GLYCOLIPID PATTERN OF STOMACH TISSUE OF A HUMAN WITH THE RARE BLOOD GROUP A,p

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1. Introduction

Globotriaosylceramide and globotetraosylceramide (globoside) have been known for a long time as major glycolipids in human erythrocytes and in many human and mammalian tissues [1,2] (table 1). These two glycolipids were recently identified as the P^k and P antigens, respectively, by hemagglutination inhibition studies [3,4]. Also the third antigen of the P system, P_1 [5], was shown in a similar way to be a glycosphingolipid [6], table 1. In contrast to the P_1 antigen which is a minor component as most antigens are, P^k and P antigens are directly chemically detectable in lipid extracts. The immunochemical results concerning P^k and P antigens [3,4] could be confirmed therefore by two independent studies of glycolipids prepared from p and P^k erythrocytes, respectively [7,8]. As expected, the p erythrocytes apparently lacked the P^k and P glycolipids [7,8]. The P^k erythrocytes lacked the P glycolipid and had ~5-times the amount of P^k glycolipid compared to normal [7], possibly explaining that these cells are the only erythrocytes with which anti-P^k antibodies are reacting [5,9].

This work was done to reveal whether the P^k and P glycolipids are lacking also in non-erythrocyte cells of p individuals. This is expected if the two actual glycosyltransferases are lacking. In fact, p fibroblasts are unable to synthetize P^k and P glycolipids [10], and P glycolipid may be absent in kidney of p individuals (Kościelak, personal communication). On the other hand, the glycolipid situation of p individuals has been chemically documented only for erythrocytes so far [7,8] and these cells may differ from other cells, as is known for Lewis substances [5]. Lewis glycolipids are not synthetized by red cells but are acquired from plasma [11]. In contrast, these glycolipids are present in large amounts in epithelial cells of small intestine [12,13].

2. Materials and methods

The stomach tissue was obtained from a woman,

Table 1
Structures of glycolipids[a] referred to in the text

GlcNAcβ1→3Galβ1→4GlcCer	Lactotriaosylceramide
Galβ1→3GlcNAcβ1→3Galβ1→4GlcCer	Lactotetraosylceramide
Galβ1→4GlcNAcβ1→3Galβ1→4GlcCer	Neolactotetraosylceramide
Galα1→4Galβ1→4GlcNAcβ1→3Galβ1→4GlcCer	P_1 antigen
Galα1→4Galβ1→4GlcCer	Globotriaosylceramide or P^k antigen
GalNAcβ1→3Galα1→4Galβ1→4GlcCer	Globotetraosylceramide or P antigen

[a] The semisystematic designations follow the nomenclature in Eur. J. Biochem. (1977) 79, 11–21.

aged 73, of blood group A,Rh(+),p (The patient is identical with Mrs E. K. Sweden in [5] p. 151, who underwent surgery for peptic ulcer disease.) The operation was done at the University Hospital of Lund and the tissue removed was immediately frozen and sent on dry ice to Göteborg. Adipose and ulcer tissue was removed and the macroscopically normal tissue (44.5 g) was lyophilized. The dry tissue (6.7 g) was extracted in two steps in a Soxhlet apparatus, 1 day with chloroform–methanol 2:1 (v/v) and 1 day with chloroform–methanol 1:9 (v/v). The combined extracts were evaporated to dryness and subjected to mild alkaline degradation, dialysis, DEAE and silicic acid column chromatography principally as in [14]. To obtain pure non-acid glycolipids acetylation and silicic acid chromatography were used [15].

References of glycosphingolipids with 1–12 sugars of human erythrocytes and other tissues were prepared in this laboratory. The structures of many of these glycolipids were established by mass spectrometry [16] and other methods [17].

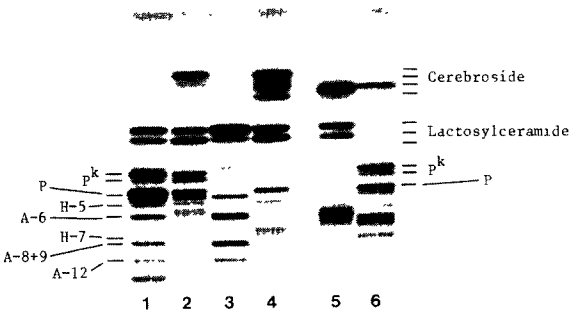

Fig.1. Thin-layer chromatogram of total non-acid glycosphingolipids obtained from erythrocyte (1) and plasma (2) of an individual of blood group A_1,Rh(+),Le(a+b–),P_1(–), erythrocyte (3) and plasma (4) of an individual of blood group A_1,Rh(+),Le(a–b–),p, and from stomach tissue of an individual of blood group A_1Rh(+),p (5) and B,Rh(+) (6). The amounts of glycolipid applied were 40 μg for (1) and 20 μg for (2–6). The erythrocyte and plasma samples were prepared from the transfusion unit (400 ml of blood) and the yields of total non-acid glycolipids were 46 mg (1), 5.0 mg (2), 26 mg (3) and 3.0 mg (4). The bands shown were coloured green with the anisaldehyde reagent and therefore all contained sugar [14]. The thin-layer plate was a precoated, silica gel 60, HPTLC plate (Merck) and the solvent chloroform–methanol–water 60:35:8 (v/v/v). The designations to the left and right indicate probable identities of the separate bands as regards number of sugars and blood group determinants. Thus, A-8 means an octaglysosylceramide with a blood group A determinant. The band with 4 sugars indicated immediately below the P glycolipid to the right may be lactotetraosyl- or neolactotetraosylceramide (table 1).

3. Results and discussion

The yield of total non-acid glycosphingolipids of A,p stomach tissue was 9.8 mg (1.5 mg/g dry wt). This is comparable to the figure (1.2 mg/g dry weight) of normal stomach tissue obtained fresh from a patient operated upon for ventricle carcinoma [18]. Fig.1 shows the thin-layer chromatogram of these two samples, and for comparison erythrocyte and plasma glycosphingolipids prepared in a similar way from A and A,p individuals have been added (in preparation). Blood and stomach tissue were from separate A,p donors.

As shown in lane 5 with the A,p stomach glycolipids no bands are found in the expected intervals for P^k and P glycolipids. These are present, however as major components in stomach tissue of the B individual (lane 6). The erythrocyte glycolipids of an A,p individual (lane 3) also lack P^k and P glycolipids in agreement with [7,8]. The faint bands shown for this sample in the 3-sugar region (travelling somewhat faster than the double band of P^k glycolipid) are not due to P^k glycolipid but to lactotriaosylceramide (table 1). In the plasma sample of this individual (lane 4) there are bands corresponding to P^k and P glycolipids. The chemical identity of these has not yet been shown. The dominating P^k and P glycolipids of normal erythrocyte are easily detected (lane 1).

The amounts of blood group fucolipids with 6, 8 and 9 sugars are considerably elevated in p (lane 3) compared to normal (lane 1) erythrocyte. Also, diglycosylceramide with longer-chain non-hydroxy fatty acids (upper of two bands) is relatively more abundant in p erythrocyte, a fact discussed in [7–9, 19]. We have shown by mass spectrometry that the fatty acids of this upper band of diglycosylceramide [7,9,19] and those of the fucolipids with 6,8 and 9 sugars (only one band each) are identical [20], that is, mainly 22:0, 24:0 and 24:1 acids.

Concerning the stomach tissue (lanes 5,6) there is no corresponding elevation of fucolipids (defined as interval below P glycolipid) as found for erythrocyte in case of p blood group. However, mono- and diglycosylceramides are relatively more abundant in A,p (lane 5) than in B (lane 6) tissue. For small intestine of man it has been shown [13] that di-, tri- and tetraglycosylceramides are confined to non-epithelial tissue, while glycolipids with 1 and with 5 or more sugars are localized in epithelial cells. If the situation is analogous in stomach tissue this would mean that

non-epithelial tissue is practically only composed of diglycosylceramide. If so, it is remarkable that this tissue is devoid of its major normal glycolipids without any apparent change in function.

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