THE CONTRIBUTION OF DIFFERENT GLYCOCONJUGATES TO THE TOTAL ABH BLOOD GROUP ACTIVITY OF HUMAN ERTHROCYTES

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1. Introduction

The presence in human erythrocytes of ABH blood group active glycosphingolipids is well documented [1]. They vary considerably in complexity from simple glycosphingolipids with 5–10 glycosyl residues to polyglycosylceramides which contain av. ~30 glycosyl residues/mol. ABH active oligosaccharides linked to the protein moiety by *N*-glycosidic [2,3] and *O*-glycosidic [4] linkages have been also described in human erythrocytes. The former seem to be quite similar in structure and complexity to those present in polyglycosylceramides. Claims have been made that the bulk of ABH activity of erythrocytes is provided either by glycosphingolipids [5,6] or glycoproteins [3,7]. Here we analyse this problem in quantitative terms.

2. Materials and methods

Polyglycosylceramides for labelling experiments were isolated from human erythrocytes as in [8]. I-active glycoprotein [9] was obtained through the courtesy of Dr Ebert. Erythrocyte ghosts were prepared as in [10] and *A*-gene specified transferase of *N*-acetylgalactosamine (EC 2.4.1.40) was isolated as in [11]. UDP-[^{14}C]GalNAc, spec. act. 61.5 Ci/mol was purchased from Amersham. Treatment of the membrane fraction with pronase (Calbiochem), 0.3 mg/ml was for 96 h at 37°C [12]. After 24 h and 48 h the reaction mixture was fortified with additional enzyme (0.15 mg/ml). Radioactivity was counted in a Packard Tri-Carb scintillation counter.

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Samples were prepared as in [13] but the incubation with Soluene-100–isopropanol mixture was for 1 h at 50°C, then for 1 h at room temperature following the addition of H_2O_2.

3. Results and discussion

Results of fractionation of membranes labelled in blood group H site are shown in fig.1. Extraction of the membranes with *n*-butanol allowed only a small

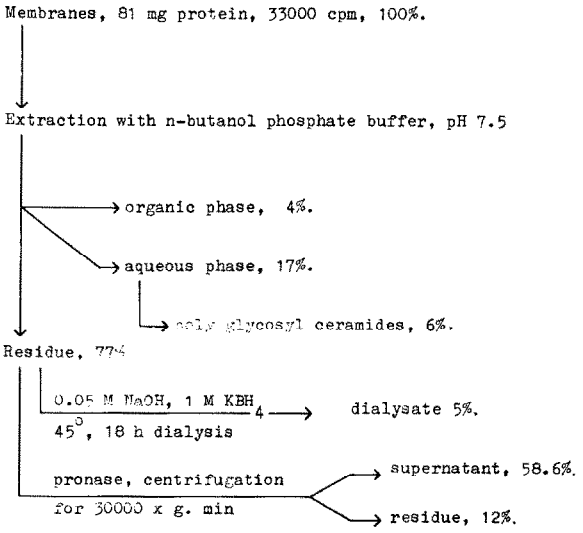

Fig.1. Fractionation of blood group O membranes labelled on the H site with [^{14}C]GalNAc using *A* blood group gene-specified transferase [8]. Labelled membranes (200 μl, 400 μg protein, 33300 cpm) were mixed with 48 ml unlabelled membranes containing 80.6 mg protein. Butanol extraction and isolation of polyglycosylceramides was performed as in [8]. Percentages refer to initial radioactivity.

portion of the initial label to be recovered thus indicating that simple glycosphingolipids represent a minor component of the total ABH activity of the membranes. Similar results were reported in [7]. The radioactivity recovered in pure polyglycosylceramides was slightly higher. This fraction, however, was obtained after all purification steps which involved acetylation of the water-soluble material, extraction with organic solvents, fractionation of the glycolipids acetates on silicic acid column, de-*O*-acetylation, extraction with chloroform–methanol [8]. Taking into account some preparative loss of this material we assume that polyglycosylceramides contribute to $\geqslant$10% of the total ABH activity of erythrocytes. The above value was confirmed in 2 additional experiments in which 10% and 12% of the initial radioactivity of the membranes, respectively, was recovered in polyglycosylceramides. In one of these experiments polyglycosylceramides were isolated by another method [5]. The bulk of the radioactivity was found in *n*-butanol–water insoluble residue. Treatment of this material with pronase resulted in the solubilisation of most of its radioactivity. The material was largely stable towards alkaline–borohydride reagent under condition when carbohydrate–protein linkages of the *O*-glycosidic type are split [14]. Most likely it represents polyglycosyl peptides [2] but it cannot be excluded that some residual polyglycosylceramides are present in this fraction. In a separate experiment whole labelled membranes were treated with alkaline–borohydride and subsequently to dialysis. The radioactivity recovered in the dialysate amounted to 14%. Under similar conditions the labelled *n*-butanol-soluble material released into the dialysate only 2% of its radioactivity (0.1% of the radioactivity of whole membranes). Summing up the contribution of different glycoconjugates to the total ABH blood group activity of human erythrocytes should be as follows: simple glycosphingolipids, ~5%; polyglycosylceramides, ~10–15%; alkali-labile glycoconjugates, ~5–15%; alkali-stable glycoproteins, ~65–75%.

The distribution of radioactivity after SDS gel electrophoresis of the labelled membranes is shown in fig.2. The fastest and faintest spot corresponds to simple glycosphingolipids, the intermediate one to polyglycosylceramides and the slowest and most intense one possibly to glycoproteins. Similar results were reported in [7,8]. It is interesting that the hypothetical glycoprotein band exhibits a similar mobility to I-active glycoprotein [9]. The latter was reported

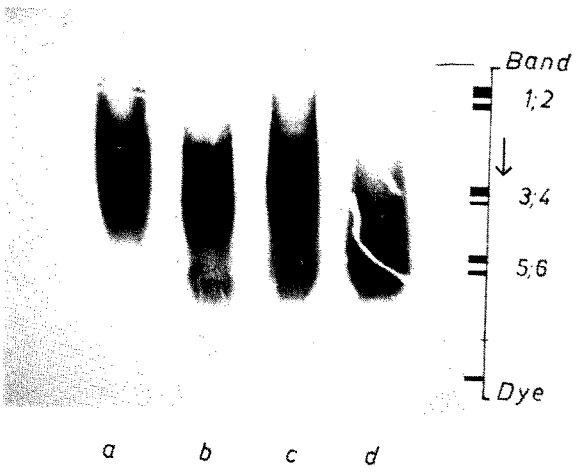

Fig.2. Fluorographic pattern of labelled membranes separated by electrophoresis in SDS–polyacrylamide gel slab [8]. (a) I glycoprotein [9], 57 μg in 100 μl 1324 cpm; (b) membranes, i phenotype 105 μg protein in 100 μl, 1962 cpm; (c) membranes, I phenotype 90 μg protein in 100 μl, 1884 cpm; (d) polyglycosylceramides 14 μg in 50 μl 1529: The photograph was overexposed to visualise simple glycosphingolipids. Numbers to the right indicate protein bands [10].

ABH-inactive but in this study we found it was labelled with [^{14}C]GalNAc when exposed to *A* enzyme. On a weight basis it was, however, a 5-fold less efficient substrate than polyglycosylceramides (see legend to fig.2). Presumably a sparse distribution of immuno-determinant groups in the glycoprotein molecule prevents this substance from manifesting its blood group activity in serological tests [15]. Theoretically the contribution of different glycoconjugates to the total ABH activity of erythrocytes as measured by the enzymic method may not necessarily reflect their reactivities with specific antibodies. The reason is that the *A* enzyme has a smaller molecular weight [16] than either IgM or IgG and thus may be able to penetrate deeper into the membrane structure. The fact that the numbers of blood group specific sites on erythrocytes as determined by enzymic [7] and antibody [17] methods are quite similar suggests, however, that this reservation is probably not valid.

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