Characterization of Novel Amino Acid Fucosides*

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The structures of FL4b and of two other related amino acid fucosides have been determined by a combination of methylation analysis and enzymatic digestion. Additionally, the anomeric configurations of the carbohydrate moieties of FL4a, previously shown to be glucosyl(1 → 3)fucosyl1 → threonine (Steiner, S., Via, D. P., Klinger, M., Larriba, G., Sramek, S., and Laine, R. (1978) in Glycopolymers and Glycolipids in Disease Processes (Walborg, E. F., Jr., ed.) pp. 378-403, American Chemical Society, Washington, D. C.) have been determined by enzymatic digestion. The results indicate that the structures are: FL3a, fucose/threonine; FL3b, fucosylol → serine; FL4a, glucosylfucosylol → threonine; and FL4b, glucosylfucosyl1 → fucosylol → serine. FL4a, which appears to have the same structure as a component from human urine (Hallgren, P., Lundblad, A., and Svensson, S. (1975) J. Biol. Chem. 250, 5312-5314), and FL4b are highly unusual in that they contain fucose in a nonterminal position. The fucosyl-serine linkages found in compounds FL3b and FL4b is a novel structure.

Previous studies in this laboratory have demonstrated that radioisotopically labeled fucose is incorporated into a series of low molecular weight compounds (1, 2) in normal rat cells. It was also shown that the level of one of these components, FL4a, is markedly reduced in NRK cells transformed by murine sarcoma virus. Respess and co-workers demonstrated that a lower ratio of FCZ to FL3 could be positively correlated with alterations in the cellular morphological phenotype. For example, butyrate-induced cellular flattening of MSV-NRK cells to a more normal phenotype is accompanied by an increase in the level of FL4a relative to FL3a (1). The connection between butyrate-induced changes in FL4a/FL3a and "oncogenicity" has not been established.

We have previously determined the amino acid and carbohydrate composition of four of these low molecular weight compounds obtained from rat tissues (4). The compositions were found to be: FL3a, fucose/threonine, 1:1; FL3b, fucose/threonine, 1:1; FL4a, glucose/threonine, 1:1:1; and FL4b, glucose/threonine, 1:1:1. By gel filtration chromatography, the apparent molecular weight of FL3a and FL3b was estimated to be approximately 250, and the apparent molecular weight of FL4a and FL4b was estimated to be approximately 500 (4). These results are consistent with the interpretation that FL3a and FL3b have 1 mol each of fucose and amino acid and that FL4a and FL4b have 1 mol each of glucose, fucose, and amino acid. Furthermore, by use of gas chromatography-mass spectroscopy, the sequence and linkages of the components of FL4a were shown to be glucosyl(1→3)fucosyl1→threonine (2). The determination of the sequence of carbohydrates, the position of the linkage of FL4b, and the anomeric configurations of the carbohydrate linkages of FL3a, FL3b, FL4a, and FL4b are reported here.

EXPERIMENTAL PROCEDURES

RESULTS AND DISCUSSION

Methylation Analysis—The carbohydrate sequence and linkages of FL4b were determined by GC-MS analysis of partially methylated alditol acetate derivatives of the two constituent carbohydrates. A mass chromatogram scan for ion m/z 262 yielded a single peak with a retention time of 5.88 min (Fig. 1A). Likewise, scanning for m/z 264 also revealed a single peak with a retention time of 5.4 min (Fig. 1B). The m/z 264 is a prominent ion expected for a terminal hexose, and was observed with authentic glucosylfucosylol → fucosylol → threonine (Glc-Fuc-Thr) (2). The m/z 262 is a prominent ion expected for 3-linked deoxyhexose and was also previously observed with authentic Glc-Fuc-Thr (2). Mass spectra of the m/z 264 and the m/z 262 peaks were consistent with the presence of a terminal hexose and an internal 3-linked deoxyhexose, respectively (Fig. 2). These mass spectra are comparable to the spectrum of the terminal hexose and 3-deoxyhexose obtained from the PMAA of the carbohydrate residues of authentic Glc-Fuc-Thr (2). Moreover, totally permethylated FL4b yielded essentially the same mass fragments as authentic Glc-Fuc-Thr (figure not shown; see Ref. 2) with the prominent ion being m/z 187 which indicates a terminal hexose (2).

The mass spectra of the PMAA derivatives of FL3a and FL3b were also recorded. Mass chromatograms for ion r/z 234 and m/z 118 revealed a major peak with a time of 4.14 min for both compounds. A mass spectrum of this peak showed ions characteristic for a terminal deoxyhexose (Fig. 3).

* Portions of this paper (including "Experimental Procedures" and Figs. 4-7) are presented in miniprint at the end of this paper. Mini-print is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20014. Request Document No. 81M-246, cite author(s), and include a check for $2.40 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

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‡ The abbreviations used are: NRK, normal rat kidney; MSV-NRK, murine sarcoma virus-transformed NRK; GC-MS, gas chromatography-mass spectroscopy; PMAA, partially methylated alditol acetate derivatives.

§ A. Respess, L. S. Kucera, and M. Waite, personal communication.
Characterization of Novel Amino Acid Fucosides

7933

Fig. 1. Mass chromatograms of partially methylated alditol acetate derivatives of the carbohydrate moieties of FL4b. The instrumental conditions were: Finnigan 3300-6110 methane-chemical ionization mode: 1 torr ion source; pressure source temperature 60 °C; ionizing electron energy, 150 eV; transfer lines, 250 °C. Gas chromatography was performed on a 1-m column of 3% OV-210, with the temperature programmed from 140 to 220 °C at a rate of 6 °C/min. The mass chromatograms of the PMAAs of FL4b were obtained by monitoring for the major ions at m/z 262 (A) and m/z 264 (B) at a rate of one scan every 2 s.

Fig. 2. Mass spectra of major peaks from mass chromatograms of PMAA's of FL4b. A, mass spectrum of major peak, retention time 5.88 min, in Fig. 1A. B, mass spectrum of major peak, retention time 5.40 min in Fig. 1B.

Enzymatic Degradation of the FL Components—To determine the anomeric configuration of the glucose and fucose residues, the FL components were subjected to treatment with the appropriate glycosidases. Attempts to cleave the terminal glucose of intact FL4a or FL4b with α- or β-glucosidases were not successful. The charge of the amino acid residue could have interfered with the enzymatic cleavage, similar to the experience of Spiro with the glycopeptide glucosyl-galactosyl-hydrotolysine (9). Therefore, the disaccharide moiety of FL4a and FL4b was cleaved from the amino acid by partial acid hydrolysis and analyzed by gel filtration (Bio-Gel P-2). A single peak which eluted between lactose and raffinose gave an elution pattern similar to that observed for fucosyl-mannose (10). Furthermore, reduction of the disaccharide followed by acid hydrolysis resulted in quantitative release of the radioactivity as fucitol, whereas acid hydrolysis alone yielded fucose. This result indicates that the fucose moiety is at the reducing end and in turn supports the GC-MS data that the fucose moiety is internal and is likely attached to the amino acid. Moreover, in control experiments, reduction followed by hydrolysis of oligosaccharide chains with norreducing fucose residues resulted in quantitative release of the radioactivity as fucose. When the disaccharide from FL4a or FL4b was subjected to β-glucosidase treatment, there was quantitative release of the label as fucose (Fig. 4). α-Glucosidase treatment had no effect on the chromatographic mobility of the disaccharide moiety of FL4a or FL4b. These data, in conjunction with the results of methylation linkage analysis, support the interpretation that the glucose moiety of FL4a and FL4b is linked β1 → 3 to fucose.

The enzymatic determination of the anomeric configuration of the fucose-amino acid linkage in FL4a and FL4b required the prior removal of the terminal glucose residue from both compounds. The removal was accomplished by one round of Smith degradation; FL4a was converted to a compound with the same chromatographic mobility as FL3a (fucosyl-threonine) as seen in Fig. 5. Likewise, FL4b was converted to a compound with the same mobility as FL3b (fucosyl-serine; data not shown). When subjected to α-L-fucosidase treatment, the Smith degradation product of FL4a quantitatively yielded fucose (Fig. 6). The same was true of the Smith degradation product of FL4b (data not shown). Similarly, when authentic FL3a and FL3b were subjected to α-L-fucosidase treatment, fucose was quantitatively released (Fig. 7). Hence, the fucose moieties of FL3a, FL3b, FL4a, and FL4b appear to be α-linked to the amino acid.

Studies have been presented in which four novel amino acid fucosides obtained from rat liver have been further characterized. The proposed structures for the compounds are: FL3a, fucosylα1 → threonine; FL3b, fucosylα1 → serine; FL4a, glucosylβ1 → fucosylα1 → threonine; FL4b, glucosylβ1 → fucosylα1 → serine. FL3a, FL3b, and FL4a were not previously described in nature. FL4a is likely to be the amino acid fucoside isolated from normal human urine and characterized by Hallgren and co-workers (3). Although the precursor(s) of these components has not been established, preliminary studies from this laboratory have demonstrated that...
Characterization of Novel Amino Acid Fucosides

mild base/borohydride treatment, i.e. β-elimination, of a fucose-labeled glycoprotein fraction results in the release of a disaccharide component which contains fucitol and is chromatographically indistinguishable from authentic glucosylβ1 → 3fucitol (2). Moreover, pulse experiments with [3H]fucose are consistent with the disaccharide-containing glycopeptide being a metabolic precursor of FL4a (2). More recently, it has been found that the disaccharide obtained from the glycoprotein fraction is sensitive to β-glucosidase, strongly supporting the idea that the disaccharide is glucosyl-fucose, and in turn is a precursor of the FL4 components. The β-elimination studies also revealed the release of fucitol from the fucoprotein fraction, thereby suggesting that the fucose is attached to serine or threonine. Hence, it seems reasonable to speculate that the FL components are generated from fucoprotein, and in turn, that the decreased level of FL4a in transformed cells is due to altered metabolism of that protein(s).

*M. M. Klinger and S. M. Steiner, unpublished observations.

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SUPPLEMENTARY MATERIAL TO
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Materials: Fucosyl rat liver were harvested from Wistar-Furusho Biologicals (Rags, Mm: 1) and delipidated with 4% acetic acid. Chromatography was performed using a Waters HPLC system equipped with a UV detector at 254 nm.

EXPERIMENTAL PROCEDURES

Materials: Fucosyl rat liver were harvested from Wistar-Furusho Biologicals (Rags, Mm: 1) and delipidated with 4% acetic acid. Chromatography was performed using a Waters HPLC system equipped with a UV detector at 254 nm.

Methods: The fucosylated fucose was isolated from rat liver and purified by column chromatography on Sephadex G-25. The purified fucose was then subjected to thin-layer chromatography in various solvent systems.

Preparation of rat liver fucose: The liver was homogenized with an equal volume of cold ethanol and the supernatant was collected and diluted with water.

Chromatography-mass Spectroscopy: The isolated fucose was subjected to HPLC-mass spectrometry analysis. The chromatogram showed a single peak at retention time of 15 minutes.

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