Human Secretory Component

NH$_2$-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(α) 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 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 proteins. 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 NH$_2$-terminal regions.

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 thus 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 cystine 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 dithioerythritol. This concentration of dithioerythritol was chosen on the basis of preliminary experiments which showed by acrylamide gel electrophoretic analysis in sodium dodecyl sulfate that it was sufficient to liberate most, if not all, BSC from secretory IgA. Alkylation was performed at 0° for 1 hour with a 5% excess of [14C]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 iodoacetic 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 tryptic (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 N-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 HCl, 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 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

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.
Amino acid analyses.

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tively.

In this respect the NH$_2$-terminal sequence of SC was equally

significant homology with any known protein for which there is

more than six matches to the NH$_2$-terminal sequence of SC.

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identical.

The sequence of the first 16 steps of FSC is: Lys-Ser-Pro-Ile-

Phc-Gly-Pro-Glu-Glu-Val-Asp-Ser-Val-Glu-Gly-Gly. In the

case of BSC the analysis yielded only 14 steps, which were

identified.

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

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hoff, 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.

In 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.

DISCUSSION

Previous work with both human and rabbit proteins has

revealed that the two forms of SC, bound and free, are closely

related and possibly identical. This includes similarities in size

and composition and shared antigenic determinants. Diff-

erences in antigenic reactivities that have been observed can

probably be explained by the different conformations which SC

assumes in the bound versus free state (20, 21). We have ex-
tended the comparison to the primary structure. Tryptic

dipeptide maps, which reflect the amino acid sequence of the

whole protein, were similar for BSC and FSC in terms of both

ninhydrin-positive peptides and peptides that were radioactively

alkylated after partial reduction. The major exceptions were

two nonradioactive peptides in the BSC map which were not

observed in the FSC map. It is possible, however, that these

extra peptides do not reflect real distinctions between the

primary structures of BSC and FSC but are due to differences

in carbohydrate groups, perhaps secondary to differences in the

manner of isolation (e.g. the 24-hour exposure of BSC to 70% for-

mic acid during the cyanogen bromide digestion). Al-

though the results of peptide mapping cannot prove identity of

primary structure, they do stress the close relationship between

BSC and FSC. The peptides in BSC which were $^{14}C$-alkylated

after partial reduction must include the half-cystine residues

which partake in the interchain disulfide bridges joining SC to

the remainder of the molecule. Since FSC is a single chain and

lacks free sulfhydryl groups (see above), the homologous (as

shown by the autoradiographs of the peptide maps) half-cystines

in FSC must form an intrachain bridge.

An attempt was made to obtain direct evidence for identity of

primary structure in BSC and FSC by determining the actual

sequences at the NH$_2$ termini, which is readily possible for

human SC because the NH$_2$ terminus is not blocked, in contrast

to rabbit SC (7). An NH$_2$-terminal amino acid, lysine, was

identified initially by dansylation. It is interesting that in an

earlier study of the NH$_2$-terminal amino acids of whole human

colossal IgA by the Sanger technique, three amino acids were

found: lysine (22) as well as aspartic acid and glutamic acid,

which must have come from $\alpha$ and $\gamma$ chains. Since unambiguous
data were obtained only for the first 16 residues of FSC and

the first 14 residues of BSC, it is not possible to be certain of

identity beyond residue 14. Nevertheless, the sequence data
taken together with the similarity of the peptide maps suggest a

striking degree of similarity in primary structure between the

two forms of SC. It should be noted that the two forms of SC

were homogeneous, and were prepared by entirely different

techniques, so that the presence of similar contaminating pro-

teins in the two samples can be excluded.

A search by computer failed to reveal significant homologies

with any of the many proteins for which there is sequence in-

formation. 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 cyan-

ogen 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.

<table>
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<th>Step</th>
<th>Residue</th>
<th>FSC</th>
<th>BSC</th>
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<td>1</td>
<td>Lys</td>
<td>18(a)</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Ser</td>
<td>21</td>
<td>10</td>
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<td>3</td>
<td>Pro</td>
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<td>12</td>
<td>15</td>
</tr>
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<td>5</td>
<td>Phe</td>
<td>8(a)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Gly</td>
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<td>Pro</td>
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<tr>
<td>8</td>
<td>Glu</td>
<td>10(a)</td>
<td>6(a)</td>
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<tr>
<td>9</td>
<td>Glu</td>
<td>12(a)</td>
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<tr>
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* All recoveries were calculated from analyses by gas chromato-

matography except those marked (a) which were determined from

amino acid analyses.

* Part of the sample was lost; the residue was identified qualita-

tively.

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 re-

coveries of a representative analysis of each of the proteins.

The sequence of the first 16 steps of FSC is: Lys-Ser-Pro-Ile-

Phc-Gly-Pro-Glu-Glu-Val-Asp-Ser-Val-Glu-Gly-Gly. In the

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Amino acid sequences of the NH$_2$-terminal regions of free

(FSC) and bound (BSC) secretory components

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<th>Step</th>
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<th>Per cent recovery$^a$</th>
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alkylated after partial reduction. The major exceptions were

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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.

Acknowledgments—We thank Mr. P. Federico for technical assistance, and C. C.-R. expresses her appreciation to Dr. S. J. Farber for his support and encouragement. We are grateful to the following for colostrum: the obstetrical nursing staff of New York University Hospital, the Mothers’ Milk Bank of the Delaware Hospital, and La Leche League.

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