THE ELECTROPHORETIC HETEROGENEITY OF CARCINOEMBRYONIC ANTIGEN

An immunochemical analysis

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1. Introduction

Carcinoembryonic antigen (CEA) has been isolated from perchloric acid (PCA) extracts of colonic carcinoma, their metastases to the liver [1,2] and, in limited amounts, from PCA extracts of normal colon [3]. Purified CEA has been shown to be a glycoprotein containing a significant amount of carbohydrate by weight [1,2]. CEA shows a 7–8 S sedimentation coefficient in the ultracentrifuge and av. mol. wt 200 000 as judged by gel filtration and sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) [4].

Purified CEA, when assessed by PAGE, isoelectric focusing, or SDS–PAGE, shows considerable electrophoretic heterogeneity, often giving a single broad migrating zone [5]. Neuraminidase treatment reduces the extent of polydisperity observed by PAGE and isoelectric focusing, but not completely [5]. SDS–PAGE heterogeneity is more difficult to assess. Electrophoretic mobility differences observed by this technique could be due to differences in the polypeptide molecular weight, unequal binding of SDS or differences in the content and composition of covalently-bound carbohydrate. We have noted a striking and reproducible bimodal distribution of molecular species when commercially-available ^{125}I-radiolabeled CEA (^{125}I-CEA) was analyzed by SDS–PAGE. We have attempted to utilize this difference in SDS–PAGE mobility to determine first, whether both species were reactive with specific antisera, and, second, whether this system could be used to study subtle differences in CEA antigicity. CEA antigenic heterogeneity has been proposed [6]; several CEA standards and their corresponding antisera were compared by radioisotopic competition studies. Their results suggested that isoantigens of CEA exist, and available antisera are probably polyspecific for these variations.

Commercially-available ^{125}I-CEA standard was compared by immunoprecipitation with three different anti-CEA sera, and the precipitates were analyzed by SDS–PAGE. The results suggest that the SDS electrophoretic bimodal mobilities cannot be explained by isoantigenic differences and that both molecular species characterized by SDS–PAGE are precipitable with anti-CEA.

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2. Materials and methods

2.1. *Antisera*

Antisera produced in goats against CEA were obtained from several sources. Two different preparations of Roche anti-CEA were obtained, one from the commercially-available kit (QC no. 77436, Hoffman-LaRoche, Nutley NJ) and another (183-TXI) kindly supplied by Drs D. Haagensen (Duke University) and J. Primus (Hoffman-LaRoche). Hope goat anti-CEA (Ace 67-70) was obtained from Drs M. Egan and C. Todd (City of Hope National Medical Center, Duarte, CA), and Montreal goat anti-CEA was sup-

plied by Dr P. Gold (McGill University Medical School, Montreal). An aliquot of the Hope anti-CEA was absorbed with a perchloric acid extract of a normal human spleen homogenate as suggested by Dr M. Egan.

Rabbit anti goat IgG was purchased from Miles Laboratories, Inc. (Elkhart, IN). Each lot was titrated by quantitative immunoprecipitation against goat IgG and used in assay systems at equivalence.

2.2. *Carcinoembryonic antigen (CEA) sources*

Three preparations of Roche ^{125}I-CEA were obtained from Hoffman-LaRoche (QC no. 0317-09086, QC 0109-02256 and QC 0173-05187).

2.3. *Immunoprecipitation*

Precipitation of ^{125}I-CEA was performed in calf serum (Grand Island Biological Co.)-coated 6 × 50 mm test tubes as suggested by Dr Peter Cresswell (personal communication). Generally, 5 μl undiluted antiserum was added to 50–100 μl ^{125}I-CEA, and sufficient rabbit anti-goat IgG was added to achieve maximal precipitation of the goat IgG (~ 100 μl, depending on the titer of the antiglobulin). This mixture was incubated at 4°C overnight.

The precipitates were centrifuged at 1000 rev./min for 5 min in an IEC table-top centrifuge and washed 3 times in Dulbecco's phosphate-buffered saline, pH 7.0 (Grand Island Biological Co.). A final wash was done in distilled water. The initial mixture, wash supernatants and final pellet were counted for ^{125}I.

The final pellet was dissolved in 100 μl sample buffer containing SDS, β-mercaptoethanol and bromophenol blue tracking dye as in [7], and heated in a boiling water bath for 2–5 min. Analysis was performed by SDS–PAGE utilizing a discontinuous buffer system [7].

2.4. *SDS–PAGE*

The immune precipitates dissolved as above or 10–50 μl ^{125}I-CEA in sample-reducing buffer (total 100 μl) were applied to polyacrylamide gel-containing tubes of a Buchler Polyanalyst Disc Electrophoresis Apparatus (Buchler Instruments, Ft. Lee NJ). The conditions for discontinuous PAGE, electrophoretic stacking and polypeptide separation have been detailed [7]. Generally, 3% polyacrylamide stacking gels and either 5% or 7.5% resolving gels were used.

Following electrophoresis, the polyacrylamide gels were removed from the tubes, frozen and sliced with a Bio-Rad 190 gel slicer (Bio-Rad Laboratories, Richmond, CA). Sections of 1 mm were loaded into 12 × 75 mm disposable test tubes and counted for ^{125}I on a Searle 1185 series gamma counter (Des Plaines IL).

Protein standards were simultaneously electrophoresed, and these gels were stained with Coomassie brilliant blue. Rabbit muscle myosin was prepared in our laboratory as in [8]. Human transferrin and hen ovalbumin were purchased from Sigma Chemical Co. (St Louis MO), and β-galactosidase was obtained from Worthington Biochemical Corp. (Freehold NJ).

3. Results

Preliminary experiments were performed to establish conditions where the radiolabeled antigen was in slight excess with respect to anti-CEA. The amount of antiserum used was designed to give 30–60% precipitation of radiolabeled CEA (table 1), a range chosen to optimize the detection of antigenic variability. Hope anti-CEA was used before and after absorption with a perchloric acid extract of normal human spleen homogenate. The titer of the Hope antiserum fell 2-fold following absorption due to dilution (data not shown), and a correspondingly greater amount of

Table 1
% ^{125}I-radiolabeled CEA precipitated with three different antisera for use in SDS–PAGE analysis[a]

Anti-CEA	Mean % cpm precip. ± SD	No. exp.
Roche	34.3 ± 8.7	10
Hope unabs.	55.3 ± 0.4	2
Hope abs.[b]	54.7 ± 2.8	2
Montreal	50.5 ± 1.6	2
Normal goat serum	1.9 ± 0.1	2

[a] Preliminary titration experiments were performed to establish conditions where radiolabeled antigen was in slight excess with respect to specific antibody (data not shown)

[b] Hope anti-CEA was assayed before and after absorption with a perchloric acid extract of human spleen homogenate (section 2)

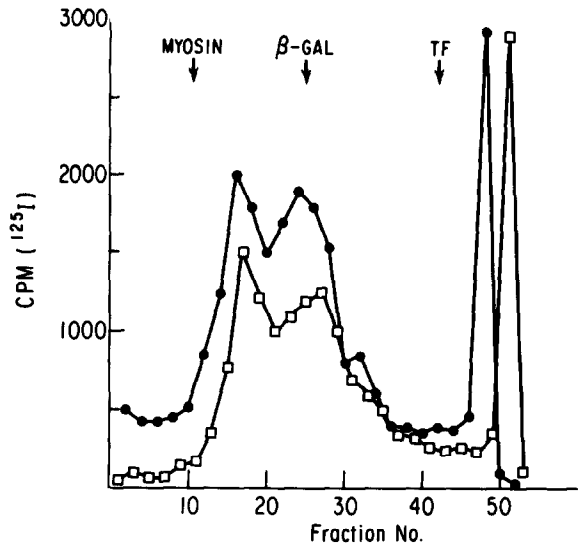

Fig.1. SDS–PAGE analyses of ^{125}I-CEA (□–□) and immune precipitated ^{125}I-CEA (●–●). 50 μl SDS-treated labeled CEA was run directly. Another 50 μl aliquot was precipitated with Montreal anti-CEA and rabbit anti-goat IgG. The immune precipitate was dissolved in SDS buffer and treated as described in section 2. 5% resolving gels were used, and a calibration curve of mobility versus polypeptide molecular weight was established with myosin heavy chain (mol. wt 210 000), β-galactosidase (mol. wt 135 000) and human transferrin (mol. wt 79 000). The ^{125}I cpm/1 mm slice are plotted versus the fraction (slice) number.

Table 2
Apparent molecular weights of ^{125}I-radiolabeled CEA as assessed by SDS–PAGE[a]

Gel system	First peak mol. wt ± SD (× 10^{-3})	Second peak mol. wt ± SD (× 10^{-3})	No. samples analyzed[b]
5.0% acrylamide	173.9 ± 13.4	130.5 ± 8.2	22
7.5% acrylamide	173.7 ± 11.0	138.0 ± 4.2	4

[a] The apparent molecular weight was calculated from standard curves relating the SDS–electrophoretic mobility of standard proteins to known polypeptide molecular weights. This method is not generally valid for glycoproteins containing significant covalent carbohydrate by weight

[b] Data from specific immune precipitation analyses and from untreated CEA analyses are included, and represent experiments performed over 1 year. Three Roche ^{125}I-CEA samples are included in these results

absorbed Hope anti-CEA was used in subsequent studies.

Figure 1 shows representative data for SDS–PAGE analysis of Roche ^{125}I-CEA on 5% polyacrylamide gels [7], and for a similar analysis of an SDS-solubilized CEA anti-CEA immune precipitate. Montreal anti-CEA was used in this illustration, however, identical data were obtained with Roche, absorbed Hope

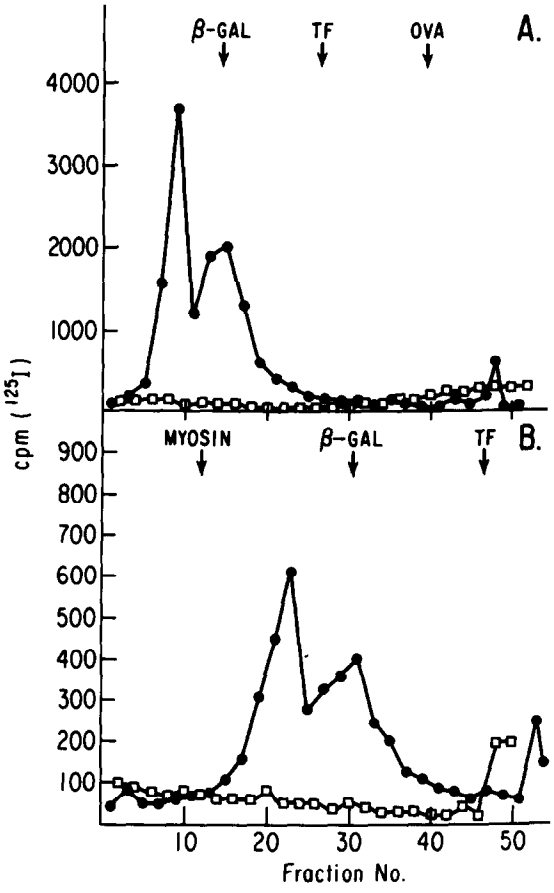

Fig.2. SDS–PAGE analyses of immune precipitated ^{125}I-CEA. Precipitates were formed with ^{125}I-CEA, Roche anti-CEA (●–●) or normal goat serum (□–□), and rabbit anti-goat IgG. The precipitates were washed, counted (see table 1), solubilized with SDS sample reducing buffer and treated as described in section 2. Either 7.5% (A) or 5% (B) polyacrylamide resolving gels were used. Calibration curves were established from the migration of myosin heavy chain, β-galactosidase, human transferrin and hen ovalbumin (mol. wt 45 000). The ^{125}I cpm/1 mm slice are plotted versus the fraction (slice) number.

and unabsorbed Hope anti-CEA. Figure 2 shows SDS–PAGE profiles of ^{125}I-CEA precipitated with Roche anti-CEA and with normal goat serum as a control. The bimodal distribution of immune precipitated species was observed on both 5% and 7.5% polyacrylamide gels, and was indistinguishable from the results for SDS-treated ^{125}I-CEA run alone. Although CEA is heavily glycosylated, there did not seem to be a large effect of polyacrylamide concentration (over a limited range) on apparent molecular weight (table 2).

4. Discussion

SDS–PAGE analyses of Roche ^{125}I-CEA consistently gave a bimodal pattern of electrophoretic mobilities (fig.1). We hypothesized that these species might be antigenically distinct, in that Roche ^{125}I-CEA and CEA standards were distinct [6] from other available CEA standards by radioimmune competition assay. We established conditions which gave a similar antigen-to-antibody ratio utilized in [6], and compared ^{125}I-CEA with specifically-precipitated ^{125}I-CEA on SDS–PAGE. Regardless of the source of anti-CEA, we could not distinguish any differences in ^{125}I-CEA and immune-precipitated material by this method. Radiolabeled material in both peaks of the bimodal distribution seem equally reactive with all anti-CEA sera tested (fig.1, 2A, 2B and data not shown) in that the ratio of the two peaks was always the same.

The possibility that the bimodal distribution results from an artifact of the chloramine T oxidation method for labeling CEA seems unlikely. In unpublished results, we have determined that CEA labeled by either the chloramine T oxidation method [9] or by the gentle acylating method [10] is identical. We conclude that the differences in SDS–PAGE mobility are not due to contaminating molecules or to antigenic variants, but are related to physical differences (polypeptide molecular weight or molecules of SDS bound per molecule of CEA) or to chemical differences (content and composition of the covalently-bound carbohydrate).

Acknowledgement

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References

[1] Rogers, G. T. (1976) Biochim. Biophys. Acta 458, 355–373.
[2] Terry, W. D., Henkart, P. A., Coligan, J. E. and Todd, C. W. (1974) Transplant Rev. 20, 100–129.
[3] Fritsche, R. and Mach, J.-P. (1977) Immunochemistry 14, 119–127.
[4] Coligan, J. E., Lautenschleger, J. T., Egan, M. L. and Todd, C. W. (1972) Immunochemistry 9, 377–386.
[5] Slayter, H. S. and Coligan, J. E. (1974) Biochemistry 13, 3365–3371.
[6] Vrba, R., Alpert, E. and Isselbacher, K. J. (1975) Proc. Natl. Acad. Sci. USA 72, 4602–4606.
[7] Maizel, J. V. (1971) in: Methods in Virology (Maramorosch, K. and Koprowski, H. eds) vol. 5, pp. 180–246, Academic Press, New York.
[8] Perry, S. V. (1955) in: Methods in Enzymology (Colowick, S. P. and Kaplan, N. O. eds) vol. 2, pp. 582–588, Academic Press, New York.
[9] Hunter, W. M. (1973) in: Handbook of Experimental Immunology, 2nd edn (Weir, D. M. ed) pp. 17.1–17.36, Blackwell Scientific, Oxford.
[10] Bolton, A. E. and Hunter, W. M. (1973) Biochem. J. 133, 529–539.