5 mg firefly desiccated tails were extracted by homogenization in 1 ml of 100 mM arsenate buffer at pH 7.4. The resulting homogenate was centrifuged at 6000 × *g* for 10 min in the cold and the supernatant was collected and used within one day.

Tubes containing 1.6 ml of distilled water were immersed in a boiling water bath to which 0.4 ml of 30% (v/v) chicken erythrocytes in Solution K were added. The resulting 2 ml suspension was subsequently incubated for 5 min, and ATP was determined in the supernatant. Samples of 0.01 ml of the supernatant were introduced into 20-ml scintillation vials containing 40 μmoles arsenate buffer (pH 7.4) and 20 μmoles $MgSO_4$ in a final volume of 0.8 ml. The luciferase reaction was initiated by the addition of 0.2 ml of the firefly tails extract. Luminescence was measured in a scintillation counter (Packard). The reaction was linear with ATP concentration in the range of $1 \cdot 10^{-9}$–$1 \cdot 10^{-10}$ mole of ATP.

Protein determination

Protein was determined according to Lowry *et al.*[13].

Materials

Prymnesin, a toxin from *Prymnesium parvum*[16], was obtained from Makor Chemicals Ltd, Jerusalem, Israel. Phospholipase C (*Cl. perfringens*, EC 3.1.4.3) was obtained from Worthington Chemical Corp. (1-2 units/mg protein). Firefly desiccated tails were obtained from Sigma Co. All other reagents were of analytical grade.

RESULTS

ATP-dependent hemolysis of chicken erythrocytes

When fresh chicken erythrocytes were incubated with 2–4 μg of phospholipase C preparations for 60 min at 37 °C, hemolysis was proportional to enzyme concentration and reached about 60–100%. When ATP was depleted from the cells by incubation with either respiratory inhibitors or with uncouplers of oxidative phosphorylation they became less susceptible to the hemolytic acitvity of these preparations. While about 100% hemolysis was obtained with fresh erythrocytes, only 15% hemolysis or no hemolysis was obtained by phospholipase C after ATP was depleted from the erythrocytes with dinitrophenol or with NaCN and NaF, respectively (Fig. 1). This process was reversible in the sense that the inhibition

of hemolysis in ATP-depleted cells could be reversed by increasing the internal ATP pool of the erythrocytes.

The hemolysis was performed in the absence of bivalent metals, under which conditions the hydrolysis of membrane phospholipids did not take place. The hemolysis was caused by the hemolytic activity of these preparations.

As can be seen in Fig. 2 the extent of hemolysis was highly dependent on the ATP content of the cells. About 70% hemolysis was obtained when the phospholipase C preparation was incubated with fresh chicken erythrocytes which contained about 0.8 μmole of ATP per ml of packed cells. After 4 h of incubation with NaCN and NaF, the ATP content decreased to about 0.15 μmole ATP per ml of packed cells and the hemolysis to about 35%. When at this point the cells were washed free from the inhibitors and incubated for 4 h in ATP restoration medium, the ATP content increased to 0.7 μmole per ml of packed cells and hemolysis reached the original value (Fig. 2).

Similar results were obtained when restoration was performed after 9 h of incubation in ATP depleting medium. After 18 h of incubation in the presence of glycolytic and respiratory inhibitors, the erythrocytes were completely depleted of ATP and practically resistant to lysis. It is unlikely that the inhibition was due to a specific effect of NaCN and NaF because a variety of glycolytic and respiratory inhibitors exhibited similar effects, namely incubation of chicken erythrocytes in their presence resulted in inhibition of hemolysis. As can be seen in Table I, hemolysis

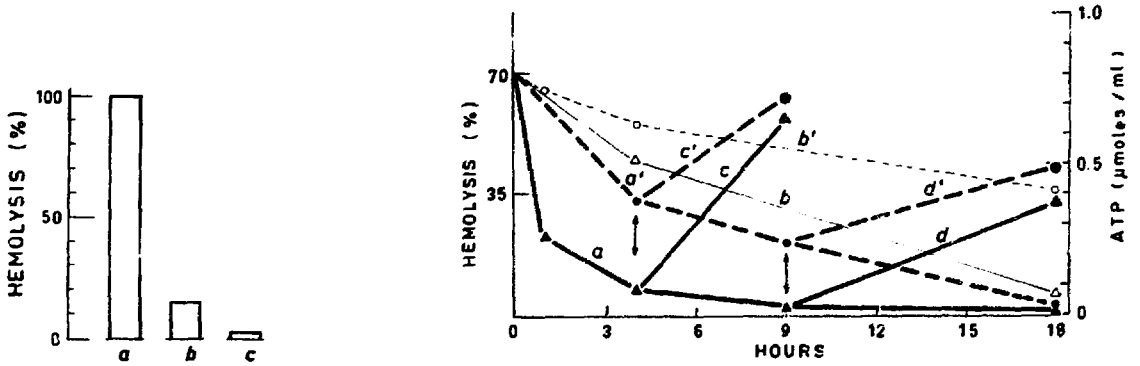

Fig. 1. Inhibition of hemolysis in ATP-depleted cells. Depletion of ATP was performed by incubation of 100 ml of 2% (v/v) chicken erythrocytes, suspended in Solution K to which KCN and NaF or dinitrophenol were added, at 37 °C for 24 h. At the end of the incubation period, cells were centrifuged and washed five times with Solution K, at room temperature. The final pellet was suspended in Solution K to give 2% (v/v), and hemolysed by the phospholipase C preparation as described before[16]. The extent of hemolysis during the depletion never exceeded 5%. Concentrations: Phospholipase C, 0.7 μg/ml; dinitrophenol, 0.05 mM; NaCN, 1 mM; NaF, 20 mM. a, Fresh cells; b, cells depleted of ATP by dinitrophenol; c, cells depleted of ATP by KCN and NaF.

Fig. 2. The correlation between ATP level and hemolysis by phospholipase C preparations. 400 ml of 2% (v/v) chicken erythrocytes were depleted of ATP by incubation with NaF and NaCN, and hemolysed by phospholipase C as described in Fig. 1. At the points indicated by arrows, samples of about 70 ml were removed, washed 5 times in Solution K and the final precipitate was suspended in ATP restoring medium (see Methods), and incubated again at 37 °C. Phospholipase C concentration was 0.7 μg/ml. a, ATP content in cells incubated with NaF+NaCN; a', hemolysis of cells incubated as in a; b, ATP content of cells incubated in Solution K only; b', hemolysis of cells incubated as in b; c, d, ATP content of cells after incubation in restoring medium; c', d', hemolysis of cells incubated as in c, d.

was inhibited almost completely after 20 h incubation of cells in the presence of inhibitors, and no significant difference could be observed between their effect. Cells in which ATP was depleted by either iodoacetic acid or iodoacetamide failed to undergo hemolysis after incubation in ATP-restoring medium, as opposed to cells incubated with NaCN and NaF or deoxyglucose (Table I). This is probably attributable to irreversible inhibition of glycolysis. Washed cells incubated for 18 h at 37 °C in the absence of inhibitors showed in principle the same tendency as cells incubated in the presence of inhibitors. The ATP of these cells was depleted and the extent of phospholipase C-induced hemolysis decreased, but at a slower rate (see Fig. 2).

It should be pointed out that ATP-depleted human erythrocytes have been shown to be more sensitive to osmotic shock hemolysis as compared to fresh cells[14]. Therefore, it was of interest to study whether the above-mentioned inhibition of hemolysis in ATP-depleted chicken erythrocytes is a phenomenon which will be shared by many hemolytic agents or is restricted only to hemolysis by phospholipase C preparations (*Cl. perfringens*).

Indeed, as is demonstrated in Fig. 3, only hemolysis by phospholipase C preparations was decreased after ATP depletion while other lytic agents showed the opposite tendency. As can be seen in that figure, hemolysis by prymnesin, lysolecithin, osmotic shock and by the Sendai virus increased considerably in ATP-depleted cells, while the opposite was true for hemolysis induced by phospholipase C preparations.

TABLE I

INHIBITION OF HEMOLYSIS INDUCED BY PHOSPHOLIPASE C PREPARATIONS IN CELLS INCUBATED IN THE PRESENCE OF VARIOUS RESPIRATORY INHIBITORS

ATP was depleted as described in Methods by incubation of cells suspended in Solution K which contained the various respiratory inhibitors. Phospholipase C concentration was 1 μg/ml. The degree of hemolysis in fresh cells was 79%.

System	*Hemolysis* (%)		
	3 h in ATP depleting medium	*20 h in ATP depleting medium*	*17 h in ATP restoring medium of cells depleted for 3 h*
Solution K	61	—	62
1 mM KCN	58	2	71
20 mM NaF	59	12	61
1 mM KCN+20 mM NaF	52	0	65
1 mM iodoacetic acid	28	6	7
5 mM iodoacetamide	71	3	3
5 mM deoglucose	30	12	60
1 mM KCN+1 mM iodoacetic acid	22	3	2
5 mM Inosine+5 mM iodoacetamide	25	4	2
5 mM glucose+5 mM iodoacetamide	46	4	2

It should be added here that external ATP failed to replace the effect of the internal pool of ATP. ATP-depleted cells remained resistant to hemolysis by phospholipase C preparations even after 4 mM Mg–ATP were added to the incubation medium.

The differential effect of bivalent cations on hemolysis in fresh and ATP-depleted cells

Bivalent cations such as UO_2^{2+}, Mn^{2+}, and Ca^{2+} are known to inhibit hemolysis induced by several lytic agents[9,15].

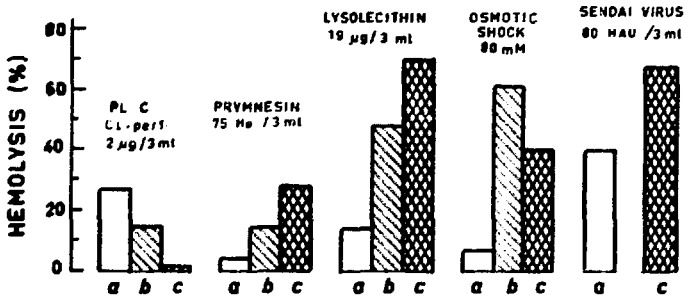

Fig. 3. The effect of various lytic agents on fresh and ATP-depleted cells. ATP was depleted and hemolysis measured as described in Figs. 1 and 2. a, fresh cells; b, cells incubated at 37 °C for 24 h in Solution K; c, cells incubated at 37 °C for 24 h in ATP depleting medium. (see Methods.) PLC, phospholipase C; Ho, hemolytic units; HAU, hemagglutinating units.

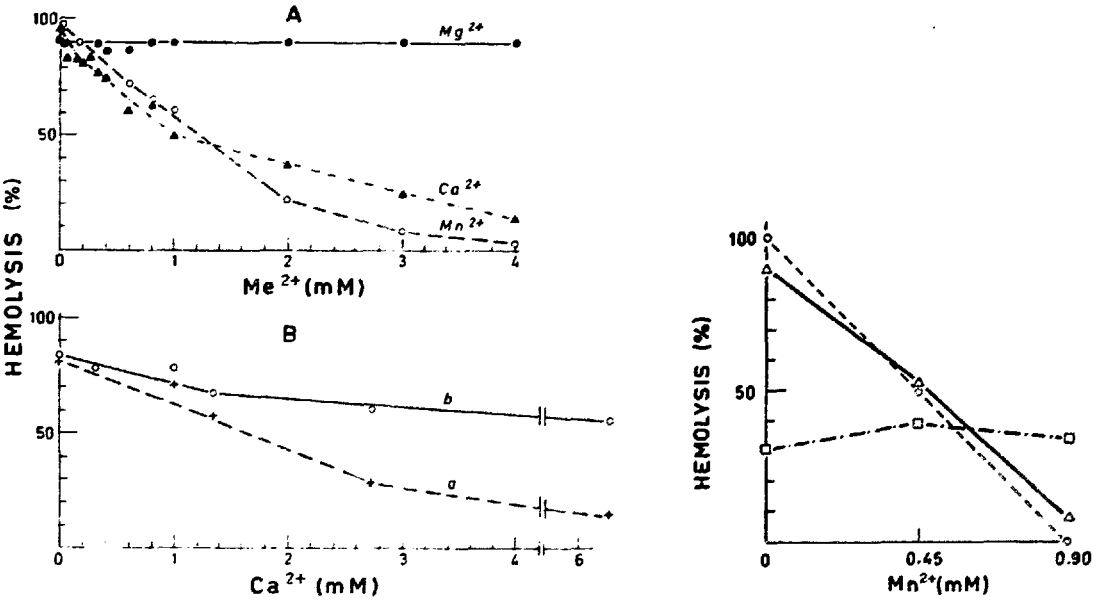

Fig. 4. The effect of bivalent metals on prymnesin-induced hemolysis of chicken red blood cells.
(A) The effect of Mg^{2+}, Ca^{2+} and Mn^{2+}.
(B) The effect of Ca^{2+} on prymnesin-induced hemolysis in ATP-depleted cells. ATP was depleted and hemolysis performed as described under Methods and in Figs 1 and 2. a, cells incubated for 2.5 h at 37 °C in Solution K. b, cells incubated in ATP depleting medium for 2.5 h at 37 °C. Hemolysis was performed in the presence of 50 hemolytic units/ml of prymnesin.

Fig. 5. The effect of Mn^{2+} on hemolysis induced by phospholipase C preparations., ○—○ fresh cells; △—△, cells incubated for 6 h at 37 °C in Solution K; □—·—□, cells incubated for 6 h at 37 °C in ATP depleting medium. Phospholipase C concentration was 1.3 μg/ml.

The inhibition of hemolysis by the above cations is due probably to a fixation of the erythrocyte membrane, which prevents leakage of hemoglobin, and not to an inactivation of the lytic reagent[1]. Hemolysis of chicken erythrocytes by prymnesin is also reduced by bivalent cations such as Mn^{2+} and Ca^{2+}, while Mg^{2+} has no effect (Fig. 4A).

It is worthwhile mentioning that no enzymatic activity has yet been detected in preparations of prymnesin. Its hemolytic effect is attributed to its physical properties[16].

Bivalent cations became less effective as inhibitors of virus-induced hemolysis when added to ATP-depleted cells as was shown previously[9,17]. In the present work it was also found that Ca^{2+} up to 6 mM has little or no effect on prymnesin-induced hemolysis in ATP-depleted cells, whereas it considerably inhibited hemolysis in fresh or control cells (compare Figs 4A and 4B). Despite the fact that bivalent cations stimulate the hydrolytic activity of phospholipase C preparations[2], they considerably reduce hemolysis caused by these preparations in fresh cells (ref. 18 and Fig. 5). As was shown in the present work, hemolysis by phospholipase C preparations was highly inhibited in depleted cells, but surprisingly, as is demonstrated in Fig. 5, its extent was not reduced by bivalent cations. As can be seen in that figure, about 30% hemolysis was obtained in ATP-depleted cells as compared to 100% hemolysis in fresh erythrocytes. In the presence of 0.9 mM Mn^{2+}, hemolysis was reduced practically to zero in fresh erythrocytes, whereas in the depleted cells, hemolysis was even slightly stimulated .So in the presence of 0.9 mM Mn^{2+} a situation appeared in which hemolysis in ATP-depleted cells is higher than in fresh cells, whereas in its absence the reverse phenomenon was observed (Fig. 5). The slight stimulation of hemolysis by Mn^{2+} in depleted cells is probably due to a cation-dependent activity of these preparations which might be associated with its hydrolytic activity.

If the minor stimulation of hemolysis by Mn^{2+} is indeed due to the hydrolytic activity of these preparations, the effect should be more pronounced in the presence of Ca^{2+}. As was shown previously and in the present work, Ca^{2+} was found to

TABLE II

Ca^{2+}-DEPENDENT HEMOLYSIS BY PHOSPHOLIPASE C PREPARATIONS IN ATP-DEPLETED CELLS

Depletion and restoration were performed, and hemolysis was determined as described under Methods. Phospholipase C was added to give a final concentration of 1.6 μg/ml.

System	*Hemolysis* (%)			
	Nonheated phospholipase C		*Heated phospholipase C*	
	$-Ca^{2+}$	$+5mMCa^{2+}$	$-Ca$	$+5mMCa^{2+}$
(1) Fresh cells	45	59	1	1
(2) Depletion, 8.5 h	17	48	3.5	14.5
(3) Depletion, 17 h	7	60	1	36
(4) ATP restoration of (2)	67	52	0	0

be an excellent activator of hydrolysis of membrane phospholipids by *Cl. perfringens* preparations, but less effective than Mn^{2+} in blocking hemolysis[1,2].

Indeed, Table II demonstrates that 5 mM Ca^{2+}, at a concentration which activates hydrolysis of phospholipids by phospholipase C, greatly stimulates hemolysis in depleted cells. In the absence of Ca^{2+}, hemolysis decreased from 45% in fresh cells to 7% in depleted cells, while in its presence practically no change in the degree of hemolysis was observed.

When phospholipase C preparations are heated for 10 min at 56 °C a preferential inactivation of the hemolytic activity takes place while its hydrolytic activity remains intact[1]. Hemolysis of fresh chicken erythrocytes by the heated enzyme, either in the presence or in the absence of bivalent metals, was virtually absent (Table II). However, the heated enzyme was able to hemolyse ATP-depleted cells, but only in the presence of Ca^{2+}. The data presented in this table show that in the presence of 5 mM Ca^{2+} no hemolysis occurred in fresh erythrocytes after incubation with the heated enzyme, while 36% hemolysis was obtained with depleted cells. In the absence of Ca^{2+} hemolysis was practically zero in both fresh and depleted cells. Moreover, as shown in Table II and Fig. 6, repletion of the ATP

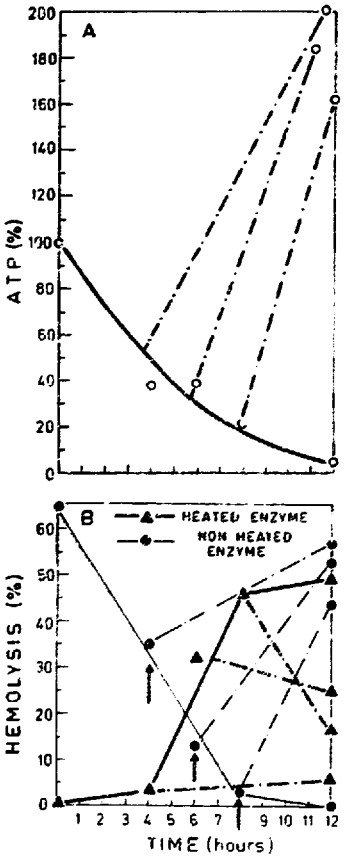

Fig. 6. The behavior of heated and nonheated phospholipase C preparations on cells during depletion and restoration of ATP. (A) ATP was depleted, restored and determined as described under Methods and Figs 1 and 2. ATP content of fresh cells was considered as 100%. (B) Heating of phospholipase C preparations and hemolysis were performed as described under Methods. Concentration of the heated and nonheated enzyme was 1.6 μg/ml. ●, hemolysis by the nonheated preparation (in the absence of Ca^{2+}). △, hemolysis by the heated preparation. Hemolysis was performed in the presence of 5 mM Ca^{2+}. At the times indicated by arrows, samples were removed for restoration of ATP as described in Figs 1 and 2.

content restores the hemolytic effect of the nonheated preparation and inhibits hemolysis by the heated preparation. By incubation of the depleted erythrocytes in ATP restoring medium they become again resistant to the Ca^{2+}-dependent hemolysis by the heated enzyme and sensitive to hemolysis by the nonheated preparation. Thus the hemolytic activity of these preparations, which is expressed in the absence of bivalent cations, seems to act under conditions opposite to those required for its hydrolytic activity. The first causes hemolysis only in fresh cells while the latter, which exists in the heated enzyme, hemolyzes only depleted cells.

Although the heated enzyme was fully active in hydrolyzing micellar phospholipids or membrane phospholipids of hemolyzed cells, it failed to hydrolyze them in intact chicken erythrocytes[1]. However, as is shown in Table III, the heated enzyme does hydrolyze membrane phospholipids in intact ATP-depleted cells. Only 6% of membrane phospholipids were hydrolyzed by the heated enzyme when incubated with fresh cells, whereas 38% and 61% of membrane phospholipids were hydrolyzed by this preparation after 12 and 24 h of ATP depletion, respectively. Hemolysis under the above conditions reached 1% in fresh cells and 64% and 62% in the depleted cells.

TABLE III

HYDROLYSIS OF PHOSPHOLIPIDS BY HEATED PHOSPHOLIPASE C

Depletion and restoration were performed as described in Figs 1 and 2. Incubation with phospholipase C took place in the presence of 5 mM Ca^{2+} in an erlenmeyer flask containing, 75 ml of 2% (v/v) washed fresh chicken erythrocytes. After 60 min of incubation at 37 °C with the enzyme, the cells were centrifuged for 40 min at 37000 × *g*. Hemolysis was assayed by determining the adsorbance of the supernatant at 540 nm. The pellet obtained was resuspended in 5 ml of Solution K and phospholipids were extracted from the suspension according to Burger *et al.*[10]. Phospholipase C was added to give a final concentration of 1.6 μg/ml.

System	*Hemolysis* (%)	*Phospholipids hydrolysis* (%)
Fresh cells	1	6
Depletion, 12.5 h	64	38
Depletion, 24 h	62	61

DISCUSSION

Experiments described in the present work raise the possibility that the chicken erythrocyte membrane undergoes structural changes which are dependent on the metabolic state of the erythrocyte. Several lines of evidence have shown that phospholipase C preparations (*Cl. perfringens*) contain at least two different activities, both of them leading eventually to hemolysis of the cells[1]. These activities have been used in the present work as an indicator for detecting the ATP-dependent structural changes of the erythrocyte membrane.

One of the activities of phospholipase C (*Cl. perfringens*) preparations is a hemolytic activity whose action is independent of the presence of bivalent cations.

Hemolysis of chicken erythrocytes by this hemolytic activity was greatly reduced in ATP-depleted cells. This was probably not due to degradation of the hemolysin sites, since the inhibition was found to be reversible. This reversibility cannot be explained by a rapid resynthesis of the protein of the hemolysin sites, because mature chicken erythrocytes are incapable of protein synthesis. Moreover, cycloheximide and chloramphenicol had no effect on the reversibility, and cells became sensitive again to hemolysis after ATP was restored even in the presence of the above inhibitors (Gazitt, Y., unpublished results).

The second activity, which also led to hemolysis, and might be associated with hydrolysis of phospholipids, is absolutely dependent on bivalent cations and especially on Ca^{2+}. The fact that by heating of the phospholipase C preparations in the presence of Ca^{2+} we have succeeded in blocking the first hemolytic activity while the second remained active, enabled us to study the second activity without interference by the first. The heated enzyme failed either to hemolyse or to hydrolyse membranes of fresh chicken erythrocytes. As already reported[4,18], the fact that hydrolysis did not occur might be explained by the fact that the membrane phospholipids are masked and thus inaccessible to the heated nonhemolytic enzyme. However, the heated enzyme could hemolyse ATP-depleted chicken erythrocytes in the presence of Ca^{2+}. Interestingly, this process was also reversible and the heated enzyme failed to hemolyse cells in which ATP was restored.

The hemolytic action of the heated enzyme might be attributed to an activity which hydrolyses membrane phospholipids. Hydrolysis of phospholipids by most of the known phospholipases is highly dependent on bivalent cations and especially on Ca^{2+} (refs 1 and 2). Indeed, the heated enzyme hydrolyses membrane phospholipids from ATP-depleted cells only in the presence of Ca^{2+}. However, it should be mentioned here that another highly active bacterial phospholipase C, that of *B. cereus*, failed to hydrolyse (or to hemolyse) membrane phospholipids either of fresh or ATP-depleted intact cells (Gazitt, Y., unpublished results). Therefore, the possibility that lysis of ATP-depleted cells by the heated enzyme is due to an additional activity, yet unknown, should not be dismissed.

Similar to the case with the fresh cells[2], hydrolysis of membrane phospholipids in ATP-depleted cells by the heated enzyme could also be the consequence of hemolysis which exposes the buried phospholipids. The hemolytic activity might be a prerequisite for hydrolysing membrane phospholipids and not its cause. Nevertheless, whatever the activity of the heated enzyme might be, it hydrolyses phospholipids only of ATP-depleted cells, and not of fresh cells.

Thus, it might be speculated that another hemolytic site exists on the erythrocyte membrane, which is cryptic in the fresh cells and exposed in the depleted cells. Reversible masking of membrane phospholipids might account for all the above observations.

ATP might cause conformational changes in the erythrocyte membrane by phosphorylation of its proteins or phospholipids. It was shown by Redman and Colvin[19] that human erythrocyte phospholipids might be phosphorylated when incubated in hypotonic media. ATP-dependent conformational changes as measured by ORD have been demonstrated by Graham and Wallach[20]. Of most interest is a recent report concerning cyclic AMP-dependent phosphorylation of human erythrocyte membrane[21]. Such modifications may alter the mobility

of the phospholipid phase within the membrane to permit a higher degree of hemolysis after interaction with the enzyme preparations. Alternatively, metabolically induced changes in the K_m of phospholipids may increase their susceptibility.

Masking and unmasking of sites located on the surface of chicken erythrocyte membrane whose changes are regulated by a phosphorylation–dephosphorylation mechanism seems also an appropriate explanation for the results described above. Experiments are under way to reveal such phosphorylation under the conditions described in the present work.

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