Human Secretory Component

NH₂-TERMINAL AMINO ACID SEQUENCES AND PEPTIDE MAPS OF THE FORM OCCURRING IN EXOCRINE IMMUNOGLOBULIN A AND THE FREE FORM*

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SUMMARY

Secretory component is found either covalently linked to exocrine immunoglobulin A or in a free form unassociated with other protein. Previous work has demonstrated considerable similarities between the two forms in terms of size, composition, and antigenic determinants. The comparison has now been extended to the primary structure of bound and free secretory components obtained from human colostrum. Their tryptic peptide maps are similar; the amino acid sequences of the first 14 residues, as determined by an automated sequenator, are identical; and they show no structural homologies with any other proteins. The data support the concept that secretory component is a unique protein species, a fraction of which forms a complex specifically with immunoglobulin A.

Secretory immunoglobulin A, the principal class of immunoglobulin in mucous membrane secretions, contains four different kinds of polypeptide chains: H(\alpha) and L chains (like all immunoglobulins) plus a J chain (like all polymeric immunoglobulins) and a distinct chain termed secretory component (for a review, see Tomasi and Grey (1)). The first three kinds of polypeptide chains are synthesized by plasma cells while SC \( I \) is made in a different type of cell, namely the lining epithelial cells of mucous membranes (2). Thus, secretory IgA presents an unusual instance of a protein whose constituent subunits are made in two different types of cell. The exact anatomical site in mucous membranes where human SC is coupled covalently to form the fully assembled secretory IgA molecule has not been definitely established; nor is it known if there are any specific regulatory mechanisms which influence the synthesis of the diverse components.

Combination of SC with IgA is specific since SC is not normally found associated with any other protein in secretions or serum (3, 4). However, not all the SC in secretions occurs as a part of secretory IgA; a portion exists in a free state uncoupled to other protein. These two forms of SC, referred to as bound secretory component (BSC) and free secretory component (FSC) have been isolated and compared in both human (5, 6) and rabbit (7), and were found to be similar in terms of size (71,000 daltons for human SC), composition, and antigenic determinants. More detailed comparison of the two forms of SC requires information on their primary structure. Toward this aim we now report results of peptide mapping studies and amino acid sequence determinations of their NH₂ terminal region.

METHODS

Isolation of BSC and FSC—Completely reduced and alkylated BSC was isolated from secretory IgA derived from human colostrum as previously described (6). This method takes advantage of the fact that SC lacks methionine, which makes it possible to use cyanogen bromide (8) to fragment the other components of the secretory IgA molecule while affecting the SC portion. FSC was purified according to the method described by Kobayashi (5). Preparations of BSC and FSC were pure according to immunological criteria and also by disc electrophoresis in urea and electrophoresis in sodium dodecyl sulfate. For amino acid sequence studies, FSC was completely reduced and alkylated (6, 9).

Reduction and Radioactive Alkylation—When only easily reduced cysteine residues were to be radioactively alkylated, reduction of solutions of secretory IgA, 10 mg per ml, in 1 M Tris chloride, pH 8.2, was done for 90 min at room temperature with 0.0025 M diithioerythritol. This concentration of diithioerythritol was chosen on the basis of preliminary experiments which showed by acrylamide gel electrophoretic analysis in sodium dodecyl sulfate (6, 10) that it was sufficient to liberate most, if not all, BSC from secretory IgA. Alkylation was performed at 0° for 1 hour with a 1% excess of [\(^{14}\)C]iodoacetic acid (Amersham Searle or New England Nuclear) containing 0.38 mCi per mmole. The same conditions were used for partial reduction and alkylation of FSC except that the specific activity of the iodoacetate acid was 5 times as great. Partially reduced and radioactively alkylated protein was next well dialyzed versus first 0.1 M NaCl and then water, after which it was lyophilized. It was then completely reduced at room temperature at a concentration of 20 mg per ml with 0.02 M dithioerythritol in 7 M guanidine-1 M Tris, pH 8.2, for 1 hour, followed by alkylation at 0° for 30 min with a 5% excess of iodoacetamide.

In two instances, samples of BSC and FSC for the sequenator...
were totally reduced with 0.02 M and 0.01 M dithioerythritol, respectively, in 7 M guanidine and alkylated with [14C]iodoacetic acid.

**Peptide Maps**—These were done on amounts of 1 to 2 mg (Whatman No. 1 filter paper) or 4 to 5 mg (Whatman No. 3MM filter paper) of tryp tic (TPCK trypsin, Worthington) digests of protein that had been previously mildly reduced and alkylated with [14C]iodoacetic acid and then completely reduced and alkylated with iodoacetamide. The procedure of Katz et al. (11) was used except that the solvent for chromatography was the butanol-acetic acid-water-pyridine system of Waley and Watson (12) and staining was done with cadmium ninhydrin reagent (13). Autoradiographs were made with Kodak RP/S-54 x-ray film.

**Amino Acid Sequence Studies**—The NH2-terminal amino acid of completely reduced and alkylated BSC and FSC was determined after dansylation in 8 M urea (14). Dansyl amino acids were identified by thin layer chromatography on polyamide sheets (15, 16). Automatic amino acid sequence determination was performed with a Beckman model 890 Sequencer by the method of Edman and Begg (17). The "slow" peptide program (Beckman 032671) was used in all but one of the studies. Released thiazolinone derivatives were converted to phenylthiohydantoins with 1 N HCl at 90° for 10 min and were identified as such or as the silylated derivative by gas chromatography using a Hewlett-Packard gas chromatograph (18). The results of gas chromatography were confirmed at each step by thin layer silica gel chromatography (19) or by amino acid analysis after hydrolysis with a Spinco model 121 automatic amino acid analyzer, or both. Hydrolysis was carried out with HCI, and in several instances with HI so as to identify threonine and serine which are converted to α-aminobutyric acid and alanine, respectively. To ascertain the possible presence of half-cystine, aliquots from each step of the sequence analysis of samples that had been [14C]-alkylated after complete reduction were counted in a liquid scintillation counter. Most yields were calculated from the gas chromatographic analyses, except for several which were calculated from the amino acid analyses.

**RESULTS**

**Peptide Maps**—Examples are shown in Fig. 1. Maps from BSC and FSC were strikingly similar. Although there were quantitative differences in the staining intensity of several of the peptides, the only clear-cut differences were two extra peptides in the maps of BSC. This conclusion was confirmed by a map of a mixture containing equal amounts of BSC and FSC peptides (not shown), which was the same as either form of SC alone, excepting the two peptides peculiar to BSC. Some 45 to 50 peptides were observed in each map when samples of 4 or 5 mg were used; this is consistent with the number expected from the lysine and arginine content of SC (5, 6). By autoradiography four peptides were found to be significantly radioactive as the result of [14C]alkylation after partial reduction, and again these were identical in the maps of BSC and FSC (as confirmed by an autoradiograph of the map of the mixture). With prolonged exposure several additional minor radioactive, ninhydrin-negative peptides could be observed. In the case of native FSC, an attempt to demonstrate free sulfhydryl groups failed. An aliquot that was exposed to [14C]iodoacetic acid without prior reduction did not incorporate radioactivity. Thus, the radioactive peptides in the map of FSC (and probably also BSC) were derived from cystine peptides in the native protein.

Only a small amount of ninhydrin-positive core material remained at the origin and this was not radioactive.

**NH2-terminal Amino Acid**—Lysine was identified by the dansyl technique. A Bence-Jones κ protein used as a control was negative for bis-dansyl lysine.

**NH2-terminal Amino Acid Sequence**—Four samples of FSC and two of BSC were examined. The results were optimal when 5 to 7 mg of protein were run and less clear-cut when

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**Fig. 1. Peptide maps of secretory component.** These tryptic peptide maps of BSC (left) and FSC (right) were done on protein that had been mildly reduced and alkylated with [14C]iodoacetic acid and then completely reduced and alkylated with iodoacetamide. The origin is at the lower right; chromatography is from right to left; and the cathode is at the top. Arrows indicate two peptides in the BSC map which were absent from the FSC map.

Radioactive peptides are marked with the letter R. These particular maps were done on small amounts (1.5 mg) of protein in order to maximize the discreteness of the individual spots. Many of the peptides were accordingly too faint to photograph well; therefore, the spots were circled on the original papers. When larger amounts of sample were mapped, several additional peptides could be observed.
more than 10 mg were used. Recoveries at each step varied somewhat from run to run, and were based on the weight of the material applied. Since the samples of SC were not first dried to constant weight, the actual recoveries were undoubtedly greater (in our hands at least 2-fold). Table I lists the recoveries of a representative analysis of each of the proteins.

The sequence of the first 16 steps of FSC is: Lys-Ser-Pro-Ile-Phe-Gly-Pro-Glu-Glu-Val-Asp-Ser-Val-Glu-Gly-Gly. In the case of BSC the analysis yielded only 14 steps, which were identical.

Homology with Other Proteins—A computer search was made to detect possible homologies between human SC as compared with the more than 600 sequences in the Protein Sequence Data Deck 72 and its Supplement 1 (W. C. Barker and M. O. Dayhoff, National Biochemical Research Foundation, Washington, D. C.). There were no segments (regardless of location) with more than six matches to the NH$_2$-terminal sequence of SC. This respect the NH$_2$-terminal sequence of SC was equally similar to several kinds of unrelated proteins. Thus, there is no significant homology with any known protein for which there is sequence information.

### Table I

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<th>BSC</th>
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* All recoveries were calculated from analyses by gas chromatography except those marked (a) which were determined from amino acid analyses.

† Part of the sample was lost; the residue was identified qualitatively.

Amino acid sequences of the NH$_2$-terminal regions of free (FSC) and bound (BSC) secretory components

A search by computer failed to reveal significant homologies with any of the many proteins for which there is sequence information. Thus, at present it is not possible to speculate on the evolutionary origin of SC. Perhaps when more sequence information is forthcoming on other mucinous glycoproteins, relationships will become apparent. The lack of homology to immunoglobulin H and L chains, even though BSC is an integral part of an immunoglobulin molecule, is not surprising in view of the fact that SC is synthesized in a different type of cell from immunoglobulins (epithelial cell versus plasma cell).

The amino acid compositions of human BSC and FSC have been reported (5, 6). The absence of methionine formed the basis of our procedure, with cyanogen bromide, for isolating BSC (6). As previously noted, since all our preparations of BSC were derived from cyanogen bromide-treated secretory IgA, the presence of a methionine residue near the NH$_2$ terminus of BSC could not be excluded. This possibility is now rendered unlikely since the NH$_2$ termini of BSC (after exposure to cyanogen bromide) and FSC (no exposure to cyanogen bromide) have the same sequence.

On the basis of data obtained in a number of laboratories, the synthesis and assembly of secretory IgA have been envisioned in general terms as follows (1). An IgA dimer plus J chain is elaborated by plasma cells in mucous membranes; this IgA molecule subsequently forms a complex (exact anatomical site unknown) with SC produced in the overlying epithelial cells to form secretory IgA. The excess SC produced by the epithelial cells is also secreted and appears in the mucous membrane...
fluids as FSC. The data presented here on the structural similarities between BSC and FSC are fully consistent with this scheme. In the future it will be important to define the role of SC in secretory IgA function and also to learn whether FSC has an independent function as well.

Addendum—Since completion of this manuscript, Drs. J. D. Capra and T. B. Tomasi have informed us that the NH₂-terminal stretch of bovine FSC closely resembles the human, with only 2 differences in the first 15 residues.

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