A bovine epithelial membrane protein that binds polymeric immunoglobulin and has a structure related to that of bovine secretory component

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A protein of M_r 94000 was isolated from detergent-solubilized bovine intestinal, liver and mammary-gland membranes. It binds immunoglobulin M and also undergoes proteolytic fragmentation in a similar manner to bovine secretory component (M_r 74000). The affinity-purified membrane protein is therefore most likely to be the bovine epithelial receptor for polymeric immunoglobulin. A structural model is proposed.

INTRODUCTION

Although IgA is an important component of epithelial external secretions, it originates from lymphoid cells in the interstitium below the epithelial layer. After synthesis in and secretion from lymphoid cells the IgA, in the form of a J-chain-linked dimer, is apparently bound to a specific receptor on the basolateral surface of the epithelial cell. The immunoglobulin–receptor complex is then transported in a vesicle across the cell to the apical surface, where the J-chain-linked dimer is released still bound to the extracellular part of the receptor. This offers an explanation as to why secretory IgA is always found to be associated with another protein that has long been known as secretory component (SC). J-chain-linked IgM polymers can behave in a similar manner, although physiologically this seems to be of less importance. Various aspects of the epithelial transport of polymeric immunoglobulin have been described by Brandtzaeg (1974, 1978), Brown *et al.* (1977), Crago *et al.* (1978), Kühn & Kraehenbuhl (1979, 1981) and Mullock *et al.* (1983).

Mostov *et al.* (1984) isolated and sequenced a cDNA clone for the rabbit epithelial polyimmunoglobulin receptor and pointed out that the translated amino acid sequence was homologous with those of immunoglobulins. Work from our laboratory (Beale & Hopley, 1985) showed that bovine SC can be proteolytically cleaved into a series of fragments related in size and disulphide content to immunoglobulin domains. It appeared that bovine SC contains five of these domains, in agreement with the proposal of Mostov *et al.* (1984) for the rabbit polyimmunoglobulin receptor. Further work (Beale, 1985a) showed that the binding of bovine SC to polymeric immunoglobulin shields some of the proteolytic sites, so that fragmentation of SC is arrested mainly at the three-domain stage. The results enabled a putative model to be proposed for the interaction between SC and IgM and IgA dimers. The present paper reports the isolation of a bovine epithelial membrane protein that binds IgM and yields similar proteolytic fragments to

those obtained from bovine SC. It is therefore most likely to be the bovine epithelial receptor for polymeric immunoglobulin.

MATERIALS AND METHODS

Materials

Nonidet P-40, Triton X-100 and SDS were obtained from BDH Chemicals (Poole, Dorset, U.K.). Sodium deoxycholate, n-octyl glucoside and phenylmethanesulphonyl fluoride were from Sigma Chemical Co. (Poole, Dorset, U.K.). Decanoyl-N-methylglucamide was prepared as described by Hildreth (1982). CNBr-activated Sepharose 4B was from Pharmacia (Hounslow, Middx., U.K.). Bovine SC was prepared as previously described (Beale & Hopley, 1985). Rabbit anti-(bovine SC) IgG was prepared from immunized rabbit sera by the conventional methods of $(\text{NH}_4)_2\text{SO}_4$ precipitation and DEAE-cellulose chromatography (see Williams & Chase, 1967). It was coupled to CNBr-activated Sepharose according to instructions supplied by Pharmacia.

Preparation and solubilization of cells and membranes

Bovine intestinal epithelial cells were prepared by the method of Weiser (1973) as modified by Sepúlveda *et al.* (1982). Membranes were prepared from perfused bovine mammary gland and liver tissue by homogenizing and centrifuging through a sucrose density gradient as described by Kühn & Kraehenbuhl (1979). Solubilization was carried out at 4 °C with 1% or 2% (w/v) detergent in 0.15 M-NaCl/10 mM-sodium phosphate buffer, pH 7.2, containing 1 mM-phenylmethanesulphonyl fluoride and 50 mM-iodoacetamide. After 30 min the mixture was centrifuged at 100000 *g* for 45 min. Portions of the supernatants were boiled in 2% (w/v) SDS in the presence of 2-mercaptoethanol and subjected to SDS/polyacrylamide-gel electrophoresis as described by Laemmli (1970) and Richardson & Feinstein (1978). Protein was located by the silver stain method of Oshawa & Ebata (1983).

Abbreviations used: SDS, sodium dodecyl sulphate; SC, secretory component.

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Isolation of SC-like membrane proteins

The method of Kühn & Kraehenbuhl (1981) was followed, with several modifications. The solubilized membranes were absorbed with rabbit polyclonal IgG coupled to Sepharose in accordance with the manufacturer's instructions and suspended in detergent. After gentle rotation at 4 °C for 16 h the Sepharose complex was gently spun down. The supernatant was then absorbed with rabbit polyclonal IgG-anti-(bovine SC)-Sepharose by gentle rotation for 16 h at 4 °C. The Sepharose complex was gently spun down or packed into a 5 ml column and washed four times with detergent. Bound protein was then eluted with detergent in 0.1 M-glycine/HCl buffer, pH 2.8, and the eluate dialysed against detergent in 0.15 M-NaCl/10 mM-sodium phosphate buffer, pH 7.2, containing 0.5% (w/v) NaN₃. Some analytical samples were eluted by boiling the Sepharose complex in 2% (w/v) SDS.

Eluted protein was labelled with ¹²⁵I by using the chloramine-T method as described by Kühn & Kraehenbuhl (1981) or the lactoperoxidase method as detailed by Beale & Hopley (1985). In each case 100 µCi of Na¹²⁵I was used per µg of protein. Protein was measured by using the Bio-Rad Protein Assay kit in accordance with the manufacturer's instructions.

Eluted labelled protein was then absorbed with human monoclonal IgM coupled to Sepharose in accordance with the manufacturer's instructions and suspended in detergent. After gentle rotation at 20 °C for 2 h, the Sepharose complex was gently spun down or packed into a 5 ml column and washed four times with detergent. Bound protein was eluted with detergent in 1 M-KSCN/50 mM-sodium phosphate buffer, pH 7.0, as described by Underdown *et al.* (1977). Some analytical samples were eluted by boiling the Sepharose complex in 2% (w/v) SDS.

Eluates were examined by SDS/polyacrylamide-gel electrophoresis (Laemmli, 1970; Richardson & Feinstein, 1978) and proteins located by silver staining (Oshawa & Ebata, 1983) or by autoradiography. In some experiments gels were sliced and their radioactivities counted on a Packard γ-radiation counter.

Fragmentation studies

Proteolysis with trypsin at 37 °C and pH 8.3 was performed by previously described methods (Beale & Hopley, 1985; Beale, 1985a) except that 0.1% (w/v) detergent was included in the digestion buffer. Digestion products were identified by using SDS/polyacrylamide-gel electrophoresis. Gels were stained with Coomassie Brilliant Blue as described by Richardson & Feinstein (1978) and scanned densitometrically on a Helena Junior Quick Scan instrument. Gels were also sliced and their radioactivities counted on a Packard γ-radiation counter.

RESULTS

Solubilization of membranes

Samples solubilized with deoxycholate, Nonidet P-40, Triton X-100, n-octyl glucoside or decanoyl-N-methylglucamide showed no major differences in protein banding when examined by SDS/polyacrylamide-gel electrophoresis. Membranes from liver and mammary gland gave at least 20 bands irrespective of the detergent used. Intestinal cells gave rise to similar but slightly fewer

Fig. 1. SDS/polyacrylamide-gel electrophoretograms of affinity-purified and ¹²⁵I-labelled bovine membrane proteins detected by autoradiography

(a)–(c) Intestinal-cell (a), mammary-gland (b) and liver (c) membranes solubilized in 1% Nonidet P-40, absorbed on IgG-anti-(bovine SC)-Sepharose and IgM-Sepharose and eluted with 2% SDS. (d) Liver membranes solubilized in 1% decanoyl-N-methylglucamide, absorbed on IgG-anti-(bovine SC)-Sepharose and IgM-Sepharose and eluted with 1% decanoyl-N-methylglucamide in 1 M-KSCN/50 mM-sodium phosphate buffer, pH 7.0.

bands. Since n-octyl glucoside and decanoyl-N-methylglucamide are non-ionic, do not absorb at 280 nm and have high critical micelle concentrations, they were chosen for preparative experiments. Also, after initial solubilization and absorption with 1% solutions of these two detergents the concentration could be lowered to 0.1% without loss of protein. This was the concentration used in binding and fragmentation studies.

Isolation of SC-like membrane proteins

Material eluted from rabbit IgG-anti-(bovine SC)-Sepharose by 1% detergent in 0.1 M-glycine/HCl buffer, pH 2.8, gave rise to several protein bands, including one with M_r 94000. Elution with 2% SDS released substantially more protein. Reabsorbed material eluted from IgM-Sepharose with 2% SDS still gave rise to several bands, but the one with M_r 94000 was the most significant. Intestinal-cell, liver and mammary-gland membranes gave similar proteins (Figs. 1a–1c). Elution with 1% decanoyl-N-methylglucamide in 1 M-KSCN/50 mM-sodium phosphate buffer, pH 7.0, gave similar results (Fig. 1d).

In control experiments with bovine SC it was found that elution with 2% SDS impaired subsequent binding to IgM. Hence SDS was not used in preparative experiments. Elution with 0.1 M-glycine/HCl buffer, pH 2.8, had only a small effect on subsequent binding, and elution with 1 M-KSCN/50 mM-sodium phosphate buffer, pH 7.0, had no significant effect.

¹²⁵I-labelling of SC by the chloramine-T method had a serious effect on binding to IgM, and so this method was

Fig. 2. Densitometric scans of stained SDS/polyacrylamide-gel electrophoretograms and radioactivity of gel slices of 37 °C tryptic digests of a mixture of ^{125}I -labelled affinity-purified bovine liver membrane protein and unlabelled bovine SC

Results were obtained with unbound mixture at zero time (profile 1) and ^{125}I distribution (profile 2), digested for 10 min (profile 3) and ^{125}I distribution (profile 4), digested for 25 min (profile 5) and ^{125}I distribution (profile 6), and digested for 1 h (profile 7) and ^{125}I distribution (profile 8). Results were also obtained with mixture bound to IgM-Sepharose and digested for 2 h (profile 9) and ^{125}I distribution (profile 10).

used only in a few initial experiments. Labelling by the lactoperoxidase method had no significant effect on binding, although less label was incorporated. This was the method used in most experiments.

Fragmentation experiments

Profiles 1 and 2 in Fig. 2 show the results obtained by protein staining and counting the radioactivity of gel slices for the undigested mixture of unlabelled SC and affinity-purified ^{125}I -labelled membrane protein. Most of the labelled protein has M_r 94000, although a significant amount coincides with the position of SC. Radioactivity in the rest of the gel is relatively minor and may represent degradation products formed during the preparation of the membrane protein.

Profiles 3 and 4 in Fig. 2 give the results of digesting the SC/membrane protein mixture with trypsin for 10 min. Protein staining (profile 3) shows that SC fragments have been formed exactly as described by Beale & Hopley (1985), who reported that fragments Q and R consist of three immunoglobulin-like domains, fragments A and T of two such domains and fragments B and C of one domain. Profile 4 shows that the membrane protein has given rise to fragments that coincide exactly with those obtained from SC. Samples digested for 25 min (profiles 5 and 6) and 60 min (profiles 7 and 8) also display exact coincidence between radioactive maxima due to membrane-protein fragments and protein staining due to SC fragments. The build-up of stainable and radioactive material at the bottom of the gels is due to the formation of products of smaller size than one domain.

Profiles 9 and 10 in Fig. 2 show that the binding of the SC/membrane protein mixture to IgM-Sepharose induces a partial resistance to fragmentation, so that even after

2 h digestion fragments Q and R were still obtained in substantial amounts. Profiles 3 and 4 show that unbound SC/membrane protein mixture reached a similar stage of fragmentation after only 10 min digestion. These results are in full agreement with the much more extensive study carried out by Beale (1985a) on the effect of binding bovine SC to human and bovine IgM coupled to Sepharose and in free solution.

DISCUSSION

Kühn & Kraehenbuhl (1981) absorbed detergent-solubilized rabbit liver and mammary-gland membranes with goat IgG-anti-(rabbit SC)-Sepharose and human IgM-Sepharose and obtained proteins of M_r 120000, 116000, 95000 and 91000. In the present work a very similar protocol was used with bovine intestinal, liver and mammary-gland membranes and gave rise to proteins of M_r 94000 and 74000, the latter being coincident with SC.

Mostov *et al.* (1984) reported that the rabbit receptor mRNA gave two translation products of M_r 90000–95000 and two of M_r 70000–71000. The theoretical M_r from the translated cDNA sequence and including the signal sequence is 82041 (Mostov *et al.*, 1984). The discrepancy between theoretical and observed M_r values is most probably due to glycosylation for the higher values and to post-translational cleavage for the lower ones.

Of particular interest in the present work is the behaviour of the bovine membrane protein towards proteolysis relative to that of bovine SC. Fragments produced from both proteins coincided exactly at every stage of the digest. Hence the major part of the bovine membrane protein must have a similar structure to that of bovine SC, namely five immunoglobulin-like domains

(Beale & Hopley, 1985). This is in complete agreement with the predictions of Mostov *et al.* (1984) for the rabbit receptor.

The translated cDNA sequence for the rabbit receptor suggests that the protein is likely to consist of some 540 amino acid residues involved in the five immunoglobulin-like domains followed by about 90 amino acid residues that in part show some homology with immunoglobulins. Then comes a putative transmembrane region of some 20 residues followed by a long cytoplasmic tail of over 100 residues. Fragmentation of the bovine protein could therefore have given rise to some fragments of different size from those of fragments produced from SC. No such products were observed in the present work, indicating that the part of the receptor not involved in the five immunoglobulin-like domains is extensively cleaved within 10 min of tryptic digestion. The apparent ease of degradation of this part of the membrane protein probably accounts for the presence of significant amounts of material having the same M_r as SC in all membrane protein preparations, even though proteinase inhibitor was present during membrane solubilization.

The present results also show that the bovine membrane protein becomes partially resistant to proteolysis by trypsin when bound to polymeric immunoglobulin, as does SC (Beale, 1985a). However, this resistance only affects the part of the molecule involved in the immunoglobulin-like domains. Even there, two of the domains appear to be relatively easily removed to leave a resistant three-domain fragment still bound to IgM. It has already been argued (Beale & Hopley, 1985; Beale, 1985a) that optimal binding of bovine SC to polymeric immunoglobulin requires at least three of the immunoglobulin-like domains. The same seems to apply to the bovine membrane receptor protein.

The present work, together with previous results (Kühn & Kraehenbuhl, 1981; Mostov *et al.*, 1984; Beale & Hopley, 1985; Beale, 1985a,b), enables a putative model of the polyimmunoglobulin receptor to be proposed as shown in Fig. 3. The molecule can be divided into four regions. Region 1 contains the five immunoglobulin-like domains identical with those in SC and responsible for binding J-chain-linked IgM and IgA dimers. The two terminal domains are probably linked by a disulphide bridge. Region 2 can be regarded as a connecting piece that may well impart some flexibility to the molecule. Region 3 is a hydrophobic transmembrane section that is probably helical, and region 4 contains the long hydrophilic cytoplasmic tail.

There now seems to be little doubt that SC is the major extracellular part of the epithelial receptor for polymeric immunoglobulin and almost certainly provides the whole of the binding region. Indeed, Kühn & Kraehenbuhl (1981) found that their affinity-purified rabbit epithelial membrane protein and rabbit SC had similar binding affinities for IgA dimers. Hence a detailed study of the interaction between SC and IgM and IgA dimers should provide a true picture of how the epithelial receptor specifically recognizes polymeric immunoglobulin.

The M_r of the bovine membrane protein is greater than that of SC by 20000. Although the exact nature of this difference has not been established, at least part of it can be attributed to the presence of an additional hydrophobic region, since the membrane protein requires the presence of detergent to keep it in solution whereas SC does not.

Fig. 3. Putative structural model of the bovine epithelial receptor for polymeric immunoglobulin

The molecule can be divided into four regions. Region 1 comprises the five immunoglobulin-like domains identical with those in bovine SC and responsible for the specific binding of J-chain-linked IgM and IgA polymers. The terminal domains are probably linked by a disulphide bridge. Region 2 is a connecting piece that might provide molecular flexibility. Region 3 is a transmembrane section. Region 4 is a cytoplasmic tail. The model is based on the results given in the present paper as well as those reported by Kühn & Kraehenbuhl (1981), Mostov *et al.* (1984), Beale & Hopley (1985) and Beale (1985a,b).

Some of the difference could be due to additional glycosylation, but this is likely to be small, since bovine SC is already considerably glycosylated (Labib *et al.*, 1976; Beale & Hopley, 1985).

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