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FUSION OF HUMAN ERYTHROCYTES INDUCED BY URANYL ACETATE AND RARE EARTH METALS

SABITA MAJUMDAR [a], RICHARD F. BAKER [a] and VIJAY K. KALRA [b]

[a] *Department of Microbiology and* [b] *Department of Biochemistry, University of Southern California, School of Medicine, Los Angeles, CA 90033 (U.S.A.)*

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Summary

Incubation of human erythrocytes with either uranyl ions (UO_2^{2+}) or rare earth metals (La^{3+}, Nd^{3+}, Sm^{3+}, Eu^{3+}, Tb^{3+}, Dy^{3+} and Yb^{3+}) at 37°C for 30—45 min resulted in the fusion of erythrocytes. Redistribution of membrane-associated particles was observed using colloidal-iron charge labelling and freeze-fracture electron microscopy. The fusion of erythrocytes induced by these agents, unlike Ca^{2+}, did not exhibit the absolute requirement for phosphate. Moreover, agglutination and fusion by these agents was observed in neuraminidase-treated erythrocytes in contrast to Ca^{2+}- and phosphate-induced fusion. Inhibitors of intrinsic transglutaminase activity partially inhibited (35—45%) the fusion induced by UO_2^{2+} suggesting that cross-linking of membrane proteins results in protein-free areas of lipid where fusion may be initiated.

The fusion of membranes has been well recognized in a large number of in vivo cellular and subcellular processes such as mitosis, fertilization, phagocytosis, etc. Sendai virus has been used as a tool to induce cell fusion [1—5] by many investigators. However, a number of problems are inherent in the use of viruses to shed light on the molecular mechanism of membrane fusion. Firstly, the active component in the virus responsible for cell fusion has not been identified and therefore the potential of a particular virus as a fusogenic agent cannot be assayed [6]. Secondly, it has been observed that the introduction of inactivated viruses can cause metabolic alterations in the cells and stimulate interferon production [7—9] despite complete elimination of viral infectivity. Recently, chemical agents such as lysophosphatidylcholine, retinol, dimethyl-

sulfoxide, poly(ethyleneglycol) and the cationic ionophores have been used as fusogenic agents [10–14]. Studies of Zakai et al. [12] and Baker and Kalra [14] have shown that human erythrocytes can be induced to fuse in the presence of both phosphate and Ca^{2+}. In these studies it was observed that pretreatment of cells with phosphate was absolutely essential for the fusion process induced by Ca^{2+}. However, the molecular mechanism of cell fusion induced by Ca^{2+} and phosphate is not clear.

Studies were undertaken to determine whether lanthanide cations and UO_2^{2+} which are isomorphic substitutes for Ca^{2+} in some biological systems [15], and competitive inhibitors of mitochondrial Ca^{2+} binding and transport [16], affect the fusion process in human erythrocytes. Moreover, the trivalent lanthanides can be used as probes for assessing coulombic interactions during the fusion process since they have very similar chemical properties and their ionic radii and free energies of hydration vary in a graded sequential manner [17]. We report that the rare earth metals and UO_2^{2+} promote the fusion of human erythrocytes. The fusion of erythrocytes by these agents did not require the presence of phosphate as has been observed with Ca^{2+}-induced fusion of human erythrocytes [12,14].

Blood was drawn in heparin from human volunteers and used on the same day or stored in acid/citrate/dextrose media for up to 1 week. It was washed three times with isotonic saline (pH 7.0, 300 mosM). A packed cell volume of 0.1 ml was resuspended to 10% hematocrit with different metal ions in isotonic saline. These cells agglutinated in a few minutes when uranyl acetate (5–10 mM) or lanthanide cations (La^{3+}, Nd^{3+}, Sm^{3+}, Eu^{3+}, Tb^{3+}, Dy^{3+}, Yb^{3+}) (5–10 mM) in saline were added. After 5 min at room temperature the cells were incubated at 37°C for varying periods of time (10–60 min). Fusion was observed after 30 to 60 min at 37°C (Fig. 1) as observed by light microscopy and scanning electron microscopy. The fusion of erythrocytes induced by uranyl acetate (10 mM) and lanthanide ions (10 mM) did not require the presence of phosphate as has been observed for Ca^{2+}-induced fusion in human and chicken erythrocytes [14,18]. It is pertinent to mention that, at low concentrations (0.25–0.75 mM) of uranyl acetate, agglutination was observed but no fusion occurred. Table I shows the effect of various lanthanide ions and UO_2^{2+} on the agglutination and fusion of erythrocytes. Among the lanthanide ions, La^{3+} and Yb^{3+} were the most effective for promoting red cell membrane fusion. Nd^{3+}, Sm^{3+}, Dy^{3+} and Tb^{3+} were moderately effective in inducing fusion. However, no correlation was observed between ionic radii of metal ions and the efficiency of fusion. The fusion efficiency was greater with uranyl acetate than with La^{3+} and Yb^{3+}. Fusion induced by lanthanide ions occurred at both pH 5.0 and 7.0. However, uranyl acetate precipitated above pH 5.0. Therefore, all fusion studies with this ion were carried out at pH 4.5–5.0.

Since sialic acid residues of glycoproteins of erythrocytes have been shown to play an important role in the agglutination and cell fusion induced by Ca^{2+} [18], studies were undertaken to examine whether neuraminidase treatment affected the agglutination and fusion efficiency induced by uranyl acetate. It was observed that neuraminidase treatment of erythrocytes did not affect either the agglutination or fusion induced by uranyl acetate as observed by phase contrast microscopy.

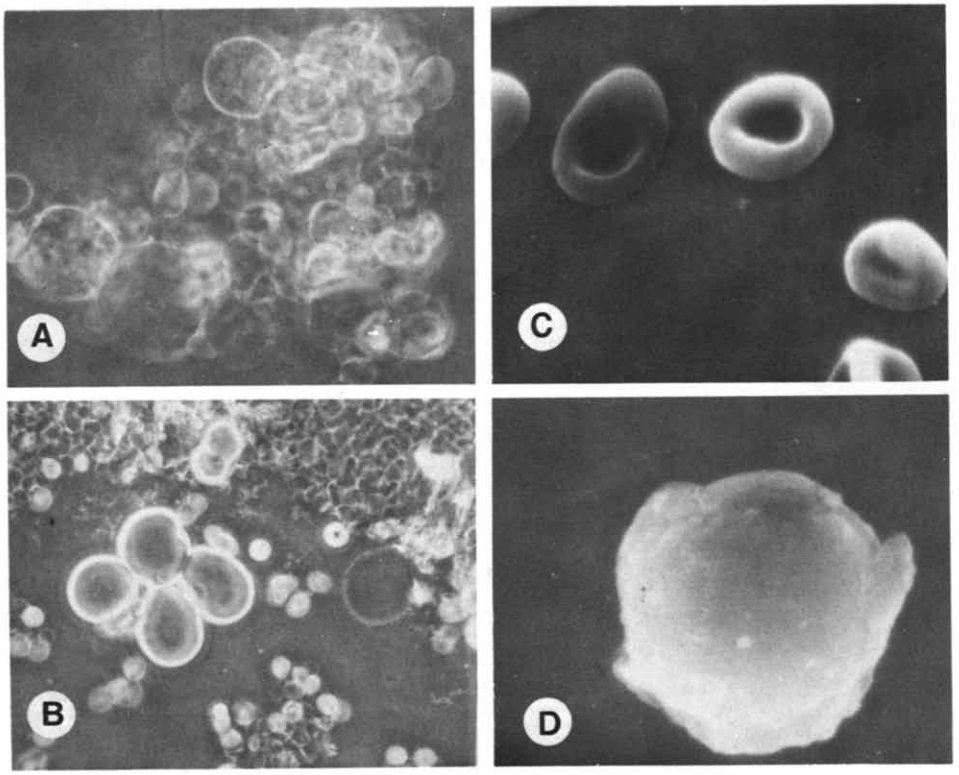

Fig. 1. Human erythrocytes fused by uranyl acetate and lanthanum chloride. Erythrocytes were washed and fused as described in the legand to Table I. Phase contrast micrographs (×500) of cells incubated at 37°C for 30 min: (A) with 10 mM uranyl acetate in saline (pH 4.5, 300 mosM); (B) with lanthanum chloride (10 mM) in saline (pH 5.0; 325 mosM) showing fusion. Scanning electron micrographs (×5000) of; (C) untreated erythrocytes; (D) cells treated with uranyl acetate (10 mM).

TABLE I

AGGLUTINATION AND FUSION OF HUMAN ERYTHROCYTES IN THE PRESENCE OF LANTHANIDES AND UO_2^{2+}

Fresh human blood stored up to 1 week in acid/citrate/dextrose was washed three times with 10 times its volume in isotonic saline. A packed cell volume of 0.1 ml was resuspended to a 10% hematocrit with the indicated metal ion (10 mM) in isotonic saline. Extensive agglutination was observed in all samples except Ca^{2+}-treated cells. After gently vortexing each set, an aliquot of 20 μl was taken from each sample and examined under the phase contrast microscope for agglutination and then the slides were sealed and incubated in an oven at 37—40°C for 30—40 min. The remaining sample was incubated in a water bath at 37°C for 60 min. Agglutination and fusion were estimated by phase contrast microscopy. Agglutination: +, clumps of 5—10 cells; ++, clumps of 10—25 cells; ++++, clumps of more than 100 cells. Fusion: ±, one fused cell in every 30 fields; +, one fused cell in every 20 fields; ++, one fused cell in every 10 fields; +++, one fused cell in each field; ++++, more than two fused cells in each field (×200).

Ion	Ionic radii * (Å)	Agglutination	Fusion
La^{3+}	1.016	++++	++
Nd^{3+}	0.995	++++	+
Ca^{2+}	0.990	—	—
UO_2^{2+}	0.97	++++	++++
Sm^{3+}	0.964	++++	±
Eu^{3+}	0.950	++++	+
Tb^{3+}	0.923	++++	+
Dy^{3+}	0.908	++++	+
Yb^{3+}	0.858	++++	++

* CRC Handbook of Chemistry and Physics, 54th edn., p. F194, 1974.

Studies have established that one of the common steps in fusion by various agents is an aggregation of membrane-associated proteins [19] in the fusing membranes. As shown in Fig. 2, aggregation of these particles, as determined

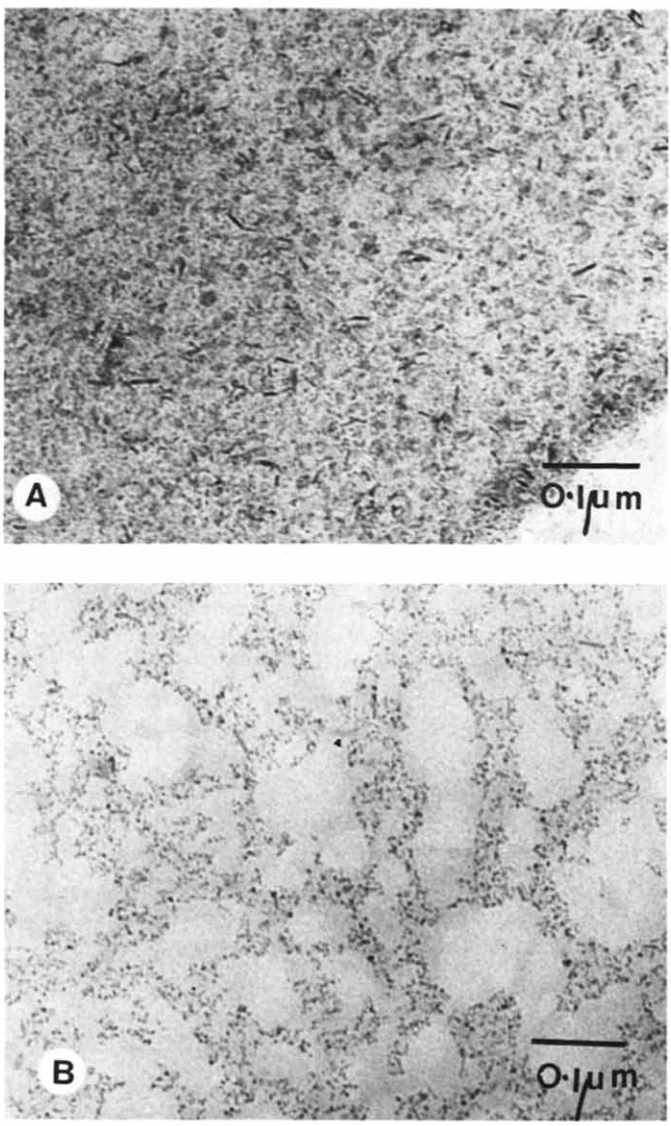

Fig. 2. Electron micrographs of human erythrocyte membrane labelled with colloidal iron hydroxide before and after uranyl acetate treatment. Washed human erythrocytes were resuspended to a 20% hematocrit in isotonic saline. One drop of this suspension was hemolysed on an air/water interface. After standing for 15 min, the membranes were picked up on a 200 mesh formvar-coated copper grid and immediately floated for 30 min on a drop of 10 mM uranyl acetate/saline. The control was floated on isotonic saline. Both sets were incubated for 20 min at 37°C in an oven. The membranes were then fixed by floating on 2% glutaraldehyde in phosphate-buffered saline (pH 7.4, 300 mosM) for 10 min, washed in redistilled water and floated on 5% bovine serum albumin for 1 min before staining with colloidal iron hydroxide for 4 min [20]. The grids were washed with distilled water, air-dried and examined with a Philips 300 electron microscope. A shows a typical random distribution of the charge label on control cells whereas B shows aggregation of membrane-associated protein induced by uranyl acetate.

by colloidal-iron charge labelling [20], was observed in erythrocyte ghosts which were treated with uranyl acetate. Freeze-fracture studies also revealed membrane-associated protein aggregation (data not shown). The aggregation of membrane-associated proteins could be due to either the removal of spectrin or cleavage of spectrin-actin complex as has been suggested by Elgsaeter et al. [19]. Studies of Lalazer and Loyter [21] and Sekiguchi and Asano [22] have shown that fusion of human erythrocytes induced by Sendai virus was inhibited by antibody to spectrin suggesting that membrane cytoskeleton structure was involved in the membrane fusion process. It has been shown that increased levels of Ca^{2+} cause protein cross-linking by activation of an intrinsic transglutaminase [23].

When histamine and cystamine (10 mM), known inhibitors of Ca^{2+}-induced transglutaminase activity in erythrocyte ghosts [23], were added 10—15 min prior to the initiation of fusion by uranyl acetate, the fusion capacity in erythrocytes decreased by 30—45%. This was ascertained by measuring the number of cells taking part in fusion over a given area of slide-coverslip preparation for both control and cystamine-treated cells. Thus, intrinsic transglutaminase may be involved in the cross-linking of membrane proteins resulting in protein-free areas of lipid where fusion may be initiated.

Studies have shown that UO_2^{2+} can open salt bridges [24] at the interface of a lecithin monolayer by bringing about condensation of the monolayer and hence, greater hydrophobic interactions between the alkyl chains of the lecithin molecules. Whether UO_2^{2+} initiates fusion at protein-free areas of phospholipids due to either the condensation of phospholipids in the outer leaflet of the bilayer or by production of fusogenic lipids such as 1,2-diacylglycerol [25] which may affect the organization of both the acyl chains and the polar head groups of phospholipids in the membrane is not clear at the present time.

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