THE ADVERSE EFFECT OF NEURAMINIDASE ON THE ANALYSIS OF CELL SURFACES BY BOROHYDRIDE TRITIATION

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1. Introduction

Of the many techniques [1,2] developed to label surface components of cell membranes, two in particular have proved to be the most popular. One is the lactoperoxidase-catalysed iodination procedure using H_2O_2 directly [3] or H_2O_2 generated the by glucose/glucose oxidase system [4]; here, accessible tyrosine residues in external proteins are labelled with ^{125}I. The other is the galactose oxidase/sodium borohydride procedure where terminal galactose or *N*-acetylgalactosamine residues of carbohydrate chains are first oxidised by galactose oxidase then tritiated by reduction with NaB^3H_4. In this latter method neuraminidase is almost invariably added along with the galactose oxidase [5–8] as the removal of sialic acid increases the number of terminal galactose residues and hence the uptake of tritium on reduction.

However, removal of sialic acid also affects the electrophoretic mobility of glycoproteins and in this report we assess the significance of this effect. Most surface proteins labelled by galactose oxidase/borohydride are also iodinated by lactoperoxidase and as iodination is not significantly affected by removal of sialic acid the lactoperoxidase system may be used to assess the effect of neuraminidase on electrophoretic mobility. Using the erythrocyte surface as a model we find that neuraminidase treatment has a deleterious effect on the mobility and resolution of labelled glycoproteins in sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis (PAGE). We suggest that this undesirable consequence of neuraminidase needs to be carefully considered when the enzyme is used to enhance galactose oxidase/borohydride tritiation of cell surfaces.

2. Experimental

Blood was collected by venipuncture from healthy human male and female adults using heparin as an anticoagulant. White cells were removed by passing the blood through an α-cellulose:microcrystalline cellulose (2:1, w/w) column [9] in Hepes-buffered isotonic saline (133 mM NaCl, 4.5 mM KCl, 10 mM Hepes (pH 7.4 = buffer A)) and the erythrocytes washed 3 times with the same buffer.

Intact cells were iodinated with ^{125}I by lactoperoxidase catalysis and their membranes prepared by hypotonic lysis as in [10]. The ghosts were suspended in 50 mM acetate buffer (pH 5.5) containing 0.1 mM $CaCl_2$, 2 μl/ml of neuraminidase (Behring Institute, Marburg, 1 unit/ml) added and samples removed at varying times, quenched in ice and the insoluble ghosts centrifuged off. The supernatant was analysed for free sialic acid [11] and the pellet prepared for electrophoresis. Total sialic acid content was determined after its release by hydrolysis of the ghosts in 0.1 N sulphuric acid at 80°C for 25 min.

Cells for tritiation were suspended (2×10^9/ml) in buffer A, 5 units galactose oxidase (Sigma Chemical Co.) added and the suspension incubated for 30 min at 37°C and the cells washed twice in the buffer. The washed erythrocytes were suspended in 0.5 ml buffer and 0.66 mCi NaB^3H_4 added and after 30 min at 18°C the cells spun off, washed 3 times in buffer and prepared for electrophoresis. Neuraminidase treatment of the cells was carried out prior to galactose oxidase treatment by incubation in 50 mM acetate buffer

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(pH 6.0) containing 2 μl/ml neuraminidase + 0.1 mM $CaCl_2$ + 0.17 M NaCl. The cells were then washed in buffer A and tritiated.

Membranes were prepared for electrophoresis by addition of 1 vol. 0.125 M Tris–HCl (pH 6.8) containing 10% (w/v) SDS, 20% glycerol, 0.002% bromophenol blue, 1 mM EDTA and 100 mM dithiothreitol to 1 vol. membranes. This mixture was then heated at 100°C for 5 min. The polypeptide constituents of the ghosts were separated by SDS–PAGE in the discontinuous buffer system of Laemmli [13] and a two-dimensional system comprising of buffer system from

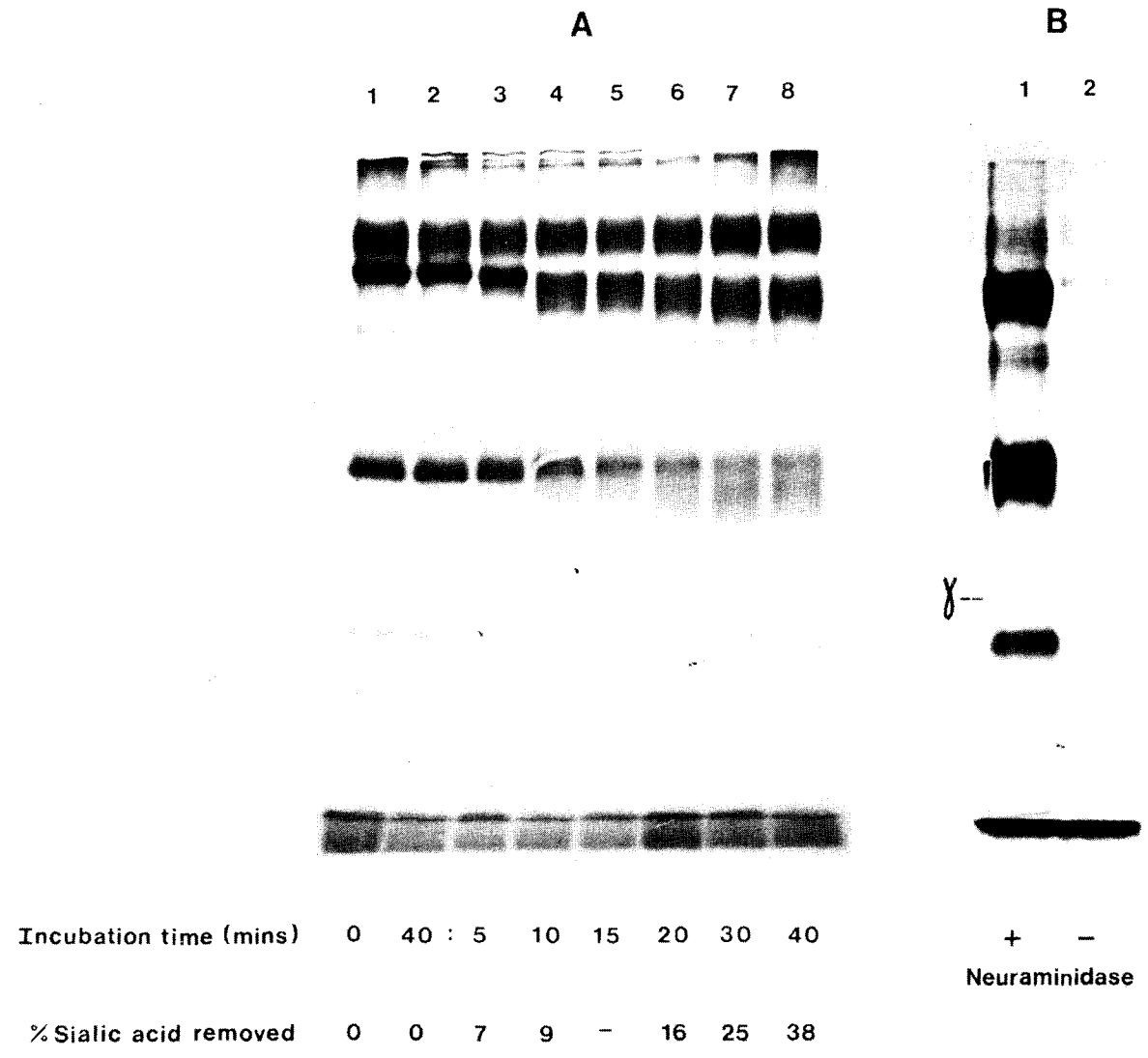

Fig.1. Fluorographs to show the effects of neuraminidase on the labelling of the surface proteins of erythrocytes. (A) Intact erythrocytes were radioiodinated by lactoperoxidase catalysis and the ghosts incubated for varying times with neuraminidase and the proteins fractionated by SDS–PAGE on a 11.5% acrylamide gel with a Laemmli buffer system (lanes 3–8); lane (1) unincubated control; lane (2) sample incubated in the absence of neuraminidase as a control for endogenous protease activity (none is apparent). (B) Fluorograph of membranes from erythrocytes tritiated by galactose oxidase/NaB^3H_4 with (lane 1) and without (lane 2) neuraminidase. With the exception of component γ, which is tritiated but not iodinated, the tritiated neuraminidase pattern is very similar to the iodinated membranes after neuraminidase digestion. It will be noted that neuraminidase causes the stongly labelled bands to subdivide and smear, while minor bands disappear (presumably because of smearing).

[13] in conjunction with the buffer in [10]. The total polypeptide content of the membranes was determined by staining the gels with Coomassie brilliant blue R250. Iodinated polypeptides were detected by autoradiography of the dried gels. Tritiated glycopeptides were detected by fluorography after impregnation of the gels with scintillant [14], before drying and exposing them to pre-flashed X-ray film [15].

3. Results

The effect of neuraminidase digestion on cell membranes pre-labelled with ^{125}I is shown in fig.1,2. Sharp glycopeptide bands become more diffuse on neuraminidase treatment and the apparent M_r-value of most of the labelled components falls with neuraminidase digestion. This effect of neuraminidase treatment is not due to proteolysis as controls incubated for 40 min at 37°C under the same conditions have the same glycopeptide pattern as unincubated membranes (fig.1A).

The pattern produced by labelling intact cells with galactose oxidase and sodium borohydride is shown in fig.1B. In the absence of neuraminidase the level of tritiation is very low but those bands which are revealed are sharp. Neuraminidase increases tritium uptake several fold but also causes the bands to broaden as they do after neuraminidase treatment of lactoperoxidase labelled cells. This effect is clearly demonstrated in 2-dimensional gels where not only are the strongly labelled components diffuse but some minor components are no longer discernable (fig.2).

Comparison of results obtained by iodination with those derived by tritiation is only valid if the same glycoproteins are labelled by both methods. The identity of the various components is not strictly relevant to the present argument (see [16,17] for a full discussion) suffice it to point out that only one component labelled by tritiation is not also iodinated (the

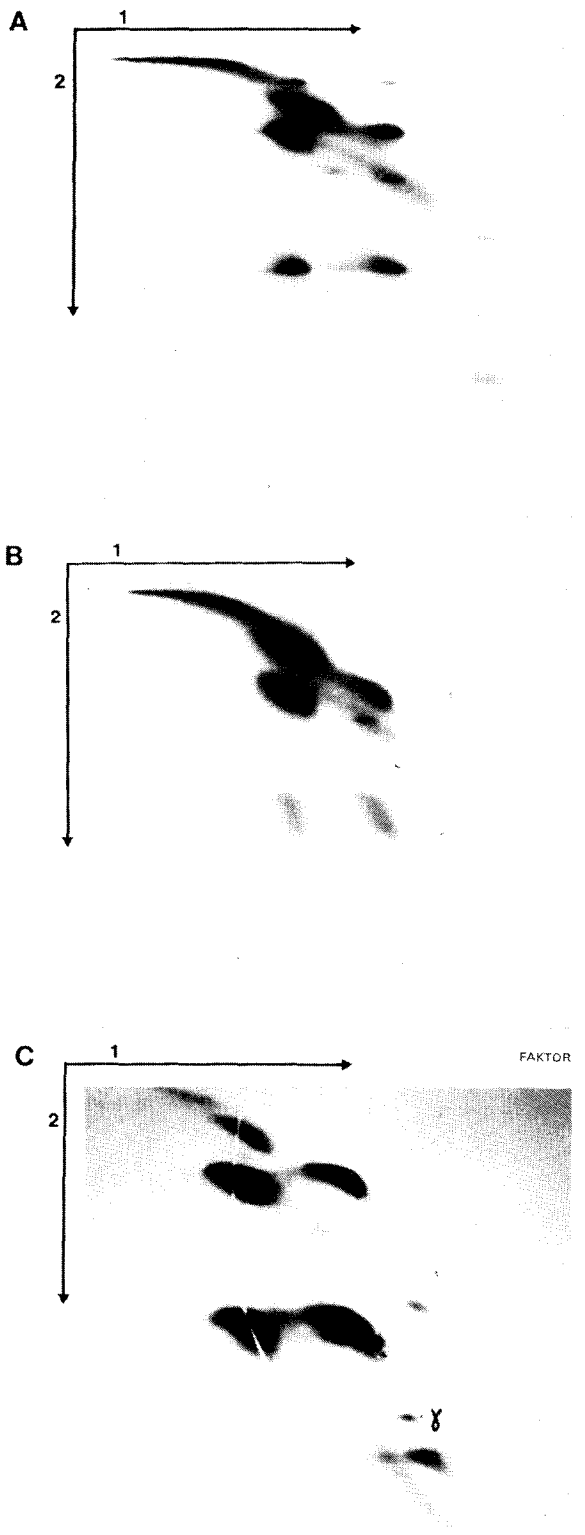

Fig.2. The effect of neuraminidase on the electrophoresis of erythrocyte surface proteins as shown by 2-dimensional SDS-PAGE. The enzyme causes spots to smear and minor components to disappear: (A) lactoperoxidase iodination of erythrocytes; (B) ghosts from (A) after neuraminidase digestion; (C) galactose oxidase/NaB^3H_4 tritiation of erythrocytes pretreated with neuraminidase; (1) buffer from [13]; (2) buffer from [12].

band designated γ in the nomenclature of [16]) and there is relatively less radioactivity associated with band 3. As the vast majority of labelled components are common to both methods it appears that at least in erythrocytes, and perhaps in most cells, very few surface proteins are unglycosylated and, conversely, that cell surface glycoproteins also have an accessible peptide sequence on the exterior.

Our experiments have been performed on the relatively simple, but exhaustively studied, erythrocyte. However, it is probable that similar undesirable effects of neuraminidase treatment exist when other more complicated, less well-known, cell surfaces are analysed. We therefore suggest that when neuraminidase is used to promote the effect of galactose oxidase these factors have to be weighed against the advantage of increasing the incorporation of tritium.

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