Biochimica et Biophysica Acta, 552 (1979) 225—237

BBA 78329

ISOLATION OF GOLGI FRACTIONS FROM COLCHICINE-TREATED RAT LIVER

II. ELECTROPHORETIC CHARACTERIZATION

KIMIMITSU ODA *, YUKIO IKEHARA * and KEITARO KATO

Department of Physiological Chemistry, Faculty of Pharmaceutical Sciences, Kyushu University 62, Higashi-ku, Fukuoka 812 (Japan)

(Received August 29th, 1978)

Key words: Secretory process; Glycosylation; Colchicine treatment; Ethanol treatment; Golgi subfractionation; (Rat hepatocyte vesicle)

Summary

1. Intact Golgi fractions, three from colchicine- or ethanol-treated rat livers and two from a control, were analyzed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. All the fractions showed very similar electrophoretic profiles with 33 protein bands, some of which, especially albumin, had rather higher density in the secretory vesicle fraction than those in the cisternal fraction.

2. Using albumin as the content marker, the Golgi fractions were subfractionated into membranes and contents by freezing-thawing and sonication followed by centrifugation. Distribution of galactosyltransferase among these membrane preparations showed that this enzyme was more enriched in the Golgi cisternal membranes than in the secretory vesicle membranes.

3. All the membrane preparations from the Golgi complex showed very similar patterns on electrophoresis, which were distinctly different from those of microsomal membranes and of plasma membrane. Furthermore, all the Golgi content subfractions had similar protein components, most of which were also found in serum. The microsomal contents, however, showed a considerably different pattern from those of the Golgi contents.

4. From these results it could be concluded that the secretory vesicles are indeed a member of the Golgi complex despite their different appearance and morphology.

---

* Present address: Department of Biochemistry, Fukuoka University School of Medicine, Nishi-ku, Fukuoka 814, Japan.

Abbreviations used: SDS, sodium dodecyl sulphate; VLDL, very low density lipoprotein. In the Golgi fraction $\rho < 1.006$ g/ml; otherwise $\rho = 0.94$—1.006 g/ml.

Introduction

In the preceding paper [1] we reported the isolation of three fractions of the Golgi complex from rat livers treated with colchicine, rather than the ethanol treatment originally developed by Ehrenreich et al. [2]. The specific activity of galactosyltransferase was shown to be higher in GF-3 than that in GF-1. This result, together with morphological evidence, indicates that galactosyltransferase is enriched in the Golgi cisternae (GF-3) and that attachment of galactose to carbohydrate chains of macromolecules mainly takes place in the Golgi cisternae. However, the possibility that the lower specific activity of GF-1 is due to a higher content of secretory proteins cannot be ruled out. In fact, Bergeron et al. [3] reported that removal of the VLDL content of Golgi elements resulted in little difference in activities among the three Golgi membrane fractions. To answer the above problem, we attempted to separate Golgi fractions into membrane and content fractions and examined the distribution of Galactosyltransferase among them.

It has been proposed that membranes of secretory vesicles as well as Golgi cisternae are derived from endoplasmic reticulum membranes, transformed during passage through the Golgi apparatus and destined to become plasma membrane [4]. This concept is first based on morphological evidence showing a gradual progression of membrane thickness and osmium stainability from endoplasmic reticulum-like to plasma membrane-like across the stacks of Golgi cisternae [5,6]. Analyses of biochemical constituents such as marker enzyme [7] and lipids [8] contained in these subcellular membranes support this concept.

In the present study we analysed the protein composition of different subcellular fractions by SDS-gel electrophoresis before and after separation of individual fractions into membranes and contents, and compared electrophoretic profiles of each in relation to membrane differentiation and secretory processes.

Materials and Methods

Preparation of subcellular fractions. The Golgi fractions were isolated from colchicine-treated or non-treated rat livers as described in the preceding paper [1]. Microsomes were obtained by the same procedure as described for the isolation of the Golgi fractions [1] except that the bottom layer under 1.15 M sucrose after the final sucrose density centrifugation was collected as the microsomal fraction. Plasma membrane was isolated by the method of Ray [9].

Subfractionation of the Golgi fractions into membrane and content. Each frozen Golgi fraction (2 mg/ml protein concentration) was once thawed and centrifuged at 105 000 × *g* for 60 min. After the first supernatant was removed, the pellet was suspended in 40 mM veronal buffer (pH 8.6) using a glass homogenizer, sonicated three times with an Umeda probe sonicator (Umeda Electric Co., Tokyo, Japan) at maximum power for 30 s and then centrifuged at 105 000 × *g* for 60 min. The resultant pellet was suspended in 0.25 M sucrose as a membrane fraction. As a content fraction, the first and second supernatants were combined and concentrated with collodion bags (Sartorius-

Membranfilter GmbH., Göttingen, F.R.G.). Microsomal contents were released from the intact microsomal fraction with 0.05% sodium deoxycholate according to the method of Kreibich et al. [10]. Determinations of galactosyltransferase activity and protein were done as described previously [1].

Electrophoresis. SDS-polyacrylamide gel electrophores was performed by the method of Fiarbanks et al. [11] using 5.6% polyacrylamide containing 0.1% SDS (gel size; 0.8 × 13 cm). Each sample was suspended to give a final protein concentration of 1.0 to 1.5 mg/ml in a solution containing 1% SDS/10 mM Tris-HCl (pH 8.0)/1 mM EDTA and 1% β-mercaptoethanol, and then heated in boiling water for 3 min. As a tracking dye, Coomassie Brilliant Blue R-250 was used. Electrophoresis was carried out at 5 mA/gel for 30 min and then at 8 mA/gel until the tracking dye reached 10 cm from the top of the gel (4—5 h). Gels removed from columns were stained for protein by the method of Fairbanks et al. [11]. Destained gels were scanned at 569 nm with a Toyo densitometer (Densitorol DMV-2, Toyo-Roshi Co., Osaka, Japan).

Materials. UDP-galactose was obtained from Sigma Chemical Co., St. Louis, MO, and UDP-[^{14}C]galactose was from New England Nuclear, Boston, MA. Acrylamide gel reagents and sodium dodecyl sulfate were purchased from Nakarai Chemicals, Kyoto, Japan and other chemicals used were of reagent grade.

Results

Electrophoretic comparison of Golgi fractions in SDS-polyacrylamide gel electrophoresis

Fig. 1 shows electrophoretic profiles in SDS-polyacrylamide gels in which Golgi fractions isolated from colchicine-treated rat liver (gels A—C) were compared with those from ethanol-treated liver (gels D—F). Each fraction of the Golgi complex was resolved into 33 protein bands (some additional bands could be seen in subfractions of membrane and content as described later), and almost all of the bands were common to all three Golgi fractions, irrespective of either treatment with colchicine or ethanol, although there were some differences in intensity of individual bands. This result shows that colchicine did not cause any significant change in protein components of Golgi fractions compared with ethanol and that GF-1 and GF-3 were composed of almost the same protein components, in spite of apparent morphological differences [1]. The most prominent feature of the gel patterns is that band 18 (marked intensity) which corresponds to serum albumin in mobility as shown in Fig. 5, increased from GF-3 to GF-1 in both cases, suggesting that albumin is more concentrated in the secretory vesicles than in the Golgi cisternae. * In addition to band 18, bands 7, 16 and 28 also increased their intensity from GF-3 to GF-1, and such

* Band 18 was identified to be albumin (and/or proalbumin) by the following experiment. Either of the Golgi fractions was solubilized with 1% Triton X-100 and applied to an anti (rat serum albumin) Sepharose 4B column. A fraction not adsorbed to the column and an adsorbed fraction which was eluted with 8 M urea from the column were analyzed by SDS-gel electrophoresis, which showed that the former contained no band 18, while the latter contained only band 18 (Ikehara and Oda, unpublished results).

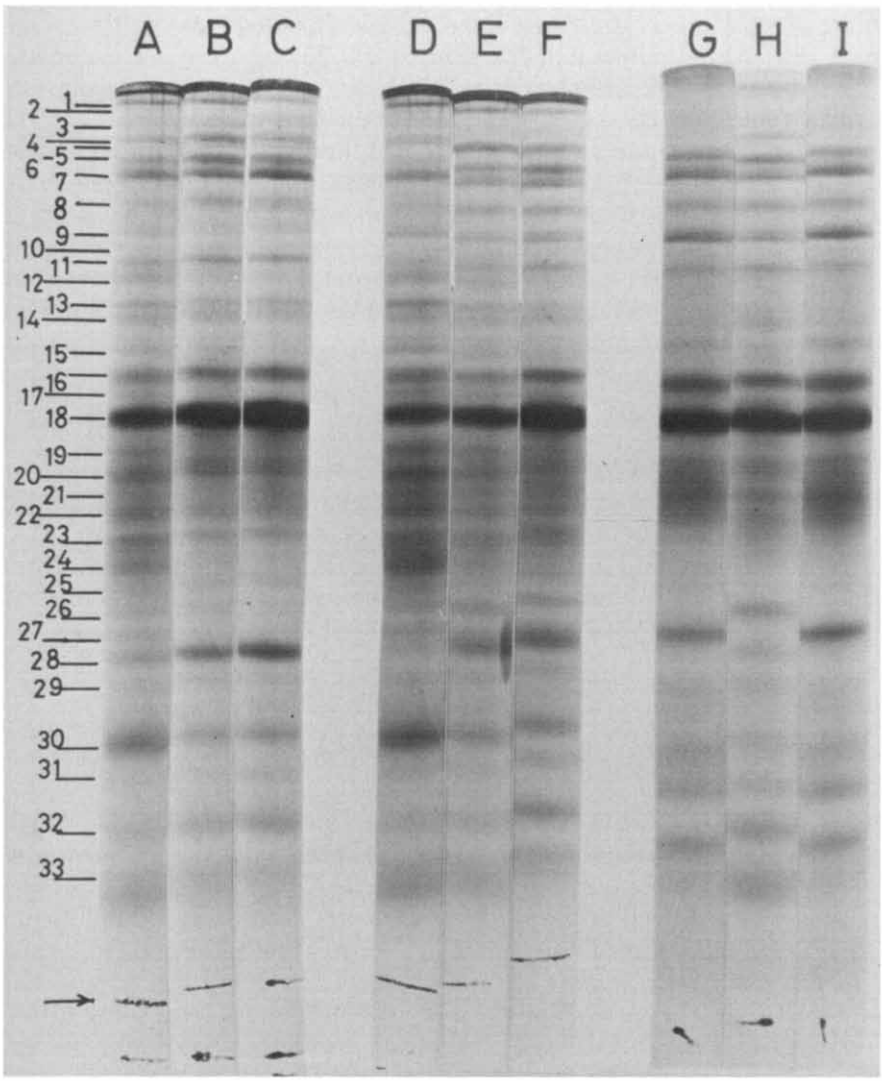

Fig. 1. Electrophoretic comparison of intact Golgi fractions isolated from the control rat liver and livers treated with colchicine or ethanol. The following Golgi fractions were isolated and 40 μg of each sample was subjected to SDS-gel electrophoresis as described in Materials and Methods: Gels A, B and C were GF-3, GF-2 and GF-1, respectively, isolated from colchicine-treated rat liver; gels D, E and F were GF-3, GF-2 and GF-1, respectively, from ethanol-treated rat liver; gels G, H and I were Golgi-total, -light, and -heavy, respectively, from control rat liver. An arrow indicates the position of tracking dye.

polarity of these bands among fractions was more evident in the case of colchicine treatment than in the case of ethanol treatment. In the latter case possible contamination of VLDL-loaded cisternae into the secretory vesicle fraction as suggested in the preceding paper [1] might minimize the distinct polarity between GF-3 and GF-1. The apparent polarity of these proteins among three fractions would suggest the validity of colchicine treatment for for subfractionation of Golgi elements.

Two Golgi fractions isolated from non-treated rat livers (gels G—I in Fig. 1)

were also compared with those from colchicine-treated rat liver. Both fractions, GF-L and GF-H, also contained characteristic bands (bands 6, 7, 16 and 18) as well as other bands common to the Golgi complex. However, GF-H contained albumin to an extent comparable to GF-2 rather than GF-3. This result, together with the high activity of galactosyltransferase, demonstrates the purity of this fraction. The relatively low content of albumin in GF-3 is compatible with the enzyme profile of this fraction which suggested considerable contamination with plasma membranes and microsomes [1].

Comparison of the three Golgi fractions with plasma membranes and microsomes indicates that there are marked differences in electrophoretic patterns among these fractions (Fig. 2). Especially, band 18 is common to all the Golgi fractions but not seen in the plasma membrane, and found in the microsomal fraction only as a minor band, thus serving as a useful marker for the Golgi fraction in hepatocytes. Furthermore, electrophoretic comparison of

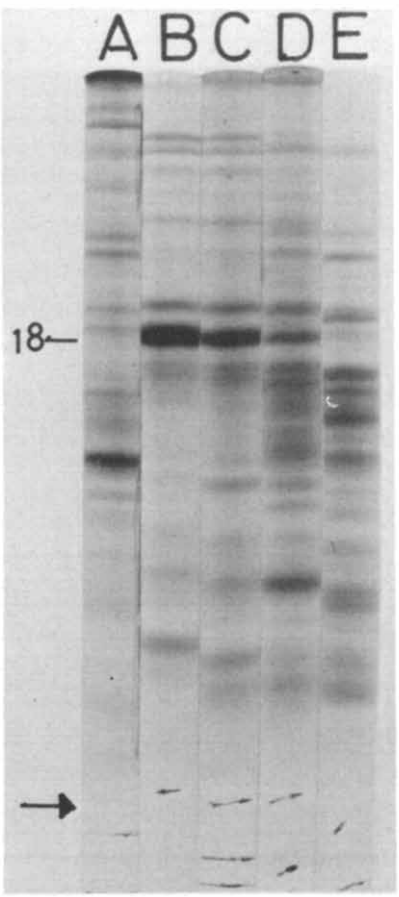

Fig. 2. Comparison of different subcellular fractions in SDS-gel electrophoresis. Three Golgi fractions from colchicine-treated rat liver, plasma membrane and microsome were isolated as described in Materials and Methods, and 40 μg of each sample was applied to SDS-gel electrophoresis. Gel A, plasma membrane; B, GF-1; C, GF-2; D, GF-3; E, microsome. Band-18 corresponds to serum albumin and an arrow indicates the position of tracking dye.

the different subcellular fractions indicates that the secretory vesicles should be included in the Golgi complex, though they are morphologically quite different from the Golgi cisternae and are often seen remote from Golgi stacks, especially in the proximity of the sinusoidal face of the plasma membrane.

Separation of the Golgi fractions into membranes and contents

Golgi fractions of hepatocytes contain large quantities of secretory proteins such as albumin, VLDL and fibrinogen, which make it difficult to compare membrane proteins. To elucidate the relationship among different subcellular structures, it seems favorable to separate organelle fractions into subfractions of membranes and contents and then compare them separately. Although a

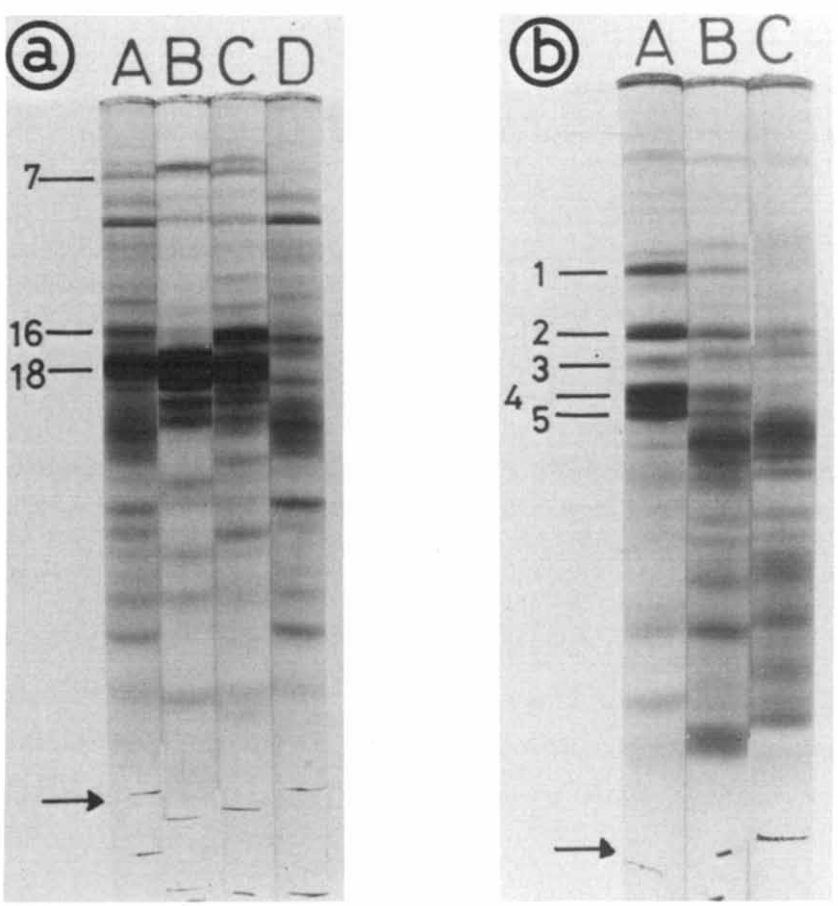

Fig. 3. (a) Comparison of subfractions of the Golgi-heavy fraction in SDS-gel electrophoresis. Intact Golgi-heavy fraction (gel A) isolated from control rat liver was once frozen, thawed and centrifuged. After the first supernatant (gel B) was removed, the pellet was suspended in veronal buffer (pH 8.6), sonicated and then centrifuged. The second supernatant (gel C) was removed and the final pellet was resuspended in 0.25 M sucrose as a membrane fraction (gel D). Each sample with 40 μg protein was loaded on gel. Band-18 corresponds to serum albumin. (b) Comparison of subfractions of the microsomal fraction in SDS-gel electrophoresis. The suspension of intact microsome (gel B) isolated from colchicine-treated rat liver was adjusted to a 0.05% sodium deoxycholate according to Kreibich et al. [10], and centrifuged to separate into membrane (gel C) and content (gel A). Each sample with 40 μg protein was loaded on gel. Bands 1—5 indicate four major proteins in the microsomal content.

complete separation of any organelle into membranes and contents has been a problem, in the present study we used albumin as a marker for the separation of Golgi content. Fig. 3a shows an electrophoretic comparison of intact GF-H and its subfractions, membranes and contents. A frozen sample of intact GF-H fraction (gel A) was thawed and centrifuged. The resultant supernatant (gel B) consisted mainly of albumin, indicating that freezing-thawing was effective in releasing albumin across the Golgi membrane. The pellet obtained from the centrifugation was resuspended in a hypotonic solution, sonicated and re-centrifuged. This treatment released the remaining albumin, together with other secretory proteins, into the second supernatant (gel C). It is interesting to note that in contrast to albumin, band 16 was released only by sonication, indicating its probable association with Golgi membrane. The electrophoretic profile of the membrane fraction (gel D) shows the absence of band 18 (albumin) and demonstrates a reasonable separation of membranes from contents. Fig. 3b shows a comparison of the intact microsomes with its subfraction separated by the method of Kreibich et al. [10]. Proteins released with 0.05% sodium deoxycholate had comparatively high molecular weights (gel A). In contrast, membrane constituent proteins (gel C) distributed over a comparatively low molecular weight region. Among five major bands found in the content fraction, band 3 which corresponds to band 18 (albumin) in the Golgi fractions was not so prominent. The relatively low concentration of albumin in the microsomal content is in marked contrast to its abundunce in the Golgi fraction.

Distribution of protein and galactosyltransferase between membranes and contents of the Golgi fractions

Table I shows protein distributions between the subfractions, membranes and contents, of the Golgi fractions. 20% of the total protein of GF-1 was recovered in the membrane fraction, while 60% of the total protein of GF-3 was recovered in the membrane fraction, in which a possible contamination of plasma membrane and microsome partly accounts for the high recovery in the membrane fraction. A slightly lower value (55%) was obtained in the case of GF-H. GF-2 gave a value intermediate between GF-1 and GF-3, and this is compatible with the morphological observation that GF-2 is composed of secretory vesicles and Golgi cisternae [1]. On the other hand, it has been reported that only 15% of the total protein in the microsomal fraction was

TABLE I

DISTRIBUTION OF PROTEIN BETWEEN GOLGI MEMBRANE AND CONTENT FRACTIONS

Values represent percentages of the total protein in individual Golgi fractions (means ± S.E.).

Fraction	Number of experiments	Membrane (%)	Content (%)	Recovery (%)
GF-1 *	5	20.3 ± 1.3	71.5 ± 3.3	90.1 ± 4.4
GF-2	4	32.4 ± 3.0	57.0 ± 4.9	90.0 ± 7.5
GF-3	4	58.2 ± 3.2	36.4 ± 2.6	94.0 ± 6.9
GF-H	3	54.5 ± 1.5	37.9 ± 10.5	96.3 ± 3.8

* GF-1, 2 and 3 were from the colchicine-treated rats.

TABLE II

DISTRIBUTION OF GALACTOSYLTRANSFERASE ACTIVITY AFTER SEPARATION OF GOLGI FRACTIONS INTO MEMBRANE AND CONTENT FRACTIONS

Fraction	Intact		Membrane		Content				Recovery (%)
					1st Sup.		2nd Sup.		
	Spec. act. *	Total activity (%)	Spec. act.	Total activity (%)	Spec. act.	Total activity (%)	Spec. act.	Total activity (%)	
GF-1 **	99	594 (100)	79	93 (15.7)	23	39 (6.6)	16	39 (6.6)	171 (28.8)
GF-2	131	1,546 (100)	176	709 (45.9)	22	52 (3.4)	27	121 (7.8)	882 (57.1)
GF-3	256	3,891 (100)	255	2,430 (62.5)	19	23 (0.6)	45	213 (5.5)	2,666 (68.6)
GF-H	630	4,353 (100)	928	3,396 (78.0)	42	56 (0.8)	53	131 (3.0)	3,538 (82.3)

* Specific activity (spec. act.) was expressed as nmol/h/mg protein. Values in parentheses represent percentages of the total activity recovered in each fraction.

** GF-1, 2, and 3 were from the colchicine-treated rats.

released as content with 0.05% sodium deoxycholate [10]. The low percentage of membrane protein in GF-1 compared with GF-3 or GF-H, raises the question of whether the polarized distribution of galactosyltransferase among the intact Golgi fractions [1] is due to the different quantity of secretory proteins in each Golgi fraction, and that there might be no polarity of the distribution of the enzyme among the membrane fractions. To answer this question, the specific activity and recovery of galactosyltransferase was determined for the membrane and content fractions. As shown in Table II, the membrane fraction of GF-H had 1.5-fold higher specific activity than the intact GF-H and had about 80% of the total activity, indicating that galactosyltransferase in the Golgi cisternae is indeed associated with membranes and different from such soluble form as found in milk [13] or serum [14]. The finding that the specific activity of the membrane fraction of GF-1 is only one third and one tenth of those found in GF-3 and GF-H membranes respectively may corroborate previous result that there exists a polarity in distribution of the enzyme between the secretory vesicles and the Golgi cisternae. However, the low recoveries of enzyme into subfractions do not permit a final conclusion on this point. Unlike the enzyme in other Golgi-derived membranes, the specific activity of the enzyme in GF-1 membrane was lower than that in the intact fraction. Distribution of the enzyme activity in the combined supernatants from GF-1 was larger than those of other Golgi fractions, especially of GF-3 and GF-H. These results suggest that galactosyltransferase of the secretory vesicles may be loosely associated with membranes, or partially soluble. In addition, the much poorer recovery of the activity in the subfractions of the secretory vesicles may suggest that galactosyltransferase becomes more labile after solubilization compared with a form tightly associated with the Golgi cisternal membrane.

Electrophoretic comparison of membrane fractions

Gel electrophoretic patterns of the membrane fractions show that the plasma membrane is composed of proteins with higher molecular weight than those in the microsomal membrane, while membrane proteins of the Golgi complex distribute in a broad range of molecular weight (Fig. 4).

As shown in gels B—D in Fig. 4, very similar electrophoretic patterns were obtained among the membrane preparations of three Golgi-derived fractions from colchicine-treated rats. However, some differences were observed among the electrophoretic patterns of the three Golgi membranes; in particular the intensities of bands 28, 30 and 32 increases from GF-3 to GF-1. Although GF-H membrane from non-treated rat liver also showed a similar pattern to those of the three Golgi membranes from colchicine-treated rat liver, the only difference observed is that GF-H membrane has prominent proteins corresponding to

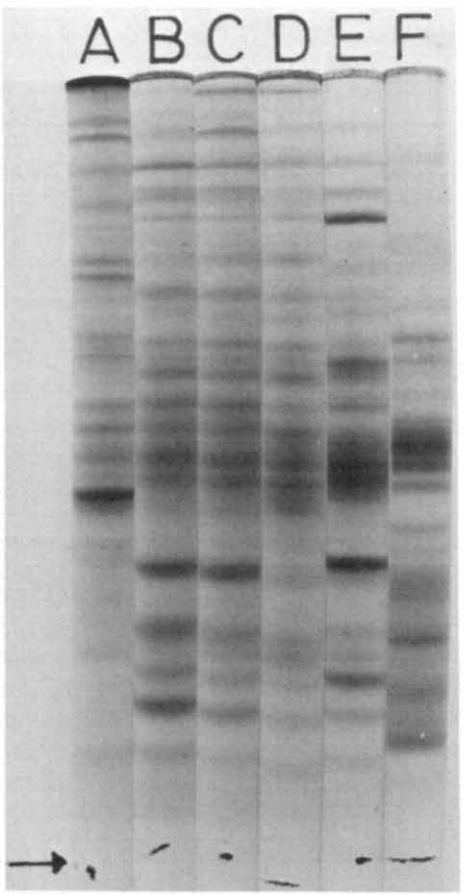

Fig. 4. Electrophoretic comparison of membrane fractions prepared from different subcellular fractions. Subcellular membranes were prepared from colchicine-treated rat liver except for plasma membrane and Golgi-heavy fraction, which were isolated from control rat liver, and 40 μg protein of each sample was subjected to SDS-gel electrophoresis. Gel A, plasma membrane; B, GF-1; C, GF-2; D, GF-3; E, Golgi-heavy; and F, microsomes.

bands 9 and 27, which are very weak in GF-3 as well as in other subfractions (Fig. 4).

Electrophoretic comparison of content fractions

To investigate the functional relationship in the secretory process between the endoplasmic reticulum and the Golgi complex, content fractions prepared from these organelles were compared by SDS-gel electrophoresis. As shown in gels B—E in Fig. 5, similar patterns were observed among the contents of Golgi fractions isolated from colchicine-treated or untreated rat livers. This result demonstrates that there an intimate relationship exists between the secretory vesicles and the Golgi cisternae with respect to transport of secretory proteins.

Comparison of Golgi contents with serum proteins (gel F in Fig. 5) showed that most of the proteins in Golgi contents were also found in serum, confirming that the secretory vesicles contain serum proteins, as had been assumed from morphological evidence that the secretory vesicles fuse with the sinusoidal

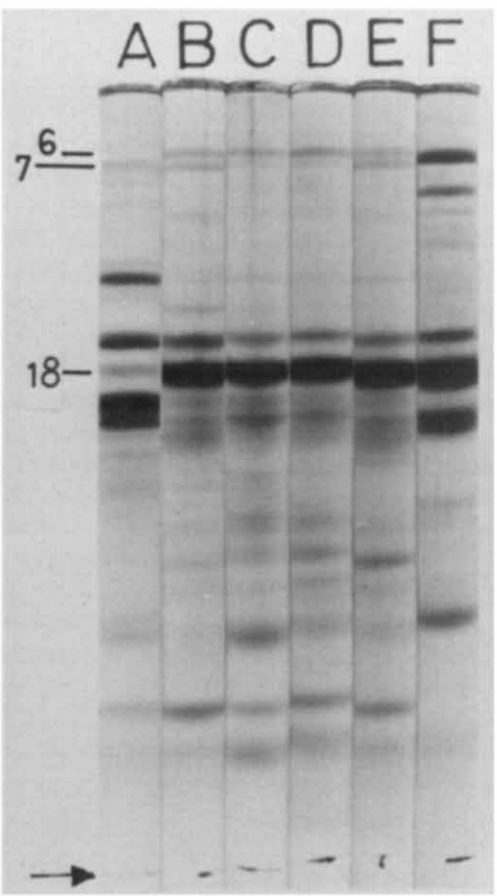

Fig. 5. Electrophoretic comparison of content fractions prepared from different subcellular fractions. Content fractions were prepared from colchicine-treated rat liver except for Golgi-heavy fraction, which was from control rat liver, and 40 μg protein of each sample was subjected to SDS-gel electrophoresis. Gel A, microsome; B, Golgi-heavy; C, GF-3; D, GF-2; E, GF-1; F, rat serum.

face of the plasma membrane and discharge their contents into blood. However, some proteins were more prominent or only seen in serum. When Golgi contents were compared with microsomal contents (gel A in Fig. 5), there was a clear difference between them, especially in the relative amount of albumin, which was a major protein in the Golgi contents but not at all in the microsomal content.

Discussion

Separation of Golgi fractions into membranes and contents was carried out by freezing and thawing, followed by sonication under hypotonic conditions. Since albumin as a content marker was concentrated in the Golgi fractions, lack of albumin in membrane fractions (Fig. 3a) showed the gross separation of membranes from contents. However, this criterion is not always satisfactory because possible adsorption of proteins to the membrane is not ruled out even by complete release of albumin from the membrane fraction [15]. In this respect Ehrenreich et al. [2] reported that a French press was useful in separating the Golgi fractions into membranes and contents using VLDL as marker, in which protein recoveries in the content and membrane fractions of GF-1 were 56.4 and 34.5% respectively. The recovery of 71.5% of content protein in GF-1 obtained in the present study is greater than the above value, indicating that our method used for separation of contents from membranes is satisfactory.

Other subcellular membranes showed quite different electrophoretic patterns compared to those of Golgi membranes (Fig. 4). However, with regard to the relation of the secretory vesicles to the plasma membrane, it seems more reasonable to compare the secretory vesicles with the sinusoidal face of the plasma membrane, since a site of fusion with the secretory vesicles is restricted to the sinusoidal face of the plasma membranes. Although many isolation procedures of plasma membranes have been reported, subfractionation of the sinusoidal region of plasma membranes has as yet been only partially successful [16,17]. Thus electrophoretic analysis could not reveal a clear relationship of membrane constituent proteins between the secretory vesicles and the plasma membrane. Nonetheless, the presence of insulin binding activity [18], and asialoglycoprotein binding activity [19,20] as well as adenylate cyclase [21] and 5-nucleotidase [22] in the Golgi fractions suggests a close relationship between the Golgi complex and plasma membranes. Recently Elder et al. [23] reported the presence of free polyribosomes which were functionally associated with the Golgi elements and isolated together with the Golgi fraction. They showed that some plasma membrane proteins may be synthesized on these polyribosomes and inserted into membranes at the Golgi region. These data strongly suggest that rearrangement or differentiation of the membrane components may occur at the step of the formation of secretory vesicles. One contribution of the Golgi complex to the components of plasma membranes must be the glycosylation of the constituents. It is likely that glycosyltransferases located in the Golgi complex carry out the glycosylation of membrane proteins [24] and lipids [25].

Comparison of the Golgi contents with serum proteins clearly showed the

function of the Golgi complex in a hepatocyte; segregation and transport of serum proteins. In particular the marked similarity between the Golgi cisternae and the secretory vesicles means that the Golgi cisternae are also directly involved in the secretion process. When the Golgi contents were compared with microsomal contents, it was shown that albumin, which was a major component in Golgi fractions was not a major one in the microsomal contents. Since more than 10% of nascent polypeptides synthesized on membrane-bound polyribosomes are albumin [26,27], it seems strange that there are four major proteins in microsomal contents with higher concentration than albumin. One possible explanation is that once discharged into the cisternal lumen of endoplasmic reticulum, albumin is promptly transferred to the Golgi complex and its concentration in endoplasmic reticulum is relatively low, whereas other major proteins migrate more slowly and accumulate in the cisternal lumen. Thus, it is likely that there might exist some mechanism to segregate serum proteins such as albumin from other proteins of microsomal contents.

Although the passage of albumin through the Golgi complex into blood has been investigated extensively [12,28—30], the existence of a precursor of serum albumin, proalbumin, in the microsomes [31] gave us another interest in the Golgi complex in relation to intracellular conversion of proalbumin into serum albumin. Applying the subcellular fractionation method of Ehrenreich et al. [2] to the ethanol-treated rat liver, we recently showed that the conversion of proalbumin occurred in the Golgi complex particularly in the secretory vesicles [32,33]. This finding demonstrates a new function of the Golgi complex, i.e. it functions in modification of macromolecules not only by glycosylation but also by limited proteolysis. Conversion of proalbumin, however, was strongly inhibited in the liver treated with colchicine [32,34], suggesting that there are some functional differences between the secretory vesicles in the ethanol-treated liver and those in colchicine-treated liver. Further studies on the identification and characterization of the proteolytic enzyme involved in the conversion will give a clue not only to a control mechanism of albumin secretion but also to the formation of secretory vesicles [35].

Acknowledgements

This work was supported in part by a grant from the Ministry of Education, Science and Culture of Japan.

References

1 Oda, K., Ikehara, Y., Ishikawa, T. and Kato, K. (1979) Biochim. Biophys. Acta 552, 212—224
2 Ehrenreich, J.H., Bergeron, J.J.M., Siekevitz, P. and Palade, G.E. (1973) J. Cell Biol. 59, 45—72
3 Bergeron, J.J.M., Ehrenreich, J.H., Siekevitz, P. and Palade, G.E. (1973) J. Cell Biol. 59, 73—89
4 Morré, D.J., Keenan, T.W. and Mollenhauer, H.H. (1971) in Advances in Cytopharmacology (Clementi, F. and Ceccarelli, B., eds.), Vol. 1, pp. 159—182, Raven press, New York
5 Yamamoto, T. (1963) J. Cell Biol. 17, 413—421
6 Grove, S.N., Bracker, C.E. and Morré, D.J. (1968) Science 161, 171—173
7 Cheetham, R.D., Morré, D.J. and Youghans, W.N. (1970) J. Cell Biol. 44, 491—500
8 Keenan, T.W. and Morré, D.J. (1970) Biochemistry 9, 19—25
9 Ray, T.K. (1970) Biochim. Biophys. Acta 196, 1—9
10 Kreibich, G., Debey, P. and Sabatini, D.D. (1973) J. Cell Biol. 58, 436—462

11 Fairbanks, G., Steck, T.L. and Wallach, D.F.H. (1971) Biochemistry 10, 2606—2617
12 Peters, T.B., Fleischer, B. and Fleischer, S. (1971) J. Biol. Chem. 246, 241—244
13 Brew, K., Vanaman, T.C. and Hill, R.L. (1968) Proc. Natl. Acad. Sci. U.S. 50, 491—497
14 Fraser, I.H. and Mookerjea, S. (1976) Biochem. J. 156, 347—355
15 Redman, C.M. and Cherian, M.G. (1972) J. Cell Biol. 52, 231—245
16 Wisher, M.H. and Evans, W.H. (1975) Biochem. J. 146, 375—388
17 Toda, G., Oka, H., Oda, T. and Ikeda, Y. (1975) Biochim. Biophys. Acta 413, 52—64
18 Bergeron, J.J.M., Evans, W.H. and Geschwind, I.I. (1973) J. Cell Biol. 59, 771—776
19 Riordan, J.R., Mitchell, L. and Slavik, M. (1974) Biochem. Biophys. Res. Commun. 59, 1373—1379
20 Pricer, W.E. and Ashwell, G. (1976) J. Biol. Chem. 251, 7539—7544
21 Cheng, H. and Farquhar, M.G. (1976) J. Cell Biol. 70, 671—684
22 Little, J.S. and Widnell, C.C. (1975) Proc. Natl. Acad. Sci. U.S. 72, 4013—4017
23 Elder, J.H. and Morré, D.J. (1976) J. Biol. Chem. 251, 5154—5068
24 Ikehara, Y., Oda, K. and Kato, K. (1977) J. Biochem. 81, 349—354
25 Keenan, T.W., Morré, D.J. and Basu, S. (1974) J. Biol. Chem. 249, 310—315
26 Takagi, M., Tanaka, T. and Ogata, K. (1969) J. Biochem. 65, 651—653
27 Ikehara, Y. and Pitot, H.C. (1973) J. Cell Biol. 59, 28—44
28 Glaumann, H. and Ericsson, J.L.E. (1970) J. Cell Biol. 47, 555—567
29 Redman, C.M., Banerjee, D., Howell, K. and Palade, G.E. (1975) J. Cell Biol. 66, 42—59
30 Bergeron, J.J.M., Borts, D. and Cruz, J. (1978) J. Cell Biol. 76, 87—97
31 Judah, J.D. and Nicholls, M.R. (1971) Biochem. J. 123, 649—655
32 Ikehara, Y., Oda, K. and Kato, K. (1976) Biochem. Biophys. Res. Commun. 72, 319—326
33 Oda, K., Ikehara, Y. and Kato, K. (1978) Biochim. Biophys. Acta 536, 97—105
34 Dorling, P.R., Quinn, P.S. and Judah, J.D. (1975) Biochem. J. 152, 341—348
35 Quinn, P.S. and Judah, J.D. (1978) Biochem. J. 172, 301—309