Vol. 115, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1983 Pages 206-211

THE DIMERIC STRUCTURE OF CARCINOEMBRYONIC ANTIGEN (CEA)

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Received July 12, 1983

SUMMARY: The results of SDS-polyacrylamide gel electrophoresis and cross-linking experiments indicated that carcinoembryonic antigen is a dimer composed of two identical or closely similar noncovalently bound subunits, dissociating on heating in the presence of SDS. The dissociation is reversible upon the detergent removal.

Carcinoembryonic antigen (CEA) was originally found in 1965 by Gold and Freedman (1,2) in human colonic carcinoma and in foetal tissues. Since that time CEA has been a subject of wide interest of biochemists and clinicians and is one of the best characterized human tumor-associated antigens (3,4). CEA is a glycoprotein containing 50 - 60% carbohydrates and shows some heterogeneity in size, charge and interaction with lectins, most probably due to the heterogeneity of the carbohydrate portion. The apparent molecular weight of CEA, determined by gel filtration (5,6) or SDS-polyacrylamide gel electrophoresis (6-9), is in the range 170,000 - 210,000. These values are concordant with sedimentation coefficients 6.8 - 8.0 S found for various CEA preparations (10 - 12). Moreover, the isolation of higher (about 300,000) or lower (120,000 - 140,000) molecular weight variants of CEA has been reported (5,9,12-14), but their structural relation to CEA with molecular weight of about 200,000 has not been elucidated. It has been generally accepted that CEA molecule consists of a single polypeptide chain

CEA - carcinoembryonic antigen, NCA - nonspecific cross-reacting antigen, SDS - sodium dodecyl sulfate, DMSI - dimethyl suberimidate.

0006-291X/83 $1.50

containing about 600 amino acid residues (3,9,15-17). The polypeptide chain of CEA seems to be homogeneous, since uniform and single sequence of 24 amino acid residues of the amino-terminal portion of various CEA preparations was found (15,18-20). CEA contains 12 half-cystine residues forming 6 intramolecular disulfide bridges (21).

We have recently found that nonspecific crossreacting antigen (NCA, one of several known glycoproteins immunologically related to CEA) of molecular weight 100,000 - 120,000 is composed of subunits of an apparent molecular weight 50,000 (22). It prompted us to study the problem of an eventual subunit structure of CEA. The results presented here led us to the conclusion that CEA molecule also is a dimer composed of two noncovalently bound subunits.

MATERIALS AND METHODS

CEA was purified from liver metastases of colon adenocarcinoma by a conventional procedure, including extraction with perchloric acid, gel filtration and ConA-Sepharose 4B affinity chromatography (23). The purified CEA was homogeneous in the Sephadex G-200 gel filtration (it was eluted at a typical position between V_0 and IgG) and in SDS-polyacrylamide gel electrophoresis.

SDS-polyacrylamide gel electrophoresis was carried out in the system of Weber and Osborn (24).

SDS was removed with Bio-Beads SM-2 (Bio Rad, USA), replacing the column method of Fox et al. (25), by the batch method (22). Briefly, the samples (0.1 % CEA solution in the sample buffer containing 1 % SDS) were diluted 5-fold with water and treated with Bio-Beads SM-2 for 1 h at 20° with gentle rotation. The supernatant was aspirated and the effectiveness of this procedure was controlled by the colorimetric determination of SDS (26). Using 4 mg wet beads per 0.1 mg SDS was sufficient for removal of over 99% of the detergent.

CEA was cross-linked with dimethyl suberimidate (DMSI, Sigma, USA) under conditions providing the cross-linking of oligomeric proteins predominantly within oligomers (27).

RESULTS

The starting point of our experiments was the observation that the electrophoretic mobility of CEA depended on the prior treatment of samples. CEA submitted to electrophoresis directly after dissolving in the 1% SDS-containing buffer migrated more slowly than the sample preheated for 5 min at 100°C (Fig. 1, A and B). The transformation of CEA into the faster-migrating form also occurred at lower temperatures, but required a longer time. After preincubation of the electrophoretic samples for 1 h at 37°C both bands were produced (not shown), after 12 h at 37°C or 4 days at room temperature CEA was totally transformed into the faster-migrating form (Fig. 1E). The effect of SDS on CEA could be re-

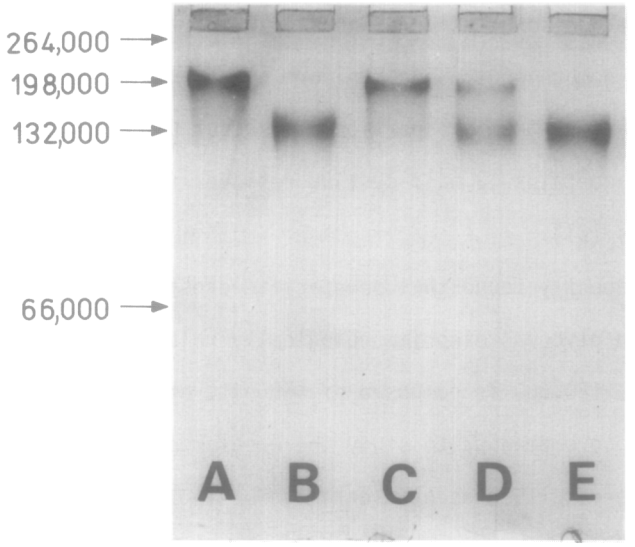

Fig. 1. SDS-polyacrylamide gel electrophoresis of CEA. Electrophoretic samples: 30 μg CEA in 30 μℓ sample buffer (containing 1% SDS). A, sample untreated; B, sample heated for 5 min at 100°C; C, sample heated for 5 min at 100°C, treated with Bio-Beads SM-2 to remove SDS, dried in vacuum desiccator over P_2O_5, and redissolved in 30 μℓ 1% SDS and directly applied to the gel; D, CEA cross-linked with DMSI (at CEA and DMSI concentration 2 mg/mℓ and 10 mg/mℓ, respectively), than diluted twice with sample buffer containing 2% SDS and incubated for 12 h at 37°C; E, CEA incubated for 12 h at 37°C. Arrows indicate the positions of molecular weight markers (Sigma, USA, Product No. A 9392 containing monomer, dimer, trimer and tetramer of cross-linked bovine albumin). Electrophoresis was done on 1.4 mm-thick 5% slab gel. Gel was stained with Coomassie Brilliant Blue R-250.

versed by an exhaustive removal of the detergent. CEA samples preheated for 5 min at 100°C in 1% SDS were treated with various amounts of Bio-Beads SM-2, (see Materials and Methods). The detergent-deprived samples were dried in vacuum desiccator over P_2O_5, redissolved in 1% SDS and submitted directly to electrophoresis. When less than 1% of the initial amount of SDS was detectable in Bio-Beads SM-2-treated samples, CEA was almost totally re-transformed into the "slow" form (Fig. 1C), but when about 5% of SDS was left, a low degree of reversibility was observed, i.e. the upper band was less intense than the lower one (not shown). All effects observed were independent of the presence of 2-mercaptoethanol which neither changed the position of electrophoretic bands of CEA (in agreement with the literature data (8,12,17), nor affected the transformation of its "slow" form into the "fast" one.

The results obtained, which were identical for 4 independent CEA preparations, suggested that CEA molecules, similarly as those of NCA (22), are compos-

ed of noncovalently bound subunits which dissociate in the presence of SDS. However, the difference in electrophoretic mobility of both forms of CEA was smaller than expected for dimer and monomer. CEA, similarly to other glycoproteins, shows an anomalous migration in SDS-polyacrylamide gel electrophoresis and its apparent molecular weight determined by this method depends on the concentration of the gel (8). In our experiments an apparent molecular weight of the "slow" and "fast" form of CEA was found to be, respectively, 220,000 and 153,000 in 5% gel, or 180,000 and 132,000 in 7% gel. It was considered whether the increase in electrophoretic mobility of CEA after preincubation with SDS occurred in fact due to the dissociation of CEA into subunits, or due to other reasons, e.g. changing the shape of the molecules or increased loading of CEA with SDS. The formation of two distinct electrophoretic forms of CEA and the lack of intermediate zones was in favour of the subunit structure concept.

To further substantiate this conclusion we applied the method of protein cross-linking with dimethyl suberimidate (DMSI), a procedure used to effectively evaluate the number of subunits in oligomeric proteins (27). The DMSI-treated CEA, dissolved in SDS-containing buffer and directly submitted to electrophoresis, showed one band located exactly at the same position as the "slow" band of an untreated CEA sample, confirming that no intermolecular cross-linking occurred. However, two bands, of the "slow" and "fast" form of CEA, were observed when the DMSI-treated CEA was incubated before electrophoresis in 1% SDS for 12 h at 37°C (Fig. 1D), i.e. under conditions providing a complete dissociation of CEA untreated with DMSI (Fig. 1E). The only reasonable explanation of this result was that CEA molecules have the dimeric structure and that this portion of dimers which was internally cross-linked with DMSI was not able to dissociate.

DISCUSSION

The subunit structure of CEA may have excaped attention, since the dissociation gives a relatively small increase in electrophoretic mobility of CEA. The effect of heating in SDS is hardly seen when the gels of higher polyacrylamide concentration (7 - 10%) are loaded with larger amount of CEA and broad zones

are formed. The anomalously slow electrophoretic migration of CEA monomer in relation to the dimer may result from the shape of the molecules. The rigorous method of molecular weight determination, taking into account hydrodynamic properties of CEA molecules in dimeric and monomeric form, should elucidate this problem. The opinion about monomeric character of CEA molecule has been based on the unchanged apparent molecular weight (shown in SDS-polyacrylamide gel electro phoresis and gel filtration) after reduction of disulfide bonds (8,12,17,21) and after treatment with 6M guanidine HCl (6,9). This view also was supported by the yields of amino acid derivatives obtained during amino-terminal sequence determination of CEA (15,16). On the other hand, there are some literature data which now seem to be in favour of the subunit structure of CEA. The proteolytic degradation of CEA gave a distinctly lower number of peptides than expected from its molecular size (28,29). Hammarström and his coworkers (29) assumed that one of possible explanations would be the occurrence of the regions with similar structure more than once within CEA molecule. Similarly, studying the interaction of CEA with monoclonal hybridoma antibodies led to the conclusion that some epitopes may be present twice on the CEA molecule (30). Our conclusion on the dimeric structure of CEA is in line with these observations. Moreover, our results do not contradict the other literature data mentioned above, since subunits of CEA are not linked via the disulfide bonds, and they may be not dissociated by guanidine HCl. The dissociation of CEA in SDS suggests the predominance of hydrophobic interactions between the subunits, with possible contribution of hydrophobic blocks in the polipeptide chain, as those found in the amino-terminal portion of CEA (residues 17 - 21) (15,18-20), or in one of its tryptic peptides (28). Formation of the one electrophoretic band by the dissociated CEA, the single amino terminal sequence (15,18-20) and the results suggesting the repetition of homologous regions in the molecule (29,30) indicate that subunits of CEA are identical or closely similar. The apparent molecular weights of smaller-size variants of CEA found under some conditions (5,9,14) are similar to that shown by us for the CEA monomer. However, the relation of these variants to CEA monomer remains to be established.

ACKNOWLEDGEMENTS

This work was supported by the Polish National Cancer Program PR-6, Grant No. 2113.

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