BINDING OF SERUM POLYPEPTIDES TO THE PLASMA MEMBRANE OUTER SURFACE

G. TARONE and P. M. COMOGLIO
Dept. of Human Anatomy, University of Torino School of Medicine. 10126 Torino, Italy

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1. Introduction

Cell membrane structure has attracted increasing attention since its participation in cell growth, differentiation and metabolism was first demonstrated. Selective labelling of the outer cell surface with chemical probes (for review see [1]) or with enzymes [2–4] has proved one of the most rewarding approaches. This technique is based on the assumption that labelled molecules are membrane components exposed on the outer surface of the cell. Their correlation with the protein molecules that cross the lipid bilayer, however, is often difficult [5,6]. In the same way, attempts to show a correlation between surface antigens or lectin receptors and visibile intramembrane particles by freeze-etching of nucleated cell membranes have proved unsuccesful in many instances [7,8], though this technique has been satisfactory in red cells.

The presence of a coat on the cell surface has long been known; it has also been suggested that proteins foreign to the cell, though adsorbed to its surface, may take part in its formation [9]. For this reason, plasma membrane labelling of cells grown in vitro usually requires careful washings, and controls for the presence of serum proteins of the culture medium adsorbed on the surface [10–12].

Using trinitrobenzene sulphonate (TNBS) as probe, however, we found that a significant amount of the labelled proteins are serum derivatives tightly bound to the membrane surface in the case of hamster fibroblasts cultured with calf serum. These components are polypeptides whose molecular weight differs from that of SDS polyacrilamide electrophoretically demonstrable fresh serum proteins, though they cross-react with the latter immunologically.

This implies that, when probe labelling of cell surface proteins is performed, the non-identity in acrylamide gel electrophoresis between labelled bands solubilized from cells and bands displayed by probe-labelled native serum components is not a proper control to exclude surface adsorbtion.

Fibroblasts belonging to the BHK-C13/21 line were cultured in Eagle MEM with 10% calf serum and plated after resuspension by treatment with 0.25% trypsin at room temperature for 1–2 min. Subconfluent monolayers were carefully washed three times with Earle solution and labelled with TNBS as already described [13–15]. Under the conditions used, 96% of the bound probe was observed on the outer surface of the plasma membrane, where it formed a covalent bond mainly with the ϵ-amino groups of exposed proteins.

We had previously shown this selective labelling with a variety of techniques, including immunofluorescence with anti-probe antibodies, subcellular fractionation and comparison of molecules labelled with TNBS or surface ^{125}Iodination with lactoperoxidase [13–15]. After labelling, cells were solubilized with SDS and the TNP-labelled surface proteins were purified from unlabelled components by affinity chromatography through a column of insoluble anti-dinitrophenyl (DNP) antibodies (table 1). These cross-react with TNP-labelled proteins and are preferred to anti-TNP antibodies because the high affinity of rabbit antibodies for their specific nitrophenylated ligands prevent elution of TNP-proteins except under very strong conditions. Surface TNP-proteins were eluted from the immunoadsorbent with 10 mM DNP–glycine, or by boiling Sepharose bead aliquots in SDS–mercaptoethanol. Their SDS–polyacrylamide gel electrophoretic pattern was

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Table 1
Affinity chromatography purification of solubilized membrane proteins after selective cell surface labelling with TNBS

Membrane proteins solubilized from	Preadsorbed on	Adsorbed on	CPM applied	CPM bound	% of specific binding[c]
a Unlabelled cells	–	IgG-Sepharose[a]	4.25×10^7	0.2×10^6	0
b Unlabelled cells	–	anti DNP-Sepharose	4.1×10^7	0.2×10^6	0
c TNP-labelled cells	–	anti DNP-Sepharose	5.1×10^7	2.6×10^6	4.6
d Unlabelled cells	–	anti CWS-Sepharose	4.8×10^7	2.6×10^6	4.9
e TNP-labelled cells	anti CWS-Sepharose[b]	anti DNP-Sepharose	4.1×10^7	1.1×10^6	2.4

[a] Non-immune rabbit IgG coupled to Sepharose 4B by cyanogen bromide [19].
[b] CWS: calf whole serum
[c] CPM bound by the antibody-Sepharose column after subtraction of the CPM bound by the IgG-Sepharose.

Carefully washed BHK fibroblasts were labelled with 5 mM TNBS for 10 min at 37°C in Earle's solution buffered to pH 7.4. Cells were removed with a rubber policeman and solubilized in 1% SDS. After reduction with β-mercaptoethnaol, 50 mM dithiotreitol for 3 h at 0°C in nitrogen atmosphere and alkylation with twofold molar excess of iodoacetic acid, samples were centrifuged at 150 000 *g* for 1 h. After dialysis against 50 mM Tris-HCl pH 8.0, 0.25 M NaCl, 0.05% sodium desoxycholate, proteins were labelled with ^{125}I by chloramine T[18] and chromatographed trough the Sepharose-antibody column. Non-specific binding sites were presaturated with excess cold membrane proteins solubilized from unrelated cells.

compared with that obtained with calf serum proteins. No significant correlation between the bands was noted. An additional control was, however, made by chromatography of proteins solubilized from the cells with SDS on an immunoadsorbent consisting of anti-calf serum antibodies linked to Sepharose. As can be seen in table 1, a significant percentage of proteins was specifically bound by the immunoadsorbent. About 40% of these were TNP labelled (cf. the result of experiment *c* and *e*). Elution of the immunoadsorbent with SDS–mercaptoethanol at 100°C permitted purification of the serum components bound to the cell surface and their comparison with fresh calf serum proteins in SDS-polyacrylamide gel electrophoresis.

Fig.1 A points to substantial non-identity of the patterns as far as the molecular weight of their respective peaks is concerned. Serum proteins bound to the cell surface migrated to the gel area separating polypeptides with a mol. wt. of less than 60 000. No significant radioactivity peaks were found in this area, even when increasing calf serum concentrations were run. This suggest that the bound components on the fibroblasts surface are fragments of larger proteins, though the possibility that they are the result of selective adsorption of polypeptides present in the serum in low concentration cannot be ruled out. Such polypeptides may be bound to the plasma membrane by interaction with the polar heads of the phospholipids, or directly to the proteins exposed

Table 2
Binding of solubilized membrane proteins to Sepharose-bound serum

Sample	CPM × 10^{-6} applied	CPM × 10^{-6} bound to:	
		IgG-Sepharose[a]	Calf Serum-Sepharose[b]
Bovine Serum Albumine	50.5	0.15 (0.2%)	0.17 (0.3%)
Solubilized Membrane proteins	64.0	0.29 (0.4%)	6.40 (10%)

[a] Non immune rabbit IgG coupled to sepharose.
[b] Serum proteins bound to Sepharose via specific rabbit anti calf serum antibodies covalently linked to the beads.

Membrane proteins were solubilized from BHK cells, ^{125}I labelled and chromatographed through Sepharose columns as described in the legend to table 1.

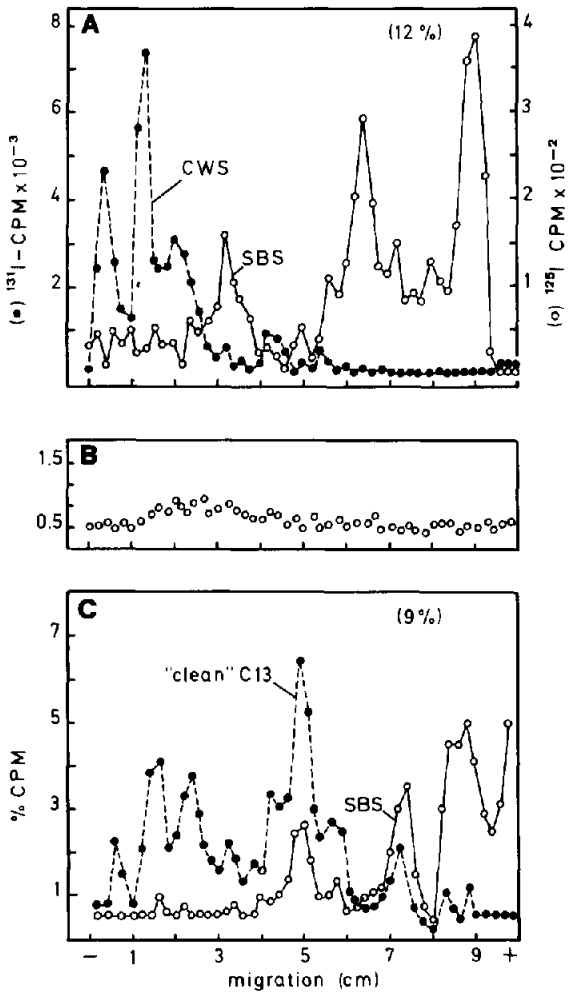

Fig.1. SDS-polyacrylamide gel electrophoresis of BHK surface components. All samples, included calf serum, were solubilized, reduced, alkylated and labelled with ^{125}I or ^{131}I as described in the legend to table 1. After being heated in 5% SDS, 0.1% β-mercaptoethanol at 100°C for 3 min, samples were electrophoresed in 12% (A) or 9% (B and C) acrylamide cilindrical gels as described in detail elsewhere [15]. ^{125}I and ^{131}I-labelled samples were co-electrophoresed in the same gel. Gels were sliced in 2 mm sections and the amount of each isotope was measured in each slice. (A) Patterns of serum components bound and eluted from the anti calf serum immunoadsorbent: (○) surface bound serum (SBS); (•) calf whole serum (CWS). (B) Control pattern of the material solubilized from unlabelled cells non-specifically bound to the anti-DNP immunoadsorbent. (C) BHK surface proteins selectively labelled with TNBS and then purified by the anti-DNP Sepharose column after being 'cleaned' by chromatography through the anti calf serum immunoadsorbent (•). Open circles (○) show cell surface bound serum polipeptides eluted from the latter column.

on the surface. The second possibility is suggested by the results shown in table 2. It will be seen that the solubilized membrane proteins themselves display a certain affinity for calf serum bound to Sepharose via specific antibodies.

Serum polypeptides bound to the cells surface can in any case be removed from TNP-labelled membrane proteins by affinity chromatography on an immunoadsorbent consisting of anti calf serum antibodies. This leads to a further simplification of the electrophoretic patter of the 'true' membrane proteins exposed on the cell surface (fig.1C), which is clearly different to that for the polypeptides derived from the serum. The biological significance of these surface bound serum polypeptides, if any, is obscure. In all events the presence of serum-derived components on the plasma membrane must be borne in mind when investigating the structure or antigen composition of the outer surface of cultured cells. Serum components are, in fact, potential targets for lectins (P.M.C., unpublished), antibodies [16], or sensitized lymphocytes [17], in addition to surface probes.

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