STRUCTURE DETERMINATION OF BLOOD GROUP TYPE GLYCOLIPIDS OF CAT SMALL INTESTINE BY MASS FRAGMENTOGRAPHY

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1. Introduction

The finding of a relatively high glycolipid content in human small intestine [1] and the fact that epithelial cells of intestine offer a system for study of the differentiation of eukaryotic cells [2], opens a unique possibility to test the postulated role of cell-surface carbohydrates in differentiation and regulation [3]. We are therefore developing specific preparative and analytical techniques for microscale characterization of complex glycosphingolipids. The present work is an application of a novel technique of mass fragmentography on a glycolipid mixture from an individual cat small intestine.

2. Experimental

One lyophilized small intestine was extracted with chloroform–methanol in a Soxhlet apparatus. The total non-acid glycolipid fraction was prepared by conventional steps like mild alkaline degradation, silicic acid and DEAE-cellulose chromatography [4], and a combinatory use of native and acetylated [5] substances. Methylation and reduction was done as in [6]. The mass spectrometer (MS 902, AEI Ltd, England) was connected on line to a data system (Instem Ltd, England). The sample probe temperature was raised linearly 1°C/min or 5°C/min and scans were taken every 2 min. A detailed description of mass scale calibration (up to *m/e* 1800) and mass fragmentography will be given elsewhere. Computerized accurate mass determination was obtained on the methylated sample in the region *m/e* 200–800. Ordinary scans were recorded on paper to check the calibration.

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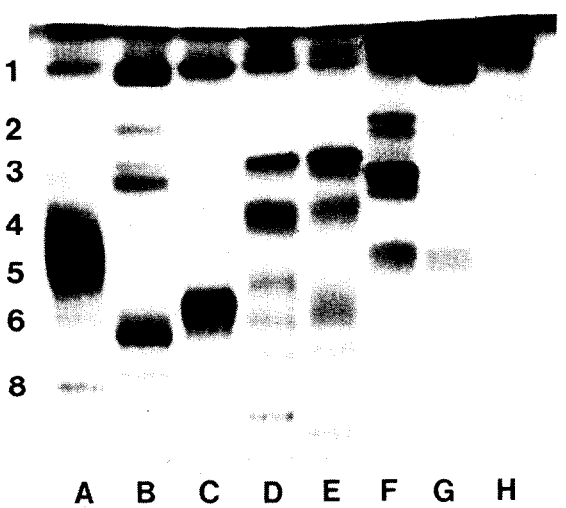

Fig.1. Thin-layer chromatogram of total non-acid glycosphingolipids (about 100 μg) of small intestine of different animals. The figure in parenthesis gives the corresponding amount in dry tissue weight. A, cat (31 mg); B, rabbit (25 mg); C, mouse (20 mg); D, white rat (23 mg); E, black and white rat (15 mg); F, guinea pig (19 mg); G, hen (53 mg); H, cod fish (330 mg). The figures to the left indicate the number of sugars of the glycolipids. Separation was done on 0.15 mm thick layer of silica gel G (Fluka) and with chloroform–methanol–water, 60:35:8 (by vol.) as solvent. Detection was done with the anisaldehyde reagent [4].

3. Results

Figure 1 shows a thin-layer chromatogram of the total non-acid glycolipids of an individual cat small intestine (A) and, for comparison, corresponding fractions of other species (B–H) to be discussed elsewhere. All spots were coloured green by the anisaldehyde reagent and therefore contained sugar [4]. The number of sugars of the glycolipids is indicated in the margin. As shown for the cat there is a major spot in the 4–5-sugar region, but also faster-moving material and a distinct slow-moving band in the 8-sugar region.

The selection of fragment peaks for reproduction (fig.2,3) from the large volume of collected data was

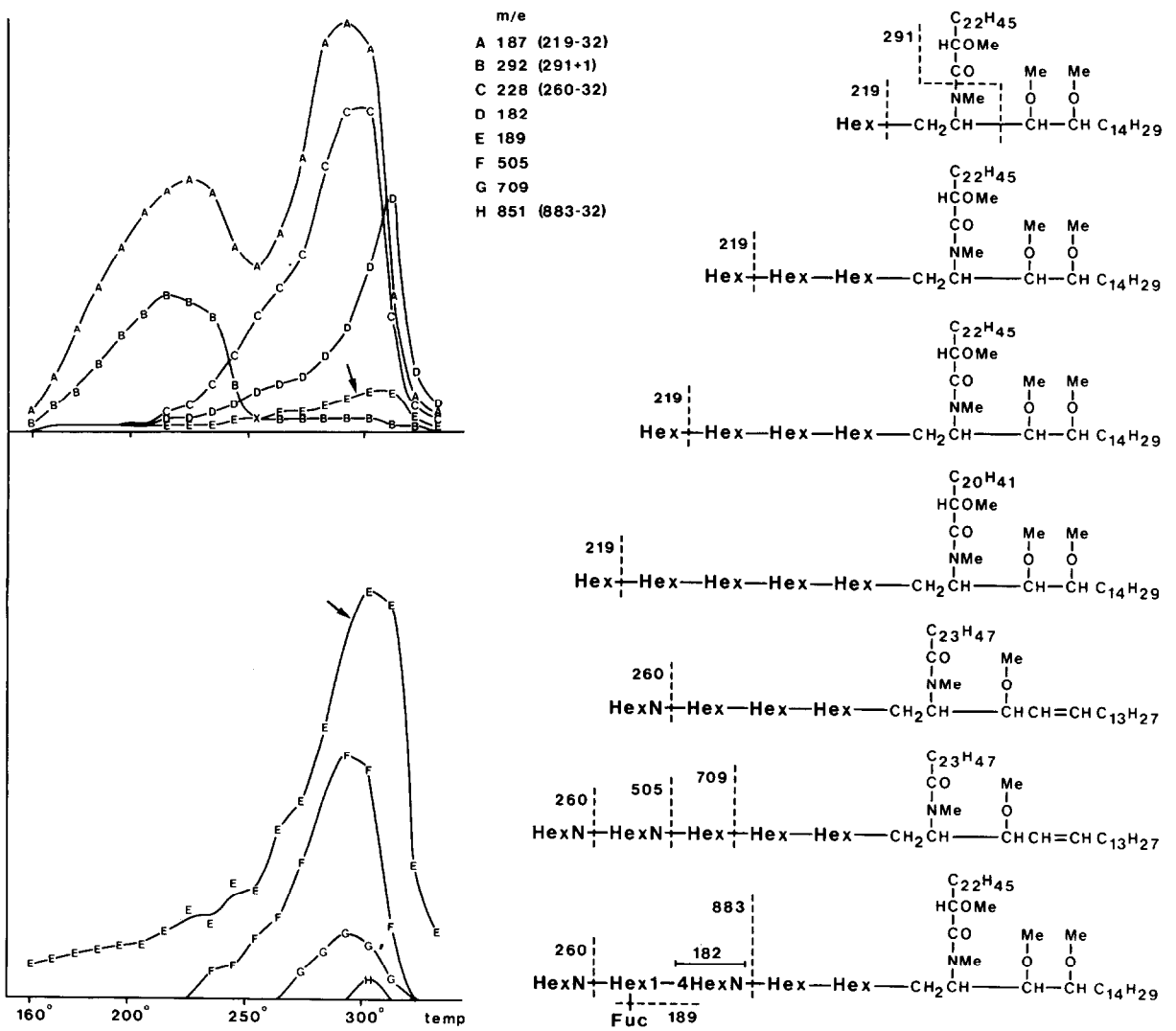

Fig.2. Mass fragmentograms and simplified formulae for interpretation concerning methylated total non-acid glycolipids of cat small intestine (30 μg). The curves shown represent relative intensities of selected ions recorded during a continuous rise in sample probe temperature (5°C/min) from 160–330°C. The arrows indicate the same curve in two reproductions with different intensity scales. Hex, hexose; HexN, hexosamine; Fuc, fucose. Electron energy 44 eV, trap current 500 μA, ion source temperature 290°C, acceleration voltage 6 kV.

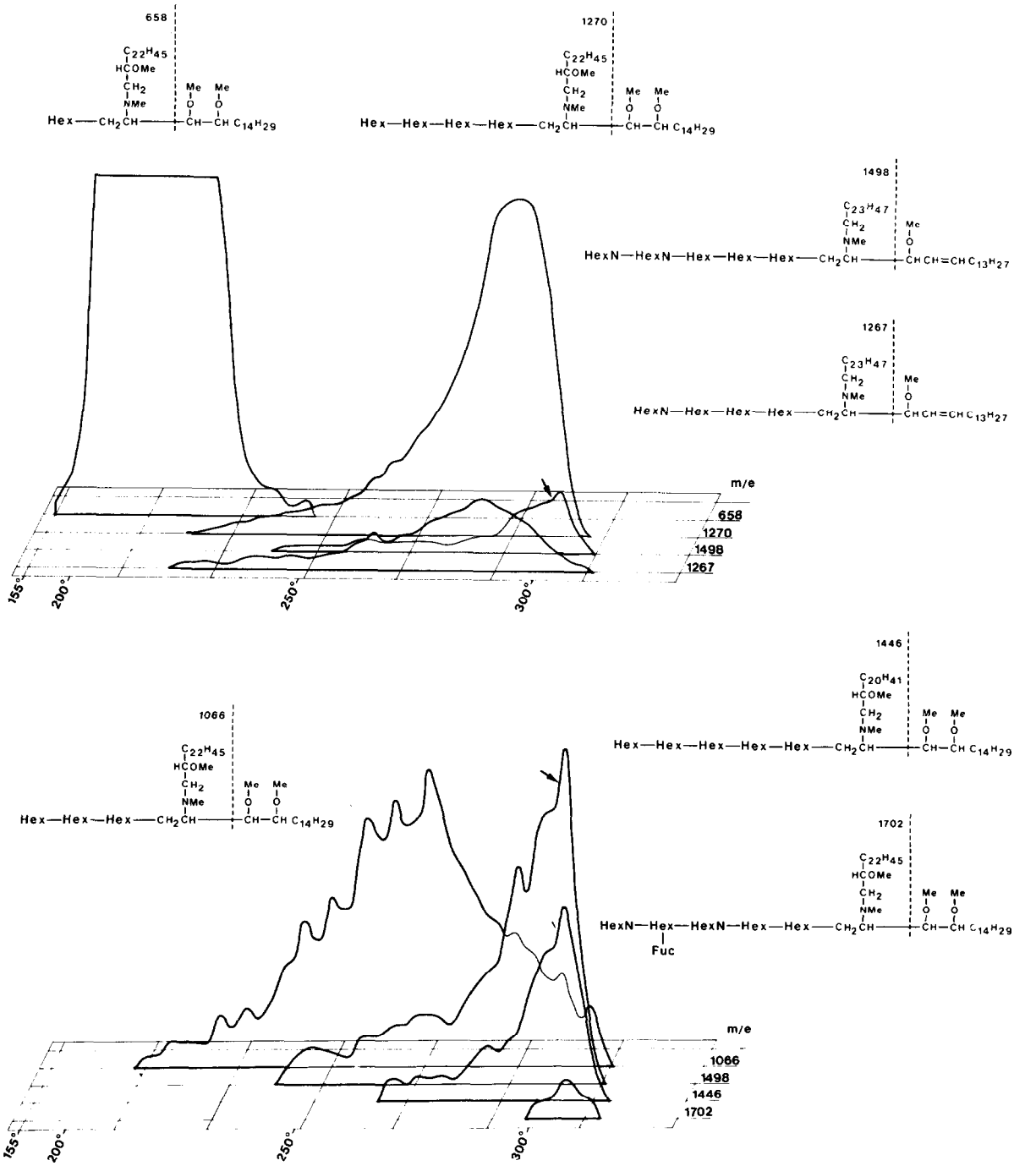

Fig.3. Mass fragmentograms and simplified formulae for interpretation concerning methylated-reduced total non-acid glycolipids of cat small intestine (200 μg). The sample temperature was raised 5°C/min in the region 150–200°C and 1°C/min in the region 200–325°C. There were 10 scans within each section of the bottom frame. Electron energy 48 eV, trap current 500 μA, ion source temperature 290°C, acceleration voltage 4 kV. For further explanations, see legend of fig.2.

based on the known fragmentation behaviour of the two types of glycolipid derivatives [7,8]. As a generalization, the methylated derivatives (fig.2) primarily provide information on the sequence of terminal saccharides and on ceramide components, while methylated-reduced derivatives (fig.3) show relatively intense peaks for the complete saccharide plus the fatty acid, in addition to sequence fragments. A combined interpretation of spectra of the two types of derivatives as appearing along the evaporation curve (155–330°C) therefore allows a rather safe conclusion concerning the number and type of sugars of individual glycolipids and most of their sequences, as well as ceramide structure. The order of evaporation in the ion source was found to be related to the mobility on the thin-layer plate. The results can therefore be summarized as shown in fig.4.

The presence of monoglycosylceramides is shown in the early part of the evaporation curve by the hexose-fatty acid peak at *m/e* 658 (fig.3), which is well separated from corresponding peaks of higher glycolipids (compare fig.1,4). A hexose part is further indicated by fragments A and B of fig.2. The major ceramide components as indicated in the formulae (24:0 hydroxy fatty acid and phytosphingosine) were supported by molecular weight ions (not reproduced).

The most abundant ion obtained at higher temperature from the reduced mixture (fig.3) is found at *m/e* 1270, which should correspond to a previously unknown glycolipid with four hexoses and 24:0 hydroxy fatty acid. This glycolipid probably makes up most of the major spot of the chromatograms (fig.1,4). Accordingly, the terminal hexose gave an abundant fragment (A) from the methylated mixture (fig.2). The major long-chain base (phytosphingosine) was concluded from *m/e* 1202 (not shown), which corresponds to the complete molecule minus the fatty acid.

In addition to these two glycolipids producing prominent curves in the fragmentograms, there is a number of additional glycolipids detectable, the conclusion of which may be based on an analogous interpretation as presented above.

There are two glycolipids with characteristics of the globo-series, namely globoside (*m/e* 1267, fig.3) and the Forssman glycolipid haptene (*m/e* 1498, fig.3). Specific sequence ions are shown in fig.2. Of interest is a separate ceramide composition (normal fatty acid)

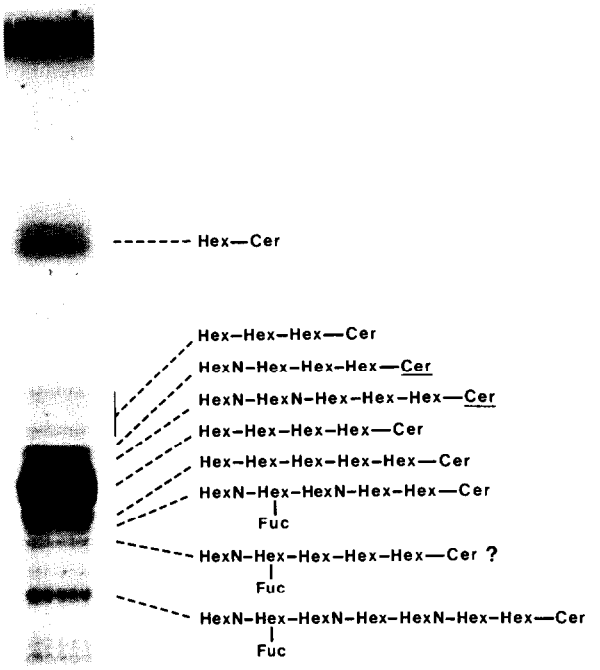

Fig.4. Thin-layer chromatogram of non-acid glycosphingolipids (30 μg) of cat small intestine and probable locations of glycolipids identified by mass fragmentography and known reference compounds. The resolution of bands is better in this case than shown in fig.1 due to a different layer (microanalytical plates precoated with silica gel 60, Merck, Darmstadt). Solvent and detection as for fig.1. Cer, ceramide with mainly hydroxy fatty acids and phytosphingosine; Cer, ceramide with mostly normal fatty acids. For other abbreviations, see legend of fig.2.

compared with all other glycolipids detected (hydroxy fatty acid and phytosphingosine).

As indicated in fig.4 and concluded from the data of fig.2,3 there are also glycolipids present with three and five hexoses, respectively, the latter representing a novel glycolipid.

In the later part of the evaporation curve there appeared fragments that are specific for blood-group A-like glycolipids as discussed before [8,9]. Figure 2 contains profiles for terminal saccharides (C,E,H) and also for *m/e* 182 (D), specific for a type 2 saccharide chain, see formula at the bottom of fig.2. From the

reduced mixture (fig.3) *m/e* 1702 is specific for an A-type hexaglycosylceramide. Also present and of somewhat higher intensity (not shown) was *m/e* 1675, which may be analogous to this hexaglycosylceramide but having the internal hexosamine exchanged for hexose (fig.4). A similar glycolipid has been detected in hog gastric mucosa [10].

Important additional information, not shown in fig.2,3 was obtained on a third A-like glycolipid by a conventional paper recording of spectra. After bleeding-off most of a 500 μg load of methylated-reduced sample for 2 h at 270°C, a rapid rise in temperature gave an almost pure spectrum of an A-like 8-sugar glycolipid. The sugar and fatty acid fragment (compare fig.3) shown at *m/e* 2137, contains 24:0 hydroxy fatty acid and the number and type of sugars, shown below in fig.4. Other ions indicative of this sequence were similar to those recorded before for a similar glycolipid of human A_1 erythrocytes [8].

Support for the presence of blood-group A glycolipids was obtained by hemagglutination inhibition in microscale of human erythrocytes. The total glycolipid fraction dissolved in 0.15 M NaCl (2 μg/μl) inhibited 4 hemagglutination units by human anti-A antisera diluted 1:2. The same inhibition was obtained by 0.05 μg/μl when the lipids were incorporated into liposomes (cholesterol:lecithin:sphingolipid, 1:1:1). No inhibition of anti-B antiserum was seen.

4. Discussion

The primary purpose for investigating small intestinal glycolipids of separate species (fig.1) was to select a suitable species for detailed study of epithelial cells in relation to differentiation [2,11]. As will be discussed elsewhere we have chosen the two strains of rat (D, E, fig.1) for detailed investigation.

The unique capacity of the present technique is evident from the detailed structural information which is obtainable from about the same amount of sample as needed for thin-layer chromatography. At least 9 separate glycolipids were specifically detected, some of them previously unknown, and several mixed-up in a major thin-layer chromatographic spot (fig.1). The method should therefore be a powerful tool in elucidating function and metabolism of glycosphingolipids, especially when analysis on small amounts of individual tissues or cells from single animals is essential.

Acknowledgements

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