FORMATION OF POLYNUCLEATE AVIAN ERYTHROCYTES BY POLYLYSINE AND PHOSPHOLIPASE C

Enne DE BOER* and Abraham LOYTER
Department of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel

Received 22 April 1971
Original figures received 25 May 1971

1. Introduction

It is well established that myxoviruses of group I induce fusion of mammalian cells thus forming homo- and heteropolykaryons [1, 2]. Fusion could be obtained in cell suspensions only after agglutination of the cells in the cold. The clumped cells fuse when transferred to 37°.

In a previous communication we showed that many polynucleate cells are formed from chicken erythrocytes under conditions in which the virus-induced hemolysis is greatly reduced by suitable bivalent cations [3]. Furthermore, we could show that fusion might take place in the absence of added virus. Calcium ions at pH 10.5 bring about the agglutination of avian and human erythrocytes in the cold, which is then followed by lysis and fusion at 37° [4].

It would seem that controlled lysis of agglutinated cells leads to fusion of membranes which are in contact with each other. Further support for this hypothesis is provided in the following study which shows that fusion of chicken erythrocytes can be obtained by a mixture of polylysine which causes agglutination [5] and phospholipase C from *Cl. prefringes*, which is known to lyse red blood cells [6]. The possibility is raised, however, that another activity besides lysis is involved in fusion.

2. Materials and methods

Red blood cells were collected from the necks of decapitated chickens and washed as previously described [3].

Hemolysis by phospholipase C was obtained by introducing 2 ml of a 2% suspension of washed cells in buffered solution containing KCl 140 mM, NaCl 5.4 mM, $MgSO_4$ 0.8 mM and tricine-NaOH pH 7.4 20 mM into a 20 ml glass flask. To the suspended cells, phospholipase C and $MnCl_2$ of various concentrations dissolved in the above buffered solution, were added to give a final volume of 3 ml. The subsequent steps were as previously described [3].

The cell fusion reaction mixture contained 0.5 ml of 5% washed chicken erythrocytes and 0.5 ml of a mixture containing 1.3 μg phospholipase C from *Cl. prefringes*, 60 μg polylysine of molecular weight 7315 and 0.5 mM $MnCl_2$ dissolved in the above buffer. The mixture was introduced into a 20 ml glass flask and was incubated in a water bath at 37° with gentle shaking for 40 min. After 40 min the suspension was cooled and samples were taken for examination in the phase microscope, or for fixation for electron microscopy.

Polylysine of molecular weight 7315 was obtained from Miles-Yeda, phospholipase C of *Cl. prefringes* was obtained from Worthington. Protein was determined by the method of Lowry et al. [7].

3. Results

The hemolytic activity of phospholipase C from

* Present address: Agricultural University, Wageningen, Holland.

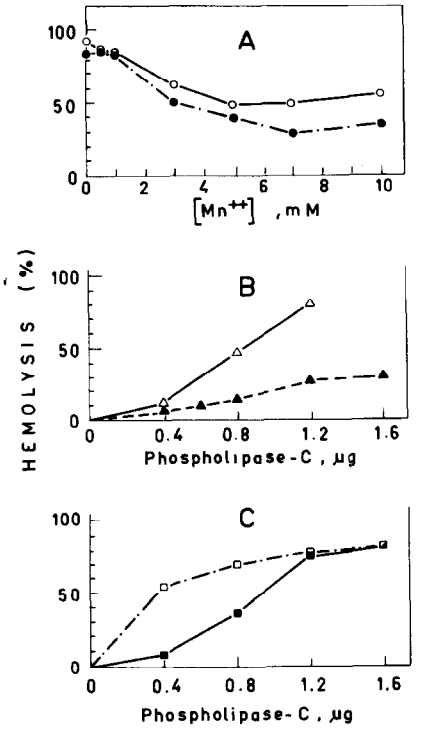

Fig. 1. Effect of $MnCl_2$ and $CaCl_2$ on phospholipase C (*Cl. prefringes*) induced hemolysis of chicken erythrocytes For experimental conditions see Materials and methods.
A) Hemolysis as a function of $MnCl_2$ concentration. ●---● 1.5 µg phospholipase C; ○——○ 1.75 µg phospholipase C.
B) Inhibition of phospholipase C induced hemolysis by $MnCl_2$. ▲- - -▲ 10 mM $MnCl_2$; △——△ without $MnCl_2$.
C) Enhancement of phospholipase C induced hemolysis by $CaCl_2$. □-·-□ 10 mM $CaCl_2$; ■——■ without $CaCl_2$.

Cl. prefringes is greatly reduced by some bivalent cations. $MnCl_2$ inhibits hemolysis in the range of 2–8 mM (fig. 1) while UrO_2 $(CH_3COO)_2$ was found to be an efficient inhibitor in the range of 0.1–1 mM. It should be mentioned that in contrast to the hemolytic activity the lipolytic activity of this enzyme (as measured by removal of phosphoryl choline from lecithin) is dependent on certain bivalent cations [8]. Indeed, not all the cations reduce the enzyme induced hemolysis, since, in fact, Ca^{2+} enhances it (fig. 1).

When polylysine, which is known to agglutinate red cells was added together with phospholipase C from *Cl. prefringes* to a suspension of chicken erythrocytes and incubated at 37° many polynucleate cells were formed after 40 min of incubation (fig. 2).

It is noteworthy that fusion takes place only in the presence of 0.3–0.7 mM $MnCl_2$ although at that concentration no inhibition of lysis occurs (figs. 1 and 3).

In the presence of EDTA (which abolishes phospholipase activity) or the absence of $MnCl_2$, fusion did not occur, although in these conditions, the extent of lysis is the same as in the presence of 0.5 mM $MnCl_2$ (fig. 3).

4. Discussion

For reasons of simplicity it appears that the fusion of erythrocyte plasma membranes may serve as a good system for elucidating the mechanism of fusion.

In contrast to virus-induced fusion in the present system, agglutination can be dissociated from lysis and fusion. In this system the correlation between fusion, phospholipase activity and hemolysis can be studied quantitatively.

Poole who has shown that lysolecithin induces fusion of avian erythrocytes at pH 5.7, claims that lysis under certain conditions will lead to fusion [9]. Indeed, it might be possible that the product of phospholipase C has detergent-like activity and induces fusion. From the present work, however, it may be inferred that hemolysis is not the only factor involved in fusion. If fusion were simply dependent upon lysis as Poole states [9] we would have observed fusion in the presence of EDTA or in the absence of $MnCl_2$ conditions in which hemolysis takes place. Nonetheless, polynucleate cells were formed only in the presence of $MnCl_2$.

Bivalent cations are activators of phospholipase C activity, and it might be possible that in addition to the hemolytic activity of the enzyme, lypolysis also plays a role in the fusion process. It is not clear at this stage whether lipolysis and hemolysis are due to the same enzyme or to different enzymes [6].

The specificity of virus agglutinins is due to the glycoproteins of the virus envelope and the plasma membrane. On the other hand, aggluti-

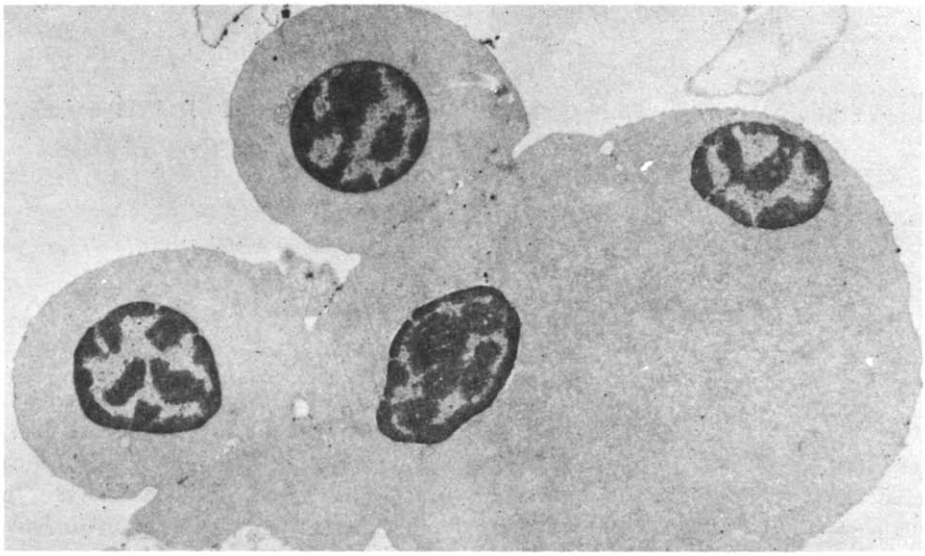

Fig. 2. Electron micrograph of fused chicken erythrocytes. Fused cells were removed after 40 min from the 37° incubation bath and centrifuged at 500 *g* for 10 min. The pellet obtained was fixed over night in the cold with 2% OsO_4 in a final concentration of 0.2 M sucrose. The fixed cells were centrifuged, dehydrated by transfer to increasing ethanol concentrations and embedded in Epon as described by Luft [10]. The sections were cut on an LKB Ultrome III.

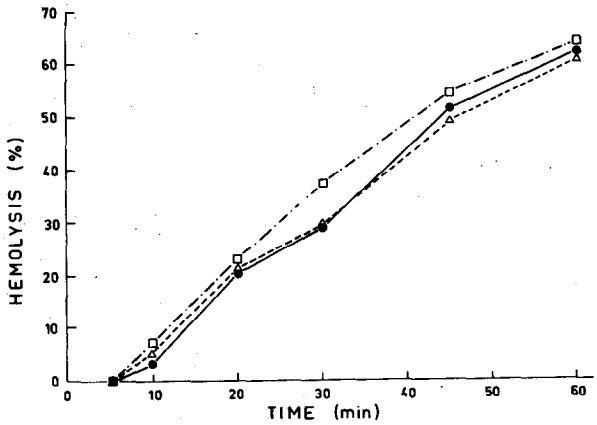

Fig. 3. Effect of EDTA on phospholipase C induced hemolyses. △- - -△ 0.5 mM $MnCl_2$; □—·—□ 2 mM EDTA; ●——● without $MnCl_2$.

nation by polycations is much less specific and is mediated by the negative charges of the membrane surface.

It seems to us that fusion by phospholipase C of cells agglutinated by polycations might be of general use in inducing fusion of cells which fail to fuse when treated with virus.

References

[1] Y. Kada, in: Current Topics in Microbiology and Immunology, Vol. 28, eds. W. Arber and W. Braun (Springer-Verlag, Berlin 1969) p. 102.
[2] A. Kohn, Virology 26 (1965) 228.
[3] Z. Toister and A. Loyter, Biochem. Biophys. Res. Commun. 41 (1970) 1523.
[4] Z. Toister and A. Loyter, Nature, submitted for publication.
[5] Y. Marikovsky and D. Danon, Biochim. Biophys. Acta 124 (1966) 154.
[6] A.C. Ottolenghi, in: Methods in Enzymology, Vol. XIV, ed. J.M. Lowenstein (Academic Press, New York, 1969) p. 188.
[7] O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem. 193 (1951) 265.
[8] A.D. Bangham and R.M.C. Dawson, Biochim. Biophys. Acta 59 (1962) 103.
[9] A.R. Poole, J.I. Howel and J.A. Lucy, Nature 227 (1970) 810.
[10] J.A. Luft, J. Biophys. Biochem. Cytol. 9 (1961) 409.