Amino-terminal Sequence of a Carcinoembryonic Antigen-like Glycoprotein Isolated from the Colonic Lavages of Healthy Individuals

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ABSTRACT

A glycoprotein has been isolated from the colonic lavages of healthy individuals that is immunologically equivalent to carcinoembryonic antigen purified from tumor tissue. The NH₂-terminal sequence of the glycoprotein from normal colon lavages is Lys-Leu-Thr-Ile-Glu-Ser-Thr-Pro-Phe-(Asn)-Val-Ala-Glu-Gly-Lys-Val-(Leu,Ile)-(Leu,Ile)-(Leu,Ile)-Val-(His,Arg?) - ?-(Leu,Ile). This is homologous to the NH₂-terminal sequence of 23 of the first 24 amino acids of carcinoembryonic antigen isolated from tumor tissue.

INTRODUCTION

CEA\textsuperscript{a} was originally described by Gold and Freedman in 0.9% NaCl solution extracts of digestive tract tumors and embryonic gut tissue (11, 12). In these initial studies it was not detectable in normal adult gut epithelial tissue. Martin and Martin (17) first raised the possibility that this glycoprotein was present in normal colonic tissue, but in greatly reduced quantities compared with malignant tissue. Others subsequently studied this question using immunological techniques (2, 13, 14, 18, 23, 24). However, results in immunological assays cannot conclusively demonstrate the structural identity of 2 substances (5, 29); chemical methods are usually required.

It is important to determine whether CEA is a constituent of normal tissue, since efforts to improve the cancer specificity of the CEA assay are based on the premise that differences exist between the glycoproteins with CEA activity in normal and tumor tissues.

We have previously reported that the amino acid composition, carbohydrate composition, amino-terminal residue, and carbohydrate linkages are identical in CEA from tumor tissue and colonic lavages of healthy individuals (7). Fritsche and Mach (9) have recently reported that the carbohydrate composition of material isolated from the mucosal scrapings of normal colons has a carbohydrate composition similar to that of CEA from tumor tissue. The NH₂-terminal sequence of 23 of the first 24 amino acids of the CEA-like glycoprotein from the colonic lavages of healthy individuals is reported here.

MATERIALS AND METHODS

CEA and Anti-CEA. CEA and goat anti-CEA were prepared as previously reported (3, 6). A final purification step for the CEA was chromatography on concanavalin A linked to Sepharose (Pharmacia Fine Chemicals, Piscataway, N. J.) (21).

Radioimmune Assay for CEA. CEA was measured by the triple-isotope, double-antibody radioimmune assay for CEA as previously described (4, 6, 22). \textsuperscript{57}Co was used in place of \textsuperscript{52}Na as the volume marker (8).

Lavage of the Colon of Healthy Individuals. Colonic saline lavages were collected either by 0.9% NaCl solution enema (6 volunteers) or by a colonic perfusion technique (8 volunteers) (10). Appropriate informed consent was obtained from volunteers prior to study. The ages of the healthy volunteers ranged from 30 to 56 years, and all had serum CEA assay values of less than 2.5 ng/ml as measured by direct radioimmune assay for CEA (10). Samples were processed as previously described (7). Briefly, individual samples were dialyzed and concentrated on a PM 10 membrane (Amicon, Lexington, Mass.), treated at 4° with an equal volume of cold 2 M perchloric acid, dialyzed, and lyophilized. Individual samples were pooled and chromatographed on Sepharose 6B and Sephadex G-200 (Pharmacia Fine Chemicals). The final purification step was chromatography on concanavalin A-Sepharose (21).

Amino Acid Sequencing. Edman degradations were performed on a Beckman Model 890C sequencer using a modified dimethylylamine program.\textsuperscript{a} One mCi of \textsuperscript{35}S]-phenyl isothiocyanate (specific activity, 145 mCi/mmole; Amersham/Searle, Arlington Heights, Ill.) was added per 16 mmoles of phenyl isothiocyanate to increase the sensitivity of the TLC procedure described below. Conversions of thiazolinone amino acid derivatives to their respective PTH derivatives were carried out in 20% trifluoroacetic acid at 55°, a procedure that does not hydrolyze the amide group of glutamine or asparagine (15). The PTH amino acid derivatives were mixed with a known amount of PTH-aminobutyric acid as an internal standard and identified and quantitated by gas-liquid chromatography (20) using a Hewlett Packard Model 5710 A gas chromatograph, and by high-pressure liquid chromatography (HPLC) using a Du Pont Model 830 liquid chromatograph with a Model 837 spectrophotometer. Both the gas chromatograph and the

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\textsuperscript{1} The abbreviations used are: CEA, carcinoembryonic antigen; TLC, thin-layer chromatography; PTH, phenylthiohydantoin; HPLC, high-pressure liquid chromatography.

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HPLC apparatus were connected to an Autolab System IV peak integrator. In addition, a polyamide TLC procedure using 2,5-bis-2-(5-tert-butylbenzoxazolyl)thiophene fluor was utilized (25).

Thin-layer chromatograms were recorded by UV photography and then allowed to expose X-ray film for 2 weeks, after which the autoradiograms were developed and compared with their respective UV photographs. The majority of sample obtained from each degradation cycle was consumed by gas chromatography and TLC analysis, since at the time of this study the HPLC was in a developmental stage in our laboratory. For this reason, the results of only 10 cycles were analyzed by HPLC and are reported here.

**Sodium Dodecyl Sulfate-Polyacrylamide Electrophoresis.** Electrophoresis was carried out using 6% polyacrylamide gels containing 0.1% sodium dodecyl sulfate in 0.1 M Tris-glycine buffer (pH 8.1). Samples were subjected to electrophoresis for 1.5 hr at 8 mA/gel at which point the dye marker, phenol red, reached the bottom of the gel. Gels were stained with 0.5% Coomassie blue in 25% isopropyl alcohol and 10% acetic acid, and destained with 10% acetic acid. Duplicate gels were stained for carbohydrate by a modification of the periodic acid-Schiff method (19). The position of the CEA activity was determined in an unstained gel that was cut into slices approximately 1 mm thick. Each slice was shaken overnight in 0.5 ml of a solution of 1 mg of gelatin per ml in 0.075 M NaCl buffered with 0.075 M sodium phosphate to pH 7.2. An aliquot of the supernatant was tested in the radioimmune assay for CEA.

**RESULTS AND DISCUSSION**

The glycoproteins isolated as previously described (7) from the colonic washings of healthy individuals migrated as a single diffuse band on sodium dodecyl sulfate-poly-
acrylamide electrophoresis, in the same position as CEA from tumor tissue. Identical bands were obtained whether the gels were stained for protein (Coomassie blue) or carbohydrate (periodic acid-Schiff). Analysis of gel slices from an unstained gel demonstrated that all of the CEA radioimmune assay activity was associated with this band (Chart 1).

The amino-terminal amino acid sequence of the CEA-like material from normal tissue shows a single sequence throughout the first 24 residues. The absolute yield of the PTH-amino acids was 45% for duplicate sequencer runs. Fifty per cent absolute yields were obtained for sperm whale apomyoglobin using the same sequencer program. The results of analysis of each degradation cycle shown in Table 1 indicate that the CEA-like glycoproteins from tumor and normal tissues have identical sequences through position 17. Positions 18 to 20 were not obtained in high enough yields to perform a distinguishing test between the pair leucine and isoleucine; however, positions 18 to 20 are all leucines in CEA from tumor tissue. Since Cycle 22 could not be identified by gas chromatography or TLC, it is likely an arginine or histidine (histidine in the case of CEA from tumor tissue). The absolute yield of PTH-asparagine at Cycle 10 was 20% less than expected, and succeeding cycles showed lower yields. This may indicate the presence of carbohydrate linked to asparagine in position 10. However, the characteristic sequence (Asn-X-Thr/Ser), where carbohydrate is attached to asparagine in other glycoproteins (16), is not present here. There was no evidence for multiple amino acid residues within a detection limit of 5% of the peak area for each amino acid identified during Cycles 1 to 24. This result, and similar results obtained in this laboratory (Refs. 26 and 27; J. E. Shively and C. W. Todd, unpublished data) differ from those by Wang et al. (28), who report that CEA has a heterogeneous amino-terminal sequence.

The single sequence obtained for the various CEA preparations and for the CEA-like glycoprotein obtained from colonic lavages of healthy individuals indicates that they possess an unblocked polypeptide chain, which is homologous for 23 of the first 24 residues. The high initial yield of PTH-amino acids obtained for the CEA from tumor tissue and normal colon lavages make the presence of significant amounts (above 10%) of additional unblocked or blocked polypeptide chains doubtful. These results provide convincing chemical evidence that for at least 23 of the first 24 NH₂-terminal amino acid residues, the CEA-like material in normal colon lavages is similar to CEA from tumor tissue (1, 26-28). These results support the growing body of immunological evidence (2, 9, 13, 14, 18, 23, 24) that has led many to believe that normal colon tissue does indeed contain a glycoprotein very similar to, and perhaps identical with, the CEA present in tumor tissue.

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