

PREVENTION OF HEMOLYSIS BY BIVALENT METAL IONS DURING VIRUS-INDUCED FUSION OF ERYTHROCYTES WITH EHRlich ASCITES TUMOR CELLS

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Received 14 January 1974

1. Introduction

Chicken erythrocytes have recently been fused to form homopolykaryons with Sendai virus in the presence of bivalent cations [1, 2] or with the aid of lysolecithin [3]. Human erythrocytes could also be fused with each other under similar conditions [4,5]. When adult chicken erythrocytes were fused with other cells only the nuclei of the erythrocyte were incorporated into the heterokaryon while the cytoplasm was lost by lysis [6]. It seemed of considerable potential interest to form heterokaryons of erythrocytes with other cells without hemolysis since this would make it possible to study the effects of erythrocyte cytoplasm on the activity of the nucleus and cytoplasm of other cell types. The aim of the present investigation was to find conditions for fusing erythrocytes with other cells without the loss of erythrocyte cytoplasm. This paper shows that the techniques developed for the formation of homopolykaryons [2,4] may be applied with slight modification to the fusion of chicken and human erythrocytes with other cells without loss of erythrocyte cytoplasm.

2. Materials and methods

The medium (solution K) used for the suspension of the cells and for the fusion process contained 5.35 mM NaCl, 135 mM KCl, 0.8 mM MgSO_4 and 20 mM Tricine-NaOH buffer pH 7.8.

Adult chicken erythrocytes were obtained as described previously [5]. Human blood, type O, aged 4–8 weeks was used in the experiments. The blood

cells of each type were washed three times with solution K. The buffy layer containing white cells was discarded and the pellet was suspended in solution K to give a concentration of 5% (v/v). Ehrlich ascites tumor cells were obtained as described previously [7]. The freshly harvested cells were freed from contaminating erythrocytes and leucocytes by washing three times in the cold (280 g for 5 min) with solution K and finally suspended at a 10% (v/v) concentration in solution K.

Sendai virus was isolated as described previously [2]. The hemagglutinin titre of the virus was determined by Salk's pattern method with 0.5 ml of 0.5% (v/v) chicken red blood cells in phosphate buffered saline at pH 7.0.

The general technique of cell fusion was as follows. The cell suspension in solution K (0.5 ml) was incubated in 25 ml scintillation counter vials for 30 min at 37°C in a rotatory shaking bath at 100 rev/min. The vials were cooled in ice and, after making up the volume to 1.0 ml with solution K containing virus and other appropriate additions, agglutination was allowed to proceed at 0° for 10 min. The suspension was then shaken at 37°C for 1 hr to facilitate fusion and, finally, cooled in ice. The fusion index was calculated as described previously [8].

For determination of the degree of hemolysis, a sample of the fusion system was centrifuged at 730 g and the absorbance of the supernatant at 540 nm was measured. The absorbance resulting from 100% hemolysis was determined on a sample of the erythrocytes used in the same experiment, suspended in water and treated with 2 drops of concentrated ammonia.

Tricine was obtained from Calbiochem. All other chemicals used were the purest commercial grade available.

3. Results

3.1. Effect of Mn^{2+} on virus-induced fusion of chicken erythrocytes with Ehrlich ascites tumor cells

Fig. 1 shows that 0.5 to 2 mM Mn^{++} prevents hemolysis of erythrocytes during virus-induced fusion of erythrocytes with each other or with Ehrlich ascites cells. Fig. 2 shows the effect of increasing virus concentration on heterokaryon formation and hemolysis at two Mn^{2+} concentrations. At 1 mM Mn^{2+} efficient heterokaryon formation with a high fusion index and virtual prevention of hemolysis was obtained over a wide range of virus concentrations. In the presence of 0.5 mM Mn^{2+} , on the other hand, both fusion and hemolysis increased with virus concentration. The heterokaryons formed between erythrocytes and Ehrlich ascites cells in presence of 2 mM Mn^{2+} contained both erythrocyte nuclei and cytoplasm [9].

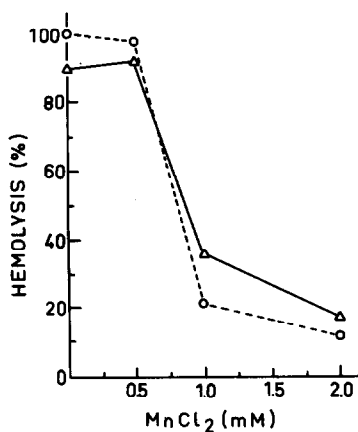


Fig. 1. Inhibition of hemolysis by Mn^{2+} during virus-induced formation of chicken erythrocyte homopolykaryons or heterokaryons with Ehrlich ascites cells. Chicken erythrocytes alone (0.5 ml 5% (v/v)), (○—○), or a mixture of 0.3 ml 5% (v/v) chicken erythrocytes and 0.2 ml of 10% (v/v) Ehrlich ascites cells, (△—△), were treated with 2450 hemagglutinating units/ml of Sendai virus in the presence of different concentrations of $MnCl_2$. For other details see Materials and methods.

3.2. Effect of UO_2^{2+} on virus-induced fusion of human erythrocytes with Ehrlich ascites tumor cells

Lysis of human erythrocytes was prevented by UO_2^{2+} when Ehrlich ascites tumor cells and human erythrocytes were incubated together in the presence of Sendai virus (fig. 3). There was a narrow optimal range of UO_2^{2+} concentration (1.3 to 1.5 mM) within which fusion was efficient and hemolysis was low. At UO_2^{2+} concentrations below about 1.2 mM erythrocytes fused with Ehrlich ascites cells but lost their cytoplasm. At a concentration of UO_2^{2+} of about 1.5 mM the lysis of erythrocytes was reduced to about 10% and fusion with Ehrlich ascites cells was observed with retention of most of the erythrocyte cytoplasm. When the UO_2^{2+} concentration was above 1.6 mM hemolysis was almost completely inhibited but the Sendai virus caused only agglutination and no fusion. Mn^{2+} (2 to 5 mM) did not prevent the hemolysis of human erythrocytes during virus-induced fusion.

4. Discussion

In the present study hemolysis during Sendai virus-induced fusion was prevented by bivalent ca-

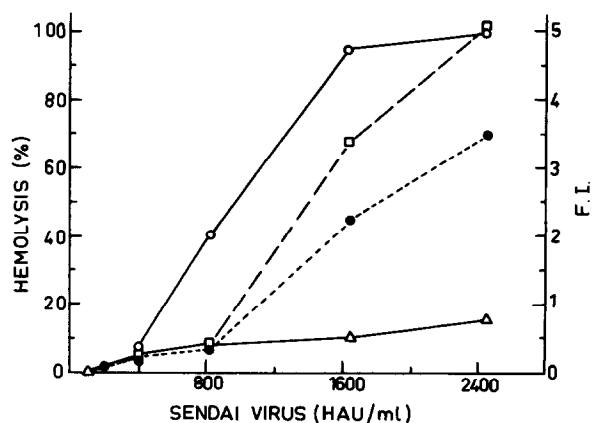


Fig. 2. Effect of Mn^{2+} concentration on virus-induced hemolysis of chicken erythrocytes and their fusion with Ehrlich ascites cells. A mixture of 0.3 ml 5% (v/v) chicken erythrocytes and 0.2 ml 10% (v/v) Ehrlich ascites cells was treated with Sendai virus under fusion conditions. Hemolysis in the presence of (○), 0.5 mM $MnCl_2$; (△), 1.0 mM $MnCl_2$. Fusion index in the presence of (□), 0.5 mM $MnCl_2$; (●), 1.0 mM $MnCl_2$.

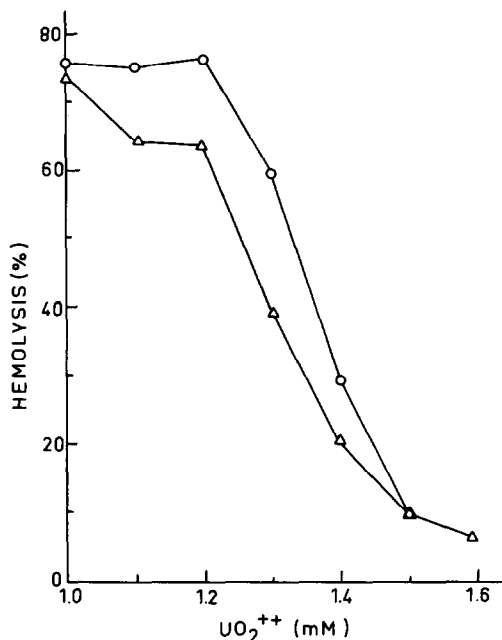


Fig. 3. Inhibition of hemolysis by UO_2^{2+} during Sendai virus-induced fusion of human erythrocytes and Ehrlich ascites cells. A mixture of 0.2 ml 5% (v/v) human erythrocytes and 0.3 ml 10% (v/v) Ehrlich ascites cells was used. (Δ), 820 hemagglutinating units/ml; (\circ), 1640 hemagglutinating units/ml of Sendai virus. For other details see Materials and methods.

tions at certain critical concentrations. Bivalent cations are known to prevent hemolysis by lytic agents [10,11] as well as the release of certain membrane-bound enzymes from erythrocytes [12]. The above results are, in fact, a logical extension of earlier experiments in which bivalent cations were shown to reduce virus-induced hemolysis and to promote the fusion of chicken [2] and human erythrocytes [4]. The findings of this paper support the previous hypothesis that bivalent metal ions facilitate fusion by inhibiting lysis [2]. It seems, however, that some degree of hemolysis is necessary for fusion since high concentrations of UO_2^{2+} , which completely inhibit hemolysis, also inhibit fusion.

The above data show that under suitable conditions both adult chicken and human erythrocytes can be fused with another type of cell with almost complete retention of the erythrocyte cytoplasm. Some of the biological properties of the heterokaryons obtained in this way are described and discussed elsewhere [9]. In earlier experiments on the virus-

induced fusion of adult chicken erythrocytes with other cells, the erythrocyte nucleus was incorporated into the heterokaryon, but the erythrocyte cytoplasm was lost by lysis [6]. Only immature erythrocytes from 3 to 5 day old chick embryos could be fused with other cells without loss of erythrocyte cytoplasm [13]. The successful fusion of whole chicken erythrocytes with cells of another type makes it possible to examine the interactions of the nuclei of both types of cells with a mixture of the two types of cytoplasm. Hitherto it has been possible to study only heterokaryons containing adult erythrocyte nuclei but not erythrocyte cytoplasm [6,13]. Another finding of the present investigation which might be useful is the fusion of human erythrocytes, which are unnucleated cells with nucleated cells. This system makes it possible to study the effect on a cell of introducing a foreign cytoplasm without the introduction of foreign nucleus. The importance of studying such cytoplasmic hybrids has been discussed by Poste and Reeve [14] who have fused enucleated cells with nucleated cells.

Acknowledgement

This work was supported in part by a grant from the Israel Cancer Association.

References

- [1] Okada, Y. (1969) in: Current topics in microbiology and immunology (Arber, W., and Braun, W., eds.) Vol. 28, p. 102, Springer Verlag, Berlin.
- [2] Toister, Z. and Loyter, A. (1973) *J. Biol. Chem.* 248, 422.
- [3] Poole, A.R., Howell, J.I. and Lucy, J.A. (1970) *Nature* 227, 810.
- [4] Perez, H. and Loyter, A. (1971) Proceedings, 41st Annual Meeting of the Israel Chemical Society, p. 78.
- [5] Bachi, T. and Howe, C. (1972) *Proc. Soc. Exptl. Biol. Med.* 141, 141.
- [6] Schneeberger, E.E. and Harris, H. (1966) *J. Cell Sci.* 1, 401.
- [7] Yanovsky, A. and Loyter, A. (1972) *J. Biol. Chem.* 247, 4021.
- [8] Okada, Y. (1962) *Exptl. Cell Res.* 26, 98.
- [9] Zakai, N., Loyter, A. and Kulka, R.G. (1973) *J. Cell Biol.* in press.
- [10] Frish, A., Gazitt, Y. and Loyter, A. (1973) *Biochim. Biophys. Acta* 291, 690.

- [11] Burt, D.H. and Green, J.W. (1971) *Biochim. Biophys. Acta* 225, 46.
- [12] Burger, S.P., Fujii, T. and Hanahan, D.J. (1968) *Biochemistry* 7, 3682.
- [13] Harris, H. (1970) in: *Cell Fusion, The Dunham Lectures*, P. 52, Clarendon Press, Oxford.
- [14] Poste, G. and Reeve, P. (1971) *Nature New Biol.* 229, 123.