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Secretion of $^{35}SO_4$-Labeled Proteins from Isolated Rat Hepatocytes†

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ABSTRACT: Sulfation is a Golgi-specific modification of secretory proteins. We have characterized the proteins that are labeled with $^{35}SO_4$ in cultures of rat hepatocytes and studied their transport to the medium. Analysis by polyacrylamide gel electrophoresis showed that of the five most heavily labeled proteins, four had well-defined mobilities—apparent molecular masses of 188, 142, 125, and 82 kDa—whereas one was electrophoretically heterogeneous—apparent molecular mass of 35–45 kDa. Judging by their relatively high resistance to acid treatment, the sulfate residues in the 125- and 35–45-kDa proteins were linked to carbohydrate. Some of the secreted proteins were sialylated. In samples of pulse-labeled cells, there appeared to be no unsialylated forms, indicating that sulfation occurred after sialylation, presumably in the trans Golgi. Kinetic experiments showed that the cellular half-life was the same for all the sulfated proteins—about 8 min— consistent with the idea that transport from the Golgi complex to the cell surface occurs by liquid bulk flow.

Proteins destined for secretion are, during or after their synthesis, transferred into the lumen of the endoplasmic reticulum. They are then transported to the cell surface via the Golgi complex. The Golgi complex consists of three functionally different compartments—the cis, medial, and trans Golgi—which the proteins pass through successively. Most secretory proteins are modified at specific sites along the secretory pathway. Thus, e.g., many glycoproteins acquire galactose, fucose, and sialic acid residues in the trans Golgi. The transport between the different compartments of the secretory pathway is mediated by vesicles which have a characteristic coat on their surface [for reviews, see Burgess and Kelly (1987) and Pfeffer and Rothman (1987)]. Recent studies suggest that secretory proteins are transported merely by the bulk flow of liquid in the transport vesicles and that organelle-specific proteins are retained by binding to receptors (Munro & Pelham, 1987; Wieland et al., 1987). Only secretory proteins destined for secretory granules (Moore et al., 1983), or for one side in polarized cells (Urban et al., 1987), seem to be actively sorted. This sorting presumably takes place in a reticular extension of the trans Golgi (Griffiths & Simons, 1986).

We use isolated rat hepatocytes to study protein secretion (Fries et al., 1984; Fries & Lindström, 1986). Hepatocytes are polarized but appear to transport newly synthesized secretory and membrane proteins only to one side (Kloppel et al., 1986; Bartles, 1987). Furthermore, hepatocytes seem to lack regulated secretion, indicating that there is no active sorting of secretory proteins in these cells. To investigate the last steps in the secretory pathway, we have in the present study made use of the earlier observation that secretory proteins may incorporate sulfate in the Golgi complex.

Sulfation of proteins has been found in every tissue and cell line so far investigated [see, e.g., Heifetz et al. (1980), Liu et al. (1985), Paulsson et al. (1985), and Griswold et al. (1986)], including rat liver (Hille et al., 1984). Sulfate groups have been shown to be linked both to tyrosine (Huttner, 1987) and to carbohydrate residues (Slomiany & Meyer, 1972; Heifetz et al., 1980; Green et al., 1986). Autoradiographic and biochemical studies of different cell types have established that the sulfation of secretory proteins occurs in the Golgi complex (Young, 1973; Fessler et al., 1986; Lee & Huttner, 1984). In a recent study of a hybridoma cell line, it was shown that sulfation takes place in the trans Golgi (Baeuerle &

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Huttner, 1987). The sulfate groups do not seem to have a general function for protein transport; inhibition of this modification has, in at least two cases, been fouund not to affect the rate of secretion (Danielsen, 1988; Hortin & Graham, 1988). Some proteins incorporate very little sulfate (Hortin et al., 1986), indicating that their sulfation is not relevant to their function. Here we describe for the first time the secretory proteins that are sulfated in cultures of rat hepatocytes. Furthermore, we show that the sulfation occurs after sialylation and that all the major labeled polypeptides are transported to the cell surface at the same rate.

Experimental Procedures

Materials. Culture media and newborn calf serum were purchased from Labassco, Stockholm, Sweden; saponin (white pure) was from Merck, Darmstadt, FRG; and rabbit antibodies against rat serum proteins were from Dakopatts, Glostrup, Denmark. Human fibronectin and protein A bearing *Staphylococcus aureus* particles were gifts from Å. Lundquist, KabiVitrum, Stockholm, and N.-P. Nilsson, Pharmacia, Uppsala, Sweden, respectively. Antibodies against rat liver heparan sulfate proteoglycan, prepared as described by Woods et al. (1984), were kindly donated by Dr. L. Kjellén of the Swedish University of Agricultural Sciences, Uppsala. Neuraminidase from *Vibrio comma* (cholerae) was purchased from Behringwerke, Marburg, FRG, and ^{14}C-methylated molecular mass standards were from Amersham International, Amersham, England. For velocity centrifugation experiments, ovalbumin (grade III) and bovine serum albumin (A-4378) were from Sigma and aldolase and catalase from Pharmacia.

Isolation of Rat Hepatocytes. Male Sprague-Dawley rats weighing 175–250 g were fasted overnight and were then anesthesized with diethyl ether. The livers were perfused with collagenase according to Seglen (1976) with some modifications (Pertoft & Smedsrød, 1987). The dispersed cells were filtered through nylon gauze with a mesh size of 100 μm. Part of the resulting suspension [(40–80) × 10^6 cells in 2–4 mL] was layered over 15 mL of a solution with a density of 1.08 g/mL containing colloidal silica (Percoll; Pharmacia, Sweden) and 0.15 M NaCl. After centrifugation for 5 min at 1000*g*, the supernatant was discarded, and the pelleted cells were resuspended in a sterile solution containing 137 mM NaCl, 5 mM KCl, 1.2 mM $CaCl_2$, 0.7 mM $MgSO_4$, and 10 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid–hydrochloric acid (Hepes–HCl), pH 7.4 (buffer 3; Rubin et al., 1977). The cells were washed once by centrifugation in the same buffer and finally suspended in Dulbecco's modified Eagle's medium with 25 mM glucose. The medium lacked $NaHCO_3$ but contained 25 mM Hepes (final pH 7.4) and was supplemented with newborn calf serum (90 mL/L), glutamine (1 mM), penicillin (100 units/mL), streptomycin (0.1 mg/mL), insulin (10 milliunits/mL), and dexamethasone (0.3 μM). Portions of the cell suspension (1.0 × 10^6 cells in 3.0 mL) were added to 35-mm-diameter plastic dishes (Falcon, Becton, Dickinson & Co.) precoated with 20 μg of fibronectin. The dishes were incubated at 37 °C in air under a plastic cover, and the medium was changed after 3–4 h.

Labeling with [^{35}S]Sulfate. The cells were used 20–24 h after plating. Each dish was rinsed twice with 1.5 mL of Ca^{2+}/Mg^{2+}-free Dulbecco's phosphate-buffered saline and incubated for 15 min on a rocking device with 900 μL of Eagle's minimum essential medium lacking $MgSO_4$ and $NaHCO_3$ but containing 25 mM Hepes–HCl (final pH 7.4) and a tenth of the normal concentration of cystine and methionine. Then 100 μL of the above medium containing 0.25 mCi of $^{35}SO_4$ (22–40 Ci/mg; Amersham International, Amersham, England) was added. At the end of the labeling period, the media were collected, and *N*-ethylmaleimide (0.1 mM), phenylmethanesulfonyl fluoride (0.2 mM), and pepstatin (1 μg/mL) were added. Cell debris was removed by centrifugation [5 min at (14 × 10^3)*g*]. The cells were washed twice in 1.5 mL of ice-cold buffer 3 and solubilized with 300 μL of 50 mM 2-amino-2-(hydroxymethyl)propane-1,3-diol hydrochloride (Tris-HCl), 0.15 M NaCl, 10 g/L Triton X-100, 10 μl/mL aprotinin solution (Trasylol, Bayer, Leverkusen, FRG), and 5 mM EDTA (final pH 8.0). They were then scraped off the plates and centrifuged (like the media above), and the supernatants were collected. In some experiments, the cellular proteins were released with the same buffer containing saponin (1 mg/mL) instead of detergent.

Immunoprecipitation. Rabbit antibodies to rat serum proteins were added in excess to aliquots of cell media, solubilized cells, or fractions from density gradients; the volumes were adjusted to 470 μL with phosphate-buffered saline. After 3–4 h, the immune complexes were recovered by incubation with *S. aureus* particles and washed once in 10 mM Tris-HCl, 0.4 M NaCl, 10 g/L Triton X-100, and 5 mM EDTA (pH 7.5) and once in 10 mM Tris-HCl (pH 8.0).

Acid Precipitation. Trichloroacetic acid (TCA, final concentration 120 g/L) and RNA (250 μg) were added to aliquots of the media and the solubilized cells. After 30-min incubation on ice, the precipitates were collected by centrifugation and washed twice in 100 g/L TCA and once in cold acetone.

Polyacrylamide Gel Electrophoresis (SDS–PAGE). Slab gels of 10–15% polyacrylamide were prepared as described by Maizel (1971). Precipitated proteins were dissolved in 35 μL of the electrophoresis buffer. After electrophoresis, the gels were treated with 200 g/L 2,5-diphenyloxazole in acetic acid (Skinner & Griswold, 1983), dried, put on preflashed X-ray films, and kept at −70 °C. The relative amounts of radioactivity in the polypeptide bands were determined by scanning of the autoradiograms (fluorograms) with an Ultroscan XL densitometer (LKB, Sweden).

Enzymatic Digestion. $^{35}SO_4$-labeled serum proteins were immunoprecipitated from cell media as described above. The *S. aureus* particles were resuspended in 50 μL of 0.1 M sodium acetate, 1 mM $CaCl_2$, and 3 mg/mL sodium dodecyl sulfate (SDS) (final pH 5.5) and incubated for 3 min at 95 °C. The *S. aureus* particles were removed by centrifugation, and 50 μL of the same buffer lacking SDS but containing 10 mg/mL Triton X-100 was added. Neuraminidase was then added (250 milliunits/mL), and after 4 h at 37 °C, the proteins were precipitated with TCA and subjected to gel electrophoresis as described above.

Results

Labeling of Rat Hepatocytes with $^{35}SO_4$. Lane 1 in Figure 1 shows the electrophoretic pattern of the acid-precipitable material in rat hepatocytes that was labeled with $^{35}SO_4$. Most of the radioactivity was found in the upper part of the gel and was probably associated with proteoglycans (Stow et al., 1985). Indeed, part of the slowly migrating radioactive material could be precipitated with antibodies against the rat liver heparan sulfate proteoglycan (lane 2; it was not determined if the antibodies were added in excess). In addition to the continuous spectrum of radioactive material in the upper part of the gel, many distinct bands could be seen, particularly in the lower part. Many of these polypeptides and apparently also part of the proteoglycans (material that accumulated at the top of the separating gel) were precipitated with antibodies against rat serum proteins (lane 3). Lane 5 shows the $^{35}SO_4$-labeled proteins obtained by acid precipitation of the medium. The

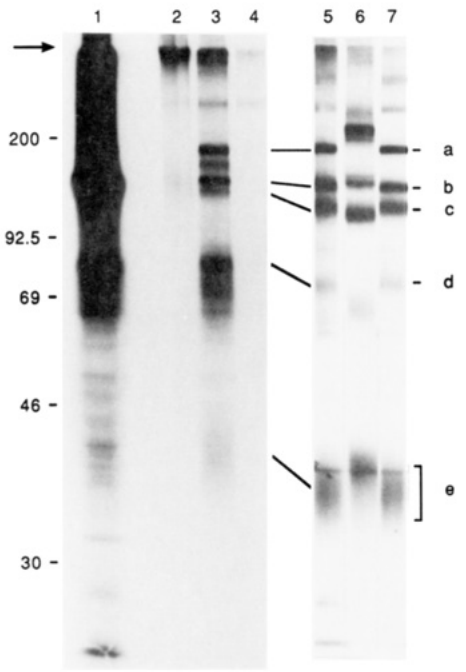

FIGURE 1: Electrophoretic analysis of sulfated secretory proteins of rat hepatocytes. Monolayer cultures of hepatocytes were labeled with $^{35}SO_4$ either for 10 min or for 2 h. To detergent extracts of the pulse-labeled cells (lanes 1–4) and to the long-term labeled medium (lanes 5–7) were added either trichloroacetic acid (lanes 1 and 5), antibodies against heparan sulfate proteoglycan (lane 2), antibodies against rat serum proteins (lanes 3, 6, and 7), or normal rabbit IgG (lane 4). The precipitates were then analyzed by SDS–PAGE; lane 6 was not treated with a reducing agent. The positions of ^{14}C-methylated standard proteins are indicated to the left with their molecular masses in kilodaltons. The arrow shows the top of the separating gel.

relatively small amount of radioactivity at the top of the separating gel showed that little proteoglycan was secreted. An almost identical protein pattern was obtained by precipitation with the antibodies against rat serum proteins (lane 7), showing that the immunization had generated antibodies against most of the major sulfated proteins. For further discussion, we have denoted the major labeled polypeptides in the medium a–e; their apparent molecular masses were 188, 142, 125, 82, and 35–45 kDa, respectively. In most experiments, there was also a 74-kDa band in the medium samples; in the analysis described below, we have treated the 85- and 74-kDa bands collectively.

In the absence of a reducing agent (lane 6), some of the major polypeptides migrated more rapidly, indicating the presence of internal disulfide bonds. Polypeptide a, however, migrated more slowly under these conditions, corresponding to a shift in apparent molecular mass from 188 to about 230 kDa. In many cases, such a shift has been shown to be due to a polypeptide being part of a complex joined by disulfide bonds. However, this behavior has also been observed for at least one protein consisting of only polypeptide chain (Saito & Sinohara, 1985a).

Sedimentation Analysis of $^{35}SO_4$-Labeled Secretory Proteins. To determine if the sulfate-labeled polypeptides detected by gel electrophoresis were separate proteins or part of larger complexes under nondenaturing conditions, we studied their sedimentation properties in phosphate-buffered saline. A sample of the medium from $^{35}SO_4$-labeled cells was layered on a sucrose gradient and centrifuged for 16 h. The gradient was then fractionated, and the secretory proteins were recovered with antibodies to rat serum proteins and analyzed by gel electrophoresis (see Figure 2A). The relative amounts

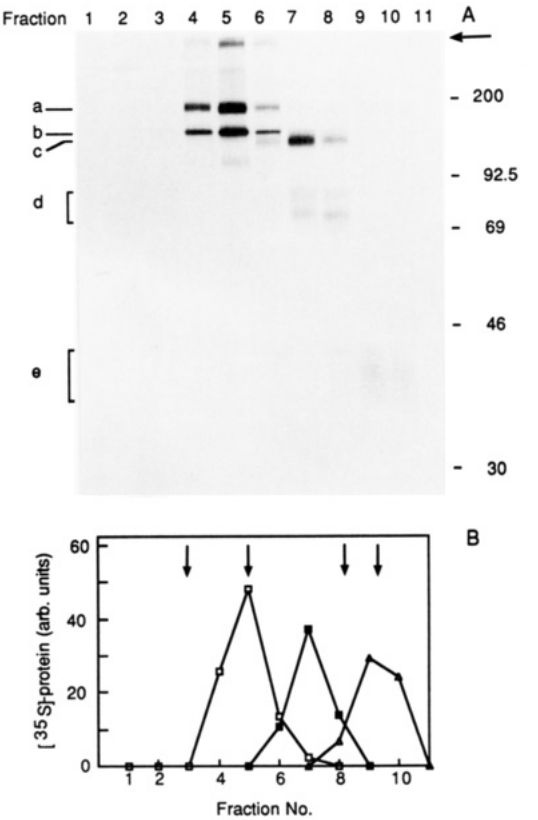

FIGURE 2: Velocity centrifugation of medium from $^{35}SO_4$-labeled rat hepatocytes. Medium from cells labeled for 2 h with $^{35}SO_4$ (250 μL) was layered on a sucrose gradient (5–20% w/w) in a tube fitting a Beckman SW 50.1 rotor. After centrifugation at $(1.2 \times 10^5)g$ for 16 h, 11 fractions were collected from the bottom of the gradient, and the serum proteins in each fraction were recovered by immunoprecipitation. Panel A shows a fluorogram of the electrophoresis gel used for separating the precipitated proteins. The bands discussed in the text are indicated by a–e. The horizontal arrow shows the top of the separating gel. The equivalent of the medium of 0.25×10^6 cells (labeled with 0.25 mCi of $^{35}SO_4$ in 1 ml) was used for the electrophoresis, and the film was exposed for 14 days. Panel B shows the relative amounts of bands b (□), c (■), and e (△) in the fractions. The arrows indicate the positions of ovalbumin (43 kDa; 3.6S), bovine serum albumin (69 kDa; 4.4S), aldolase (158 kDa; 7.4S), and catalase (232 kDa; 11.3S), which were centrifuged in parallel.

of the major polypeptides in the fractions were determined by densitometric scanning of the fluorogram of the dried gel (Figure 2B); for simplicity, the distribution of only three of the polypeptides is shown. Comparison with standard proteins centrifuged in parallel (positions indicated with arrows) showed that the polypeptides a–e sedimented like globular proteins with molecular masses of 150, 150, 81, 64, and 36 kDa, respectively. These values are similar to those obtained by polyacrylamide gel electrophoresis and indicate that the major sulfated polypeptides are separate proteins. Similarly, the *s* values of these proteins could be estimated: 6.7, 6.7, 4.8, 4.2, and 3.2 S, respectively.

Acid Resistance. Sulfate groups linked to carbohydrate residues are generally more resistant to acid hydrolysis than are those bound to tyrosine residues (Huttner, 1984). Resistance to acid may be assessed by comparing the amounts of radioactivity in a protein band in an electrophoresis gel before and after acid treatment (Huttner, 1984). The result of such an analysis is shown in Figure 3. The upper tracing shows a densitometric scan of a fluorogram of the normal electrophoretic pattern of the secretory proteins that are labeled with $^{35}SO_4$ in rat hepatocytes. The lower tracing was obtained from an identical sample run in parallel, which was treated with 1.0 M HCl at 95 °C for 1.5 min before the gel was

Table I: Characteristics of Major Sulfated Proteins[a]

protein	molecular mass (kDa)		sedimentation coefficient (S)[c]	N-linked carbohydrate[d]	carbohydrate-linked SO_4[e]
	denatured[b]	nondenatured[c]			
a	188 ± 8	150 ± 14	6.7 ± 0.6	yes	no
b	142 ± 5	150 ± 14	6.7 ± 0.6	yes	no
c	125 ± 5	81 ± 12	4.8 ± 0.4	yes	yes
d	82 ± 3; 74 ± 4	64 ± 8	4.2 ± 0.2	yes	no
e	38 ± 3	36 ± 6	3.2 ± 0.4	no	yes

[a] The values given are the means ± the deviations, from two experiments. [b] Estimates from SDS–PAGE (Figure 1). [c] Estimates from velocity centrifugation (Figure 2). [d] Based on sensitivity to tunicamycin (see text). [e] Based on resistance to acid (Figure 4).

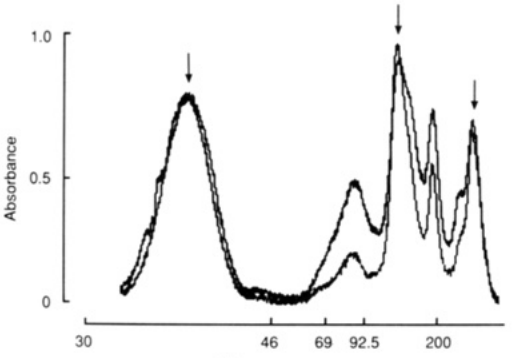

FIGURE 3: Acid resistance of $^{35}SO_4$-labeled proteins. Two samples of medium from cells labeled with $^{35}SO_4$ were analyzed by SDS–PAGE. One of the lanes was cut out and treated with 1 M HCl at 95 °C for 1.5 min. Both lanes were then processed for fluorography, and the resulting film was scanned with a densitometer. The upper and lower tracings show the control and the acid-treated lanes, respectively. The positions of ^{14}C-methylated standard proteins are shown on the abscissa with their molecular masses in kilodaltons. The arrows mark the proteins which have acid-resistant sulfate groups.

processed for fluorography. The radioactive sulfate groups of polypeptides c and e (see Figure 1) as well as a protein that hardly entered the separating gel were resistant to the acid treatment (marked with arrows).

To determine the nature of the carbohydrates on the sulfated proteins, we incubated the cells with tunicamycin, a drug that inhibits N- but not O-linked glycosylation. Increase in the electrophoretic mobility or disappearance of the sulfate label of a protein after treatment with this drug would show the presence of N-linked carbohydrates. We found that the mobilities of bands a, b, and d increased, that band e was unaffected, and that band c disappeared (data not shown). These properties of the sulfated proteins, as well as some of those described in the previous sections, are summarized in Table I.

Sialidase Treatment. The acquisition of sialyl residues is a late intracellular modification of secretory proteins. For rat hepatocytes, it has been shown by immunoelectron microscopy that the corresponding transferase resides specifically in the trans Golgi (Roth et al., 1985). We wanted to determine if the sulfate groups were added onto proteins that were already sialylated or not. To ascertain if the sulfated proteins contained sialic acid residues, we incubated them with sialidase and saw if the treatment resulted in an increase of their electrophoretic mobilities. Using this assay, we found that several of the major sulfated proteins were sialylated (Figure 4). At the positions in the gel where the major proteins in the cell sample ran after sialidase treatment, there appeared to be no or little labeled protein in the untreated sample; this was also so if the cells were labeled for only 4 min (not shown).

Time Course of Secretion. To determine the time course for the secretion of the sulfated proteins, we initially performed pulse–chase experiments. We then found, however, that the incorporation of $^{35}SO_4$ could not be stopped fast enough after the pulse. We therefore did our kinetic analysis with cells that

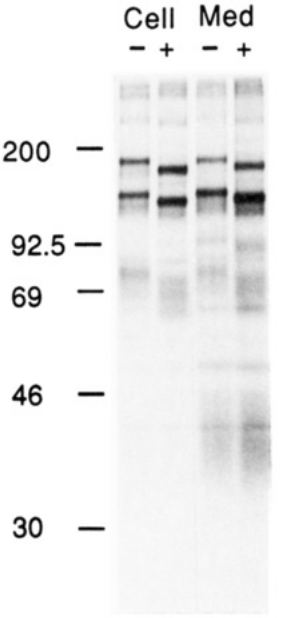

FIGURE 4: Sialidase treatment of $^{35}SO_4$-labeled proteins. Secretory proteins were isolated from cells and medium by immunoprecipitation. Following the dissociation of the precipitates, the proteins were treated with sialidase and analyzed by SDS–PAGE and fluorography. Samples treated with enzyme are indicated with (+) and control samples with (−).

were continuously labeled for different times. The resulting fluorograms (see Figure 5) showed that the cellular level of the labeled bands increased only during the first 15–20 min and then remained constant. In contrast, the intensity of the bands in the medium increased steadily for at least 100 min.

Comparison of the protein patterns of cell and medium samples showed that a diffuse band in the 35–45-kDa region, which was a minor component in the cells, was a major one in the medium. Interestingly, a large number of equally spaced sharp lines could be discerned within the diffuse band (not apparent after reproduction). This pattern could also be seen within the 60–80-kDa region in the cell samples. Possibly, a protein with this molecular mass is the precursor of the 35–45-kDa protein.

To obtain a more quantitative picture of the secretion of the sulfated proteins, we scanned the fluorograms with a densitometer. Figure 6 shows the relative amounts of all the polypeptides in the 100–200-kDa region in the different samples from the cells and the media. This graph shows that incorporation of $^{35}SO_4$ started after a lag of about 3 min, increased for 20–30 min, and then leveled off. Half-maximal labeling occurred after about 11 min. If the lag in labeling is taken into account, these data show that the cellular half-life of the sulfated proteins was about 8 min. The labeled proteins began to appear in the medium about 12 min after the addition of $^{35}SO_4$, indicating that the minimum time required for transport from the site of labeling to the cell surface was 9 min. The same transport characteristics were also found for

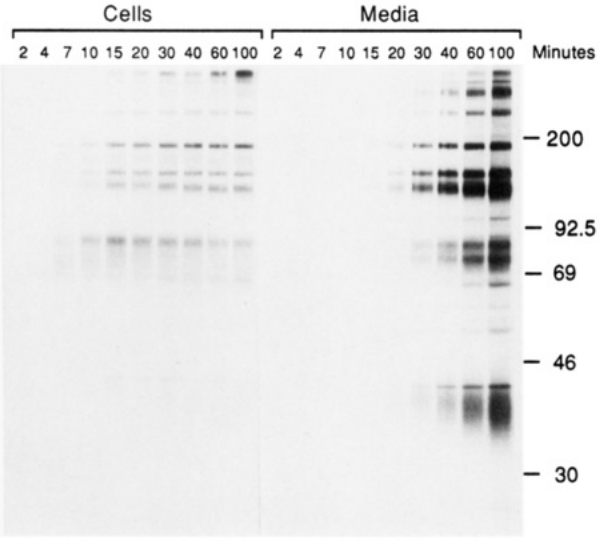

FIGURE 5: Time course of secretion of $^{35}SO_4$-labeled proteins. Monolayer cultures of hepatocytes were labeled with $^{35}SO_4$ for the length of time indicated. The media were collected and the cells permeabilized with saponin. Antibodies against rat serum proteins were added to equivalent volumes of the media and the cell permeabilisates (75% of total). The immunoprecipitates were subjected to SDS–PAGE followed by fluorography. Shown is a fluorogram obtained after a 7-day exposure. Treatment of cells with saponin releases only soluble proteins (Wassler et al., 1987). Accordingly, little proteoglycan appeared at the top of the separating gel in cell samples (cf. lane 3 in Figure 1).

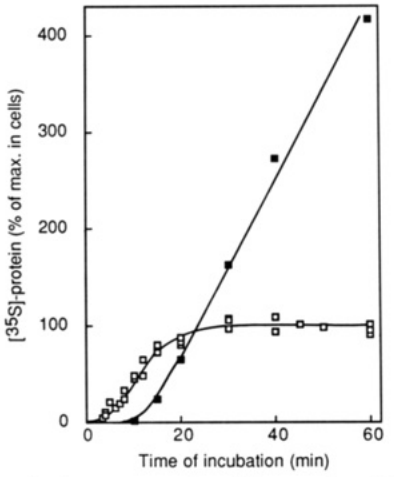

FIGURE 6: Quantitative representation of the secretion of $^{35}SO_4$-labeled proteins. From labeling experiments performed as described in Figure 5, the relative amounts of radioactivity in the 100–200-kDa polypeptides in cell (□) and medium (■) samples were determined by densitometric scanning of the fluorograms.

individual polypeptides as well as for the total radioactive material along the whole length of the gel (data not shown).

Discussion

The purpose of this study was to use the secretory proteins that are labeled with $^{35}SO_4$ in rat hepatocytes to analyze the protein transport from the Golgi complex to the cell surface. Since these proteins had not previously been described, a partial characterization was needed. By velocity centrifugation, we established that the major labeled proteins were separate entities and not part of large complexes. We also obtained evidence that the major labeled proteins were sulfated either on N- or O-linked carbohydrates or on tyrosine residues. Taken together, these observations show that the sulfated proteins constitute a heterogeneous class of proteins and can be considered as representative of the hepatic secretory proteins.

The serum proteins that are labeled after injection of $^{35}SO_4$ into rats have previously been described. Two of the most prominent radiolabeled polypeptides were identified as α_2-macroglobulin and fibrinogen (Hille et al., 1984), both of which are produced by hepatocytes. From our experiments with specific antisera (unpublished observations) and sedimentation analysis (see Figure 2), we conclude that these proteins are not the major sulfated proteins secreted by isolated rat hepatocytes. After the completion of this study, we realized that the properties of protein a (see Table I) were similar to those reported for α_1-inhibitor 3 (Esnard & Gauthier, 1980; Saito & Sinohara, 1985b; Geiger et al., 1987; Lonberg-Holm et al., 1987), the second most abundant protein in rat serum (Lonberg-Holm et al., 1987). We have recently found that antibodies against α_1-inhibitor 3 (kindly provided by Dr. F. Gauthier) precipitate protein a. One of the other major sulfated proteins (protein e) has a characteristic electrophoretic heterogeneity, reminiscent of that observed for a chondroitin sulfate proteoglycan produced in a pituitary tumor cell line (Burgess & Kelly, 1984). Indeed, recent biochemical studies have shown that the hepatic protein belongs to the same class of proteins (Marcks von Würtemberg and Fries, submitted for publication).

It was recently shown that the sulfation of an IgM produced in a hybridoma cell line occurred after its sialylation (Baeuerle & Huttner, 1987). However, the measured time courses for the secretion of this protein when labeled with [^{3}H]galactose (which is added before sialic acid) or $^{35}SO_4$ were the same, indicating that both modifications occurred in the same compartment. Apparently, galactosyl and sialyl residues are transferred more rapidly than sulfate groups, and all these reactions are much faster than the transport of the IgM molecule to the cell surface. We report here that in rat hepatocytes sulfation also occurs after sialylation. In these cells, sialyltransferase has been localized by immunocytochemical techniques to the trans Golgi and its network (Roth et al., 1985). Whether the sulfate-transferring enzyme has the same cellular distribution remains to be determined.

The time course we obtained for the secretion of the sulfated proteins agrees with the one observed earlier for [^{3}H]fucose-labeled proteins in rat liver (Sztul et al., 1983). These studies show that proteins labeled in the trans Golgi appear in the medium only after a time lag of about 10 min. The time required for a medium-sized protein to diffuse a distance similar to the dimension of a Golgi cisterna (1 μm) is less than 1 min, even in the presence of high concentrations of protein (Gershon et al., 1985). The time lag therefore probably represents the time required for the transport vesicles to form, migrate, and fuse with the plasma membrane. Our kinetic experiments show that half of the sulfated proteins present in the cells at any one moment is secreted within a time which is of about the same length as the minimum time required to transport a newly sulfated protein to the cell surface (the lag time). This finding indicates that a large proportion of the sulfated proteins in hepatocytes are in transport vesicles. Labeling of the proteins with $^{35}SO_4$ should therefore be a useful tool for the identification of these vesicles, for which there is as yet no isolation procedure.

We found that the major $^{35}SO_4$-labeled proteins in hepatocytes are transported to the cell surface with similar, if not identical, rates. This finding is in keeping with earlier but less precise results on the kinetics at which different secretory proteins (pulse-labeled with [^{35}S]methionine) pass through the

Golgi complex (Lodish et al., 1983; Fries et al., 1984). These observations are consistent with the proposal that unregulated protein secretion occurs by bulk flow, without any interaction with receptors (Burgess & Kelly, 1987). For the transport of proteins from the endoplasmic reticulum to the Golgi complex, widely different rates have been recorded (Lodish et al., 1983; Fries et al., 1984). These differences have been suggested to be due to differential binding of the secretory proteins to resident ER proteins (Rothman, 1987). It is possible that future studies will reveal secretory proteins which, owing to binding to membrane proteins in the Golgi complex, are transported to the cell surface at rates lower than those described in this paper.

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