IS SECRETORY COMPONENT DISULFIDE-BONDED TO BOTH MONOMER SUBUNITS IN HUMAN SECRETORY IgA DIMER? *

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Received November 19, 1974

SUMMARY

Human secretory IgA dimer and complexes formed *in vitro* with $[^{125}I]$-secretory component and dimeric IgA myeloma proteins, were cleaved with CNBr in 70% formic acid, for 18 hours at 4°C. The molecular size of the principal products resulting from the CNBr digests was determined by gel filtration on columns of 4% agarose in 5.0 M guanidine-HCl, pH 4.1. Under these conditions, the molecular weight of the principal fragment(s) containing secretory component was 150,000-230,000, depending on the protein studied. These results were incompatible with a model in which secretory component was joined to both 7S IgA monomer subunits and suggested that secretory component was disulfide-bonded to only one of the monomer subunits in human secretory IgA dimer.

INTRODUCTION

Secretory IgA, the major Immunoglobulin in the secretions of man and most animal species, is composed of subunits derived from two different cell types. The principal product of the IgA-producing mucosal plasma cell is a dimeric molecule consisting of heavy (H=α), light (L=κ or λ) and J chains with the following structural formula: $(H_2L_2)_2 \cdot J$ ** (1-2). Secretory component (SC) appears to be synthesized in mucosal epithelial cells (3-4) and current hypotheses suggest that the IgA dimer combines with SC while crossing the mucosal epithelium to form a composite molecule $(H_2L_2)_2 \cdot J \cdot SC$, which is then secreted into the lumen of mucosal glands or organs (5-6).

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* Supported by the Medical Research Council of Canada.

** Nomenclature for Immunoglobulins corresponds to that recommended by the World Health Organization. H=heavy chain; L=light chain; J=J chain; SC=secretory component; IgA=Immunoglobulin A.

In man, SC is apparently linked to the IgA dimer, $(H_2L_2)_2 \cdot J$, through disulfide bonds although the actual location of these bonds has not been established (7-8). Most reviews on the structure of secretory IgA have assumed that SC is disulfide linked to both monomer (H_2L_2) subunits (9-11). However, direct evidence for this assumption is lacking. Available data indicate that SC is linked to the α chain since Fc fragments isolated from rabbit (12) and human (13) secretory IgA have been found to contain SC. Mestecky et al (14) reported that following CNBr cleavage of intact secretory IgA dimer, a fragment consisting of J chain and the C-terminal octapeptide of the α chain could be split away from a larger fragment containing SC, α, and light chains. The large molecular weight fragment was not extensively characterized. On the basis of their data these authors concluded that SC was not disulfide bonded to J chain.

The experiments reported here were designed to obtain more definitive evidence to support a model of secretory IgA dimer in which SC disulfide-links both monomer subunits. Our strategy was to cleave human secretory IgA dimer with CNBr and to determine the molecular size of the principal products by gel filtration of the digest on a column of 4% agarose equilibrated with 5.0 M guanidine-HCl (8,15). Similar experiments were also performed with secretory IgA dimers prepared in vitro by forming complexes with serum IgA dimers isolated from myeloma sera, and $[^{125}I]$-labelled free SC isolated from human whey. In the experiments with $[^{125}I]$-SC-IgA complexes, the molecular size of SC-containing fragments could be determined by counting radioactivity in the column effluents following gel filtration of CNBr digests of the in vitro secretory IgA dimers.

Under the acidic conditions employed, CNBr selectively cleaves peptide bonds at methionine residues (16). Since SC is devoid of methionine residues (17) and the α chain contains only 2 methionines in the constant region, 9 and 40 residues from its C-terminus (18-19), it was anticipated that the principal product isolated from CNBr digestion of intact secretory IgA dimer would be a

dimer-like molecule consisting of SC joined to two monomer-like subunits. From a consideration of available sequence data (18-21), it was estimated that the minimum molecular weight of the CNBr-derived dimer would be approximately 276,000 for a secretory IgA dimer of the IgA1 subclass. This was based on the assumption that the variable regions of both heavy and light chains might be cleaved away in addition to the C-terminal 39 residues of the α chain, leaving 4 α chain fragments of approximately 40,000 molecular weight, 4 light chain fragments having a molecular weight of 11,250, and one mole of SC (M.W. = 71,000). In the case of secretory IgA dimers of the IgA2 ($Am_2(1)$) subclass with dissociable light chains, a minimum expected molecular weight for the putative CNBr-derived dimer would be 231,000. The data obtained are not consistent with the current models for Secretory IgA and suggest that a single SC may be disulfide-linked to only one of the monomer subunits in the secretory IgA dimer.

MATERIALS AND METHODS

Human secretory IgA dimer obtained from colostral whey, and serum IgA dimers obtained from IgA myeloma sera, were prepared as previously described (22). Free human SC prepared according to published procedures (23), was labelled with $[^{125}I]$ by the iodine monochloride technique (24). Complexes of $[^{125}I]$-SC with dimeric IgA myeloma proteins were formed by incubating 5 ug of $[^{125}I]$-SC with 30 mg of purified IgA myeloma dimer for 1 hour at 23°C in PBS (0.01 M potassium phosphate, 0.15 M NaCl, pH 7.4). The mixture was applied to a Sephadex G-200 column in PBS and the first fraction containing the IgA myeloma dimer and the $[^{125}I]$-SC-IgA complexes was collected and concentrated by ultrafiltration. CNBr cleavage of native secretory IgA, or IgA myeloma dimer containing $[^{125}I]$-SC-IgA complexes was carried out with 17 mg of IgA and 34 mg of CNBr in 70% formic acid for 18 hours at 4°C. The digest was then dialysed against 5.0 M guanidine-HCl, 0.01 M sodium acetate, pH 4.1, and applied to a column of Sepharose 4B equilibrated with the same buffer. In some experiments, the CNBr digests were diluted 10 x in water, lyophilized, and taken up in the guanidine

buffer. Under the latter conditions, an additional fraction eluting close to the total column volume was recovered which was lost when the digest was dialysed prior to application to the column. The elution volumes were determined by weighing the effluent fractions as recommended by Fish et al (15). The molecular weights of the digest products were estimated by comparing their elution volumes to that of proteins of known molecular weight. In a separate series of experiments, iodoacetamide at a final concentration of 0.001 M was incorporated into both digest and column solvents to prevent the possibility of disulfide interchange. There was no difference in the results obtained with or without iodoacetamide.

RESULTS

The absorbance profile obtained when a CNBr digest of human secretory IgA dimer was filtered on Sepharose 4B is shown in Figure 1. The first major

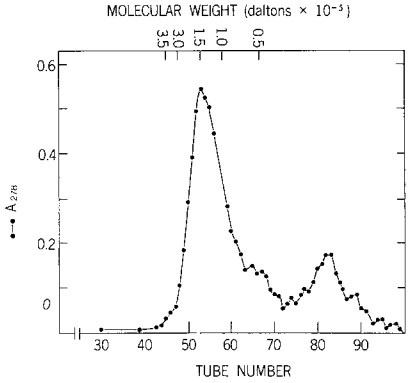

Figure 1. Gel filtration of a CNBr digest of 17 mg of human secretory IgA dimer on Sepharose 4B in 5 M guanidine-HCl, 0.01 M sodium acetate, pH, 4.1. The column was 1.6 x 90 cm and the flow rate was 6 ml/hr. The molecular weight axis shown was taken from a standard curve constructed by determining the elution volumes of a series of protein markers including: human secretory IgA dimer (M.W. = 385,000), human IgG (M.W. = 150,000), human serum albumin (M.W. = 65,000) and lysozyme (m.W. = 14,300).

fraction had a peak elution volume consistent with a modal molecular weight of 150,000, substantially less than that expected if SC were disulfide-linked to both monomer subunits in the intact dimer (See Above). In a control experiment, incubation of secretory IgA in 70% formic acid without CNBr, followed by gel

filtration in Sepharose 4B resulted in the elution of a major peak in an identical position to intact secretory IgA dimer. Immunodiffusion experiments with the pooled 150,000 molecular weight fraction dialysed against PBS, revealed the presence of both α chains, κ and λ chains, and SC. This fraction appeared to be heterogeneous as evidenced by the slight shoulder on the descending portion of the peak and a slightly greater peak width than that observed when IgG and monomer IgA monoclonal proteins were filtered on the same column. The second major fraction, eluting at tube #82 (Figure 1) had a modal molecular weight of approximately 10,000 and was presumed to be a fragment originating from the heavy or light chain variable regions.

Due to the inherent heterogeneity of secretory IgA and the difficulties in determining the individual elution profiles of SC- and α chain-containing fragments in the above experiment, additional experiments were carried out with homogeneous dimeric IgA myeloma proteins containing a small proportion of molecules complexed to $[^{125}I]$-SC (See Methods). Such complexes evidently form disulfide bonds between the $[^{125}I]$-SC and IgA myeloma dimer since they do not dissociate in solvents such as 5 M guanidine (25-26). The results obtained when such a mixture was treated with CNBr and analysed by Sepharose 4B gel filtration in guanidine are shown in Figure 2. The products resulting from cleavage of the IgA myeloma dimer were identified by the optical density profile. The elution profile of the radioactivity allowed identification of the fragment containing the $[^{125}I]$-SC. In the experiment shown in Figure 2, the first major fraction obtained following CNBr digestion of a dimeric IgA(λ) myeloma protein eluted with a molecular weight of 130,000 (absorbance profile). The radioactivity eluted as a major peak with an estimated molecular weight of 210,000. If the $[^{125}I]$-SC were disulfide-bonded to two of the monomer subunits in the complex then the radioactivity would have eluted at a position consistent with a molecular weight of 331,000 (2 x 130,000 + 71,000). The molecular weight observed (210,000), was very close to that expected if the $[^{125}I]$-SC were disulfide-bonded to only one monomer subunit, i.e., 201,000 (130,000 + 71,000).

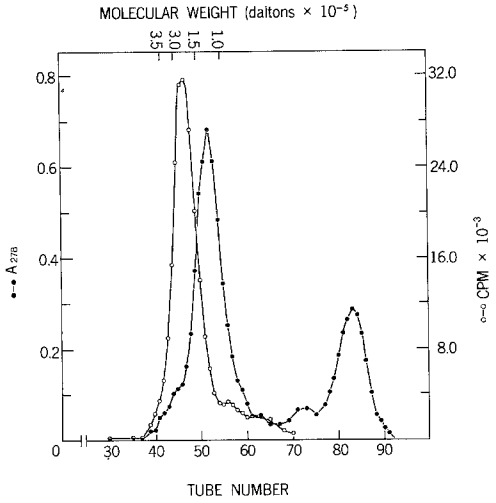

Figure 2. Gel filtration of a CNBr digest of 17 mg of an IgA(λ) myeloma dimer containing a small proportion of molecules complexed to $[^{125}I]$-SC. The conditions were similar to those listed in the legend of Figure 1.

Similar experiments were carried out with two other IgA(κ) myeloma dimers complexed to $[^{125}I]$-SC. In the latter cases the CNBr-derived IgA monomer eluted with a molecular weight of 150,000 and the $[^{125}I]$-SC-containing fraction eluted with a molecular weight of 230,000. Thus, the molecular weight of the SC-containing fragment was approximately 80,000 greater than the principal monomer-like fragment, suggesting that SC was disulfide-linked to only one monomer subunit in the secretory IgA complex.

DISCUSSION

The data presented in this paper are not consistent with a model for human secretory IgA in which SC is disulfide-linked to both monomer subunits. Experiments were carried out at acid pH and in the presence of alkylating agents, and thus it is unlikely that the second monomer subunit became separated from SC by disulfide interchange. Although we cannot rule out the possibility that the CNBr behaved atypically and cleaved a peptide bond in SC or α chains at a residue other than methionine, we and other (17) have not found evidence for such a cleavage. Complete reduction and alkylation of the CNBr derived monomer

fragment followed by gel filtration, confirmed that the principal product of the α chain had a molecular weight of 40,000-42,000 and that SC had an identical molecular weight (± 5,000) to intact secretory component. The results obtained would also be compatible with a model of secretory IgA containing 2 moles of SC per mole of IgA dimer, each molecule of SC being bonded to only one IgA monomer subunit. This is unlikely since several laboratories have reported only one mole of SC per IgA dimer (1-2).

It was of interest that complexes formed *in vitro* with $[^{125}I]$-SC and IgA myeloma dimers behaved in a similar fashion to native secretory IgA. This suggests that such in vitro complexes are similar in structure to native secretory IgA.

ACKNOWLEDGMENTS

The author would like to thank Mr. Joseph DeRose for his careful technical assistance, Miss Donalee McMillan for typing the manuscript and Dr. Allan Knight for his continued encouragement.

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