Methylation Analysis of the Carbohydrate Portion of Carcinoembryonic Antigen

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INTRODUCTION

Since its initial isolation in 1965 (9), CEA has been the subject of numerous studies (8, 20). In spite of these investigations, the detailed structures of both the carbohydrate and protein portions of the molecule remain largely undefined. Recent studies (5–7, 11) utilizing methylation analysis and periodate oxidation techniques have yielded information about the types and relative amounts of the various carbohydrate structural units in CEA. Information on the nature of the anomeric linkages of certain sugar residues in CEA has been provided by lectin binding studies (11).

In this study, CEA was isolated from 4 individual tumors, and the carbohydrate structural units present in each preparation were determined by methylation analysis. On the basis of these data, constraints have been placed on the structure of the carbohydrate moiety of CEA.

MATERIALS AND METHODS

Preparation of CEA. CEA was purified as described previously (4) from the liver metastases of primary colon tumors.

Carbohydrate Analysis. Neutral sugars were quantitated by the gas chromatographic procedure of Clamp et al. (2). Amino sugars were determined on the amino acid analyzer by using a modification (6) of the method of Liu and Chang (13) on a Beckman Model 121H amino acid analyzer. Sialic acid was measured by the method of Warren (22).

Methylation Analysis. Samples were methylated by the method of Hakomori (10), in which the methylsulfinyl anion was used to generate the polysaccharide aldehyde before the addition of methyl iodide. The methylsulfinyl anion was prepared as described by Sandford and Conrad (16).

Acetolysis, hydrolysis, reduction, and acetylation of the permethylated polysaccharides were performed using the procedures described by Stellner et al. (18).

Identification of the partially methylated alditol acetates was carried out according to the method of Björndal et al. (1) for the neutral sugar derivatives and according to the method of Stellner et al. (18) for the amino sugar derivatives. A Varian Model 2740 gas chromatograph equipped with a flame ionization detector connected to a Dupont Model 21-492B double-focusing mass spectrometer was used for these analyses. A 6-ft column (2 mm inside diameter) of 3% ECNSS-M coated on Gas Chrom Q (Applied Science Laboratories, Inc., State College, Pa.) was used to separate the alditol acetates derived from the methylated sugars. Isothermal runs at temperatures between 150–160 °C were used for the neutral sugars and at 180 °C for the amino sugars. Mass spectra of the carbohydrate derivatives were taken over a mass range of 35 to 600 atomic mass units using an ionizing potential of 70 eV. Scans were taken every 9 sec, and the spectra were processed using a Dupont 21-094B disc-based data system.

RESULTS

The results of methylation analysis of CEA samples isolated from 4 different tumors are shown in Table 1. The quantity of each carbohydrate derivative was determined as follows. The peak areas from gas chromatography of the individual methylated, acetylated derivatives of a particular carbohydrate (e.g., mannose) were totaled, and the fraction of the total for each was determined. The amount of each derivative was obtained by multiplying this fraction by the total amount of this carbohydrate present in the CEA preparation. Corrections made for differences in response factors as described by Sweet et al. (19) were found to be insignificant and therefore were not used. For comparison, the results of Hammarström et al. (11) are also shown. All CEA samples were approximately 50% by weight carbohydrate.
DISCUSSION

Although the relative amounts of the various carbohydrate structural units found in our 4 CEA preparations were often very similar to those reported by Hammarström et al. (11), there were several distinct differences. For example, in 3 of our 4 CEA preparations we observed much larger amounts of N-acetylglucosamine and galactose at the non-reducing termini than did Hammarström et al. (11) in their preparation. It is possible that the differences observed among the 5 CEA samples may reflect merely the frequently observed heterogeneity of the carbohydrate portions of glycoproteins (15).

Methylation results indicated that all the fucose residues in CEA were terminal. Complete removal of N-acetylneuraminic acid with neuraminidase indicated that all of this sugar was terminal (3, 6). In each of the 5 CEA preparations compared, fucose and N-acetylneuraminic acid account for more than one-half of the sugar residues at the nonreducing termini.

Periodate oxidation studies (6) showed that N-acetylneuraminic acid was linked to galactose since prior treatment of CEA with neuraminidase led to increased destruction of galactose by periodate. To our knowledge, the terminal fucose residues of mammalian glycoproteins have never been found linked to mannose. In CEA, therefore, the fucose residues may be linked only to galactose and/or N-acetylglucosamine. There are not enough galactose linkages available to accommodate all the fucose, especially since a substantial portion of the nonterminal galactose is linked to N-acetylneuraminic acid. It would appear, therefore, that a large proportion of the fucose residues in CEA is linked to N-acetylglucosamine.

Three-quarters of the mannose residues in all the CEA samples studied were linked to 3 other sugar residues. The linkages of these branching mannose units are of great importance in defining the carbohydrate structure of CEA. It is a general characteristic of mannose residues in mammalian glycoproteins to be linked only to other mannose residues or hexosamines (12). On the basis of this observation, 4 possible linkage configurations for the branching mannose residues in CEA are illustrated in Chart 1.

Branching mannose units such as those illustrated in Chart 1,a and b, cannot be present to any great extent, since they would require a much higher ratio than that observed of nonbranching to branching mannose. A large proportion of structural units such as those illustrated in Chart 1,c and d, would put the ratio closer to that actually observed. The type of branching mannose unit illustrated in Chart 1c would require a minimum of 3 N-acetylglucosamines for every branching mannose. A linkage configuration such as that shown in Chart 1d would not require such
a large excess of N-acetylglucosamine to branching mannos.

On the average, the ratio of N-acetylglucosamine to branching mannose is close to 3. In one of our CEA preparations and in the preparation of Hammarström et al. (11), this ratio was less than 3. This would imply that at least some of the branching mannose units were linked to other branching mannose units such as in Chart 1d (or some variation thereof). The structure proposed for a glycopeptide from porcine thyroglobulin contains branching mannose units of this type (21).

Further delineation of the carbohydrate structure of CEA will require methylation and periodate studies on purified glycopeptides. This work is now in progress.

REFERENCES


The experimental values for N-acetylglucosamine may be erroneously low as a result of the well-known difficulty in cleaving the glycosidic linkages of hexosamines that have already been deacetylated (14, 17).

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