Journal of Cellular Biochemistry 24:307–317 (1984)
Protein Transport and Secretion 241–251

Rat Liver Membrane Secretory Component Is Larger Than Free Secretory Component in Bile: Evidence for Proteolytic Conversion of Membrane Form to Free Form

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Secretory component is a receptor for polymeric immunoglobulins on epithelial cells and hepatocytes that facilitates transport of polymeric immunoglobulins into external secretions. Little is known about the transcellular migration of secretory component-polymeric IgA complexes or the membrane forms of secretory component. We therefore examined rat bile and liver membranes to identify and compare the various molecular species of secretory component. Bile or liver membrane proteins were electrophoresed in sodium dodecyl sulfate-polyacrylamide gels and electrophoretically transferred to nitrocellulose membranes. Protein profiles on blots were probed with antisecretory component antiserum, and the immunoreactive bands were visualized by indirect immunoperoxidase staining. Bile collected in the presence of proteolytic inhibitors showed an immunoreactive doublet band (M_r = 82,000 and 78,000) in the molecular weight range of free secretory component. By contrast, free secretory component in bile collected in the absence of proteolytic inhibitors and purified by affinity chromatography migrated as a single protein with an M_r = 70,000. Both components of the free secretory component doublet bound dimeric IgA when blots were probed with human dimeric IgA. Crude liver membranes prepared in the presence of proteolytic inhibitors showed two immunoreactive secretory component-containing bands, M_r = 107,000 and 99,000, whereas membranes prepared without proteolytic inhibitors showed two smaller immunoreactive bands; one of these proteolytically severed proteins comigrated with the 82,000-dalton free secretory component in bile. These results indicate that membrane forms of secretory component are present in rat liver. The observations that the membrane secretory component is larger than biliary free secretory component and yields biliary SC-like forms of secretory component upon proteolysis support the hypothesis that free secretory component in bile is a proteolytic product of larger liver membrane-associated secretory component.

Key words: secretory component, bile, IgA, immunoblot

Abbreviations used: HRP, horseradish peroxidase; pIgA, polymeric IgA; PAGE, polyacrylamide gel electrophoresis; PI, proteolytic inhibitors; sIgA, secretory IgA; SC, secretory component; SDS, sodium dodecylsulfate.

Received June 7, 1983; revised and accepted November 29, 1983.

Secretory component (SC) is a glycoprotein product of various kinds of epithelial cells and, in some species, of hepatocytes [1–3]. SC appears in external secretions, including bile, in a free state and in association with the polymeric forms of immunoglobulins [4–6]. During the external secretion of polymeric IgA (pIgA), the immunoglobulin molecules bind to SC on the basolateral surfaces of the epithelial cells or the sinusoidal surface of hepatocytes [7–9]. The SC-pIgA complexes then are internalized and transported across the cells in endocytic vesicles and subsequently released at the apical surface, yielding the secretory form of the IgA (sIgA) [7]. The transfer of pIgA across hepatocytes into bile consequently results in the clearance of pIgA from the circulation [10–12].

It is speculated, although not reported, that the ectoplasmic portion of the membrane-SC molecule, which binds pIg, is proteolytically severed during intracellular transport or on the luminal surface of the transporting cells to produce free SC or SC-pIg complexes. Membrane forms of SC have been detected in the liver and mammary gland of the rabbit [13,14] and in a human colonic carcinoma cell line [15]. The membrane-associated SC is synthesized as a transmembrane protein and is presumed to be the precursor of free SC [13–15].

The transcellular migration and biliary secretion of free SC reportedly occur independent of its ligand [13] and may be the primary event in the transport of pIg into bile. Free SC in rat bile has been characterized as a single 70-kilodalton (kd) protein [8]. The presence of a liver membrane-associated SC in the rat has been demonstrated immunohistochemically [14], but the SC has not been characterized biochemically or compared with the free SC in the bile. Therefore, we have examined the molecular forms of SC in bile and liver membranes of the rat through use of immunoblotting techniques. The results indicate that (1) SC in liver membranes occurs as two proteins, both larger than free SC in the bile; (2) membrane-associated SC prepared in the absence of proteolytic inhibitors is proteolytically degraded to produce a biliary SC-like protein; (3) free SC exists in rat bile as a protein doublet that is higher in molecular weight than the single free-SC protein previously reported [6].

MATERIALS AND METHODS

Biological Material

Liver. Rat liver was obtained from Sprague Dawley rats. Rats were bled by cardiac puncture, and the livers were removed, minced, and homogenized in 5 vol of cold 50 mM Tris buffer (pH 7.8), containing 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and soybean trypsin inhibitor (10 mg/liter) (proteolytic inhibitors [PI]; Sigma, St. Louis, MO). Crude membranes prepared by acetone precipitation [17] were detergent extracted with 1% sodium dodecyl sulfate (SDS) in 10 mM Tris, pH 7.8. In some experiments, crude membranes were homogenized in 100 mM Na_2CO_3 prior to SDS extraction. PI were omitted in designated experiments.

Bile. Bile was collected via polyethylene cannula from the common bile duct of sodium pentobarbital-anesthetized rats. Bile either was collected without PI and stored at −20° until analysis or was collected into Laemmli sample buffer [18] containing PI and immediately analyzed.

Immunoreagents

Rat free SC. Free SC was prepared from concentrated and dialyzed bile by column chromatography (Sephadex G200; Pharmacia, Piscataway, NJ) and affinity

chromatography against rat myeloma IgA [9] conjugated to Sepharose beads (Pharmacia). Free SC was eluted from the IgA-affinity column with 3 M NaSCN, dialyzed against phosphate-buffered saline, and concentrated by vacuum dialysis.

Rabbit antiserum to rat SC. Rabbit antiserum was prepared by multiple injections of the animals with the affinity-purified rat SC. Goat antiserum to rat SC was kindly provided by Dr. J.P. Vaerman, Brussels. The anti-SC antisera were exhaustively absorbed with rat serum to remove anti-IgA activity and were affinity purified using a rat bile protein-Sepharose (Pharmacia) column; antibodies were eluted from the column by 3 M NaSCN, pH 7.2 The anti-SC antibodies produced two immunoprecipitin lines against rat bile (representing free SC and sIgA) in immunoelectrophoretic analysis and did not react with rat serum proteins in immunoblots.

Human polymeric IgA. IgA myeloma protein was kindly donated by Dr. B. Underdown of Toronto. Trace contaminants were removed from the protein by passage through a column of Sepharose coupled to a rabbit antiserum prepared against serum from a patient with selective IgA deficiency. (Antihuman Ig light chain antibodies had been removed from the antiserum by absorption with human IgG before coupling to Sepharose.)

Antiserum to human IgA. Antiserum to human IgA was prepared in rabbits by multiple injections with the myeloma IgA. The antiserum was depleted of antibodies to Ig light chains by passage over a column of Sepharose linked to serum from an IgA-deficient patient and was affinity purified using a myeloma IgA-Sepharose column. The eluted anti-IgA antibodies were digested with pepsin and chromatographed over Sephadex G-150 [19]. Fab′ fragments were collected and conjugated with horseradish peroxidase (HRP) [20].

Swine antirabbit Ig conjugated to HRP. HRP-conjugated swine antirabbit Ig was purchased from Accurate Chemical and Scientific Corp., Westbury, NY.

Rabbit antigoat Ig conjugated to HRP. HRP-conjugated rabbit antigoat Ig was prepared as previously described [7].

Electrophoretic Techniques

Polyacrylamide gel electrophoresis (PAGE). Bile or liver membrane proteins were electrophoresed in 8.5% polyacrylamide slab gels for 16 hr at 5 mA constant current [18]. Protein samples were loaded onto the gel in a manner that would allow staining of one portion of the gel with Coomassie blue R250 and immunoblotting of the identically loaded and electrophoresed remainder of the gel. Molecular weight markers obtained from Biorad (Richmond, CA) were used to estimate the molecular weights of SC bands.

Immunoblotting. Protein profiles in SDS-PAGE gels were electrophoretically transferred for 4 hr at 100 mA onto nitrocellulose membrane [21]. Completeness of transfer was monitored by Coomassie staining of the polyacrylamide gel used for transfer and amido black (0.1%) staining of the blots. After the transfer, blots were washed briefly in 10 mM Tris, pH 7.4, containing 0.9% NaCl, 0.05% nonidet-P40, and 0.01% SDS (blotting buffer). The blots were then soaked in blotting buffer plus 3% bovine serum albumin (BSA; Sigma, RIA grade) for 1 hr at 37°C to coat remaining protein binding sites.

For immunodetection of SC, the blots were first overlayed with either the rabbit anti-SC or goat anti-SC antiserum (diluted 1:100 in BSA-blotting buffer) for 16 hr at room temperature with agitation. The blots were washed with blotting buffer (three 1-hr washes with agitation) and overlayed with HRP-conjugated swine antirabbit Ig

or HRP-conjugated rabbit antigoat Ig (detecting antibodies) diluted in BSA-blotting buffer plus 10% homologous serum and 2% O.C.T. compound (Tissue-Tek II, Miles, Naperville, IL). The blots were incubated for 1.5 hr at room temperature with agitation. Unbound antibodies were removed by four 15-min washes with blotting buffer, and immunoreactive bands were visualized by use of diaminobenzidine (25 mg/100 ml in 50 mM Tris buffer (pH 7.6), plus 2 drops of 30% H_2O_2) as a reaction product [20]. Blots were then washed with running tapwater for 30 min and photographed using a No. 80A Wratten gelatin filter.

To determine the ability of transferred SC to bind pIgA, human pIgA (90 μg/ml) in BSA-blotting buffer was applied to the blots for 16 hr at room temperature with agitation. Bound human pIgA was detected by reacting the blots with HRP-conjugated rabbit antihuman pIgA as described above for immunodetection of SC.

RESULTS

Protein profiles from SDS-PAGE gels were effectively transferred to nitrocellulose membranes as judged by Coomassie staining of gels after transfer and by comparing amido black staining of blots with corresponding Coomassie-stained, pretransfer gels. All blots probed with nonimmune serum plus detecting antibody or with detecting antibody alone showed no immunoreactive bands. The addition of O.C.T. (which contains elvanol resin, dimethylbenzylammonium chloride, and polyethylene glycol) to the detecting antibody solution resulted in diminished nonspecific binding of HRP-conjugated antibodies to albumin-coated blots and enhanced contrast of the immunoreactive bands (to be described in detail elsewhere).

Rat Bile SC

Affinity-purified SC from rat bile collected without PI showed two weakly Coomassie-stained bands (Fig. 1, lane A1). The slower-migrating band, M_r = 70,000, reacted with anti-SC antisera (Fig. 1, lane B1). The more prevalent faster-migrating band comigrated with rat serum albumin and did not react with anti-SC antisera; it was presumed to be a contaminant of the purification technique.

Bile collected in the presence of PI showed several protein bands on the Coomassie-stained gel (Fig. 1, lane A3). When these proteins were blotted and probed with rabbit or goat anti-SC antisera and detecting antibody, a doublet band (M_r = 82,000 and 78,000; Table I) migrating as higher molecular weight protein than affinity-purified SC was observed (Fig. 1, lane B3; the doublet is better resolved in Fig. 2, lane B, and in Fig. 4, lane B2). The slower-migrating component of the SC band (M_r = 82,000) always appeared more intensely stained with Coomassie blue and more immunoreactive with anti-SC antiserum than did the faster migrating component. Neither component reacted with antirat IgA antibodies (not shown). In addition to the SC doublet, about four protein bands of higher molecular weight (greater than M_r = 200,000) were present in the gel and blots. These bands were reactive with the anti-SC antiserum (Fig. 1, lane B3) as well as with antirat IgA antiserum (not shown). The high molecular weight bands were absent from gels and blots when samples were reduced with 2-mercaptoethanol (Fig. 2, lane D).

When bile was collected without PI and stored at −20°C for several months, in comparison to bile collected in the presence of PI, there was a loss of high molecular weight proteins and an increase in the amount of lower molecular weight proteins

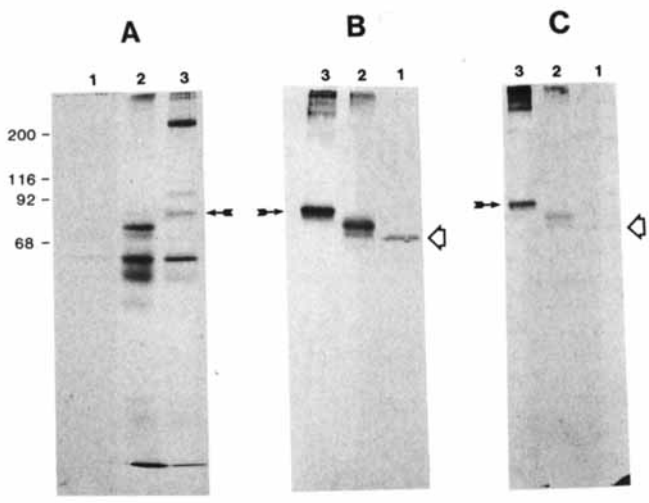

Fig. 1. Photograph of SDS-PAGE gel (8.5%) of rat bile proteins. A) Proteins were stained with Coomassie blue. B, C) Immunoimmunoblots of proteins shown in A. B) Blot was probed with anti-SC antibodies followed by HRP-swine antirabbit IgC (detecting) antibodies. C) Blot was probed with human pIgA followed by HRP-rabbit antihuman pIgA. Lane 1, affinity purified free SC prepared in the absence of PI; lane 2, frozen bile collected in the absence of PI; lane 3, fresh bile collected in the presence of PI. Small, solid arrows point to free-SC doublet. Large, open arrows point to immunoreactive affinity purified free SC. Migration of molecular weight markers (in a separate lane, not shown) are indicated (kd).

TABLE I. Apparent Molecular Weights of PI-Protected Nonreduced and Reduced Free Bile SC and Liver Membrane-Associated SC in SDS-PAGE*

	Bile free SC		Membrane SC	
	Slower-migrating band	Faster-migrating band	Slower-migrating band	Faster-migrating band
Nonreduced	82 ± 1.5 (10)[a]	78 ± 1.4 (10)	107 ± 1.8 (5)	99 ± 3.5 (5)
Reduced	88 ± 1.0 (10)	82 ± 1.8 (10)	115 (1)	105 (1)

*Apparent molecular weights ($\times 10^{-3}$) ± standard deviation ($\times 10^{-3}$) were estimated from migration of known molecular weight standards.
[a]Numbers in parentheses refer to number of individual gels analyzed.

(Fig. 1, lane A2 vs A3). Blots of the frozen bile proteins, when probed with either of the anti-SC antisera, showed an SC doublet band that migrated between that observed in fresh PI-protected bile and SC purified in the absence of PI (Fig. 1, lane B2). A few smaller immunoreactive fragments also were noted. When affinity-purified SC was mixed with fresh bile containing PI prior to SDS-PAGE and blotting, the immunoreactive staining profile showed the characteristic SC doublet as well as the single 70-kd purified SC (not shown). This observation confirmed that the M_r difference between PI-protected and non-PI-protected SC was not due to excessive protein loading of the gels. It appeared, therefore, that SC in the bile was subject to

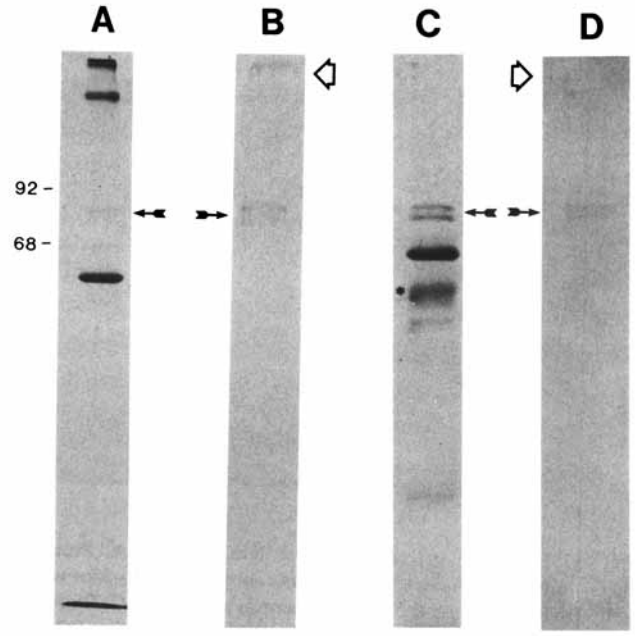

Fig. 2. Photograph of SDS-PAGE gels (8.5%) and immunoblots of nonreduced and reduced bile proteins. Lanes A and C) Bile proteins were stained with Coomassie blue. Lanes B and D) Immunoblots of proteins shown in Lanes A and C, respectively, reacted with anti-SC antibodies followed by detecting antibodies. Lanes A and B) Bile proteins were not reduced with 2-mercaptoethanol. Lanes C and D) Bile proteins were reduced with 2-mercaptoethanol prior to gel electrophoresis. Small, solid arrows depict free-SC doublet. Large, open arrows show presence of immunoreactive high molecular weight proteins in nonreduced bile (lane B) and absence of reactivity in reduced bile (lane D). Asterisk denotes the appearance of proteins in the molecular weight range of Ig heavy chains in reduced bile. Migration of molecular weight markers (in a separate lane, not shown) are indicated (kd).

stepwise, selective proteolytic degradation during in vitro storage or purification when either was done in the absence of PI.

When blots of fresh bile collected with PI or of frozen, stored bile were probed with human pIgA, the immunoglobulin bound to the SC doublet in a pattern identical to that of the anti-SC binding (Fig. 1, lanes C2 and C3 vs B2 and B3). Human pIgA also bound to affinity-purified SC (Fig. 1, lane C1). The detecting antibody alone (antihuman IgA-HRP) did not react with SC or other bile proteins. Unexpectedly, human pIgA bound to the high molecular weight SC-associated protein bands in bile. That the human pIgA bound with all the various free-SC molecules in the differently processed samples indicated that the proteolytic degradation that occurred in the absence of PI was not sufficient to alter the IgA binding ability.

When bile proteins were reduced with 2-mercaptoethanol prior to SDS-PAGE, the protein profile, as judged by Copomassie staining, was changed. Most of the higher molecular weight proteins disappeared, while Ig heavy and light chains appeared (Fig. 2, lane A vs C). The lower band of the SC doublet seemed to have increased in intensity in the reduced bile; however, this lower band has been shown to be transferrin (data not shown). The reduced SC proteins migrated with slightly larger M_r values than did the nonreduced proteins (Table I); the reduced free-SC

doublet was difficult to resolve by Coomassie staining (Fig. 2, lane C) and was weakly reactive anti-SC antisera (Fig. 2, lane D).

Rat Liver Membrane SC

Crude rat liver membrane proteins were analyzed by SDS-PAGE, blotted to nitrocellulose membranes and probed with anti-SC antiserum. Membranes that had been prepared in the presence of PI contained two major immunoreactive bands (M_r = 107,000 and 99,000) that were larger than the free-SC doublet present in bile (Fig. 3); the upper band (M_r = 107,000) occasionally appeared as a doublet (Fig. 3, lane B). Reduction of the membrane-SC proteins with 2-mercaptoethanol yielded two immunoreactive bands that were slightly larger in apparent molecular weight than the nonreduced immunoreactive membrane bands (Table I). The liver membrane SC bands were capable of binding human pIgA when blots were probed with human pIgA (Fig. 3, inset, lane 3). Both SC bands also remained after washing the membranes with 100 mM Na_2CO_3 (Fig. 3, inset, lane 1 vs 2), although minor immunoreactive bands that were probably proteolytic fragments of the larger membrane forms were lost (Fig. 3, inset, lane 1 vs 2, asterisks). When membranes were prepared in the absence of PI, two distinct SC protein bands, which were smaller in M_r than those observed when PI was present during preparation, were seen. The faster-migrating band of these proteolytically cleaved membrane-SC proteins comigrated with the slower-migrating free-SC band present in rat bile (Fig. 4).

DISCUSSION

This study has characterized the molecular species of SC in bile and liver membranes of the rat. The results support the concept that SC in bile is a proteolytic product of liver membrane SC. The study also demonstrated that immunoblots can be used for detecting single proteins in complex mixtures not only by antibody binding, but through use of ligand-receptor interaction as well.

SC of two markedly different molecular weights was present in rat bile. The first of these, in bile protected by proteolytic inhibitors, was a doublet of 78,000 and 82,000 daltons. The SC doublet was reactive with anti-SC antiserum and was capable of binding pIgA; however, the doublet did not react with antirat IgA antisera. Therefore, it was considered to be free SC. When SC was purified from bile unprotected by proteolytic inhibitors, the free-SC doublet disappeared and was replaced by a single 70,000-dalton protein with pIgA-binding ability. Thus, we assume that the 70,000-dalton protein is a proteolytic product of the larger free-SC doublet. Others have reported that free SC in rat bile is a 70,000-dalton protein [6]. Since the PI added to bile during collection was not added until the bile had entered the collection tube, it is likely that free SC in bile exists in the 78,000- and 82,000-dalton doublet form in vivo. It is not known yet whether this SC is a dissociation product of SC-pIgA complexes after their secretion into bile or is secreted by the liver independent of IgA. However, we have observed in unpublished work with the isolated perfused rat liver that the liver can secrete a free-SC doublet in the absence of IgA. In frozen bile unprotected with PI, the appearance of free-SC proteins intermediate in molecular weight between free SC collected with PI and free SC purified in the absence of PI suggests that the proteolysis that proceeds in vitro occurs in a stepwise manner. Susceptibility of free SC to proteolysis in vivo and in vitro has been described [22].

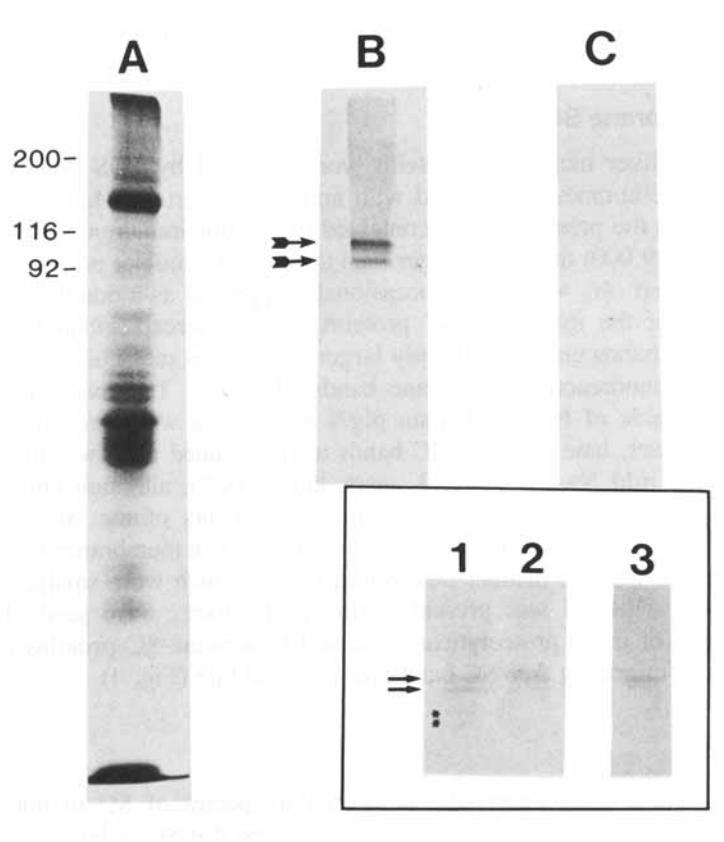

Fig. 3. Photograph of SDS-PAGE gel (8.5%) and immunoblots of rat liver membrane proteins. Lane A) Rat liver membrane proteins prepared in the presence of PI were stained with Coomassie blue. Lanes B and C) Immunoblots of proteins shown in lane A. Lane B) Blot was probed with anti-SC antibodies followed by detecting antibodies. Lane C) Blot was probed with nonimmune antibodies followed by detecting antibodies. Inset photograph shows anti-SC blots of liver membranes prepared in the absence of Na_2CO_3 (lane 1) and after being washed with 100 mM Na_2CO_3 (lane 2) and liver membranes probed with pIgA and HRP-rabbit antihuman IgA are shown in lane 3. Asterisks depict immunoreactive bands that are not present in Na_2Co_3-washed membranes (these bands have similar mobility to the immunoreactive bands shown in Figure 4 from liver membranes prepared in the absence of proteolytic inhibitors). Solid arrows depict immunoreactive-SC proteins in liver membranes. Migration of molecular weight markers (in a separate lane, not shown) are indicated (kd).

The fact that both bands of the free-SC doublet in bile are capable of binding pIgA is evidence that both have this functional characteristic of SC. It was to be expected that the rat SC would bind to heterologous (human) pIgA, since species cross-affinity between free SC and Ig polymers is well documented [23]. The relationships between the two bands of the SC doublet are not defined, however. Whether the bands represent separate gene products, as has been shown for rabbit SC doublets [13], or proteins related through a precursor-product scheme is unknown.

In addition to the free-SC doublet, rat bile contained a molecular species of SC that migrated in SDS-PAGE in the range of proteins with much higher molecular weight (M_r greater than 200,000) than free SC. These heavy molecular weight

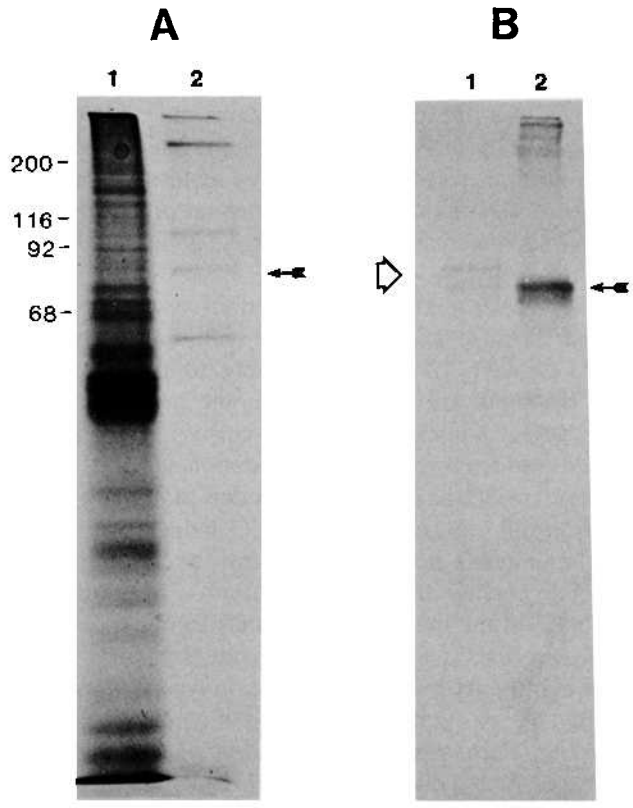

Fig. 4. Photograph of SDS-PAGE gel (8.5%) and immunoblot of rat bile and liver membrane proteins. A) Bile and liver membrane proteins were stained with Coomassie blue. B) Immunoblot of proteins shown in A; blot was probed with anti-SC antibodies followed by detecting antibodies. Lane 1, rat liver membrane proteins prepared in the absence of PI; lane 2, rat bile collected in the presence of PI. Small, solid arrows show free-SC doublet in the bile. Large, open arrow points to immunoreactive-SC proteins present in rat liver membranes. Migration of molecular weight markers (in a separate lane, not shown) are indicated (kd).

proteins reacted with anti-IgA antibodies as well as with anti-SC antibodies and were susceptible to reduction with 2-merceptaethanol. Thus, they are presumed to be, at least in part, dimers or larger polymers of IgA bound to SC, ie, secretory IgA. Polymers of sIgA in rat bile have been described [6].

After reduction of bile with 2-merceptoethanol, the only SC bands observed were in the molecular weight range of free SC. Since the reduced SC bands were only weakly immunoreactive, interpretation of the forms associated with IgA was difficult. However, this result suggests that the SC associated with the high molecular weight biliary proteins are of the same M_r as the free SC proteins. Whether both forms are associated with IgA remains to be clarified. The presence of two SC bands after reduction of rat biliary sIgA has been described [6]; however, only one of the bands was in the 80-kd range. In our experiments, the reduced free-SC and membrane-SC bands migrated with a slightly larger M_r than nonreduced free-SC proteins.

This increase in M_r may be attributed to an altered conformation of the molecule induced by reduction of intrachain disulfide bonds.

The ability of the high molecular weight SC-containing proteins to bind human pIgA in Western blots was unexpected and remains unexplained. We suggest three possible explanations for this occurrence: (1) aggregates or polymers of free SC capable of binding pIgA were present in the heavy molecular weight material; (2) the human pIgA nonspecifically interacted with either rat pIgA or sIgA in the bile; (3) dimers and trimers of sIgA are capable of binding an additional IgA dimer to produce tetramers and pentamers of sIgA.

The SC present in liver membrane preparations also was found to be a doublet. That both membrane forms remained associated with the membrane following treatment with 100 mM Na_2CO_3 [24] and were only solubilized after treatment with detergent suggests that both are integral membrane proteins. Each of the doublet bands was about 20,000-daltons larger than the corresponding free-SC bands in bile and was susceptible to proteolysis during preparation in vitro, yielding a band that migrated like biliary free-SC bands. However, one SC band in the PI-unprotected membrane preparation still was larger than free SC in the bile. This may have resulted from a portion of the protein's being protected from proteolysis through a particular orientation in a vesicle created during homogenization of the liver. We assume that one, and possibly both, of the membrane-SC bands can be cleaved to yield the free-SC doublet observed in bile. Both of the membrane-SC bands were also capable of binding pIgA, although the intensity of the reaction was always very weak. Perhaps the membrane SC undergoes significant alteration of conformation during SDS-PAGE to inhibit pIgA binding. These results are consistent with the hypothesis that free SC in the bile is a proteolytic product of transmembrane SC in the liver. It has been theorized that the ectoplasmic portion of membrane-bound SC is proteolytically clipped to produce free SC and a residual membrane-anchoring fragment. In our studies, the 20,000-dalton extension of biliary SC observed in the liver membrane SC might be the anchoring fragment of the molecule. Recent studies on a human colonic carcinoma cell line [15] have shown that a large intracellular transmembrane SC is a precursor of secreted free SC; perhaps a similar relationship between membrane forms and secreted forms of SC exists in the rat liver. Free-SC doublets and membrane-SC doublets have been described also in rabbit liver and mammary gland [13,14]. Although no information concerning precursor-product relationships of the membrane forms and secreted forms of SC was reported in that species, peptide and N-terminal analyses of the proteins suggested that they differ only in molecular weight, perhaps as the result of modifications of the C-terminal end of the molecule [13–15].

The finding that the rat liver membrane SC bands were susceptible to in vitro proteolysis during preparation indicates that the proteolytic enzyme(s) required to cleave membrane SC to free SC are present in liver tissue, although the cleavage prevented by proteolytic inhibitors in vitro may not be at the same site as that observed under normal physiological conditions. In addition, it is not known whether the in vivo cleavage of membrane SC to free SC or SC-pIg occurs within the hepatocyte or by proteolytic enzymes present in bile.

It had been predicted that a membrane form of SC is present in rat liver [3]. The results presented in this communication document that, indeed, two membrane forms of SC are present. Moreover, the liver membrane forms of SC are larger than

free SC proteins in the bile, and the size difference likely is due to the presence of a membrane-anchoring fragment on the membrane form. Our data extend the recent observations of Mostov and Blobel [13], who showed a precursor-product relationship between a membrane form and secreted form of SC in a human colonic carcinoma cell line. We provide evidence that a proteolytic event converts rat liver membrane SC to a form of SC like that in bile. In addition, our data are derived from nonneoplastic tissues and are the first to contrast liver membrane forms and biliary forms of SC.

ACKNOWLEDGMENTS

We wish to thank Ms Evelyn Lee and Ms Doris Gaskin for excellent technical assistance and Ms Jean Gilbert and Ms Kathy Hunt for superior secretarial assistance. We would also like to thank Drs Dennis Ahnen and John Vierling for helpful advice.

This work was supported in part by the Veterans Administration, National Institutes of Health Grant CA17342 through the National Large Bowel Cancer Project, and American Cancer Society Postdoctoral Fellowship PF-1957.

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NOTE ADDED IN PROOF

While this paper was in press, Sztul et al. (J Cell Biol 97:1582, 1983) identified an 80,000 dalton form of SC in rat bile; and 94,000 and 116,000 dalton forms of SC in Golgi membranes from rat liver.