The Distinguishing Characteristics of the Plasma Membrane are its Exposed Proteins

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The exposed proteins of the plasma membrane of normal human lymphocytes and platelets were labelled by using the lactoperoxidase macromolecular probe system. The labelled components were separated into molecular-weight classes by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis. In contrast with the report by Tanner *et al.* (1974), a comparison of the two cell types showed that the major labelled components in both cell types were glycoproteins and were not identical. It is concluded that the exposed proteins are probably the most distinguishing characteristic of the plasma membrane of differentiated cell types.

Although it is reasonable to believe that the plasma membrane, like other subcellular organelles and membranes, has a similar composition and contains many common protein components, this is difficult to establish. The plasma membrane of most cells is very difficult to isolate and purify (DePierre & Karnovsky, 1973; Neville & Kahn, 1974) and efforts to evaluate common features have not been definitive. One approach to a comparison of the plasma membranes that does not require the isolation and purification of the plasma membrane is to use the lactoperoxidase labelling procedure. With this macromolecular probe only the exposed membrane proteins are labelled. In this way it is possible to compare the exposed proteins of the plasma membranes of different cells (Morrison *et al.*, 1974).

Tanner *et al.* (1974) have compared whole-cell proteins in human platelets and human lymphocytes. Their data suggested that the relative amounts of different molecular-weight classes of the major peptides are similar in the two cells. They also reported that all the major surface proteins on the platelet and the lymphocyte detected by lactoperoxidase-catalysed iodination are common between the cells and concluded that these plasma-membrane proteins are not directly involved in each cell's unique functions.

In our laboratory, we have undertaken to compare the surface proteins of a variety of cell types. In contrast with the data of Tanner *et al.* (1974), we have found that the exposed surface proteins of plasma membranes are probably unique to each cell type and reflect the differentiated function of the cell. It is the purpose of this note to present our results comparing platelets and human lymphocytes which show that different proteins are present on the exposed surface of the plasma membrane of these two cells.

Methods

Platelets were isolated from platelet-rich plasma obtained from freshly drawn blood (Mohammad & Glenn, 1974). The platelet-rich plasma was made 10 μ M in ADP, and the aggregates were collected by centrifuging at 100g for 30s. The pellet was rinsed twice with 0.9% NaCl without resuspending it; it was then suspended in 0.9% NaCl containing 1 mg of potato apyrase/ml (Sigma Chemical Co., St. Louis, Mo., U.S.A.) and 2 mM-CaCl₂. The solution was incubated at 37°C for 10min and then the platelets were collected by centrifuging at 1000g for 12min. This pellet was diluted to 10⁹ platelets/ml with Mg²⁺-free Tyrode's solution (Tyrode, 1910) and incubated for 15min at 37°C before iodination. To 10ml of this platelet suspension was added 1.25 mCi of carrier-free ¹²⁵I as iodide (Schwarz-Mann, Orangeburg, N.Y., U.S.A.) and 2.7 nmol of lactoperoxidase (Morrison & Hultquist, 1963). Four 20 μ l portions of 10 mM-H₂O₂ were added at 30s intervals. The platelets were then spun down at 2000g for 10min and washed once with Mg²⁺-free Tyrode's solution. Sodium dodecyl sulphate [4 vol. of a 2% (w/v) solution] was added to this pellet which was immediately mixed and heated at 100°C for 3min. The solubilized sample was diluted 1:10 with a solution of 1% (w/v) sodium dodecyl sulphate–1% (v/v) β -mercaptoethanol–4 M-urea–1 mM-EDTA and again heated at 100°C for 3min before electrophoresis.

Lymphocytes were isolated from freshly drawn blood by defibrinating the blood to remove all the platelets and then settling most of the erythrocytes by making the blood 1.2% in dextran-500 (Sigma). The leucocyte-rich serum was incubated on nylon fibre columns (Leucopak; Fenwal Laboratories, Morton Grove, Ill., U.S.A.) at 37°C for 45min. The nylon fibre was exhaustively washed with distilled

water and dried before it was used. The lymphocytes and remaining erythrocytes were washed from the column with Dulbecco's phosphate-buffered saline (Dulbecco & Vogt, 1954) at 37°C. After lysis of the erythrocytes with a buffered NH_4Cl solution (Roos & Loos, 1970), the lymphocytes were washed three times in phosphate-buffered saline. More than 98% of the cells were viable according to Nigrosine Black extrusion (Martin *et al.*, 1961) and less than 1% of the cells were polymorphonuclear cells as determined from Wright stained smears (Wright, 1902). The cells were diluted to 2×10^8 cells/ml with phosphate-buffered saline and iodinated by adding 1 mCi of ^{125}I and 20 nmol of lactoperoxidase for each ml of cell suspension. Then ten $10\mu\text{l}$ portions of 1 mM- H_2O_2 were added at 1 min intervals. The lymphocytes were spun down at 500g for 5 min and washed three times with phosphate-buffered saline. The final pellet was diluted 1:20 with water, mixed and immediately heated at 100°C for 3 min. To 1 ml of this suspension was added $5\mu\text{l}$ of bovine pancreas deoxyribonuclease I (5 mg/ml; Sigma). After a 30 min incubation at 37°C an equal volume of a solution of 2% (w/v) sodium dodecyl sulphate - 2% (v/v) β -mercaptoethanol-2 mM-EDTA-8 M-urea was added. This mixture was immediately heated at 100°C for 3 min.

The polypeptides in the samples were separated by electrophoresis on 12 cm-long gels (Leonard, 1970). The gels were laterally sliced into 1.5 mm slices and their radioactivity was measured in a γ -spectrometer. Some gels were stained for protein in 0.025% Coomassie Brilliant Blue in 50% (v/v) methanol-10% (v/v) acetic acid. The molecular weights were determined from the position of known marker proteins incorporated into the sample. These proteins were *Escherichia coli* β -galactosidase (Sigma), bovine serum albumin (Sigma) and bovine erythrocyte carbonic anhydrase (Sigma).

Results and discussion

Whole platelets and whole lymphocytes were solubilized and proteins separated into molecular-weight classes by electrophoresis on sodium dodecyl sulphate-polyacrylamide gels. Plate 1 shows that the peptides in these cells are different. In particular the lack of major peptides above 130 000 daltons for the lymphocyte contrasts with the four or five major peptides present in this region for platelets. The absence of major peptides above 130 000 daltons has also been observed when membranes were isolated from human lymphocytes (Lopes *et al.*, 1973). This contrasts with the results of Tanner *et al.* (1974), who found several major peptides above 130 000 daltons in the lymphocyte similar to those in the platelet. The reason for this discrepancy is not immediately

apparent. However, the separation of platelets from lymphocytes by differential centrifugation has been noted to yield lymphocytes contaminated by an equal number of platelets (Sirchia *et al.*, 1972).

The idea that platelets and lymphocytes have similar distributions of proteins in the cell is very attractive. Both cell types arise in the bone marrow from a common precursor and both are found circulating in peripheral blood. However, morphologically, platelets and lymphocytes are quite different.

The lymphocyte, for example, has a large nucleus, relatively little cytoplasm, few mitochondria and other organelles, whereas the platelet has no nucleus and is rich in organelles and granules.

Fig. 1. Distribution on gel electrophoresis of the components labelled by lactoperoxidase of intact lymphocytes (a) and intact platelets (b)

The protein polypeptides were separated as described in the text in sodium dodecyl sulphate-polyacrylamide gels. Each gel contained the marker proteins (1) β -galactosidase and (2) bovine serum albumin, whose positions are indicated by broken lines. Polyacrylamide gels were laterally sliced into 1.5 mm slices and counted for radioactivity in a γ -spectrometer.

EXPLANATION OF PLATE I

Polypeptides of whole lymphocytes and whole platelets

The polypeptides contained in whole lymphocytes (a) and whole platelets (b) were separated into molecular-weight classes by sodium dodecyl sulphate–polyacrylamide-gel (5%, w/v) electrophoresis. The arrows indicate the position of the marker proteins (1) β -galactosidase, (2) bovine serum albumin and (3) carbonic anhydrase. The proteins were stained with Coomassie Brilliant Blue.

It would be surprising therefore in comparing all the proteins in the cells to find the same proteins in anything like the same quantitative distribution.

The components labelled with lactoperoxidase were separated into molecular-weight classes as shown in Fig. 1. The labelling patterns for the two cell types are quite different. In particular, the major labelled peak in lymphocytes is 138 000 daltons, whereas the major labelled peak in platelets is 106 000 daltons. The positions of the labelled peaks were established from the position of known molecular-weight marker proteins and by double-labelling. In the latter case, ^{125}I -labelled lymphocyte proteins were separated by electrophoresis on the same gel with ^{131}I -labelled platelet proteins and the radioactivity in the gel slices was determined on a dual-channel γ -spectrometer. Both methods gave identical results for the relative position of labelled peaks between the two cell types.

The labelled components designated I, II and III in platelets are glycoproteins. This is most convincingly shown for isolated membranes from labelled platelets (Phillips & Agin, 1973). Gels stained for carbohydrate show three well-resolved glycoproteins. Slicing and radioactivity counting of these gels showed that the third glycoprotein corresponds to the most heavily labelled component in intact platelets. It should be noted that the glycoproteins are not always easily identified on gels of solubilized whole platelets (Phillips & Agin, 1973) by periodate-Schiff staining. The absence of the periodate-Schiff stain led Tanner *et al.* (1974) to conclude erroneously that the major labelled component contained little or no carbohydrate.

The major labelled component in lymphocytes has an apparent molecular weight of 138 000 on 5% gels and 110 000 on 7% gels. This anomalous behaviour on sodium dodecyl sulphate-polyacrylamide gels is characteristic of glycoproteins (Phillips & Morrison, 1971; Segrest *et al.*, 1971). In contrast, Tanner *et al.* (1974) suggested that in human platelets the most heavily labelled component contains very little or no carbohydrate.

Platelets incorporate much more label into surface components than do lymphocytes when each are iodinated under comparable conditions. Only a small contamination of lymphocytes by platelets would be sufficient to obtain a platelet-labelling pattern for lymphocytes. Defibrination of the starting blood sample ensured that our lymphocyte preparations were free of platelets (Harris & Ukaejiofo, 1970).

In conclusion, our data show that the major peptides of the platelet and lymphocyte are quite different. This is consistent with the very different morphologies of these cells. Further, our data show that the major exposed surface proteins labelled by

lactoperoxidase are different for these cells. This is consistent with the very different functions of these cells. The lymphocyte and platelet interact with the extracellular environment through their plasma membranes. Lymphocytes are stimulated to proliferate by cross-linking exposed glycoproteins on their surface (Novogrodsky & Katchalski, 1972, 1973*a,b*). The major labelled protein of the lymphocyte may be such a glycoprotein. The platelet-release reaction is central to their unique role in haemostasis. The hydrolysis of labelled component II on the platelet by thrombin induces the release reaction (Phillips & Agin, 1974*a,b*, 1975). Thus the major labelled surface components of the lymphocyte and platelet are involved in the different functions of these cells and are not identical. The exposed proteins are probably the most distinguishing characteristic of the plasma membrane of differentiated cell types.

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