Biogenesis of the Polymeric IgA Receptor in Rat Hepatocytes. II. Localization of Its Intracellular Forms by Cell Fractionation Studies

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ABSTRACT In the companion paper (Sztul, E. S., K. E. Howell, and G. E. Palade, *J. Cell Biol.*, 100:1248–1254), we have shown that pulse labeling of hepatic proteins with [^{35}S]cysteine can be obtained in vivo in intact rats. Soluble label clears the plasma in ~5 min, and incorporated label reaches peak values in the liver ~20 min after injection. In the present study, we show that the 105,000-mol-wt protein (105K), kinetically the earliest intracellular form of secretory component (SC), is the predominant form found, between 5 and 20 min postinjection, in homogeneous rough microsomal fractions. The second kinetically defined form, i.e., 116K, is the predominant species present in relatively homogeneous, light Golgi fractions in which it appears at ~15 min, and peaks at ~25 min, postinjection. The third kinetically defined form, 120K, is found 30 min after injection as the major SC species (albeit still accompanied by its immediate precursor, 116K), in a sinusoidal plasmalemmal fraction isolated by immunoadsorption to anti-SC-coated Sepharose beads.

These findings lead to the following conclusions: (*a*) SC is synthesized on polysomes attached to the rough endoplasmic reticulum (ER) membrane; (*b*) it is partially translocated across the ER membrane and core glycosylated co-translationally to give a 105K peptide; (*c*) 105K moves from the ER to the Golgi complex where it is terminally glycosylated to give the 116K form; (*d*) the latter moves to the sinusoidal plasmalemma where it appears together with the final mature form, 120K. Kinetic evidence indicates that the vesicular carriers involved in the transport of SC from the Golgi complex to the sinusoidal plasmalemma, and from the latter to the biliary front of the hepatocytes, are present in a Golgi heavy fraction and a crude carrier vesicle fraction from which they remain to be isolated, purified, and characterized.

The pathway followed by the polymeric IgA receptor (i.e., the secretory component [SC])[1] within hepatocytes can be delineated, and the subcellular compartments involved in its processing can be identified, by using cell fractionation procedures in the framework of a pulse-chase experiment. Toward this end, we pulse-labeled living rats with [^{35}S]cysteine and isolated, at selected, postpulse intervals, a series of cell fractions that represent subcellular components known to be involved in the synthesis and transport of proteins to the plasmalemma (reviewed in reference [26]). By analyzing these preparations, we have identified the major SC form present in each fraction, defined the kinetics of transport of each SC form into and out of each fraction, and in so doing, identified most of the compartments in which SC incurs major postranslational modifications.

MATERIALS AND METHODS

All materials and methods used in the experiments reported in this study are listed or described in the companion paper (34). Procedures applied specifically for experiments to be reported in this paper are given below.

General Protocol: Adult, male Sprague-Dawley rats (Charles River Breeding Laboratories Inc., Wilmington, MA) received under light ether anesthesia 2 mCi [^{35}S]cysteine by injection into the saphenous vein. At chosen

[1] *Abbreviations used in this paper:* ER, endoplasmic reticulum; GF, Golgi fraction; K, × 1,000 mol wt (e.g., 105K, 105,000-mol-wt [form]); SC, secretory component; SRA, specific radioactivity; TM, total microsomal fraction.

intervals postinjection, the livers were removed (under ether anesthesia) and homogenized in 0.25 M sucrose to give a 30% (wt/vol) homogenate. The latter was centrifuged (in a Beckman preparative ultracentrifuge, Beckman Instruments, Inc., Palo Alto, CA) to yield, after 10 min at 7,000 g_{av}, a supernate and a pellet that contained cells, cell fragments, nuclei, mitochondria, and other large particles. The supernate was recovered and centrifuged for 90 min at 105,000 g_{av} to give a final supernate and a pellet designated total microsomal fraction (TM). This fraction comprises all subcellular components involved in SC synthesis and transport, i.e., rough and smooth microsomes (derived from the endoplasmic reticulum [ER]), Golgi elements, vesicular carriers and vesicles derived from the plasmalemma. The TM fraction was the starting material for the subsequent preparation of all cell fractions used in this study.

To be able to compare the results obtained on cell fractions at successive intervals after pulse, a series of three to four experiments was carried out within the same week with the same batch of [^{35}S]cysteine. A second, similar series extended the intervals examined, and an overlap (at 20 min postinjection) made possible the connection of the two sets of data.

Golgi Fractions: Two Golgi fractions, GF_{1+2} and GF_3, were isolated from the resuspended TM by a previously reported (16) modification of the procedure of Ehrenreich et al. (11). Briefly, TM fractions (~120 mg protein) resuspended to 10 ml in 1.22 M sucrose were loaded at the bottom of a discontinuous sucrose gradient (1.15, 0.86, 0.25 M) which was centrifuged for 3 h at 82,500 g_{av} in an SW27 rotor using a L5-65 Beckman centrifuge. The band that formed at the 0.25/0.86 M interface was designated the Golgi light fraction (GF_{1+2}); that found at the 0.86/1.15 M interface constituted the heavy Golgi fraction (GF_3).

We tested the reproducibility of our cell fractionation procedures by measuring the amount of protein and SC (quantitated by radioimmunoassay) in three different GF_3 preparations. No significant differences were found between these fractions, indicating comparable fractionation yields from one experiment to another.

Rough Microsomal Fraction: The residual microsomal fraction, which remained at the bottom of the centrifuge tubes after the flotation of GF_{1+2} and GF_3 components, was used to isolate rough microsomes by a modification of the procedure of Adelman et al. (2). In short, the residual microsomal fraction was adjusted to 1.4 M sucrose with 2.0 M stock sucrose solution, and 7 ml of the ensuing suspension was layered over a 2-ml cushion of 1.9 M sucrose. The load was overlaid with ~1 ml of 0.86 M sucrose, and the gradient was centrifuged at 195,000 g_{av} for ~16 h in an SW41 swinging bucket rotor. The rough microsomal fraction, which collected at the 1.9/1.4 M sucrose interface, was recovered and diluted to 0.25 M sucrose before further processing.

Plasmalemmal Fraction: A TM fraction was isolated as above at 30 and 60 min postinjection of 2 mCi [^{35}S]cysteine. The pellet was resuspended, and vesicles derived from the sinusoidal plasmalemma of the hepatocytes were isolated from the mixed vesicular population of the suspension by the following procedure: (*a*) Protein A-Sepharose beads (200 μl of 60% suspension in buffer A [2% Triton X-100, 150 mM NaCl, 2 mM EDTA, 30 mM Tris-HCl, pH 7.4]) were incubated for 60 min at 24°C with excess immune (anti-SC serum) or preimmune rabbit serum (50 μl per bead aliquot); (*b*) the coated beads were recovered by centrifugation and washed four times (by pelleting followed by resuspension) in buffer B (1% Triton X-100, 0.2% SDS, 150 mM NaCl, 5 mM EDTA, 10 mM Tris-HCl, pH 8.0) and then twice with the same buffer without detergents; (*c*) the washed beads were incubated for 2 h at room temperature with 500 μl of a TM fraction resuspended at a final concentration of 2 mg protein/ml; (*d*) after incubation, the beads were recovered by centrifugation and washed (four times, 10 min each) by gentle resuspension in 100 μl of 0.25 M sucrose, 50 mM Tris-HCl buffer, pH 7.4; the final pellet was resuspended in 50 mM Tris-HCl buffer, pH 7.4. In some experiments, a liver plasmalemma fraction (isolated as in reference 22) was used.

Processing of Cell Fractions for SDS PAGE and Fluorography: Aliquots of cell fractions, containing the same amount of proteins, were solubilized in SDS, and the ensuing lysates were processed through immunoprecipitation with anti-SC serum as in reference 34. SDS PAGE followed by fluorography of the gels or by transfer to nitrocellulose filters was performed as described (34).

Electron Microscopy of Cell Fractions: Aliquots of cell fractions were fixed in suspension for 60 min, at 4°C with 1.5% glutaraldehyde (final concentration) in 0.1 M Na cacodylate-HCl buffer, pH 7.4. The particles were pelleted at 20,000 g_{av} in an SW41 rotor for 20 min, and the pellets were postfixed for 2 h on ice in 1% OsO_4 (final concentration) in 0.1 Na cacodylate-HCl, pH 7.4. Pellet strips were processed through dehydration and embedded so as to allow sectioning of their entire depth (top to bottom). The sections were stained by routine procedures and examined in a Philips 301 electron microscope. Micrographs were taken at sufficiently high magnification (11,000–19,000) to allow a reliable identification of the subcellular components present in each fraction.

RESULTS

Characterization of Isolated Subcellular Fractions

ROUGH MICROSOME FRACTION: The degree of homogeneity of this fraction was assessed by electron microscopy (micrographs not shown). Throughout their depth, the corresponding pellets consisted of rough microsomes; morphologically recognizable contaminants were absent or present in negligible amounts. The fraction has been characterized biochemically and morphologically by Adelman et al. (2). Their results and our electron microscopic survey indicate that the vesicular components of the fraction are derived nearly exclusively from the rough ER.

LIGHT GOLGI FRACTION (GF_{1+2}): The electron microscopic survey showed that this fraction consisted primarily of Golgi-derived vesicles loaded with lipoprotein particles. Cisternal elements were few in number, and contaminants were limited to a few lysosomes and a few smooth-surfaced vesicles derived from unknown intracellular sources. The fraction has been characterized biochemically and morphologically (3, 11, 16). Its elements are derived primarily from secretion vacuoles and detached rims of distended Golgi cisternae. Other Golgi elements (cisternae) were minor constituents. This fraction is not as homogeneous as the rough microsomal fraction, but it can be considered a clean, reasonably homogeneous preparation.

HEAVY GOLGI FRACTION (GF_3): This fraction consisted primarily of Golgi cisternae, most of which had apparently lost their distended, lipoprotein-loaded rims during tissue homogenization. Golgi vesicles containing lipoprotein particles were rare. The fraction was, however, significantly contaminated by smooth-surfaced vesicles of different sizes. Some of them were probably derived from the smooth ER and the plasmalemma; others may represent intracellular or transcellular vesicular carriers. Rough microsomes were minor contaminants of the heavy Golgi fraction. GF_3 was characterized biochemically and morphologically in previous studies (3, 11, 16). Our electron microscopy survey is in full agreement with findings already published.

CRUDE CARRIER FRACTION: The vesicular elements found within the 1.15 M region of the sucrose density gradient at the end of the flotation of the Golgi fractions represent a heterogeneous population in which small smooth-surfaced vesicles and small fragments of Golgi cisternae predominate. Large smooth-surfaced vesicles of unknown derivation are present in smaller numbers and rough microsomes are relatively rare.

Pulse-Chase Conditions in Isolated Subcellular Fractions

As shown in Fig. 3 of the companion paper (34), conditions approaching pulse-chase were obtained in rat liver after an intravenous injection of [^{35}S]cysteine. To document the degree to which such conditions are obtained at the intracellular level, we investigated the labeling kinetics of total protein in isolated subcellular fractions. Rough microsomes, Golgi-, and carrier-enriched fractions were isolated (as in Materials Methods) from animals killed at various times after the administration of label and their content of radioactivity was determined. As shown in Fig. 1, sharp peaks of label were obtained at 10 min postinjection in the rough microsome fraction and at 20 min after injection in the Golgi light fraction. Radiola-

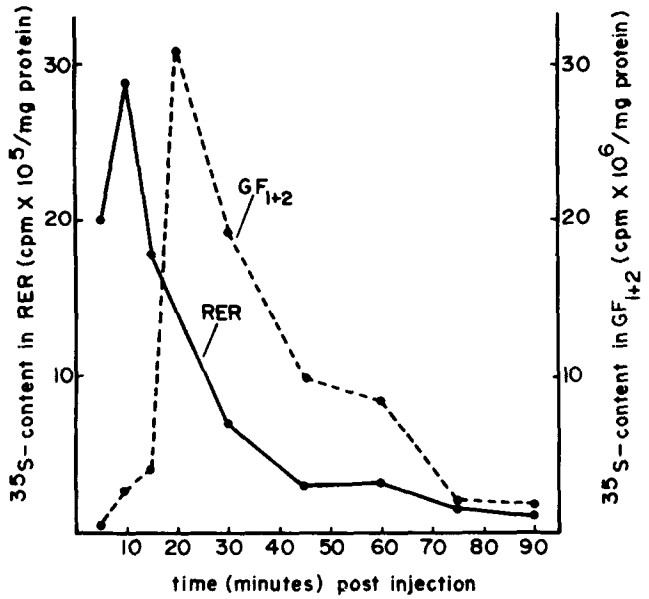

FIGURE 1 Incorporated label in liver rough ER and Golgi light (GF_{1+2}) fractions. Fractions were isolated from livers of animals sacrificed at selected times after intravenous injection of 2 mCi [^{35}S]-cysteine. The graph shows phosphotungstic acid-precipitable radioactivity normalized per gram liver (i.e., counts per minute per milligram protein in fractions derived from 1 gram wet wt liver).

beled proteins appeared in the Golgi heavy fraction (GF_3) at 5 min postinjection, increased in intensity until 15 min after injection, and then continuously declined[2] until the end of the experiment (i.e., 90 min postinjection) (Fig. 2). Comparison of the peak specific radioactivity (SRA) times in these three fractions indicates rapid transit of a radiolabeled protein cohort through each fraction, and by inference, through the corresponding subcellular compartments.

Radiolabeled proteins were detected in the mixed carrier fraction at 5 min postinjection, reached SRA peak at 10 min, and then slowly declined in intensity (not shown). The clearance of radiolabeled proteins from this fraction was much slower than from the other three, suggesting that this fraction contains multiple (temporally separate) compartments involved in the transport of hepatic proteins.

The asymmetric shape of all the SRA peaks, with the leading edge much sharper than the trailing edge, indicates that only the entrance time of the radiolabeled cohort and the time of maximum labeling can be accurately determined in each fraction. The egress time cannot be reliably established due to the extensive tail of post-peak labeling.

Kinetics of SC Intracellular Transport at the Level of Cell Fractions

Cell fractions isolated at successive intervals after administration of [^{35}S]cysteine were solubilized and the ensuing lysates were used as starting preparations for the detection of different forms of intracellular SC by immunoprecipitation with anti-SC serum. The immunoprecipitates were resolved by SDS PAGE and their components were detected by fluorography.

[2] Peak SRA of 15 min is clearly shown in the first four lanes. The last six lanes were exposed for a longer time to show persistance of labeled proteins up to 90 min postinjection.

Rough Microsomal Fraction

As shown by the fluorogram in Fig. 3, 105K—identified in the preceding paper (34) as kinetically the earliest form of intracellular SC—was detected in this fraction as the shortest interval examined, i.e., 5 min after injection. It reached half maximal value at ~10 min postinjection, peaked at 15 min postinjection, and declined first rapidly over the next 10–15 min and then slowly enough to be still detectable at 45 min postinjection, albeit at a very low fraction (~5%) of its peak value.

At 30 min after injection, 116K, kinetically the second form of intracellular SC (35), became detectable in the rough microsomal fraction; it had initially less than one fifth of the intensity of the 105K band, declined slowly from this level, and was still visible by the end of the experiment (90 min postinjection), at which time it was the only form of SC still detectable in the rough microsomal fraction.

Taken together, these results indicate that 105K was the predominant form of SC in rough microsomes, hence, in the rough ER of intact hepatocytes. It was the only form found in this fraction up to 20 min postinjection and remained the major form up to 30–45 min postinjection. Its relatively slow clearance from the rough microsomal fraction can be ascribed to imperfect chase conditions in intact animals, but it may also reflect the inherently slower transport kinetics for glycoproteins (both membrane and soluble [references 21 and 33] and our unpublished results) than for nonglycosylated proteins. The presence of the 116K form of SC in low concentration in rough microsomes could be ascribed to the contamination of this fraction by Golgi elements (see below); an alternative explanation could be membrane recycling between the ER and the Golgi compartment in which the trimming and terminal glycosylation of the 105K form occur.

Golgi Light Fraction

The major SC form detected in the light (GF_{1+2}) Golgi fraction was 116K (Fig. 3). It appeared already at 15 min after injection, reached its peak value between 20 and 30 min

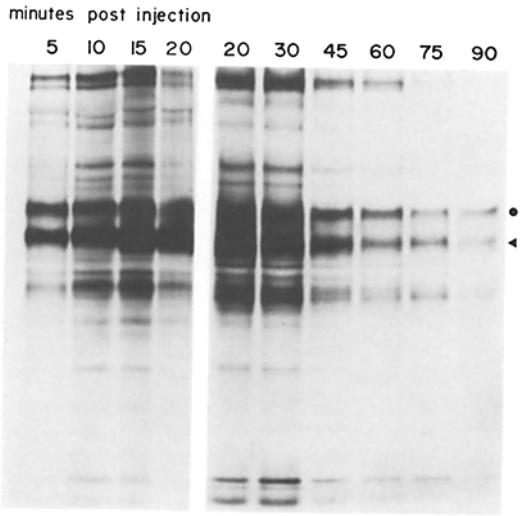

FIGURE 2 Radiolabeled proteins in Golgi heavy (GF_3) fractions. Fractions were isolated from livers of animals sacrificed at selected times after intravenous injection of 2 mCi [^{35}S]cysteine. Approximately 100 μg of protein was loaded per well at each time point. ▲, position of albumin, ●, position of transferrin.

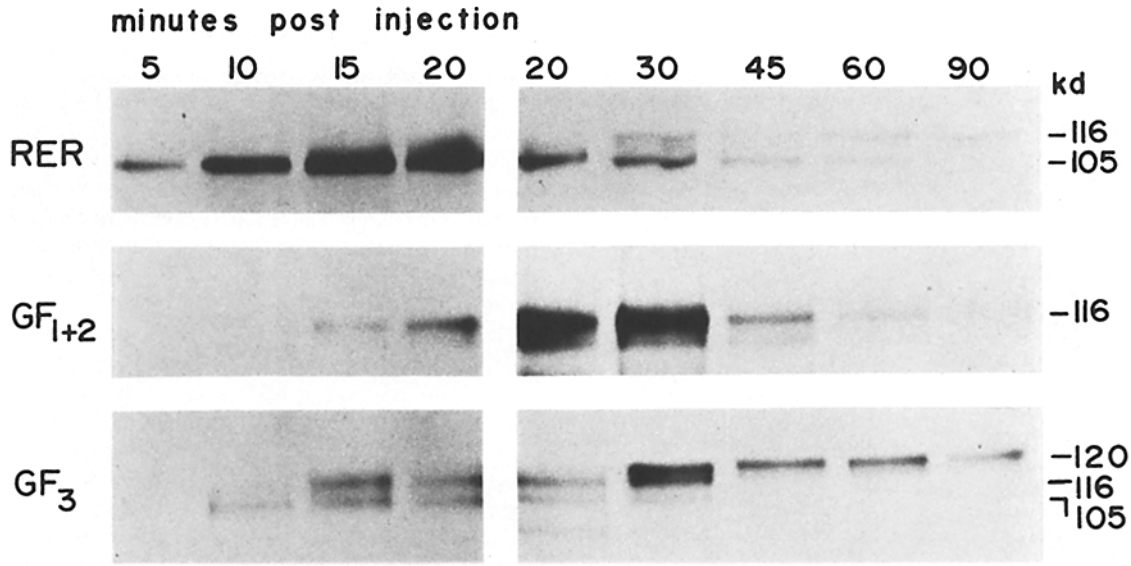

FIGURE 3 Appearance of biosynthetically labeled SC in subcellular fractions. Fractions were isolated from livers of rats sacrificed at selected time intervals after an intravenous administration of 2 mCi [^{35}S]cysteine. Shown are fluorographs of SDS PAGE electrophoretograms of polypeptides immunoprecipitated with anti-SC sera; only parts of gels containing SC are included. (*RER*) SC forms detected in rough ER fractions; (*GF_{1+2}*) SC forms detected in Golgi light (GF_{1+2}) fractions; (*GF_3*) SC forms detected in Golgi heavy (GF_3) fractions.

postinjection, lost radioactivity rapidly thereafter, but persisted as a hardly detectable, faint band to the end of the experiment, i.e., 90 min postinjection. A 105K band appeared as a minor SC form in light Golgi fractions at ~20 min after injection and was fully cleared by 45 min (like the same band in rough microsomes). At peak value, 105K amounted to only a small fraction (<5%) of the radioactivity in the 116K band.

The presence in light Golgi fractions of 105K, the major SC form found in rough microsomes, could be ascribed to either contamination or membrane recycling. The first alternative is not excluded, given the composition of the fraction; but the second alternative deserves careful consideration since (*a*) 105K was not detected in GF_{1+2} at the times of its maximal labeling (10–20 min postinjection) in rough microsomal fractions; and (*b*) its appearance in GF_{1+2} coincided with its clearing from rough microsomes at 20–30 min after injection.

Golgi Heavy Fraction

Three SC forms were found in this fraction (Fig. 3). The first, 105K, appeared at 10 min postinjection, reached a low peak at 15–20 min, and persisted at low intensity up to 30 min postinjection. At considerably lower radioactivity and with an apparent shift of ~5 min, it paralleled the kinetics of the same form in rough microsomal fractions. For these reasons, 105K in GF_3 could be ascribed to the already known contamination of this fraction by rough microsomes.

The second form, 116K, appeared in GF_3 at or before 15 min postinjection, declined slowly in activity thereafter, and was no longer detectable 45 min after injection. 116K, the predominant Golgi form of SC, appeared in GF_3 slightly ahead of its detection in GF_{1+2}, and its gradual decline in the former coincided with its gradual increase in the latter. On this basis, it can be assumed that GF_3 contains some of the first Golgi subcompartments through which SC passes along its route from the ER, and in which it is concurrently converted from the 105K to the 116K form.

The third form, 120K, kinetically the latest intracellular form of SC (35), was detected in GF_3 at 30 min postinjection and persisted while slowly declining in intensity, as the only form from 45 min to the end of the experiments, i.e., 90 min postinjection.

The intracellular origin of the 120K form found in GF_3 is uncertain. Since this form is found in purified plasmalemmal fractions (see below), its presence in GF_3 could be at least partly explained by the latter's contamination with plasmalemma-derived vesicles. But its long persistence in GF_3 up to and past the time (40 min) when newly synthesized SC begins to appear in the bile indicates that 120K must also be the SC form present in transcellular vesicular carriers.

The 120K form of SC was not detected in light Golgi fractions (GF_{1+2}), which, as already stated, appeared to contain only the 116K form. It was possible, however, that the gel in Fig. 3 did not resolve the last two forms, and that the broad band seen in that figure represented, in fact, the two unresolved components. To test this possibility, GF_{1+2} and GF_3 isolated at 30 min postinjection were run together on the same gel. The fluorograph (Fig. 4) revealed the 116K-120K doublet in GF_3 and confirmed the absence of 120K from GF_{1+2}.

In an immunocytochemical study, carried out by electron microscopy on hepatocytes in situ, we have detected SC in practically all the cisternal elements of the Golgi complexes, but not in lipoprotein-loaded vesicles located on the trans side of the Golgi stacks (17). These results suggest that in GF_{1+2} the 116K form of SC is located in Glogi cisternae rather than in lipoprotein-loaded secretory vacuoles; and that SC and secretory proteins are transported from the Golgi complex to the sinusoidal plasmalemma by different vesicular carriers.

Crude Carrier Vesicle Fraction

The SC forms found in this fraction were the same as in GF_3, except that the 120K form appeared to be present in even higher concentration (fluorograph not shown). This

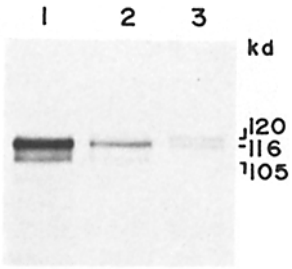

FIGURE 4 Comparison of SC forms present in GF_3 and GF_{1+2} fractions. Fractions were isolated from the liver of an animal killed 30 min post-injection of 2 mCi [^{35}S]cysteine. SC forms were immunoprecipitated, resolved by SDS PAGE, and detected by fluorography. (Lane *1*) SC forms present in GF_{1+2} fraction; (lane *2*) as in lane *1* but 1/10 of the load; (lane *3*) SC forms present in GF_3 fraction.

preparation may therefore contain a large number of plasmalemma-derived vesicles and/or a large fraction of the total population of carrier vesicles involved in the transcellular transport of SC.

Plasmalemmal Fractions

Plasmalemmal vesicles were isolated by immunoadsorption on protein A-Sepharose beads coated with anti-SC serum antibodies. The starting preparations were TM fractions prepared at either 30 min after administration of [^{35}S]cysteine, i.e., at a time when newly synthesized SC was expected to have emerged at the cell surface; or 60 min postinjection, when 116K is almost completely cleared from Golgi fractions and 120K persists as the major SC form detected within hepatocytes. As already indicated, our antiserum was raised against the biliary form of SC; hence, it was expected to recognize only the ectodomain of the membrane forms of SC. The immunoadsorption procedure takes advantage of the fact that during liver homogenization intracellular compartments and the plasmalemma form vesicles that generally retain their right-side-out orientation. Consequently, the ectodomain of SC is expected to be oriented inwards in vesicles, "microsomes," derived from the ER, Golgi complex, and various intracellular vesicular carriers, and thus inaccessible to the antibodies, but directed outwards and thereby accessible to antibodies in vesicles derived from the plasmalemma. Moreover, since vesicles derived from the biliary domain of the hepatocyte were shown to lack SC (22), the only vesicles expected to bind to the insolubilized antibody should derive from the sinusoidal and perhaps lateral domain of the hepatocytic plasmalemma.

As shown in Fig. 5, lane *1*, the starting TM fraction contained, as already established (35), all the intracellular forms of SC, i.e., 105K, 116K, and 120K. 105K and 120K were present in nearly equal amounts slightly in excess of 116K. The vesicles bound to anti-SC-coated beads (Fig. 5, lane *2*) contained both larger SC forms, in a 120K/116K ratio higher than in the starting preparation.[3] The 105K form was not detectable. The finding is in keeping with our original assumption that right-side-out microsomes can not bind to the immunobeads. When the same beads were coated with preimmune serum, no binding of vesicles occurred (Fig. 5, lanes *4* and *5*). Nonradioactive plasmalemmal vesicles isolated as in reference 23 competed effectively with the radioactive vesicles of our plasmalemmal fraction in binding to the immunobeads (Fig. 5, lanes *6* and *7*). A TM fraction isolated at 60 min postinjection predominantly contained the 120K form, with

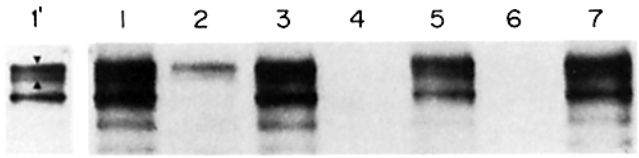

FIGURE 5 Plasmalemmal SC forms. Hepatic TM fractions were isolated from rats killed 30 min postinjection of 2 mCi [^{35}S]cysteine. Aliquots were incubated in the absence or presence of cold plasmalemma vesicles (isolated as in reference 22), with Protein A-Sepharose beads coated with preimmune or anti-SC serum. In each case, the material bound to the immunobeads or the nonbound flow-through was solubilized and the lysate was used for immunoprecipitation with anti-SC serum as given in Materials and Methods. (Lane *1'*) Same as Lane *1* except for shorter exposure to demonstrate the presence of the 116K–120K doublet; (lane *1*) the starting TM fraction isolated 30 min postinjection; (lane *2*) material bound to anti-SC-IgG beads; (lane *3*) flow-through from lane *2*; (lane *4*) material bound to preimmune IgG-beads; (lane *5*) flow-through from lane *4*; (lane *6*) anti-SC-IgG beads were incubated with ^{35}S-labeled TM vesicles and excess cold plasmalemma vesicles; material bound to immunobeads; (lane *7*) flow-through from lane *6*.

lower concentrations of 116K and 105K than at 30 min. The elements bound to the immunosupport contained 120K, traces of 116K, but no 105K.

These results indicate clearly that both 116K and 120K are present in clean plasmalemmal fractions, but they do not provide information about the site where the conversion begins: it could be the carrier vesicle that brings SC to the sinusoidal plasmalemma, or it could be the plasmalemma itself. Since 120K is phosphorylated (34), it can be assumed that, as in the case of other receptors, phosphorylation of 116K and its ensuing conversion to 120K occur concomitantly with the binding of the proper ligand $(IgA)_2$ to the receptor, but the assumption remains to be validated by appropriate tests. If this is the case, and if conversion begins rapidly upon arrival of 116K at the sinusoidal plasmalemma, then the minimum time needed for SC transport from the ER to the sinusoidal plasmalemma is ~30 min.

In the plasmalemma, the 116K seems to be converted progressively but relatively slowly into 120K, because 116K is present in clean plasmalemmal fractions at 60 min postinjection and is greatly reduced in concentration past 45 min in both TM and GF_3. 120K is still present in both fractions 90 min after injection.

To estimate the total amount of SC forms present in the plasmalemma (rather than the amounts of newly synthesized forms), we solubilized a plasmalemmal fraction isolated by the procedure of Meier et al. (22),[4] resolved its proteins by SDS PAGE, transferred the gel to nitrocellulose filters, and processed the latter through immuneoverlays with anti-SC serum followed by overlay with ^{125}I-labeled protein A and autoradiography. The results obtained (data not shown) indicate that the 116K and 120K bands appear to be present in about the same concentration in the plasmalemmal fraction, whereas 105K is absent.

The soluble, biliary form of the secretory component, 80K, was not detected by immunoprecipitation in any of the particulate cell fractions already mentioned. As already indicated, 80K is easily detected in collected bile.

[3] No attempt at full recovery of SC (116K–120K) was made in this experiment as shown by the fluorographs of the flow-through samples.

[4] This fraction contains vesicles derived from both the sinusoidal and biliary domain of the hepatocytic plasmalemma.

DISCUSSION

In the experiments reported in this paper, we have localized to specific subcellular compartments the three intracellular forms of SC described in the preceding paper (35). Relevant cell fractions were isolated at selected intervals after a labeling pulse generated in living animals by an intravenous injection of [^{35}S]cysteine. The SC forms present in each fraction were immunoprecipitated, resolved by SDS PAGE, and detected by fluorography. This approach has the advantage of providing directly applicable and physiologically relevant data on the biogenesis of hepatocytic SC, but it is affected, to a limited extent, by the fact that a perfect chase of the biosynthetic precursor used, [^{35}S]cysteine, cannot be achieved in intact animals.

Direct information on the intracellular location of the biosynthetic, macromolecular precursors of membrane proteins is rarely obtained. Their location is usually inferred from kinetic data coupled with their sensitivity to specific endoglycosidases (when the proteins happen to have N-linked oligosaccharide chains) (for examples, see references 6, 31, and 35). As a result, in most of the pertinent literature currently published, the pathway of transport for membrane proteins and the compartments in which they undergo specific postranslational modifications are assumed on the basis of indirect evidence, rather than reliably identified on the basis of direct findings. A partial exception is represented by immunocytochemical (7, 18, 31) and autoradiographic (4) studies which can help identify the compartments involved in transport, but so far have not succeeded in localizing specific precursors.

In the case of the hepatic SC, we have shown that the 105K glycopeptide, kinetically identified as the earliest biosynthetic form of the receptor (34), is the predominant form in homogeneous rough microsomal fractions. In fact, it is the only form detected therein at short intervals (<15 min) postinjection. Extrapolating from cell fractions to the situation in vivo, 105K can be recognized as the SC precursor initially inserted into the rough ER membrane of hepatocytes and co-translationally glycosylated by the addition of a still unknown number of N-linked oligosaccharide chains. In these respects, i.e., SC insertion in rough ER membranes and co-translational glycosylation, our findings validate in vivo the results obtained by Mostov et al. (25) and Mostov and Blobel (24) in heterologous reconstituted systems in which the mRNAs of different secretory components (from rabbit mammary gland and human colonic carcinoma) were translated in vitro in the absence or presence of microsomal membranes.

About 15 min after injection, 105K begins to be converted to 116K, which is the predominant SC form found in homogeneous light Golgi fractions (GF_{1+2}) and the only form detected therein at early time points. The conversion of 105K to 116K apparently involves the terminal glycosylation of its N-linked oligosaccharide chains.

At late time points, 116K appeared as a minor SC form in rough microsomal fractions, and conversely 105K was detected in light Golgi fractions as a minor component at the time when the peptide was cleared from rough microsomal fractions. The presence of these SC forms as minor components in the two cell fractions remains to be explained: it could represent an experimental artifact due to intercontamination between the fractions, or it may reflect membrane recycling at the ER–Golgi complex junction.

The 120K form of the receptor, kinetically the last intracellular SC form (34), was detected at 30 and 60 min postinjection in plasmalemmal fractions isolated by immunoadsorbtion on insolubilized anti-SC antibody. On this account, we assume that the fractions consist primarily of vesicles derived from the sinusoidal domain of the hepatocyte plasmalemma. Since the fraction contained a relatively large amount of 116K, in addition to 120K, we assume that the conversion of the former into the latter (probably) occurs on the sinusoidal plasmalemma. Therefore, the 120K is either the mature form of the secretory component that functions as polymeric IgA receptor on the sinusoidal aspect of the plasmalemma, or, as already mentioned, is the form that has just bound its specific ligand.

We have not succeeded in identifying the vesicular carriers that move SC from the Golgi complex to the plasmalemma. Our immunocytochemical tests (Howell, K. E., and E. S. Sztul, manuscript submitted for publication) show that SC can be detected in Golgi cisternae but not in the hepatic equivalent of secretion vacuoles, i.e., the large, lipoprotein-loaded vesicles usually found on the trans side of Golgi cisternal stacks.[5] Vesicles of this type are a major, but not the only, component of the light Golgi fractions (11, 16). These findings suggest that SC is present in the cisternal elements found in the light Golgi fractions and is transported from some compartment in the Golgi complex to the sinusoidal plasmalemma by other vesicular carriers than those moving secretory proteins to the same destination; they also suggest that the sorting of the two categories of proteins occurs in the Golgi complex at a step or compartment that precedes the formation of recognizable secretion vacuoles. The existence of different post-Golgi pathways for membrane and secretory proteins has been documented in a cultured pituitary tumor cell line (At T-20) (14). The hepatocyte may operate in a similar way, although in its case a secretory pathway regulated by hormones or neurotransmitters apparently does not exist. Sorting at, or past, the Golgi complex has also been suggested for membrane proteins destined for distinct plasmalemma domains (29).

We assume, on the basis of results obtained with heavy Golgi fractions (GF_3), that the vesicular carriers that move SC from the Golgi complex to the plasmalemma separate in this heterogeneous fraction together with vesicles of other origins and functions. The same heavy Golgi fraction (GF_3) apparently contains the vesicular carriers that move 120K, the mature form of SC from the sinusoidal to the biliary domain of the hepatocyte's plasmalemma. The conclusion is based on the persistance of 120K in this fraction up to and past the time (~40 min) when newly synthesized SC begins to appear as a soluble 80K protein in the bile (33). GF_3 apparently contains only a fraction of these transcellular carriers; the rest separates in the crude carrier vesicle fraction we have described. We assume that the conversion of 120K, the mature membrane form of SC, to the soluble biliary 80K form of SC occurs by proteolysis immediately upon the fusion of the carriers with the biliary plasmalemma. This assumption is based on: the absence of 120K SC from bile and the absence of the 80K form from any cell fraction.

The transcellular vesicular carriers that move $(IgA)_2$ bound

[5] In normal hepatocytes a certain (presumably small) fraction of these vacuoles are probably endosomes loaded with recovered chylomicron and very low density remnants (9, 15).

to the membrane form of SC have been the object of a number of studies that have established that the transport is rapid (<30 min for the appearance of SC $(IgA)_2$ complexes in bile) (12, 13, 19) and is effected by smooth-surfaced vesicular carriers identified topographically and kinetically by both immunocytochemistry (36) and autoradiography (5, 13, 28) using polymeric IgA either radioiodinated or conjugated to horseradish peroxidase.

Our immunocytochemical observations (Howell, K. E., and E. S. Sztul, manuscript submitted for publication) indicate that the initial uptake of $SC(IgA)_2$ complexes may involve coated pits and coated vesicles, thereby following a pattern already established in a number of cases (8, 21, 37) for ligand-mediated endocytosis. This pattern of uptake implies the sorting of $SC(IgA)_2$ from other receptors, ligands, and non-specific ingesta somewhere on the way from one (sinusoidal) to another (biliary) domain of the plasmalemma, as established for IgG transport across the intestinal epithelium in the newborn rat (1).

It should be clear from the preceding discussion that the reliable localization of the intracellular precursor forms of membrane proteins requires homogeneous cell fractions consisting essentially of one type of vesicle. With such fractions, we were able to localize the 105K, 116K, and 120K forms of SC to the rough ER, Golgi complex, and sinusoidal plasmalemma, respectively. The identification of vesicular carriers of either intracellular or transcellular type depends on the isolation of the corresponding vesicles in equally homogeneous fractions by either immunoadsorption procedures (references 17, 23, and 30 and this paper), or by density modifying methods, as in the work of Courtoy et al. (10) and Quintart et al. (27). The availability of vesicular carriers in reasonably homogeneous fractions is a prerequisite for future attempts to decipher the mechanisms involved in the regulation of vesicular carrier traffic in hepatocytes as well as in all other eucaryotic cells.

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