

On the Role of the Cell Coat Glycoproteins in the Permeability of the Cell

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The release of a part of the cell coat of Ehrlich ascites cells by chelation with ethylenediaminetetraacetic acid modifies significantly the uptake of various radioactive molecules. This phenomenon seems to be related to a change in the permeability of the cell membrane. The chemical analyses of the released material appear to indicate that glycoproteins are involved in that change and that their release modifies the structural and physiological characteristics of the cell membrane.

The glycoprotein coat of mammalian cells plays a very important role in many functional and immunological characteristics of the cell¹. There is evidence that alterations of the glycocalyx structure affect all permeability and ion-dependent processes within the cell¹. On the other hand, the chelating agent ethylenediaminetetraacetic acid (EDTA) releases the glycoprotein coating by chelation of the cell surface divalent cations².

The nature of the cell coat materials removed by EDTA and the effects of their removal on the permeability of the cell to various radioactive molecules have been studied in the present experimental work.

Materials and Methods

Eight-day old Ehrlich ascites carcinoma cells, maintained by weekly transplant into MNRI mice, were used for these experiments. Ehrlich ascites cells were labeled *in vivo* by intraperitoneal injection of [³²P]orthophosphate or [³⁵S]sulfate. The procedures for isolation, fractionation and chemical characterization of the labeled phosphocompounds and acid glycosaminoglycans have been described elsewhere^{3–5}. The studies of the uptake of radioactive molecules were carried out by incubation of cells pretreated for 30 min at 36 °C, in phosphate buffered saline PBS (0.01 M phosphate buffer in

0.15 M NaCl containing 5×10^{-3} M Mg²⁺ as MgCl₂), with isotonic sodium salt of EDTA (pH 7.2), or with 0.25% trypsin plus 0.02% DNAase at room temperature. After washed twice with cold saline and resuspended in PBS, aliquots were incubated for 30 min at 36 °C and in PBS, with L-[1-¹⁴C]glucose (10 mCi/mmol), or ⁴⁵CaCl₂ (30 μCi/μg Ca), or ^{99m}Tc-pertechnetate. After incubation, centrifugation and washed twice with cold saline the cells were counted for radioactivity in a gamma scintillation counter (for ^{99m}Tc) or in a liquid scintillation counter (for ¹⁴C and ⁴⁵Ca). Ehrlich ascites cells preincubated with PBS alone were used as a control. Samples of cells stained with ruthenium red were submitted to electronmicroscopic observation.

Results

The pretreatment of the cells with EDTA releases a considerable lower amount of protein, phospholipid phosphorus, total phosphorus and magnesium than the pretreatment with trypsin (Table I). The released amounts of calcium and glycosaminoglycans do not differ significantly in both cases. The chemical composition of the phospholipid was identical in both cases (60% of lecithin and 40% of cephalin), and the phospholipid-³²P expressed as percent of total ³²P was also similar (4.6%). The fractionation of ³⁵S-labeled acid glycosaminoglycans also showed a fairly similar pattern: Low sulfated glycosaminoglycans 53.8% and 56.4% respectively, and chondroitin sulfates plus heparan sulfates 46.0% and 43.2% respectively. The chemical composition of the EDTA-released material was as follows:

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Abbreviations: DNAase, deoxyribonuclease; PBS, phosphate buffered saline; EDTA, ethylenediaminetetraacetic acid; TCA, trichloroacetic acid.

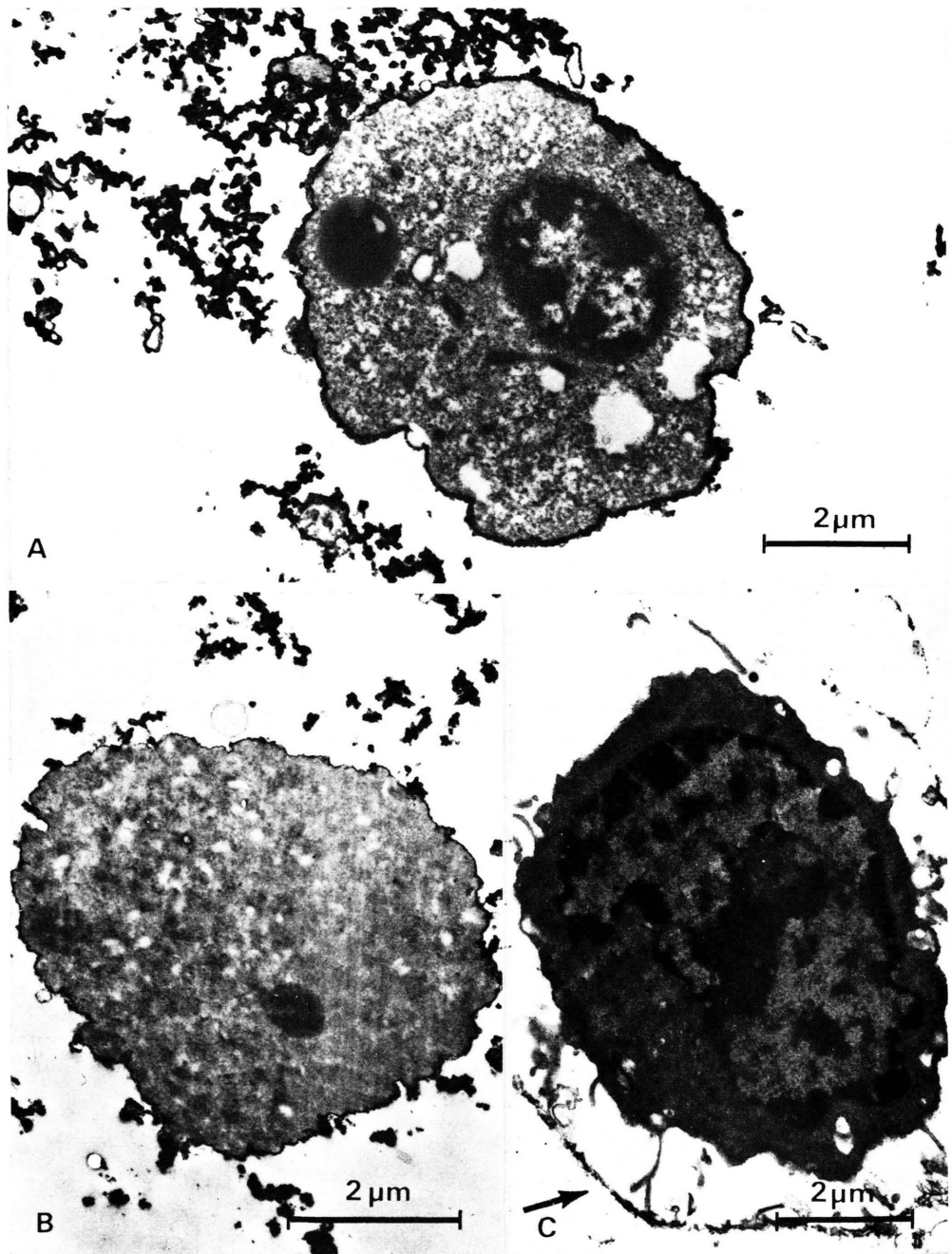


Fig. 1. A. Control Ehrlich ascites carcinoma cell stained with ruthenium red. The cell periphery reveals a continuous electron dense surface coat; B. EDTA treated cell. The cell surface still shows a thin continuous ruthenium red positive coat; C. Trypsin treated cell. An electron dense corona (arrow) which probably represents the stripped off coat encircles the cell.

Table I. Comparative composition of the cell coat material released by EDTA and by trypsin treatments of Ehrlich ascites cells (mean value $\pm$ S.E. of 4 experiments).

A	B	C	D
EDTA			
3.30 $\pm$ 0.54	147.8 $\pm$ 24.3	130.8 $\pm$ 16.7	64.3 $\pm$ 8.29
Trypsin			
6.05 $\pm$ 0.55	169.5 $\pm$ 17.2	120.5 $\pm$ 4.01	506.0 $\pm$ 81.8
Ratio total released by trypsin to total released by EDTA:			
Calcium	Magnesium	Phosphorus ³⁵ S	TCA-insoluble ³² P
Ratio			
1.15 $\pm$ 0.08	2.62 $\pm$ 0.29	5.35 $\pm$ 0.08	1.16 $\pm$ 0.09 4.40 $\pm$ 0.12

A: Percent of total cell protein.
 B: Uronic acid as percent of released by control cells.
 C: Hexosamine as percent of released by control cells.
 D: Phospholipid phosphorus as percent of released by control cells.

polysaccharide (as equimolar mixture of galactose-mannose) 1.34%; phospholipid-phosphorus 0.13%; protein-phosphorus 0.60%; DNA 1.94%; RNA 1.78%; hexosamine 0.19% and protein 58.4%.

The studies of the uptake of radioactivity indicate that the pretreatment with EDTA, contrarily to what occurs with trypsin, reduces considerably the permeability of the cell membrane to the three assayed radiocompounds (Table II). In addition to

Table II. Effects of EDTA and trypsin treatments on the Ehrlich ascites cell incorporation of various radiocompounds (mean value $\pm$ S.E. of the percent of control assay corresponding to 5 experiments).

	L-[¹⁴ C]glucose	[⁴⁵ Ca]chloride	[^{99m} Tc]pertechnetate
EDTA	74.2 $\pm$ 4.1	36.5 $\pm$ 2.5	32.6 $\pm$ 3.1
Trypsin	93.1 $\pm$ 2.8	87.6 $\pm$ 3.9	100.8 $\pm$ 4.9

this, the electronmicroscopic observations show that in comparison to control cells, the EDTA-treated cells present a thinner cell coat which is completely stripped off by trypsin treatment (Fig. 1 *).

Discussion

The rate of uptake of radioactive molecules by the cell is determined by their rates of diffusion

through the plasma membrane. Their flux into the cell can be produced by a passive phenomenon or can be mediated by mechanisms of active transport. As a consequence, when operating under similar experimental conditions, variations of the uptake of radioactivity can be used as indicators of changes in the permeability of the cell membrane.

The experimental results indicate that EDTA releases only a part of the cell coat. The higher amount of protein and phospholipid released by trypsin suggests that the hydrolytic cleavage takes place deep in the cell coat at the plasma membrane level. The electronmicroscopic observations corroborate this hypothesis. The higher proportion of glycosaminoglycans in the material released by EDTA appears to indicate that this chelating agent releases only the more external coat, which is presumably formed by glycoproteins bound to the lipid-protein bilayer of the plasma membrane by cationic bridges. At this respect, it has been reported that plasma membranes are the major locus of binding of circulating glycoproteins, and that this binding exhibits an absolute dependence upon the presence of calcium, and it is carried out by the sialic acid on the membrane⁶. This seems to be the external part of the cell coat released by EDTA in our experiments.

The fact that EDTA releases less cell surface material than trypsin, while it produces a higher decrease of uptake of radioactivity, indicates that a modification of the diffusion of the radioactivity, rather than a loss of cell surface binding sites, is the process involved in the inhibition of the uptake. This assumption is specially valid for L-[1-¹⁴C]-glucose and ^{99m}Tc-pertechnetate which are not significantly bound to the cell surface⁷. These experimental results appear to indicate that in the plasma membrane, and as a consequence of the EDTA-chelation which releases polar macromolecules, changes on the hydrodynamic plane of shear with possible reorientation in its structure take place. These changes produce modifications of its permeability, and consequently of the uptake of radioactive molecules. Alterations in the plasma membrane and in its permeability induced by EDTA have been already observed⁸⁻¹⁰.

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* Fig. 1 see Plate on page 700 b.

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