Identification of glycoproteins bearing human blood group A determinants in rabbit enterocyte plasma membranes

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1. INTRODUCTION

The presence or absence of human blood group A-like substances in the intestinal secretions of animals has enabled them to be classified as A^+ and A^-, respectively [1,2]. This A antigenicity is due to the terminal trisaccharide α-GalNAc-[α -Fuc-(1→2)]β -Gal- of O-linked glycans of glycoproteins [3]. The surface of intestinal absorbing cells or enterocytes in A^+ rabbits also bears the A antigeniticy [4].

The plasma membrane of enterocytes is composed of at least two regions characterized by their different morphological aspects and enzymatic equipment well adapted to their specific functions: the brush border membrane at the luminal pole of the cells and on the contra-luminal faces, the lateral and basal membranes. Both of these membranes can be purified [5]. Hydrolases constitute a major glycoprotein class of the brush border membrane that can be solubilized either by neutral detergents or papain treatment of the membrane preparations. Detergents (Triton X-100) or Emulphogen BC 720) extract intact amphiphilic proteins known as detergent-forms while papain only released hydrophilic domains located on the luminal side of the membrane. The hydrophilic protease forms (125 000–250 000 M_r) represent the major part of hydrolases and bear the active sites as well as the sugar moieties, these latter amounting up to 15–25% by wt [6].

The surface of brush border membranes also contains an abundant fuzzy coat or glycocalyx [7]; this carbohydrate-rich constituent, characterized only by electron microscopy, has been considered as the carbohydrate extension of hydrolases [8]. Basolateral membranes contain low quantities of glycoproteins, in particular hydrolases, compared to the brush border membrane [5].

Evidence is presented below for the presence of human blood group A-like determinants on all the major hydrolases. An enterocyte surface antigen, tentatively identified as the remaining glycocalyx part of the membrane preparation, has been characterized through its precipitation by an anti-human blood group A-antibody.

2. MATERIALS AND METHODS

The A^+ or A^- typing of rabbits, and brush border and basolateral membrane preparations have been described [4,5]. Immunoglobulins from the serum of guinea pigs immunized with an Emulphogen extract of membrane preparations were purified as in [9]. Rabbit anti-human blood group A immunoglobulins were prepared with the same technique from the serum of A^- rabbits immunized with human blood group A erythrocytes [2]. Immunoglobulins were labeled with [^{125}I]iodogen (Pierce) according to [10]. Crossed immunoelectrophoresis was done as in [11].

3. RESULTS

Crossed immunoelectrophoresis is a very convenient technique for resolving and identifying enterocyte plasma membrane proteins solubilized by either neutral detergents or papain [5,11]. This technique was actually used to directly identify in A^+ rabbit enterocytes the plasma membrane glycoproteins bearing the A antigenicity. For this purpose we prepared an anti-serum against a detergent

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extract of brush border membrane preparation from A^- rabbits. The resulting antibodies precipitated membrane proteins without any binding on A-like determinants. These latter could therefore be labeled provided [^{125}I]iodinated anti-human blood group A-antibody was added to the precipitating antibodies. Protein immunoprecipitates with an A-type antigenicity can be specifically revealed by autoradiography (fig.1). If the antigens were proteins from A^- rabbit membranes (fig.1a,b) no immunoprecipitate could be revealed by autoradiography. By contrast, in the case of A^+ rabbit membrane proteins, all the peaks revealed by Coomassie blue (fig.1c,d) were also revealed by autoradiography (fig.1e,f). Almost all could be identified as hydrolases [5,11], but not for peak 1. It was more heavily labeled by anti-A than the hydrolases and was undetectable after Coomassie blue staining of A^- rabbit membrane proteins (fig.1a,b). If anti-A was not added to anti-A^- rabbit brush border proteins, peak 1 could not be observed with A^+ rabbit membrane proteins as antigens either. The quantity of A determinants in peak 1 antigen, which we termed enterocyte surface antigen, was sufficient enough so that it could be precipitated by anti-A alone (fig.1g,h).

The solubilization of the enterocyte surface antigen was further studied and compared to that of hydrolases. Although similar results were obtained with both types of membranes, only those obtained with basolateral membranes are presented in fig.2 since the enterocyte surface antigen peak (no. 1) was well separated from the hydrolase peaks. The antibodies used here were raised against an Emulphogen extract of a basolateral membrane fraction repared from non-typed rabbits, and thus contained the anti-A antibody. Like hydrolases, the surface antigen was well extracted from membranes

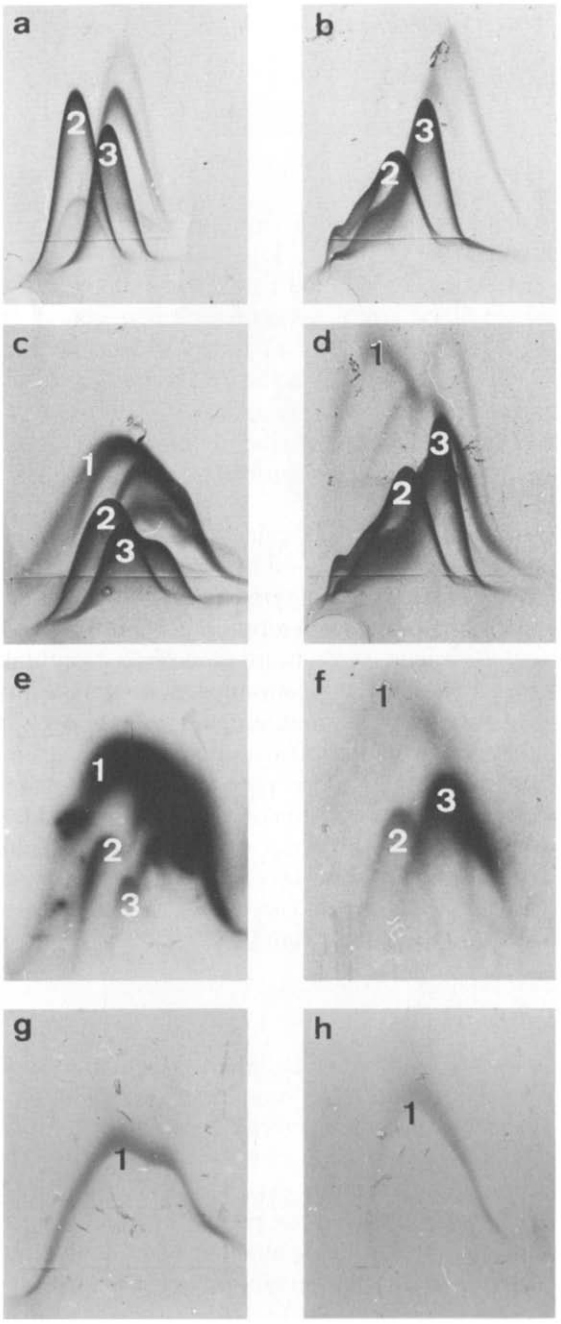

Fig.1. Crossed immunoelectrophoresis of Emulphogen-extracted proteins from brush border (left) and basolateral (right) membranes. The gel used in the second dimension (from bottom to top) contained 100 μg/ml of immunoglobulins from guinea pig sera raised against an Emulphogen extract of brush border membrane from A^- rabbit, plus 60 μg/ml of ^{125}I labeled immunoglobulins from rabbit sera raised against human blood group A erythrocytes in a–f and only anti-A immunoglobulins (200 μg/ml) in g,h. Applied samples: 6 μg and 60 μg of proteins from, respectively, brush border and basolateral membranes from A^- rabbits in a,b and A^+ rabbit in c–h. In g–h the amount of membranes proteins were raised to 20 and 70 μg, respectively. The precipitates were revealed by staining with Coomassie blue in a–d, g and h. In e and f autoradiography of c and d plates, respectively, were performed with Kodak XS-5 Omat SX-ray films.

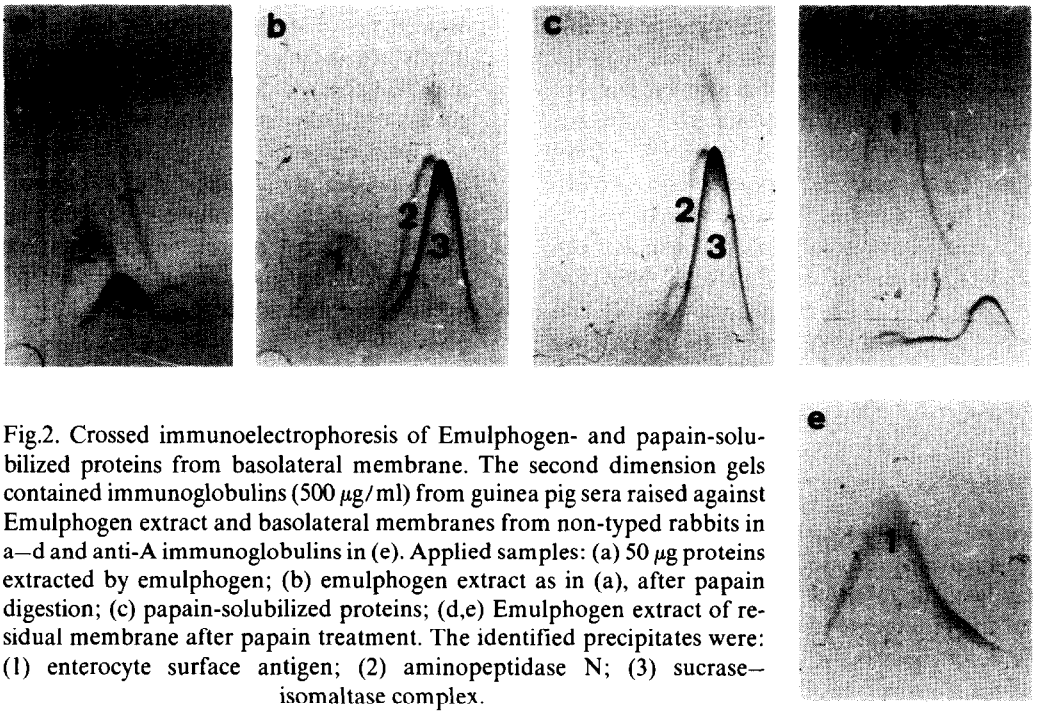

Fig.2. Crossed immunoelectrophoresis of Emulphogen- and papain-solubilized proteins from basolateral membrane. The second dimension gels contained immunoglobulins (500 μg/ml) from guinea pig sera raised against Emulphogen extract and basolateral membranes from non-typed rabbits in a–d and anti-A immunoglobulins in (e). Applied samples: (a) 50 μg proteins extracted by emulphogen; (b) emulphogen extract as in (a), after papain digestion; (c) papain-solubilized proteins; (d,e) Emulphogen extract of residual membrane after papain treatment. The identified precipitates were: (1) enterocyte surface antigen; (2) aminopeptidase N; (3) sucrase–isomaltase complex.

by detergents (fig.2a). When an Emulphogen extract was treated with papain, the hydrolases were transformed into papain forms characterized by their rapid electrophoretic migration [11] and the enterocyte surface antigen was almost completely destroyed (fig.2b). The remaining small quantity of this antigen, however, does not seem to be modified. In contrast, the surface antigen was not degraded after papain treatment of the membrane. It can even be extracted by Emulphogen from residual papain–treated membranes (fig.2c). Its more specific identification was performed by precipitation with anti-A antibody (fig.2e). These results show that enterocyte surface antigen is a glycoprotein anchored in the membrane independently from the hydrolases.

Now, it may be asked whether the surface antigen characterized in A^+ rabbits by its high content in A-like determinants is also present in the plasma membrane of A^- rabbit enterocytes. In these enterocytes, the A determinants might be replaced by human blood group H_1-like determinants since this type of antigenicity has already been found to replace the A antigenicity as pointed out by immunofluorescence studies on ultrathin frozen section of jejunum from A^- rabbits [12]. However, the H_1 determinants are by far less antigenic than the A determinants. This could help to explain why antisera raised against enterocyte plasma membrane proteins are not generally able to precipitate the surface antigen from A^- rabbit membranes (fig.1a,b). Consequently only sera containing high levels of antibodies raised against the polypeptide

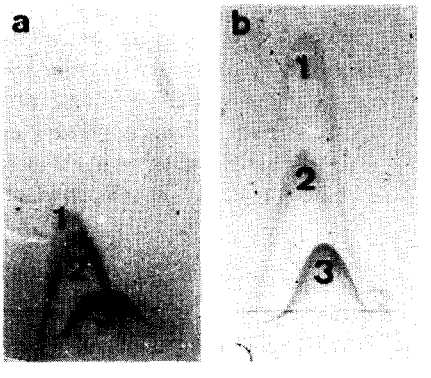

Fig.3. Crossed immunoelectrophoresis of Emulphogen-solubilized proteins (50 μg) from basolateral membranes from A^- rabbits in (a) and a mixture of 50% of 2a and 50% of 3a in (b). The second dimension gels were as in fig.2a–d.

chain of the surface antigen might precipitate this antigen from A^- rabbit membranes. This is probably the case for the serum used in the experiment reported in fig.2,3 since it precipitated 3 antigens from Emulphogen extracts of A^+ and A^- rabbit membranes (fig.2a and 3a, respectively). Fig.3b shows that in both cases the 3 peaks were immunologically identical since no additional peak was obtained when a mixture of the 2 extracts was submitted to crossed immunoelectrophoresis. It can therefore be concluded that peak 1 in fig.3a, contains the membrane enterocyte surface antigen of A^- rabbits.

4. DISCUSSION

Addition of ^{125}I-labeled anti-human blood group A antibodies during crossed-immunoelectrophoresis has allowed us to specifically label the glycoproteins of the enterocyte plasma membrane bearing A-like determinants. In fact, it was shown that all hydrolases from A^+ rabbits contained such determinants.

We found that the A antigenicity of the enterocyte surface is primarily due to the presence of the enterocyte surface antigen in the plasma membrane characterized by its high content in A determinants. The high sensitivity of the antigen to papain digestion suggests a fibrillar rather than globular structure. We tentatively identify this antigen as a constituent of the glycocalyx. An immunoelectron microscopic study has shown that the fibrillar material forming the glycocalyx of the microvilli is particularly rich in A determinants (Bernadac, A. et al., in preparation). At least the particularly long fibrillar glycoconjugates of the summit of the microvilli are very sensitive to mechanical cleavages [13]. A portion of this material may thus be lost during subcellular fractionation, but smaller fibers and the residual basal portions of broken long filaments are retained in membrane preparations. It may be expected that these membrane glycoconjugates would have a heterogeneous size distribution. It should be noted that, particularly in the case of brush border membranes, the shape and the width of the immunoprecipitate obtained with anti-A antibody (fig.2g) express a large heterogeneity of the enterocyte antigen. This observation also argues in favor of the identity of this antigen and the glycocalyx.

REFERENCES

[1] Zweibaum, A. and Bouhou, E. (1973) Transplantation 15, 291–293.
[2] Oriol, R. and Dalix, A.M. (1977) Immunology 33, 91–99.
[3] Slomiany, B.L., Murty, V.L.N. and Slomiany, A. (1980) J. Biol. Chem. 255, 9719–9723.
[4] Feracci, H., Bernadac, A., Gorvel, J-P. and Maroux, S. (1982) Gastroenterology 82, 317–324.
[5] Colas, B. and Maroux, S. (1980) Biochim. Biophys. Acta 600, 406–420.
[6] Kenny, J. and Maroux, S. (1982) Physiol. Rev. 62, 91–128.
[7] Ito, S. (1965), J. Cell. Biol. 27, 475–491.
[8] Bennett, G. and Leblond, C.P. (1977) Histochem. J. 9, 393–417.
[9] Fourcart, J., Limonne, F., Gorvel, J-P., Saint Blancard, J., Girot, P. and Boschetti, E. (1981) SSA Trav. Scient. 2, 431–433.
[10] Fraker, P.J. and Speck, J.C. jr (1978) Biochem. Biophys. Res. Commun. 80, 849–857.
[11] Feracci, H. and Maroux, S. (1980) Biochim. Biophys. Acta 599, 448–463.
[12] Gorvel, J-P. (1982) Thèse de Spécialité de l'Université Aix-Marseille I.
[13] Ugolev, A.M., Smirnova, L.F., Iezuitova, N.N., Timofeeva, W.M., Mityushova, N.M., Egorova, V.V. and Parshkov, E.M. (1979) FEBS Lett. 104, 35–38.