Preparation and Specificities of Antisera to the Amino-terminal Sequence of the Carcinoembryonic Antigen

Deepika Paul, George Flouret, Joseph T. Tomita, Kathleen Ranney, Jay Schenck, and Byron Anderson

ABSTRACT

A tetracosapeptide (peptide-24) corresponding to the amino-terminal sequence of the carcinoembryonic antigen (CEA) was synthesized and characterized. Antisera were produced to the peptide-24, and a radiomunnoassay was developed utilizing peptide-24 with a tyrosine residue on the amino-terminal end (Tyr-peptide-24). Inhibitions of anti-peptide-24,125I-Tyr-peptide-24 complex formation were done with several preparations of CEA and the normal cross-reacting antigen. The extent of cross-reactivities was low, one CEA preparation requiring a 250-fold molar quantity greater than peptide-24 to obtain the same degree of inhibition. Attempts to degrade the CEA and normal cross-reacting antigens in order to possibly expose the amino-terminal ends for reactivity with antibody did not result in any great increase in inhibitory capacity. It was concluded that either the conformations of the antigenic determinant(s) of the peptide-24 and of the amino-terminal end of CEA were sufficiently different to result in little cross-reactivity or that the amino-terminal end of CEA and/or normal cross-reacting antigen are blocked for reactivity with antibody by other portions of the molecule.

INTRODUCTION

The CEA is a glycoprotein which was initially shown to be associated with the presence of adenocarcinomas of the colon (9, 10). Further studies revealed that elevated serum levels of CEA were also found associated with cancers other than of the gastrointestinal tract as well as with nonmalignant diseases (14, 16, 18). The CEA has been shown to be immunochemically and chemically related to several glycoproteins from normal tissues and body fluids. Frisch and Mach (8) isolated CEA-reactive material from normal colon mucosa and showed that the material was similar to CEA of tumor tissue by immunological criteria and by physicochemical criteria including carbohydrate composition. Egan et al. (4) reported that a CEA-like component could be isolated from colon lavages of healthy individuals which had amino acid and carbohydrate compositions similar to that of tumor CEA and which gave inhibition curves similar to that of tumor CEA in a RIA. From the same laboratory, the colon lavage CEA-like material was shown to 2 be homologous for 23 of the first 24 amino acids of the aminoterminus sequence of tumor CEA, except that the leucine and isoleucine could not be distinguished at positions 18 to 20 and 24 (20). The physicochemical properties of the NCA purified from spleen tissues were reported by Engvall et al. (5). NCA had an amino acid composition similar to that of CEA and an identical amino-terminal sequence up to 26 residues except at position 21. However, in a report on the amino-terminal sequence of 3 CEA preparations, Wang et al. (24) showed differences in at least 2 of the first 24 positions for 2 of the CEA's as compared to the other reported sequences. Also, each of the CEA's appeared to be heterogeneous with multiple amino acids being identified at many positions.

Arnon et al. (1, 2) have shown that a synthetic peptide corresponding to the first 11 amino acid sequence of CEA could be attached to poly-L- or to BSA and that rabbit antisera could be elicited to the peptide-protein conjugates containing specificities to the peptide. Using a bacteriophage inactivation assay, they reported that pure CEA did not inhibit the antipeptide sera but that semipurified CEA and a large proportion of sera from patients with various types of adenocarcinomas were capable of inhibiting the antipeptide-peptide complex formation. The studies presented here were begun in order to ascertain if antisera could be produced to a synthetic peptide with a sequence corresponding to the first 24 amino acids of CEA and to test the specificity and cross-reactivities of those antisera. The synthesis of the peptide-24, immunizations to elicit anti-peptide-24 sera, the RIA with a Tyr-peptide-24, and inhibitions with CEA and NCA materials are reported and discussed.

MATERIALS AND METHODS

Synthesis of Peptide-24

The tetracosapeptide (peptide-24) with the sequence as that described for the amino terminus of CEA (23) was synthesized by the solid phase method (17) with minor modifications. Chloromethylated polystyrene 1% cross-linked with divinyl benzene (1.25 mEq of chlorine per g of resin) was esterified with Boc-asparagine-p-nitrophenyl ester (1.25 mEq of chlorine per g of resin) was esterified with Boc-asparagine-p-nitrophenyl ester (1.25 mEq of chlorine per g of resin) was esterified with Boc-asparagine-p-nitrophenyl ester (1.25 mEq of chlorine per g of resin) was esterified with Boc-asparagine-p-nitrophenyl ester.
in dimethylformamide, or Boc-amino acids (as the sequence required), per Boc-leucine resin using dicyclohexylcarbodiimide as the coupling reagent. The various amino acids were introduced by use of the following derivatives: Boc-lysine (benzylxycarbonyl), Boc-threonine (Bzl), Boc-serine (Bzl), Boc-glutamic acid (Bzl) and Boc-histidine (DNP). The coupling reactions were deemed complete when the resin gave a negative test with ninhydrin (13). The yield of final peptide-resin was 7.5 g. Deprotection of the latter resin (2.5 g) was accomplished in a TFA (50 ml) suspension saturated at room temperature by HBr continuously bubbled for 1.5 hr. The reaction mixture was then filtered, the resin was washed with three 10-ml portions of TFA, and the filtrate was evaporated to dryness. The residual oil was redissolved in TFA (20 ml) and evaporated to dryness, the procedure being repeated 4 times. The residue was treated with ether, and the resulting powder was washed thoroughly with additional ether, collected, and dried over P₂O₅-KOH in a vacuum yielding 1.0 g of material. DNP-peptide-24 thus obtained (200 mg) was dissolved in 2 N NaOH (0.60 ml) and H₂O (5 ml), and mercaptoethanol (2 ml) was added to the clear solution. After 1 hr, acetic acid (6 ml) was added to acidify the reaction mixture, and most of the solvent was removed by evaporation in a vacuum. The residue was dissolved in 50% acetic acid (2 ml), and the solution was applied to a Sephadex G-15 column (65 x 2.3 cm). Elution was accomplished with 50% acetic acid. Three peaks were detected by the Folin-Lowry (15) color reaction: Peak A, containing the desired peptide, and the slower eluting Peaks B and C, which were colored deep yellow and were thus related to derivatives of the DNP blocking group. Lyophilization of Peak A from glacial acetic acid yielded 145 mg of the peptide-24. For further purification, 120 mg of the partially purified peptide were subjected to gel filtration on a Sephadex G-25 column (2.9 x 115 cm) with 1% acetic acid after the sample was dissolved with 0.2 ml of 2 N HCl and 4 ml of 1% acetic acid. The Folin-Lowry color reaction showed a main peak with shoulders at either side. Fractions corresponding to the central peak were pooled, lyophilized, and purified once again on the same Sephadex G-25 column, yielding one peak. Lyophilization of the central portion of this peak yielded 40 mg of product which was homogeneous on silica gel thin-layer chromatography with optimal dilution to lower nonspecific bound radioactivity to a minimum while giving a visible precipitate on addition of AS to the reaction mixture. 50% saturation. To 0.3 ml of the appropriate dilution of anti-CEA in 12- x 75-mm disposable glass tubes were added 10 to 20 µl of 125I-labeled Tyr-peptide-24 (approximately 1.2 ng), and the tubes were incubated for 18 hr at room temperature. In the case of inhibition studies, potential inhibitors were preincubated with the diluted antiserum for 2 hr at 41° before addition of 125I-labeled Tyr-peptide-24. Total radioactivity was measured by counting the tubes in a Beckman Model 300 gamma counter. Antibody-bound 125I-Tyr-peptide-24 was separated from antibody-free 125I-Tyr-peptide-24 by a modification of the method of Farr (6): 0.3 ml of saturated AS was added to the RIA tubes; and the resulting precipitates were centrifuged at 1500 x g for 30 min, washed once with 2 ml of 50% AS, and counted.

RIA of 125I-labeled CEA (Roche Laboratories, Nutley, N. J.) with goat antiserum to CEA was carried out in an identical manner. Twenty µl of the 125I-CEA (specific activity, approximately 0.1 to 0.2 µCi/ng) were used per tube. The anti-CEA serum was diluted in normal goat serum and had a titer of 1: 40,000.
**125I-Labeling of the Tyr-Peptide-24**

The Tyr-peptide-24 was labeled by a modification of the method of Greenwood et al. (12), the reaction mixture consisting of 30 μl of 0.5 M sodium phosphate buffer, pH 7.4, 10 μg of Tyr-peptide-24 in 5 μl of 30% DMSO, 3 mCi of Na<sup>125I</sup> (Amersham/Searle Corp.), and 5 μg of chloramine-T. The reaction was allowed to proceed for 6 min with gentle stirring in ice and then halted by the addition of 10 μg of sodium metabisulfite. Finally, 100 μl each of 0.1 M KI and 1% BSA were added to the reaction mixture, and the 125I-labeled Tyr-peptide-24 was separated from the residual 125I by passage through a 10-ml Sephadex G-25 column equilibrated and eluted with 0.1% BSA. The 125I-Tyr-peptide-24 was further purified using glutaraldehyde cross-linked antiserum, prepared by the method of Avrameas and Ternynck (3). To 3 ml of antisem-24 serum was added saturated AS to give a final concentration of 35%. The precipitated proteins were resuspended and dialyzed against PBS, pH 7.0, and the protein solution was concentrated to approximately 50 mg/ml using an Amicon filter cone (Amicon Corp., Lexington, Mass.). To 1.5 ml of this solution were added 200 μl of 2.5% glutaraldehyde, dropwise with stirring. After the mixture was allowed to stand at room temperature for 1 hr, the cross-linked proteins were washed by centrifugation and resuspension with 0.1 M glycine-HCl buffer (pH 2.2) followed by PBS. The 125I-Tyr-peptide-24 was incubated with two-thirds of the cross-linked protein pellet in a centrifuge tube, with rotation, for 2 hr at room temperature and then overnight at 4°C. The mixture was centrifuged, the pellet was washed 3 times with 1 ml PBS, and the 125I-Tyr-peptide-24 was eluted with 3 washes of 1 ml glycine-HCl buffer (pH 2.2). The glycine-HCl eluates were combined, BSA was added to a concentration of 0.7%, and the mixture was dialyzed overnight in Spectrapor No. 6 (molecular weight exclusion of 2000; Spectrum Medical Industries, Inc., Los Angeles, Calif.) tubing against 5% DMSO in water. RIA with anti-peptide-24 sera, other than those used for the preparation of immunoadsorbent, showed that specific binding increased from 10% of the 125I-Tyr-peptide-24 to 60 to 70% of the 125I-Tyr-peptide-24 repurified from the immunoadsorbent.

**Precipitation of Tyr-Peptide-24 with R1051 Anti-Peptide-24 Serum**

The fraction of the R1051 antiserum containing mainly IgG was prepared by the batchwise method of Reif (19) utilizing DE-52 ion exchanger (Whatman Ltd., Maidstone, Kent, England). The IgG fraction was concentrated using a type CF25 Centrifle membrane cone (Amicon Corp.) and was chromatographed on a 1 x 10-cm column of Sephadex G-100 (Pharmacia Fine Chemicals, Piscataway, N. J.), and the protein eluting in the void volume was used in the precipitation reaction mixture. The latter IgG fraction (400 μl of a 2.1-mg/ml solution) was added to 100 μl of a 1 mg/ml solution of Tyr-peptide-24, and the mixture was left at 4°C for 48 hr. The precipitate that formed was collected by centrifugation, washed once with 1 ml of cold PBS, dissolved in 100 μl of 0.1 M NaOH, and chromatographed on the same Sephadex G-100 column as described above. Fractions (0.75 ml) were collected, and absorbance measurements were obtained at 240 nm. For comparative purposes and calculation of the quantity of Tyr-peptide-24 in the precipitate, the IgG fraction of the antiserum and the Tyr-peptide-24 were each separately chromatographed on the same G-100 column, as well as the supernatant from the precipitin reaction mixture.

**CEA's and NCA's**

Three preparations of CEA and 2 of NCA were used in these studies. CEA-I and NCA-I were purified from a liver metastasis of colon adenocarcinoma and were the generous gifts of Dr. Sten Hammarström. CEA-II and NCA-II were isolated from tissue of a liver metastasis of a colon adenocarcinoma and normal lung tissue, respectively. CEA-III was the international reference preparations of CEA kindly supplied by the WHO International Laboratory for Biological Standards, National Institute for Biological Standards and Control, London, England.

**Degradations of CEA and NCA-II**

**Protease Digestion.** CEA (300 μg) was dissolved in 150 μl of 3 mM Tris buffer, pH 8.8, and 50 μl of a 0.01-mg/ml solution of protease (type IV; Sigma) in 1 mM HCl were added. The mixture was kept for 24 hr at room temperature; then, 40 μl of protease inhibitors (2.5 mM e-aminocaproic acid and 1.3 mM p-hydroxymercuribenzoate) were added, and the solution was frozen until used.

**Base Degradation.** To 100 μl of CEA or NCA-II (300 μg) was added an equal volume of 1 N NaOH. After incubation at 37°C overnight, the solution was neutralized to pH 6.7 with 1 N HCl and then frozen.

**Chloramine-T Treatment.** To 50 μl of DMSO in a small test tube were added 65 μl of CEA (195 μg), followed by 20 μl of chloramine-T (2 mg). The mixture was shaken for 6 min in an ice bath, then 40 μl of sodium metabisulfite (4 mg) were added, followed by 15 μl of a 10% BSA solution. An additional 100 μl of 1% BSA solution was added in order to transfer the solution to Spectrapor dialysis tubing. The solution was dialyzed overnight at 4°C against 5% DMSO and frozen. For each of the degradation reactions, controls were done with all reagents omitting the CEA or NCA.

**RESULTS**

**Characterization of Anti-Peptide-24 Sera.** All the rabbit sera collected throughout the immunizations with peptide-24 and the peptide-24 conjugates were titered against 125I-Tyr-peptide-24 by the RIA described in "Materials and Methods." Sera from rabbits immunized with peptide-24-thyroglobulin showed no binding to 125I-Tyr-peptide-24 even after 4 to 6 months of immunization with the antigen preparation. Animals immunized with peptide-24-BSA began to produce measurable anti-peptide-24 antibodies 5 months after the first immunization, and those immunized with peptide-24 produced antibodies after 1 month. Sera obtained from one rabbit injected with peptide-24-BSA had titers ranging from 1:50 to 1:300 dilutions of the antisera. Sera from the other rabbit immunized with peptide-24-BSA and those rabbits immunized with peptide-24 had titers ranging from 1:800 to 1:4800. Chart 1 shows the titration curves of 2 antisera, one obtained from a rabbit immunized with peptide-24-BSA (R317) and the other from a rabbit immunized with peptide-24 (R1051). Total binding of the labeled Tyr-peptide-24 was between 65 and 75%, and the nonspecific
binding in the precipitates with preimmune sera ranged between 5 and 15%. These particular antisera were used in the inhibition experiments described below at dilutions that gave between 40 and 50% of binding of $^{125}$I-Tyr-peptide-24 without inhibitors present in the incubation mixtures.

In order to determine the sensitivity of the RIA, unlabeled peptide-24 and Tyr-peptide-24 were used as inhibitors as shown in Chart 2. Inhibition of antibody-$^{125}$I-Tyr-peptide-24 complex formation could be discerned with 0.5 ng of peptide-24, and 50% inhibition was obtained with 1.0 ng, whereas the Tyr-peptide-24 was less effective, giving 50% inhibition at 5.0 ng. This indicates that the addition of the tyrosine residue to the peptide (needed for labeling purposes) alters the affinity of the peptide-24 sequence for the anti-peptide-24 antibodies. The titers of the antisera and the percentage of inhibition obtained with unlabeled peptide-24 were reproducible throughout all the experiments performed and with different preparations of $^{125}$I-Tyr-peptide-24.

The R1051 antisera collected at several intervals during the immunization procedure formed precipitin bands on double immunodiffusion analyses in agarose gels with both the peptide-24 and the Tyr-peptide-24, whereas no precipitin bands formed between either peptide preparation and preimmune serum or nonimmune rabbit sera. The R317 antisera were not similarly tested. In order to determine whether the anti-peptide-24 serum reacted with a majority of the molecules in the Tyr-peptide-24 preparation, a precipitin reaction was performed with the IgG fraction of R1051 antiserum as described in "Materials and Methods." The Tyr-peptide-24 was used because of the absorbance in the UV spectral range of the tyrosine residue and because the Tyr-peptide-24 was the preparation radiolabeled with $^{125}$I and utilized in the RIA experiments. The precipitate that formed was dissolved in 0.1 N NaOH and chromatographed on Sephadex G-100, and the elution profile obtained is shown in Chart 3. By comparing the areas under the Tyr-peptide-24 peak to a known amount of Tyr-peptide-24 chromatographed separately on the same Sephadex G-100 column, it was estimated that at least 65% of the components of the Tyr-peptide-24 preparation were pre-

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**Chart 1.** Titration of anti-peptide-24 sera by RIA with $^{125}$I-Tyr-peptide-24 as antigen. •, Rabbit 1051 antisera; x, Rabbit 317 anti-peptide-24 sera; ---, nonspecific binding of the labeled antigens by preimmune or normal rabbit sera.

**Chart 2.** Inhibition of anti-peptide-24-$^{125}$I-Tyr-peptide-24 complex formation by peptide-24 (•) and Tyr-peptide-24 (x). Rabbit 317 antiserum was used at a dilution of 1:1600.

**Chart 3.** Gel filtration chromatography of the precipitate obtained between Tyr-peptide-24 and the IgG fraction of R1051 antiserum. The precipitin reaction and chromatography on Sephadex G-100 were performed as described in "Materials and Methods." The dissolved precipitate (•) gave 2 elution peaks, one in the void volume (IgG) and one included peak (Tyr-peptide-24). The latter peak eluted in the same region as did 50 µg of Tyr-peptide-24 applied to the column (x). The supernatant (100 µl applied) of the precipitin reaction (O) showed no detectable Tyr-peptide-24. The IgG fraction of the antiserum gave only one peak which eluted in the void volume (not shown).
cipitated under the conditions used. Thus, the majority of the Tyr-peptide-24 preparation was reactive with anti-peptide-24 antibodies.

The various CEA and NCA glycoproteins were tested for their abilities to inhibit anti-peptide-24-125I-Tyr-peptide-24 complex formation as shown in Chart 4. CEA-I was the most effective inhibitor with both the R1051 and R317 antisera giving 50% inhibition on addition of between 10 and 20 µg of the glycoprotein to the incubation mixture. Only 25% inhibition was seen with R1051 antiserum when a total of 80 µg of CEA-II was used; in the case of R317 antiserum, 20% inhibition resulted at the highest amount used, 40 µg. CEA-III showed no inhibition at up to 80 µg with R1051 antiserum. Both NCA preparations gave inhibitions slightly better than did CEA-II with R1051 antiserum; with R317 antiserum, the higher amounts of NCA's gave apparent lower values of inhibition. However, because of the intrinsic greater variability of the inhibition data at low values, those data are interpreted to mean that NCA-I and NCA-II probably result in little or no inhibition of the R317 antiserum. The limited amounts of these CEA and NCA preparations prevented testing larger amounts, although it appears that complete inhibition with CEA-I of the antibody-antigen complex formation could be obtained with approximately 200 to 300 µg.

The inhibition seen with the CEA and NCA preparations may be due either to a small cross-reactivity of the amino-terminal sequences of the glycoproteins with anti-peptide-24 antibodies or to exposed amino-terminal sequences accessible to reaction with antibody of only a small percentage of the CEA and NCA molecules in the preparation. In order to test whether degradation of the glycoproteins could result in increased accessibility of the amino terminus and enhanced reactivity with anti-peptide-24 antibodies, the CEA's and NCA's were treated by several chemical and protease degradative methods. In addition, chloramine-T treatment of the glycoproteins was attempted because between 10 and 30% of the total counts of different 125I-labeled CEA preparations were bound by the 2 anti-peptide-24 sera indicating possible exposure of the amino-terminal sequence under the conditions of the radiolabeling procedure.

The results of the inhibitions of degraded CEA's and NCA-II compared to the nondegraded substances are presented in Table 1. The inhibition values (mean ± S.E.) in the table were calculated from values where the same amounts of the various inhibitors were used. Thus, although the RIA inhibition curve is not linear with respect to amount of inhibitor added, the mean values for each of the CEA's and NCA-II and their degradation products accurately reflect their relative inhibitory activities. In all cases, the degradation procedures did not result in great increases of capacity to inhibit the anti-peptide-24-125I-Tyr-peptide-24 complex formation. For both CEA-I and CEA-II, base and chloramine-T treatments resulted in slight increases of inhibition, and CEA-III, which showed no inhibition in the nondegraded form, gave only minimal inhibition after the degradation procedures. Protease treatment of CEA-II resulted in a loss of inhibitory activity. Only a small amount of NCA-II was available for testing, and base degradation resulted in a loss of activity. In the anti-CEA-125I-CEA RIA system, chloramine-T treatment or reduction and alkylation resulted in slight losses of inhibitory activities, whereas base treatment of CEA's resulted in great losses of immune reactivity.

**DISCUSSION**

The results show that antisera can be produced to a synthetic peptide corresponding to the 24-amino acid amino-terminal sequence of CEA and that the Tyr-peptide-24 could be utilized in a RIA to assay the antisera and to ascertain the specificities of anti-peptide-24 antibodies. Both peptide-24 and peptide-24 conjugated to BSA resulted in antisera with specificities to the peptide-24, and 2 of the sera with the highest titers were selected for further cross-reactivity studies with CEA and NCA preparations. The best inhibitor was CEA-I which resulted in 50% inhibition of anti-peptide-24-125I-Tyr-peptide-24 complex formation at about 15 µg. This amount of CEA-I is 1.5 × 10^4 greater than the amount of peptide-24 needed to give the same inhibition and represents a 250-fold molar excess of the CEA-I compared to peptide-24. One practical consequence of these results is that the anti-peptide-24 RIA could not be used for the quantitation of CEA or related materials in biological specimens.
as the extent of cross-reactivity was very small. Furthermore, as shown in Chart 3, the R1051 antiserum reacts with both CEA and NCA preparations, although the R317 antiserum may not react well with NCA. Although it has been shown that CEA and NCA differ in their amino-terminal sequences at position 21 [Engvall et al. (5)], it appears that the R1051 anti-peptide-24 serum does not differentiate between the glycoproteins under the conditions of the RIA. It is possible that an antibody specificity in the anti-peptide-24 sera may selectively react with the CEA amino-terminal sequence and not with that of NCA; however, the finding that only a very small percentage of either the CEA's or NCA's interact with anti-peptide-24 sera were produced by immunization with the peptide-24 through the carboxy-terminal leucine residue. The R1051 antiserum was produced by immunization with the peptide-24 without a carrier, although again the conformation(s) of the peptide-24 (and the antibody specificities to one or more conformational amino acid sequences of the peptide-24) may be only partially similar to the same sequence of the amino terminus of CEA. In addition, the placement of the tyrosine residue (for radiolabeling purposes) at the amino terminus of the RIA system was not altered to any great extent, indicating that the immunodeterminants reactive with anti-CEA are clearly different from the amino-terminal sequence. We have not tested anti-CEA sera for reactivity with $^{125}$I-Tyr-peptide-24, although even if reactivity were seen, those antibody specificities may represent only a small proportion of the total anti-CEA antibodies and may not be significant at the dilution used in the anti-CEA RIA system.

Because the peptide-24 contains 3 glutamic acid residues in addition to the carboxy-terminal leucine residue, the carbodiimide coupling reaction of peptide-24 to BSA would yield a mixture of conjugate with respect to the attachment site to peptide-24. Thus, the R317 antiserum (rabbit immunized with peptide-24-BSA conjugate) would be expected to contain a heterogeneous mixture of antibodies to different determinants and conformational arrangements of the peptide-24. An antiserum of greater specificity and cross-reactivity with CEA may be obtained by specifically conjugating the peptide-24 with a crude CEA preparation. We have not been able to demonstrate inhibition in our anti-peptide-24 assay system using sera of cancer patients as did the latter authors. Also, we have not yet attempted to ascertain whether various anti-CEA sera may exhibit reactivity with $^{125}$I-Tyr-peptide-24, whereas Arnon et al. (1, 2) presented data that anti-CEA reacted with and was inhibited by their synthetic peptide.

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The large molar excess (250-fold for CEA-I) of the CEA and NCA preparations needed to produce partial inhibition of the anti-peptide-24-$^{125}$I-Tyr-peptide-24 complex formation may be due to a poor cross-reactivity of the amino-terminal sequence of the glycoproteins because of differences in the conformation of the sequences as compared to that adopted by the free peptide-24 in solution. Alternatively, very few of the CEA or NCA molecules may have exposed amino-terminal sequences capable of interacting with the antibodies. In order to test the latter possibility, the CEA's and NCA-II were degraded under several conditions in attempts to expose a greater percentage of amino-terminal sequences. The procedures did not result in greater inhibitory activity of CEA-I and only small increases in activity of CEA-II, and both CEA-III and NCA-II were only slightly altered in activity. If it is assumed that the degradation proce-
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