Biochimica et Biophysica Acta, 640 (1981) 398—408
© Elsevier/North-Holland Biomedical Press

BBA 79072

INHIBITORY EFFECT OF COLCHICINE ON TRANSLOCATION OF ALKALINE PHOSPHATASE TO THE PLASMA MEMBRANE CONCOMITANT TO ITS INDUCTION IN RAT LIVER *

KIMIMITSU ODA and YUKIO IKEHARA **

Department of Biochemistry, Fukuoka University School of Medicine, 34 Nanakuma, Nishi-ku, Fukuoka 814 (Japan)

(Received July 4th, 1980)

Key words: Alkaline phosphatase; Colchine effect; Enzyme induction; Subcellular translocation; Golgi complex; (Rat liver)

Summary

A single injection of colchicine (1—3 mg/kg body weight) caused a remarkable induction of hepatic alkaline phosphatase, which increased linearly in the homogenate starting at 5—6 h and reached a maximum level (14-fold of the control activity) at 20—22 h after the drug treatment. In the plasma membrane, however, the increase in specific activity and the recovery of alkaline phosphatase were greatly inhibited up to 12 h after the treatment. Such an inhibitory effect of colchicine was confirmed by a combination experiment of the drug treatment with bile duct ligation; in the plasma membrane elevation of the enzyme induced by bile duct ligation was also greatly retarded by colchicine. The subcellular distribution of the enzyme activity in livers was determined among the four groups of rats with or without bile duct ligation and/or colchicine administration taken at 8 h after each treatment. In the control and the bile duct-ligated livers, the highest specific activity was observed in the plasma membrane fraction, while in the colchicine-treated livers, with or without bile duct ligation, the highest activity was found in the Golgi fractions. These results indicate that the Golgi membranes enriched with the induced enzyme were blocked by the drug to prevent migration toward the plasma membrane, thus demonstrating involvement of the Golgi complex in the translocation route of newly synthesized alkaline phosphatase to the plasma membrane.

---

* Part of this work has been published in abstract form [18].

** To whom correspondence should be addressed.

Introduction

It is a key aspect in the biogenesis of the plasma membrane to know the intracellular pathway by which newly synthesized proteins are processed after translocation and finally assembled into the plasma membrane. The membrane-flow theory suggests that the biogenesis of the plasma membrane involves the translocation of membrane proteins from the endoplasmic reticulum to the Golgi complex and subsequently to the plasma membrane [1,2]. This would imply that continuous renewal of the plasma membrane is achieved through fusion of Golgi-derived vesicles with the plasma membrane [1—3], although direct evidence for this view has generally been lacking. On the other hand, since the work of Lacy et al. [4], many studies have demonstrated that anti-microtubular agents such as colchicine and vinblastine impair secretory functions in a variety of tissues and cultured cells [5—10]; the point of inhibition in these cases has been postulated to be in the movement of secretory vesicles from the Golgi complex to the plasma membrane [8—12]. If the intrinsic proteins and glycoproteins were to follow an intracellular route analogous to the secretory process as suggested by the membrane-flow theory, their translocation to the plasma membrane should also be affected by anti-microtubular agents.

Alkaline phosphatase (orthophosphoric-monoester phosphohydrolase, EC 3.1.3.1) is an intrinsic membrane glycoprotein and a marker enzyme for the plasma membrane [13]. The enzyme of rat liver, the basal level of which is apparently very low, is easily inducible in response to various stimuli such as bile duct ligation, partial hepatectomy, and administration of hormones, heparin, etc. [13—15]. Although the increase in the enzyme activity has been shown to require de novo protein synthesis [13—15] and to occur entirely in the plasma membrane [15], there is no direct evidence on the details of intracellular migration of the enzyme from its site of synthesis to the plasma membrane. If the assembly pathway of alkaline phosphatase were to proceed via the endoplasmic reticulum and the Golgi apparatus as suggested by the membrane-flow theory mentioned above, the translocation of the induced enzyme to its final destination on the plasma membrane would be blocked by anti-microtubular agents, resulting in little accumulation of the enzyme in the plasma membrane.

From this point of view, we have tried to explore the possible effect of colchicine on the intracellular translocation of alkaline phosphatase in rat liver. Our previous study [16], however, has demonstrated that colchicine itself has an inducible effect on alkaline phosphatase in rat liver, which was also reported by Wilfred [17]. Although this effect of colchicine, the mechanism(s) of which is not clear at present, appeared to be unfavorable to the purpose of our present study, the results described here demonstrate that colchicine exerts an inhibitory effect on the translocation of the induced enzyme to the plasma membrane.

Materials and Methods

Animals. Male Wistar rats, weighing 300—400 g, were used throughout the experiments. Under ether anesthesia, the common bile duct was exposed

and ligated according to the method of Kaplan and Righetti [14]. The animals were injected intraperitoneally with colchicine freshly dissolved in 0.9% NaCl as indicated in each experiment. All the rats used were starved for 18—20 h before being killed at 8:00—9:00 in the morning.

Preparation of plasma membranes and other subcellular fractions of livers. Plasma membranes were isolated from livers according to the method of Ray [19]. Subfractions of the Golgi complex were prepared by using the method of Ehrenreich et al. [20] with some modifications as follows. (a) Livers were crushed through stainless-steel mesh (100-mesh, Iida Seisakusho, Osaka, Japan), suspended in 0.25 M sucrose and homogenized in a Potter-Elvehjem homogenizer by hand. The resultant 20% homogenate was centrifuged at 10 000 × *g* for 10 min. (b) An additional 1.15 M sucrose layer was inserted between the microsomal suspension and 0.86 M sucrose layer as described by Redman et al. [12] and centrifuged at 73 000 × *g* for 3 h in a Hitachi RPS 25.2 rotor. Three fractions (GF-1, GF-2 and GF-3, floating at the interfaces of 0.25/0.6, 0.6/0.86 and 0.86/1.15 M sucrose, respectively) were collected by pipette, diluted with cold water to give a final sucrose concentration of 0.25 M and centrifuged at 105 000 × *g* for 60 min [21]. Rough and smooth microsomes were prepared from the total microsomes sedimenting below the Golgi cisternal fraction (GF-3) as described previously [22]. In brief, total microsomes, which were diluted to 0.25 M sucrose and centrifuged at 105 000 × *g* for 60 min, were resuspended in 0.44 M sucrose + buffer I (50 mM Tris-HCl, pH 7.4, 25 mM KCl and 10 mM $MgCl_2$) and adjusted to 1.35 M sucrose by the addition of 1.47 vol. of 2.0 M sucrose in the same buffer. A 40 ml portion of the suspension was layered over 10 ml of 2.0 M sucrose in buffer I and overlaid with 4 ml of 0.44 M sucrose in the same buffer. This discontinuous gradient was centrifuged in a Hitachi RPS 25.2 rotor at 73 000 × *g* for 12 h. The rough and smooth microsomes floating at the boundary between the 2.0 and 1.35 M sucrose layers and on top of the 1.35 M sucrose layer, respectively, were collected by pipette, diluted to 0.25 M sucrose and centrifuged at 105 000 × *g* for 60 min.

Assay of enzyme activities and protein. Alkaline phosphatase and 5′-nucleotidase were determined using *p*-nitrophenyl phosphate and 5′-AMP as substrate, respectively, as described previously [23]. Phosphodiesterase I was determined with *p*-nitrophenylthymidine 5′-monophosphate as substrate in 50 mM Ammediol (2-amino-2-methyl-1,3-propanediol) buffer, pH 10.5. 1 unit of enzyme activity is defined as 1 nmol substrate hydrolyzed per min at 37°C. Protein was determined by using the method of Lowry et al. [24] with bovine serum albumin as standard.

Chemicals. Colchicine was obtained from E. Merck and Co., Darmstadt, F.R.G., 5′-AMP from Boehringer GmbH, Mannheim, F.R.G., and *p*-nitrophenylthymidine 5′-monophosphate from Sigma Chemical Co., St. Louis, U.S.A. All other chemicals used were of reagent grade.

Results

When the dose effect of colchicine on alkaline phosphatase was examined, the induced activity levels observed in liver homogenates showed no significant

TABLE I

DOSE EFFECTS OF COLCHICINE ON THE INDUCTION OF ALKALINE PHOSPHATASE AND ITS LOCALIZATION IN THE PLASMA MEMBRANE IN RAT LIVER

Liver homogenates and plasma membranes were prepared from three rats with or without colchicine treatment and alkaline phosphatase determined. Values represent the means of the results obtained from different experiments, the numbers of which are shown in parentheses. Specific activity is expressed as nmol/min per mg protein.

Treatment	Specific activity		Ratio of specific activity (B/A)	Recovery of activity in plasma membrane (%)
	Homogenate (A)	Plasma membrane (B)		
None (10)	2.3	52.9	23.0	21.6
Colchicine				
6 h, 1 mg/kg (3)	7.1	78.1	11.0	12.4
6 h, 3 mg/kg (2)	7.0	45.8	6.5	5.7
12 h, 1 mg/kg (3)	15.5	396.5	25.6	20.5
12 h, 3 mg/kg (2)	15.1	181.0	12.0	12.9

difference between doses of 1 mg/kg and up to 3 mg/kg. In plasma membranes, however, both the specific activity and the recovery of the total activity varied depending upon the drug doses used, especially during the initial 12 h after treatment; the specific activity in plasma membranes when treated with the higher doses was much lower with poorer recovery of the induced activity than those obtained with 1 mg/kg. A typical experiment is shown in Table I, which demonstrates that the higher dose exhibited the most distinct effect on the subcellular distribution of the induced enzyme. However, since few rats sur-

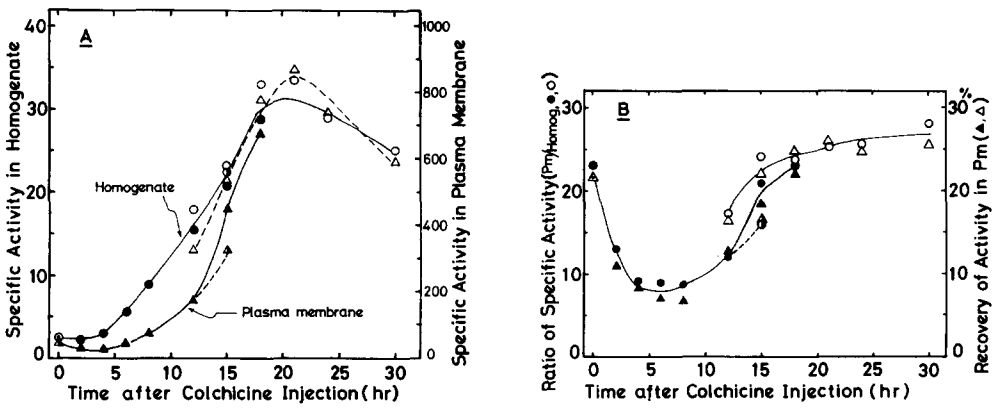

Fig. 1. Changes in activity level of alkaline phosphatase in homogenates and plasma membranes of rat livers as a function of time after colchicine administration. Rats were injected intraperitoneally with colchicine at 2 mg/kg (open symbols) or 3 mg/kg (closed symbols). Liver homogenates and plasma membranes were prepared from three rats for each experiment at the times indicated and alkaline phosphatase determined. Each point represents the average of the results obtained from two or three experiments except for the control which was from six experiments. (A) Specific activity of homogenates (○, ●) and of plasma membranes (△, ▲). The specific activity is expressed as units of nmol/min per mg protein. In one experiment, rats which had been treated with 3 mg/kg of the drug at zero time were additionally injected with 2 mg/kg at 12 h and killed at 15 h after the first treatment (◑, ◭). (B) Ratio of the specific activity of plasma membranes to that of homogenates (○, ●) and recovery of the total activity in plasma membranes (△, ▲).

vived doses higher than 3 mg/kg for periods longer than 15 h after treatment, we used 3 mg/kg as the effective dose in the following experiments, unless otherwise specified.

Fig. 1 shows changes in alkaline phosphatase levels of liver homogenates and of plasma membranes as a function of time after colchicine injection. The specific activity of homogenates started increasing at about 5 h and reached the maximum level at about 20 h, which was the same time course obtained with 1 mg/kg as reported previously [16]. The activity levels of the plasma membrane fractions, however, did not increase in parallel with those of the homogenates. As shown in Fig. 1A, not only the starting time of the increase was retarted, but also the rate of increase was considerably suppressed up to 12 h, after which the specific activity of plasma membranes increased rapidly, reaching the maximum level almost at the same time as that of homogenates. Such differences in the time courses between homogenates and plasma membranes are also clearly illlustrated in Fig. 1B, showing that the ratio of the specific activity of plasma membranes to that of homogenates as well as the recovery of the total activity in plasma membranes were greatly decreased during the initial 12 h, after which both parameters recovered to the original values. To confirm such an inhibitory effect of the drug on the enzyme localization in plasma membranes, we carried out another experiment in which rats treated with 3 mg/mg at zero time were additionally injected with 2 mg/kg at 12 h and killed at 15 h after the first treatment. As shown in Fig. 1, the additional injection significantly inhibited both the increase in specific activity and the recovery of the enzyme in plasma membrane as compared to those obtained by a single injection of 3 mg/kg at zero time. The second injection had no significant effect on the activity induced by the first injection. The results suggest that colchicine has an inhibitory effect on the translocation of the newly synthesized alkaline phosphatase to the plasma membrane.

One could argue that colchicine at the higher dose might have another unknown effect and result in a peculiar preparation of plasma membranes different from that of control livers, resulting in lower specific activity with poor recovery of alkaline phosphatase. To examine this possibility, we investigated whether there was any change in two other marker enzymes, 5′-nucleotidase and phosphodiesterase I, between the control and colchicine-treated livers. As Table II shows, little or no change was found in the specific activities of these enzymes in all prepartions, both of homogenates and of plasma membranes, and also in the yields of plasma membranes. In addition, each preparation of plasma membranes from the control and the treated livers showed identical protein profiles on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate.

It was of interest to learn whether colchicine exhibited the same effect on translocation of the enzyme induced by other stimuli. Fig. 2 shows changes in alkaline phosphatase levels in homogenates and plasma membranes as a function of time after bile duct ligation alone and after simultaneous treatments with colchicine. In the bile duct-ligated livers, the specific activity of plasma membranes rose rapidly after a lag period of about 4 h which was just behind that of the homogenate activity. Thereafter, it followed a time course nearly the same as that of homogenates (Fig. 2A). On the other

TABLE II

COMPARISON OF ACTIVITY LEVELS AND LOCALIZATIONS OF THREE MARKER ENZYMES FOR PLASMA MEMBRANES AFTER TREATMENT WITH COLCHICINE

Liver homogenates and plasma membranes were prepared from three rats for each experiment with or without colchicine treatment, and determined for three marker enzymes for plasma membrane. Values in each column represent the specific activities of homogenates (H) and plasma membranes (PM), ratios of the specific activity of plasma membranes to that of homogenates (PM/H), and recovery of the total activity in plasma membrane (R), respectively. Each value is the mean of the results obtained from two or three experiments for the colchicine-treated rats and from 10 experiments for the control. Values of the recovery of total activity are expressed as %. Specific activities are given as nmol/min per mg protein.

Treatment	Alkaline phosphatase				5′-Nucleotidase				Phosphodiesterase I				Protein recovery in PM
	H	PM	PM/H	R	H	PM	PM/H	R	H	PM	PM/H	R	
None (control)	2.3	52.9	23.0	21.6	48.6	826	17.0	15.0	296	5800	19.6	20.6	0.89
Colchicine (3 mg/kg)													
4 h	3.1	28.8	9.4	8.2	49.2	792	16.1	14.2	320	6100	19.0	16.7	0.88
8 h	8.7	75.5	8.7	6.7	52.1	930	17.9	13.7	310	5970	15.7	19.2	0.76
12 h	15.1	181.0	12.0	12.9	48.1	723	15.0	13.9	322	5010	15.5	16.7	0.92
18 h	28.8	678.8	23.6	22.0	54.3	844	15.5	14.5	267	5550	20.8	19.4	0.80

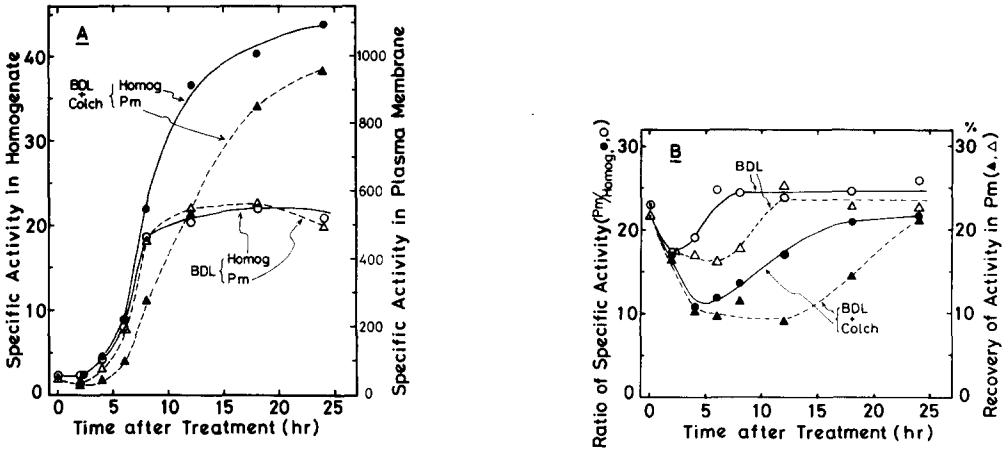

Fig. 2. Changes in activity level of alkaline phosphatase in homogenates and plasma membranes of rat livers caused by bile duct ligation alone and by its combination with colchicine treatment. Liver homogenates and plasma membranes were prepared from three rats for each experiment at the indicated times after bile duct ligation alone (BDL, open symbols) or after simultaneous treatment with 3 mg/kg colchicine (BDL + Colch, closed symbols). Each point represents the average of the results obtained from at least two experiments. (A) Specific activity (units/mg protein) of homogenates (○, ●) and of plasma membranes (△, ▲). (B) Ratio of the specific activity of plasma membranes to that of homogenates (○, ●) and recovery of the total activity in plasma membranes (△, ▲).

hand, the simultaneous injection of colchicine remarkably enhanced the elevation of the enzyme activity in homogenates caused by bile duct ligation, which was disclosed at about 8 h and became more prominent with time. The enzyme activity finally reached a maximum level which was twice that brought about by bile duct ligation alone (Fig. 2A). Localization of the induced enzyme to the plasma membrane, however, was significantly retarded as compared with that observed with bile duct ligation alone. Both the ratios of the specific activities between plasma membranes and homogenates and recoveries of the total activity in plasma membranes were greatly inhibited (Fig. 2B).

To confirm the inhibitory effect of colchicine on translocation of the enzyme, we prepared subcellular fractions from the control livers and three groups of livers obtained at 8 h after bile duct ligation, treatment with colchicine or both, and examined whether there was any fraction in which the induced enzyme was accumulated as a result of colchicine treatment (Table III). When the specific activities of rough and smooth microsome fractions were compared with that of the homogenate in each group, little difference was found in the extent of increase among the four groups, each of which showed approx. 2- and 4—5-fold higher activities in rough and smooth microsomes, respectively. Differences, however, were observed in the Golgi fractions and plasma membranes between the groups with and without colchicine treatment. In both the control and the bile duct-ligated livers, the highest specific activities were obtained in plasma membranes, whereas in the colchicine-treated livers with or without bile duct ligation, the highest activities were found in the Golgi fractions. The ratios of the specific activities of the Golgi fractions to those of homogenates or of plasma membranes were

TABLE III

COMPARISON OF ACTIVITY LEVELS OF ALKALINE PHOSPHATASE IN THE SUBCELLULAR FRACTIONS AMONG THE FOUR GROUPS OF RAT LIVERS WITH OR WITHOUT TREATMENTS

Four groups of rats, non-treated (control), bile duct-ligated (BDL), colchicine-(3 mg/kg)-treated, and simultaneously treated with colchicine (3 mg/kg) and bile duct ligation, were killed 8 h later if treated. Liver homogenates and subcellular fractions were prepared from four rats for each experiment and alkaline phosphatase determined. Enzyme units are given as nmol/min. Value represents the means of results obtained from at least two experiments. Rough and smooth microsomes and Golgi membrane fractions were prepared from total microsomes. Golgi subfractions, GF-1, GF-2 and GF-3, were prepared as described previously [20, 21]. Plasma membranes were separately prepared according to the method of Ray [19].

Fraction	Specific activity of alkaline phosphatase (units/mg protein)			
	Control	BDL (8 h)	Colchicine (3 mg/kg; 8 h)	BDL + colchicine (3 mg/kg; 8 h)
Homogenate	1.7	15.9	12.1	18.5
Total microsomes	3.2	38.8	29.5	45.3
Rough microsomes	2.7	35.1	24.7	42.6
Smooth microsomes	6.5	68.9	47.2	87.9
Golgi GF-1	3.7	134.5	174.5	383.8
Golgi GF-2	11.9	143.1	168.1	310.4
Golgi GF-3	20.9	193.3	140.7	272.3
Plasma membrane	52.9	473.2	75.5	280.9
GF-1/homogenate	2.2	8.4	14.4	20.7
GF-1/plasma membrane	0.07	0.28	2.31	1.37

increased remarkably in the colchicine-treated groups. Taken together, these results indicate that the newly synthesized alkaline phosphatase was accumulated by colchicine in the secretory vesicles as well as in the Golgi cisternae, resulting in a non-proportional increase of the enzyme in the plasma membrane.

Discussion

The present and previous studies show that the activity of alkaline phosphatase in the liver is extremely low at the basal level and can be induced to high levels by appropriate stimuli [13—17]. These observations offered the advantage of being able to trace the intracellular migration of the newly synthesized membrane protein without the necessity of labeling with radioactive precursors or specific antibody. As reported previously [16,17], a remarkable increase in alkaline phosphatase caused by colchicine is not due to catalytic activation of the enzyme but, rather, to enzyme induction, because this increase was prevented by simultaneous treatment with cycloheximide and no change in the activity was observed when the liver homogenates or plasma membranes were incubated with colchicine under various conditions [16]. Details on the induction mechanism(s), however, are not available at present.

In the present study, we demonstrate that colchicine inhibits the appearance of the induced activity of alkaline phosphatase in the plasma membrane whether the induction is caused by colchicine itself or by bile duct ligation (Figs. 1 and 2). The result is an accumulation of the activity in the Golgi

complex (Table III). These findings indicate that colchicine impairs the translocation of newly synthesized enzyme from the Golgi complex to the plasma membrane. Recent reports have suggested that colchicine most likely exerts its effect on protein secretion at the level of the Golgi complex, a more proximal site, rather than at the discharge step (membrane fusion process). This hypothesis is based on the following observations, largely obtained with polarized cells having a great capacity for storage of their secretory products. (a) Colchicine was ineffective in inhibiting either basal or chemical-induced release of stored proteins such as amylase [9], pancreatic proteins [8] and lacrymal proteins [10], although it inhibited the secretion of the newly synthesized proteins. (b) Blockade of the secretory activity by colchicine was accompanied by marked changes in the morphology of the Golgi region, an accumulation of Golgi-associated vacuoles and vesicles, together with dispersion of the dictyosomes, and by the complete disappearance of the microtubules in this region [8,9,25]. In hepatocytes, similar morphological alterations have been demonstrated to occur in the Golgi region after colchicine treatment [26,27]. These observations suggest that microtubules may be important in the maintenance of the Golgi complex. Thus, the most plausible explanation for the present observations is that colchicine disaggregation of microtubules by blocking polymerization, leading to dysfunction of the Golgi complex and diminished movement towards the plasma membrane of the Golgi-derived vesicles enriched with the induced enzyme so that any accumulation of the enzyme in the plasma membrane is inhibited.

As shown in Table I and Fig. 1, the inhibitory effect of colchicine is dose and time dependent. Also, Stein et al. [11] reported that the duration of colchicine inhibition of lipoprotein secretion was related to the dose used. With a low dose (0.5 mg/kg body weight), the inhibitory action of the drug was found to be reversible within 7 h after administration; however, at the higher dose level (5 mg/kg), the inhibitory effect was preserved for at least 7 h, although these authors did not show any result during longer periods. In our case, with 3 mg/kg, relatively rapid elevation of the activity in the plasma membrane which occurred after 12 h would reflect the release from the inhibitory action of the drug (Fig. 1). This phenomenon could be explained as follows: during a period longer than 7 h, the administered drug within cells may be gradually metabolized into an inactive form, resulting in reformation of microtubules which restore the normal function of the Golgi complex and activate the movement of Golgi-derived vesicles to and their fusion with the plasma membrane.

Both 5′-nucleotidase and phosphodiesterase I are also marker enzymes of the plasma membrane and are presumably delivered via the Golgi complex. These enzymes, however, showed no response to colchicine even after 18 h of treatment, in contrast to alkaline phosphatase (Table II). The results suggest that as opposed to the induced alkaline phosphatase, a steady-state supply to the plasma membrane of the two non-induced enzymes may be very small compared to those as pooled in the plasma membrane. In addition, a possibly slow turnover of the membrane-bound enzymes might make it difficult to detect such a small change in enzyme localization caused by colchicine, unless tracing could be made by labeling with a radioactive precursor.

In order to validate the precursor-product relationships entailed in membrane-flow mechanisms, it has to be established that newly synthesized components are found first in other subcellular membranes and, therefore, a lag occurs before such components appear in the plasma membrane itself. The previous kinetic studies using rat liver subcellular fractions showed that labeled amino acids appeared in the plasma membrane after a significant lag time during which successive activity peaks appeared first in rough and smooth endoplasmic reticulum and then in the Golgi fraction [28—30]. However, few individually recognizable plasma membrane proteins have been analyzed in detail for kinetic behavior in intracellular migration, mainly due to difficulty in isolation of any membrane proteins with a hydrophobic nature and the low abundance of individual components in the plasma membrane. Recently, the post-translational events in the biogenesis of the viral glycoprotein (G-protein) have been extensively studied [31—33] and widely accepted as a model for that of a glycoprotein located in plasma membranes, although it is not an indigenous component of plasma membranes. G-Protein is synthesized on the membrane-bound polysomes and cotranslationally integrated into rough endoplasmic reticulum, subsequently membrane-associated G-protein migrates to plasma membranes via the Golgi complex, where processing and completion of oligosaccharides occur [34]. More recently, Hauri et al. [35] have reported clear-cut evidence on the post-translational route of sucrase-isomaltase, an intrinsic glycoprotein of the intestinal plasma membrane. After [^{3}H]fucose labeling in vivo, they followed the intracellular migration of the enzyme by immunoprecipitation with the specific antibody. The results demonstrate that the precursor form of sucrase-isomaltase is transported from the Golgi membrane to the microvillus membrane where the precursor form is cleaved into the subunits of the mature form, providing experimental support for the membrane-flow hypothesis.

Although a detailed kinetic analysis is still required for final elucidation, our observation on hepatic alkaline phosphatase, an intrinsic component of the plasma membrane, is generally congruent with the evidence so far reported, as mentioned above, indicating that the Golgi complex is involved in its translocation to the plasma membranes as in the well defined secretion process [36].

Acknowledgements

We wish to thank Dr. N. Hamasaki for useful discussions. This work was supported in part by grants from the Ministry of Education, Science, and Culture of Japan.

References

1 Morré, D.J. (1977) in The Synthesis, Assembly and Turnover of Cell Surface Components (Poste, G. and Nicolson, G.L., eds.), pp. 1—83, North-Holland, Amsterdam
2 Whaley, W.G., Dauwalder, M. and Kephart, J.E. (1972) Science 175, 596—599
3 Bennett, G., Leblond, C.P. and Haddad, A. (1974) J. Cell Biol. 60, 258—284
4 Lacy, P.E., Howell, S.L., Young, D.A. and Fink, C.J. (1968) Nature 219, 1177—1179

5 LeMarchand, Y., Singh, A., Assimacopoulos-Jeannet, F., Orci, L., Rouiller, C. and Jeanrenaud, B. (1973) J. Biol. Chem. 248, 6862—6870
6 Stein, O. and Stein, Y. (1973) Biochim. Biophys. Acta 306, 142—147
7 Ehrlich, H.P., Ross, R. and Bornstein, P. (1974) J. Cell Biol. 62, 390—405
8 Seybold, J., Bieger, W. and Kern, H.F. (1975) Virchows Arch. A. 368, 309—327
9 Patzelt, C., Brown, D. and Jeanrenaud, B. (1977) J. Cell Biol. 73, 578—593
10 Chambaut-Guerin, A.-M., Muller, P. and Rossignol, B. (1978) J. Biol. Chem. 253, 3870—3876
11 Stein, O., Sanger, L. and Stein, Y. (1974) J. Cell Biol. 62, 90—103
12 Redman, C.M., Banerjee, D., Howell, K. and Palade, G.E. (1975) J. Cell Biol. 66, 42—59
13 Fishman, W.H. (1974) Am. J. Med. 56, 617—650
14 Kaplan, M.M. and Righetti, A. (1970) J. Clin. Invest. 49, 508—516
15 Pekarthy, J.M., Short, J., Lansing, A.I. and Lieberman, I. (1972) J. Biol. Chem. 247, 1767—1774
16 Ikehara, Y., Mansho, K. and Kato, K. (1978) J. Biochem. 84, 1335—1338
17 Wilfred, G. (1977) Biochim. Biophys. Acta 500, 209—212
18 Oda, K. and Ikehara, Y. (1979) Cell Struct. Funct. 4, 381 (Abstr.)
19 Ray, T.K. (1970) Biochim. Biophys. Acta 196, 1—9
20 Ehrenreich, J.H., Bergeron, J.J.M., Siekevitz, P. and Palade, G.E. (1973) J. Cell Biol. 59, 45—72
21 Oda, K., Ikehara, Y., Ishikawa, T. and Kato, K. (1979) Biochim. Biophys. Acta 552, 212—224
22 Ikehara, Y. and Pitot, H.C. (1973) J. Cell Biol. 59, 28—44
23 Ikehara, Y., Takahashi, K., Mansho, K. and Eto, S. and Kato, K. (1977) Biochim. Biophys. Acta 470, 202—211
24 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
25 Moskalewski, S., Thyberg, J., Lohmander, S. and Friberg, U. (1975) Exp. Cell Res. 95, 440—454
26 DeBrabander, M., Wanson, J.-C., Mosselmans, R., Geuens, G. and Drochmans, P. (1978) Biol. Cell. 31, 127—140
27 Reaven, E.P. and Reaven, G.M. (1980) J. Cell Biol. 84, 28—39
28 Ray, T.K., Lieberman, I. and Lansing, A.I. (1968) Biochem. Biophys. Res. Commun. 31, 54—57
29 Barancik, L.C. and Lieberman, I. (1971) Biochem. Biophys. Res. Commun. 44, 1084—1088
30 Franke, W.W., Morré, D.J., Deumling, B., Cheetham, R.D., Kartenbeck, J., Jarasch, E.-D. and Zentgraf, H.-W. (1971) Z. Naturforsch. 26, 1031—1039
31 Atkinson, P.H., Moyer, S.A. and Summers, D.F. (1976) J. Mol. Biol. 102, 613—631
32 Knipe, D.M., Lodish, H.F. and Baltimore, D. (1977) J. Virol. 21, 1121—1127
33 Rothman, J.E. and Lodish, H.F. (1977) Nature 269, 775—780
34 Tabas, I. and Kornfield, S. (1979) J. Biol. Chem. 254, 11655—11663
35 Hauri, H.-P., Quaroni, A. and Isselbacher, K.J. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 5183—5186
36 Palade, G.E. (1975) Science 189, 347—358