Differences in fragmentation between bound and unbound bovine secretory component suggest a model for its interaction with polymeric immunoglobulin

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Unbound bovine secretory component was cleaved into two-domain and one-domain fragments by trypsin within 1 h. Bovine secretory component covalently bound to bovine IgA dimer, as in secretory IgA, was much more resistant to fragmentation, which did not proceed beyond the three-domain stage even after 5 h. Bovine secretory component non-covalently bound to bovine IgM or to human IgM or IgA polymer was also relatively resistant to fragmentation, which again was largely arrested at the three-domain stage. A model for the binding of secretory component to polymeric immunoglobulin is proposed.

There has been increasing evidence that secretory component (SC) is related to the extracellular part of the epithelial receptor for polymeric immunoglobulin (Brandtzaeg, 1974, 1978; Brown *et al.*, 1977; Crago *et al.*, 1978; Kühn & Kraehenbuhl, 1979, 1981) and that the presence of SC in secretory IgA (sIgA) is an aftermath of its epithelial transport after combination with the receptor.

Translation of a cDNA sequence for the rabbit receptor indicated that the extracellular part might consist largely of five domains related to immunoglobulins (Mostov *et al.*, 1984). This was supported by reduction and fragmentation studies on bovine SC (Beale & Hopley, 1985).

The present paper shows that considerable differences in fragmentation occur between bound and unbound SC, and suggests a possible model for the interaction of SC with IgA and IgM.

Materials and methods

Materials

These were the same as those used in previous work (Beale & Hopley, 1985).

Preparation of proteins

Bovine SC and sIgA were prepared from whey by the methods of Groves & Gordon (1967) and

Butler (1971). Bovine IgM and human IgM were obtained from normal and pathological sera as described by Beale & Van Dort (1982). Human IgA dimers were prepared from myeloma serum by the method of Underdown & Dorrington (1974).

Fragmentation with trypsin

Solutions of protein (0.1%, w/v) in 0.15M-NaCl/0.1M-Tris/HCl buffer, pH 8.0, were digested with trypsin (protein/enzyme ratio 100:1) for various times at 37°C. Twice the weight of trypsin inhibitor to that of trypsin was added, and the samples were stored at -20°C.

Polyacrylamide-gel electrophoresis

This was carried out in the presence of 0.1% (w/v) SDS by the method of Laemmli (1970) as modified by Richardson & Feinstein (1978). The 12% (w/v) running gels and 3% (w/v) stacking gels were used with a discontinuous Tris/glycine buffer. Protein was detected with Coomassie Brilliant Blue, and gels were scanned densitometrically on a Helena Quick Scan Junior machine. The radioactivity in gel slices was detected by counting on a Packard γ -radiation counter.

Coupling of proteins to Sepharose

The manufacturer's (Pharmacia) instructions for the use of CNBr-activated Sepharose-4B were followed.

¹²⁵I-labelling of proteins

Solutions of protein (0.1%, w/v) in 0.15M-NaCl/50mM-sodium phosphate buffer, pH 7.2,

Abbreviations used: SC, secretory component; SDS, sodium dodecyl sulphate; PBS, phosphate-buffered saline (0.15M-NaCl/10mM-sodium phosphate buffer, pH 7.2).

were treated, per 1 ml of solution, with 100 μ Ci of Na¹²⁵I, 50 μ l of lactoperoxidase (1 mg/ml) in PBS, and 50 μ l of H₂O₂ (0.015%, v/v) in PBS, and left for 1 h at 20°C. Then 100 μ l of 1.5M-NaCl/100mM-sodium phosphate buffer, pH 7.2, containing 0.5% NaN₃ was added, and the sample was dialysed against 0.5% NaN₃/PBS to constant radioactivity.

Binding and fragmentation of SC

Samples (30 μ l) of bovine SC (0.1%, w/v) in PBS were added to 100 μ l portions of 30% (v/v) IgM-Sepharose in PBS and rotated for 1 h at 20°C. The Sepharose was gently spun down and washed with three 100 μ l portions of PBS. The pellets were resuspended in 0.15M-NaCl/0.1M-Tris/HCl buffer, pH 8.0, and sufficient trypsin was added to give 1/100 of the weight of SC.

Samples were then incubated at 37°C with gentle shaking. The digested samples were gently spun and then washed with PBS, and the pellets were reduced in SDS ready for SDS/polyacrylamide-gel electrophoresis.

In another type of experiment ¹²⁵I-SC was added to IgM or IgA dimer (0.1%, w/v) in PBS in equimolar proportions. The mixture was left at 20°C for 1 h. The pH was adjusted to 8.0, and sufficient trypsin to give 1/100 of the weight of SC was added. The samples were incubated at 37°C for various times, and then twice the weight of trypsin inhibitor to that of trypsin was added. The pH was adjusted to 7.0, and sheep [anti-(human L-chain) IgG]-Sepharose was added (100 μ l; 30%, v/v, in PBS). After rotation for 1 h, the Sepharose was gently spun down, washed with PBS and then reduced in SDS ready for SDS/polyacrylamide-gel electrophoresis.

Model building

Use was made of the following: the translated cDNA sequence for the rabbit receptor (Mostov *et al.*, 1984), its transposition on to high-resolution crystallographic models of immunoglobulin domains (Beale, 1985), the arrangement of domains in bovine SC suggested by Beale & Hopley (1985), the high-resolution crystallographic results for human IgG Fc fragment (Deisenhofer, 1981), and models of IgM, IgG and IgA published by Feinstein & Beale (1977) and Feinstein & Richardson (1981). Domains were represented as cylinders, as described by Schiffer *et al.* (1973).

Results

Fig. 1 (profile 1) shows the products obtained by digesting bovine SC with trypsin for 10 min. They are lettered in accordance with Beale & Hopley

Fig. 1. Densitometric scans of SDS/polyacrylamide-gel electrophoretograms of tryptic digests of bovine SC and sIgA

Profiles 1 and 2, SC digested for 10 min (1) and for 1 h (2). Profiles 3–5, sIgA digested for zero time (3), for 1 h (4) and for 2 h (5).

(1985), who reported that fragments Q and R contain three immunoglobulin-like domains; fragments A and T have two such domains, and fragments B and C consist of single domains. After digestion of SC for 1 h, fragments A, B and C were the only significant products (Fig. 1, profile 2).

When bovine sIgA was digested with trypsin for 1 h, some SC still remained, but there was a substantial amount of material in the position of fragment Q (Fig. 1, profile 4). After 2 h almost complete conversion of SC into fragment Q had occurred, but there were no smaller products (Fig. 1, profile 5). There was no detectable cleavage of α - or L-chains. The products after 5 h digestion showed no further change. A 2-fold increase in the amount of trypsin had no significant effect.

Fig. 2 (profiles 3–7) shows the results of digesting bovine SC bound *in vitro* to (bovine IgM)-Sepharose. To ensure complete extraction of products, the final washed pellets were boiled in SDS in the presence of 2-mercaptoethanol. This gave a protein background (Fig. 2, profile 3) that tended to mask the fragmentation results obtained by protein staining (Fig. 2, profiles 4 and 6). However, fragments Q and R seemed to be the only significant products. This was confirmed by using

Fig. 2. Densitometric scans of SDS/polyacrylamide-gel electrophoretograms and radioactivity of gel slices of tryptic digests of bovine SC, free and bound to (bovine IgM)-Sepharose.

Profiles 1 and 2, free SC digested for 15 min (1), with ^{125}I distribution (2). Profile 3, IgM-Sepharose digested for 1 h. Profiles 4-7, SC bound to IgM-Sepharose and digested for 1 h (4), with ^{125}I distribution (5), and for 2 h (6), with ^{125}I distribution (7).

Fig. 3. Densitometric scans of SDS/polyacrylamide-gel electrophoretograms and radioactivity of gel slices of tryptic digests of bovine SC, free, bound to (human IgM)-Sepharose and to human IgM and IgA in solution

Profiles 1 and 5, free SC digested for 15 min (1), with ^{125}I distribution (5). Profile 2, IgM-Sepharose digested for 1 h. Profiles 3 and 4, SC bound to IgM-Sepharose and digested for 1 h (3) and for 2 h (4). Profiles 6 and 7, 1 h digests of SC bound in solution to IgM (6) and IgA (7), then isolated with [anti-(human L-chain) IgG]-Sepharose.

^{125}I -SC and counting the radioactivity of gel slices (Fig. 2, profiles 5 and 7). Again, a 2-fold increase in the amount of trypsin had no significant effect on the result.

Similar experiments were carried out with bovine SC bound *in vitro* to (human IgM)-Sephadex. Boiling the final washed pellets in SDS in the presence of 2-mercaptoethanol again gave a protein background that tended to mask the fragmentation results. Therefore extraction was performed at room temperature with SDS only. The extract was removed from the pellet and then boiled in the presence of 2-mercaptoethanol. This gave a low protein background (Fig. 3, profile 2), but the recovery of products also seemed to be somewhat low. Even so the results (Fig. 3, profiles 3 and 4) were similar to those obtained with (bovine IgM)-Sephadex. Fragments Q and R were clearly the main products, and no smaller fragments could be detected.

To ensure that the results were not being influenced by the IgM being bound to Sephadex, bovine ^{125}I -SC was bound to human IgM and IgA polymers in solution. The complexes were digested with trypsin and then isolated with [anti-(human L-chain) IgG]-Sephadex. Fig. 3 (profiles 6 and 7) shows that the radioactivity lay mainly in the positions of fragments Q and R. There was no radioactivity corresponding to two-domain or one-domain fragments. A small amount of radioactivity detected between SC and fragment Q was probably due to a minor four-domain fragment (Beale & Hopley, 1985).

Discussion

The results presented in this paper show that bovine SC bound to bovine IgA or IgM or to human IgA or IgM is more resistant to fragmentation by trypsin than is unbound SC. When unbound, the five-domain SC is fragmented into two-domain and one-domain entities within 1 h, but fragmentation of bound SC appears to be largely arrested at a three-domain stage, at least up to 5 h digestion.

Bovine SC covalently bound to bovine IgA dimer, as in sIgA, seems to be the most resistant form, although the difference from bovine SC non-covalently bound to bovine IgM is not great. In turn, bovine SC non-covalently bound to human IgM is slightly less resistant than when bound to bovine IgM, and this seems to correlate with a difference in binding affinities (Beale & Hopley, 1985).

The loss of proteolytic sites when bovine SC binds to polymeric immunoglobulin suggests that a considerable portion of the molecule is involved,

probably at least three of the five domains. It has already been reported (Beale & Hopley, 1985) that the three-domain fragments Q and R give rise to a two-domain fragment A. Although this fragment binds to polymeric immunoglobulin (Beale & Hopley, 1985), it is less effective than SC. This indicated that optimal binding probably requires at least three of the domains of SC. Another two-domain fragment, designated as T in the previous work (Beale & Hopley, 1985), was not detected under the conditions of the present work, presumably owing to its proteolysis. This suggests that two of the domains of SC are not strongly bound and remain relatively unprotected.

Examination of molecular models of IgM (Feinstein & Beale, 1977) based on sequence homology and high-resolution X-ray crystallography of IgG fragments shows that SC cannot be placed between the Fc regions of two IgM subunits, because of their packing into a disulphide-bridged cyclic pentamer. Therefore SC is more likely to bind at the planar surface of the central (Fc)₅ disc of the IgM molecule.

Although structural information about SC is very limited, the available results (Mostov *et al.*, 1984; Beale, 1985; Beale & Hopley, 1985) suggest that SC consists of a single polypeptide chain folded into five immunoglobulin-like domains with at least one inter-domain disulphide bridge, most probably linking domains 1 and 5. In Fig. 4(a) these two domains have been slightly overlapped to allow for disulphide bonding, but the overlap does not imply domain pairing as found in immunoglobulins (Fig. 4b). Indeed, the polarities of the domains are different in the two structures.

As with immunoglobulin molecules (Fig. 4b), the peptide chain joining two SC domains would lie on one side of the plane of the molecule, and could easily become buried when SC interacts with polymeric immunoglobulin, as shown in Fig. 4(c). Examination of a molecular model of IgA (Feinstein & Richardson, 1981) shows that Fig. 4 is also applicable to the interaction of SC with IgA dimer.

Unfortunately, there is insufficient information about the J-chain to allow a model of it to be built. It has M_r of about 12000, but has no homology with immunoglobulins. However, if it formed a reasonably globular structure, it could be roughly the same size as an immunoglobulin domain. It could interact with SC and provide additional shielding of proteolytic sites.

Woodard *et al.* (1984) have produced a panel of monoclonal antibodies against human SC and sIgA. They found that most antibodies from fusions where SC had been the antigen bound preferentially to SC and rarely to sIgA. Conversely, sIgA specific antibodies from fusions where sIgA had been the antigen bound preferentially to

Fig. 4. Possible model for the interaction of SC with IgM and IgA dimer

Domains are represented as cylinders in accordance with Schiffer *et al.* (1973). (a) Possible structure for SC based on the results reported by Mostov *et al.* (1984), Beale (1985) and Beale & Hopley (1985). The five immunoglobulin-like domains are arranged so that domains 1 and 5 overlap slightly to allow for an inter-domain disulphide bridge (not shown). Broken arrows indicate the main regions of tryptic cleavage that become inaccessible after SC is bound to polymeric immunoglobulin. Full arrows indicate the main regions of tryptic cleavage that remain relatively exposed. Domains labelled Q/R form the three-domain fragments Q and R, which eventually give rise to the two-domain fragment A and the one-domain fragments B and C. Domains labelled T form the two-domain fragment T. (b) Fc regions from two J-chain-linked IgA subunits or two J-chain-linked IgM subunits (J-chain is not shown) on the basis of the results reported by Deisenhofer (1981), Feinstein & Beale (1977) and Feinstein & Richardson (1981). (c) SC-polyimmunoglobulin complex. The peptide chain joining two Fc domains lies on one side or the other of the plane of the molecule, and a similar feature is included in the SC structure. Therefore, when SC interacts with IgM or IgA dimer, the peptide chain lying between three consecutive SC domains (labelled Q/R) becomes buried and inaccessible to tryptic cleavage. The peptide chain linking two other domains (labelled T) remains relatively exposed.

sIgA and rarely to SC. Results for SC bound *in vitro* to IgA dimer were similar to those for sIgA. To comply with the model shown in Fig. 4, the epitopes recognized by most of these antibodies would need to lie within or near to the regions of interaction between SC and IgA dimer.

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