Biochimica et Biophysica Acta 937 (1988) 319–327
Elsevier

BBA 73846

A study on the intracellular transport of prothrombin, albumin and transferrin in rat

Astrid H. Kvalvaag, Ole K. Tollersrud and Liv Helgeland

Department of Biochemistry, University of Oslo, Blindern (Norway)

(Received 20 August 1987)

Key words: Intracellular transport; Prothrombin; Protein secretion; Kinetics; (Rat liver)

The intracellular transport of prothrombin in rat has been studied and compared with the transport of albumin and transferrin. The proteins were immunoisolated from plasma samples after pulse labelling with [^{3}H]leucine and the secretion kinetics were determined. The half-times for secretion ($t_{1/2}$) were approx. 30, 53 and 75 min for albumin, prothrombin and transferrin, respectively, whereas the minimal transit time for prothrombin was approx. 30 min, and those for albumin and transferrin 15–20 min. After injection of vitamin K-1 into warfarin-treated rats, the accumulated prothrombin precursor was γ-carboxylated and secreted with a $t_{1/2}$ of 37 min. This indicates that the γ-carboxylation of prothrombin in rough endoplasmic reticulum cannot account for the delay in the transport of prothrombin as compared to albumin. Comparison of the incorporation of [^{3}H]leucine and [^{3}H]glucosamine into plasma prothrombin and transferrin suggested that transferrin is secreted randomly from an intracellular pool, whereas prothrombin is transported in a more orderly sequence. Moreover, treatment of rough microsomes with 0.05% sodium deoxycholate indicated that prothrombin is more tightly associated with the membranes of rough endoplasmic reticulum than albumin and transferrin.

Introduction

Secretory proteins are synthesized in rough endoplasmic reticulum and transported by vesicles via the Golgi complex to the plasma membrane [1]. It has been shown that various secretory proteins [2–4], lysosomal proteins [5] and plasma membrane proteins [6] are transported intracellularly at different rates. The rate-limiting step in secretion is mainly the transport from rough endoplasmic reticulum to the Golgi complex [2,3,7], although variability in retention within the Golgi is also demonstrated [8,9]. Proteins accumulate in rough endoplasmic reticulum when the transport rate is low [6,10] or inhibited because of an abnormal or incomplete configuration [11–13]. To account for the different secretion kinetics, it has been suggested that individual secretory proteins have different affinities for receptors located to the luminal side of the rough endoplasmic reticulum membrane, or, alternatively, that the amount of specific receptors may be rate-limiting [10,14]. A recent study of the transport of secretory proteins in human hepatoma cells showed that the secretion of nine proteins falls into three distinct classes, suggesting that there are different receptor-mediated pathways from rough endoplasmic reticulum to the Golgi complex [4]. Furthermore, there are presumably stationary binding proteins in rough endoplasmic reticulum which specifically retard polypeptides until they have achieved a proper conformation necessary for transport [15].

Correspondence; A.H. Kvalvaag, Department of Biochemistry, University of Oslo, Box 1041, Blindern, 0316 Oslo 3, Norway.

Comparison of the secretion kinetics of various secretory proteins may supply information about the transport mechanisms involved.

Rat prothrombin is a plasma glycoprotein of M_r 75000–77000 [16], being synthesized as pre-proprothrombin [17]. The protein is modified by a post-translational carboxylation of about ten specific glutamic residues in the N-terminal part of the protein [18]. This reaction is catalyzed by a membrane-bound vitamin-K-dependent carboxylase facing the lumen of rough endoplasmic reticulum [11,19]. It is reported that the propeptide of prothrombin may be necessary for binding to the carboxylase [20–22]. By administration of the vitamin K antagonist, warfarin, in rat, secretion of prothrombin is inhibited, and undercarboxylated prothrombin accumulates in rough endoplasmic reticulum [11,23].

In the present work the intracellular transport of prothrombin, albumin and transferrin in rat during pentobarbital anaesthesia have been studied. Albumin and transferrin were included as reference proteins, as the transport of these two proteins has been studied in various systems. Experiments were performed to determine the secretion kinetics of the proteins, and to determine whether the γ-carboxylation can account for the delay in the secretion of prothrombin as compared to albumin. Differences in the transport mechanisms were also revealed by the patterns obtained by leucine and glucosamine labelling of transferrin and prothrombin, and by the distribution of the proteins between membranes and luminal fractions of rough microsomes.

Materials and Methods

Materials

L-[4,5-^{3}H]Leucine (128 Ci/mmol), L-[U-^{14}C]leucine (348 mCi/mmol) and D-[6-^{3}H]glucosamine hydrochloride (33 Ci/mmol) were purchased from Amersham International; warfarin (sodium salt) from Chemoswed AB, Sweden; TH-1 (D-cyclohexylglycyl-L-alanyl-L-arginine-*p*-nitroanilide dihydroacetate) from Nyegaard & Co, Oslo, Norway; Vitamin K-1 (Konakion) from Hoffman-LaRoche; *Echis carinatus* venom from Sigma; Protein A-Sepharose CL-4B from Pharmacia; $BaSO_4$ from Baker; Soluene and Hionic-Fluor were from Packard; rabbit anti-rat transferrin IgG and rabbit anti-rat serum albumin IgG from United States Biochemical Cooperative.

Animals

Male Wistar rats, 240–280 g, from Möllegaard, Havrup, Denmark, were used. The rats were fasted prior to the experiment only when the liver was removed for subcellular fractionation.

General procedures

The experimental procedures were started between 9:00 a.m. and 10:00 a.m. The rat was anaesthesized with 15 mg sodium pentobarbital in 0.3 ml 0.9% NaCl, intraperitoneally. Precautions were taken to keep the body temperature reasonably constant during the experiment. Vitamin K-1 (10 mg/kg body wt.), [^{3}H]leucine (800 μCi/kg body wt.), [^{3}H]glucosamine hydrochloride (800 μCi/kg body wt.), or a mixture of [^{14}C]leucine (20 μCi/kg body wt.) and [^{3}H]glucosamine hydrochloride (400 μCi/kg body wt.) were injected in a volume of 0.5 ml 0.9% NaCl through a cannula 22G/0.8 mm into the femoral vein. Samples of 200–500 μl were collected at each withdrawal of blood and equal volumes of 0.9% NaCl were injected to restore the liquid balance. To prevent coagulation, 50 μl 10 U/ml of heparin was injected into the cannula. Before each withdrawal of blood, the heparin was completely removed. For continuous anaesthesia, 2 mg pentobarbital in 0.9% NaCl was given every 40–50 min intravenously. The blood was immediately mixed 9:1 (v/v) with 0.15 M potassium oxalate as anticoagulant, and centrifuged. Plasma samples were stored at −20 °C.

Preparation of rough microsomal subfractions

The rats were starved 20 h before being killed. Rough microsomes were prepared as previously described [24]. The luminal content of rough microsomes was released by treatment with 0.05% sodium deoxycholate as described by Kreibich and Sabatini [25]. Separation of the luminal content from membranes and ribosomes was carried out according to Gogstad and Helgeland [26], with the exception that 5.5 ml of the microsomal suspension layered onto a 1.0 ml cushion of 0.25 M sucrose in a thick wall polycarbonate tube was

centrifuged for 90 min at 125 000 × *g* in a No. 65 Beckman rotor. A satisfactory separation was obtained by these conditions as determined experimentally and by theoretical calculations. The membrane fraction was solubilized by treatment with 2.5% Triton X-100 for 30 min and centrifuged at 100 000 × *g* for 90 min. All steps were carried out at 2–4 °C. The luminal and solubilized membrane fractions were kept at −20 °C.

Analytical methods

Protein was determined by the method of Lowry et al. [27] using crystalline bovine serum albumin as standard. Sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis with 10% gel was performed as described previously [28]. Albumin and transferrin were determined by single radial immunodiffusion as described by Mancini et al. [29]. Protein-bound radioactivity was determined by precipitation with cold 6% trichloroacetic acid/0.5% phosphotungstic acid/2.3% glucosamine hydrochloride/1.3% leucine and the precipitates were washed three times with the same reagent. The precipitate was solubilized in 1 M NaOH and added to the Hionic Fluor scintillation solution. ^{3}H and ^{14}C radioactivities were counted in a Packard liquid scintillation counter. Quench corrections were carried out using external standard channels ratio.

Purification of rat prothrombin and preparation of antiserum

Prothrombin was purified from rat plasma by barium citrate adsorption and ammonium sulphate precipitation, essentially as described by Grant and Suttie [16]. The protein was further purified by chromatography on a DEAE-Sephadex A-50 column using a 200 ml linear gradient (0.04–0.74 M NaCl in 0.01 M sodium phosphate buffer (pH 7.0)). The prothrombin fractions were stored at −20 °C in the presence of 1 mM phenylmethylsulfonyl fluoride. A minor contaminant was removed by preparative discontinuous polyacrylamide gel electrophoresis using a 5% separating gel in a Tris-HCl buffer system (pH 8.5). The entire procedure was carried out at 4 °C. Antiserum was raised in a 2 kg female rabbit by multiple intradermal injections spaced over the back of the animal. Four injections at 10–20 day intervals were given. About 50 μg of pure prothrombin, as judged by SDS-gel electrophoresis, was injected each time, having been emulsified with Freund's complete adjuvant in the primary injection and Freund's incomplete adjuvant in the subsequent injections. The antiserum was stored at −20 °C. The specificity of the antiserum was verified by immunoelectrophoresis.

$BaSO_4$ adsorption and elution

Oxalated plasma was adsorbed on 30 mg $BaSO_4$/ml. The mixture was kept at 4 °C for 10 min with intermittent shaking, and was then centrifuged. The pellet was washed once with 5 plasma volumes of 0.9% NaCl and then eluted twice with, respectively, 0.5 and 0.25 plasma volumes of 0.2 M trisodium citrate. The eluates were pooled.

Immunoprecipitation and immunoadsorption

(a) Immunoprecipitation. Plasma samples (see last part of paragraph) were added to 2.0 ml buffer A (0.05 M Tris-HCl/0.15 M NaCl/0.1% Triton X-100/10 mM dithiothreitol/2 mM leucine/2 mM glucosamine hydrochloride/2 mM EGTA (pH 7.4)), 80 μl of phenylmethylsulfonyl fluoride (1 mg/ml ethanol) and 200 μl antiserum against rat albumin, prothrombin or transferrin. After incubation overnight at 4 °C, the immunoprecipitate was washed twice with buffer A containing 0.4 M NaCl, solubilized in Soluene, added to the Hionic Fluor scintillation solution and counted.

(b) Immunoadsorption. 100 μl antiserum diluted with buffer A was applied to a column of 20 mg protein A-Sepharose CL-4B equilibrated with buffer A, and incubated for 30 min at room temperature with intermittent mixing. The column was washed twice with 1 ml buffer A. The sample (see last part of paragraph) was applied to the column and incubated for 30 min with intermittent mixing. The column was washed three times with 1.5 ml buffer A containing 0.4 M NaCl. The immunocomplex was eluted with 0.5 ml 1 M acetic acid, mixed with the Hionic Fluor scintillation solution and counted.

The specificity of the immunoprecipitation and immunoadsorption was confirmed by SDS-gel electrophoresis. Labelled albumin and transferrin

were sufficiently purified by either immunoprecipitation or immunoadsorption. The specificity of the anti-(rat prothrombin) serum in these techniques was investigated by studying the distribution of radioactivity in the gel pattern obtained from an immunoprecipitate using [^{3}H]leucine-labelled plasma. The only radioactivity detected was from the gel slice corresponding to a band of an apparent M_r of 85000. This SDS M_r for prothrombin is in agreement with Swanson and Suttie [31]. Immunoadsorption of prothrombin gave an extra band with $M_r \approx 67000$, corresponding to albumin, apparently being unspecifically bound to the Protein A-Sepharose. This extra band was removed when the plasma was initially adsorbed on $BaSO_4$ (results not shown).

To obtain a quantitative estimation of the radiolabelled proteins, a small excess of antiserum was used. 100 μl of the respective antisera was sufficient to precipitate 40 μg albumin, 50 μg transferrin and 4 μg prothrombin, or to adsorb 50 μg albumin, 60 μg transferrin and 6.5 μg prothrombin.

Immunoadsorption was preferred to immunoprecipitation when:
(1) The antigen solution was very dilute;
(2) It was important to reduce the amount of antiserum needed.

Quantitation of total prothrombin

Total prothrombin was determined by measuring the amidolytic activity of *Echis carinatus* venom-activated prothrombin [30]. 0.1 ml plasma diluted in 8 mM veronal-HCl/0.12 M NaCl (pH 7.4) was mixed with 0.025 ml 50 μg/ml *Echis carinatus* venom and 0.05 ml 0.1% Triton X-100 in 0.9% NaCl, and incubated at 37°C for 10 min. Triton X-100 was included to avoid unspecific adsorption of prothrombin or thrombin to the wall of the plastic test-tube (Helgeland L., unpublished data). Prewarmed (37°C) 0.575 ml 8 mM veronal-HCl/0.12 M NaCl (pH 8.4) and 0.05 ml of 2 mM TH 1 were added, and the mixture was incubated for 15 min. The reaction was stopped by 0.4 ml 40% acetic acid and A_{405} was measured. 1/2000–1/500-diluted control plasma was used for the standard curve. Control plasma was pooled plasma from six normal, unstarved rats.

Results

To determine the secretion kinetics of different secretory proteins, [^{3}H]leucine was injected into the femoral vein and blood samples were withdrawn at time intervals of 10–20 min, as described in Materials and Methods. Labelled leucine has a very high turnover in liver, and will act as a pulse [32]. The animals were kept under pentobarbital anaesthesia during the experiment. The results obtained by this experimental procedure were highly reproducible, as shown for the incorporation of [^{3}H]leucine into total plasma protein (Fig. 1).

Fig. 2 summarizes the results of experiments in which the secretion kinetics of prothrombin, albumin and transferrin were determined simultaneously. As seen from Fig. 2, [^{3}H]leucine-labelled albumin and transferrin were detected in plasma 15–20 min after injection. The time-courses are in agreement with previous reports [3,10,32]. The half-time for secretion, $t_{1/2}$, was thus 30 min for albumin and 75 min for transferrin, when it was assumed that 85% of the maximal specific radioactivity of transferrin is reached 130 min after the pulse. [^{3}H]Leucine-labelled prothrombin appeared in plasma approx. 30 min after intravenous injection of the isotope, increased rapidly, and reached

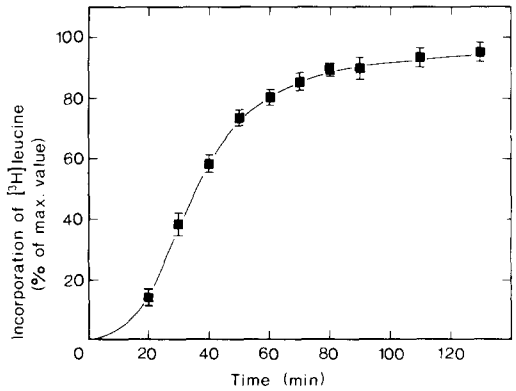

Fig. 1. Incorporation of [^{3}H]leucine into total plasma protein. 200 μCi [^{3}H]leucine was injected into the femoral vein and 300 μl blood was withdrawn at time intervals. Plasma aliquots were precipitated with trichloroacetic acid as described in Materials and Methods. The results were calculated as cpm/mg protein, and expressed as % of maximum value, assuming that 95% of total specific radioactivity was reached after 130 min. The values are mean of 11 experiments, each from one rat, ± S.D.

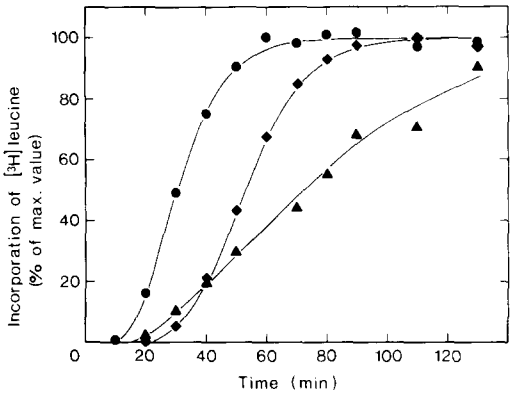

Fig. 2. Incorporation of [^{3}H]leucine into plasma albumin (●), prothrombin (◆) and transferrin (▲). Plasma from two rats (treated as in Fig. 1) was mixed and aliquots were taken for purification of albumin, transferrin and prothrombin. Albumin and transferrin were isolated by immunoprecipitation. Prothrombin was adsorbed on $BaSO_4$ prior to immunoadsorption as described in Materials and Methods. The results were calculated as cpm/mg protein, and expressed as % of maximum value. The values are mean of two experiments differing by less than 5%.

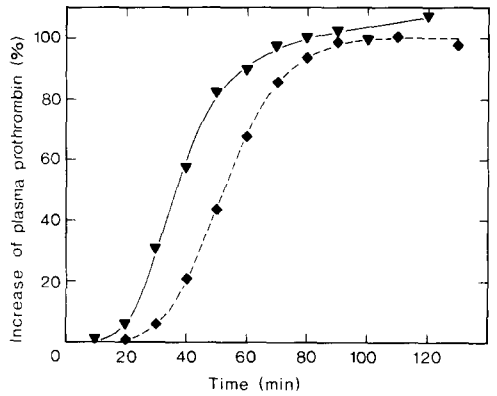

Fig. 3. Increase in plasma prothrombin after an injection of vitamin K-1 into warfarin-treated rats (▼———▼) and the incorporation of [^{3}H]leucine into plasma prothrombin in normal rats (◆------◆). 10 mg warfarin/kg body wt. was injected intraperitoneally 20 h prior to injection of vitamin K-1. 10 mg vitamin K-1/kg body wt. was injected into the femoral vein. 2–300 μl blood was withdrawn at time intervals, and plasma from two rats was mixed. The prothrombin concentration in the samples was measured as the amidolytic activity after activation by *Echis carinatus* venom as described in Materials and Methods. Specific activity was calculated as units prothrombin/mg protein, and expressed as % of the value obtained 80 min after injection of vitamin K-1 (see Results). The values are mean of two experiments differing by less than 5%. The curve showing the time-course of [^{3}H]leucine-labelled prothrombin (from Fig. 2) is included for comparison.

a plateau level after 90 min. The time-course showed resemblance with that of albumin except that the curve was displaced approx. 20 min. $t_{1/2}$ for prothrombin was thus approx. 53 min.

To investigate whether the γ-carboxylation of prothrombin in rough endoplasmic reticulum might contribute to the long minimal transit time, vitamin K was injected into warfarin-treated rats. During warfarin-treatment, prothrombin precursor accumulates in rough endoplasmic reticulum until a plateau 7-times higher than the normal level is reached (Tollersrud, O.K. and Helgeland, L., unpublished data). In total microsomes from warfarin-treated rats, a similar degree of accumulation (6–8-times) has been reported [33–35]. Upon vitamin K administration the accumulated precursor is carboxylated and secreted. The amount of prothrombin secreted under these conditions is approximately 10-times the amount secreted due to the de novo synthesis during 1 h [36,37]. Accordingly, the increase of biologically active plasma prothrombin after vitamin K injection was regarded as the transport of intracellularly accumulated prothrombin into the circulation (Fig. 3).

As shown in Fig. 3, an increase in plasma prothrombin appears 15–20 min after the injection of vitamin K; then follows a rapid increase until approx. 80 min. This amount is arbitrarily chosen as 100%, as further secretion is due to the de novo synthesis. $t_{1/2}$ after vitamin K injection is about 37 min, approx. 15 min less than the $t_{1/2}$ for normal prothrombin from synthesis to secretion (indicated for comparison from Fig. 2). This indicates that the carboxylation process alone cannot account for the retention of prothrombin as compared to albumin.

To investigate the transport of prothrombin and transferrin further, the incorporation of labelled glucosamine and leucine into proteins from rough microsomes and plasma was compared. In contrast to leucine, the turnover of free glucosamine in liver is low [38] and, in addition, the amino sugar is incorporated into glycoproteins both in endoplasmic reticulum and Golgi. To reveal the differences in the incorporation of [^{3}H]leucine and [^{14}C]glucosamine in endoplasmic

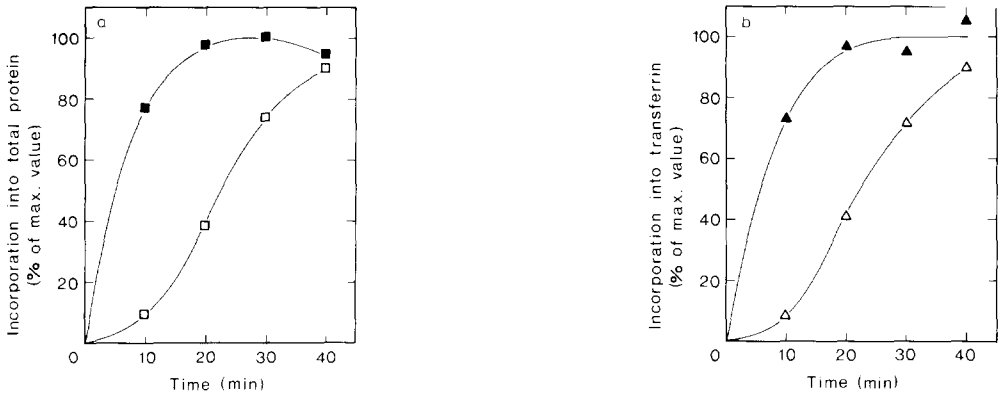

Fig. 4. Incorporation of [^{14}C]leucine (■, ▲) and [^{3}H] glucosamine (□, △) into total protein (a) and transferrin (b) in the luminal fraction of rough microsomes. 5 μCi [^{14}C]leucine and 100 μCi [^{3}H]glucosamine were injected into the femoral vein. The livers were removed at the indicated time points, rough microsomes from two combined livers were isolated, and microsomal subfractions were prepared as described in Materials and Methods. Aliquots were taken for measurement of radioactivity in total protein after trichloroacetic acid precipitation, and in transferrin after immunoadsorption. The results were calculated as cpm/mg protein, and expressed as % of maximum value.

reticulum, double labelling experiments were carried out.

Fig. 4 shows the incorporation into total luminal protein (a) and transferrin (b) from rough microsomes after double labelling with [^{14}C]leucine and [^{3}H]glucosamine. The incorporation of labelled glucosamine into total luminal protein as well as into transferrin of rough microsomes shows a 15 min delay compared to the incorporation of labelled leucine, and a maximum is not reached in 40 min. These incorporation curves are apparently characteristic for labelled leucine and glucosamine, on account of the markedly different turnover of these two compounds in liver. As the concentration of prothrombin in microsomes is very low, it was not possible to carry out corresponding experiments with this protein. It is reasonable to assume, however, that the incorporation of [^{3}H]glucosamine into prothrombin in rough microsomes also will show a 15 min delay compared to the incorporation of [^{14}C]leucine.

In Fig. 5 the time-courses of [^{3}H]glucosamine incorporated into plasma prothrombin (a) and plasma transferrin (b) are presented. The time-courses of the incorporation of [^{3}H]leucine (data from Fig. 2) are included for comparison. The two diagrams are clearly different. As shown in Fig. 5a, the incorporation of [^{3}H]leucine into plasma prothrombin is reaching a maximum after approx. 90 min, whereas a maximum for labelled glucosamine is not obtained even after 130 min. This reflects the difference in incorporation of [^{3}H]leucine and [^{3}H]glucosamine in rough endoplasmic reticulum (Fig. 4). Fig. 5a shows further that the [^{3}H]glucosamine-labelled prothrombin appears in plasma approx. 10 min before the [^{3}H]leucine-labelled protein, which is expected since this carbohydrate is also incorporated in the Golgi apparatus. As illustrated in Fig. 5b the time-courses of [^{3}H]glucosamine-labelled and [^{3}H]leucine-labelled plasma transferrin are almost identical. The appearance in plasma of [^{3}H]leucine-labelled transferrin obviously coincides with the appearance of the [^{3}H]glucosamine which has been incorporated in the Golgi. Furthermore, the fact that labelled glucosamine is incorporated approx. 15 min later than labelled leucine into transferrin in rough endoplasmic reticulum (Fig. 4b) is not reflected in the time-course of plasma transferrin.

Binding of secretory proteins to receptors in the membrane of rough endoplasmic reticulum might affect the transport pattern. The luminal content of rough microsomes was therefore released with 0.05% sodium deoxycholate according to Materials and Methods, and the association of prothrombin, albumin and transferrin with the membranes was investigated. Approx. 50% of the prothrombin from normal rough microsomes was found in the membrane fraction, compared to 20% of both albumin and transferrin. In rough micro-

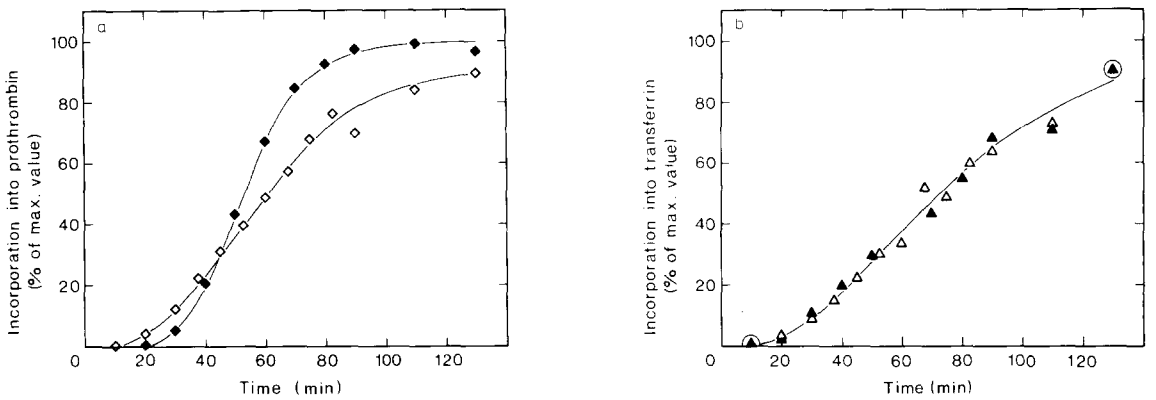

Fig. 5. Incorporation of [^{3}H]glucosamine (◇, △) and [^{3}H]leucine (◆, ▲) into plasma prothrombin (a) and transferrin (b). 200 μCi [^{3}H]glucosamine hydrochloride was injected into the femoral vein and 2–300 μl blood was withdrawn at time intervals. Plasma from two rats was mixed and aliquots were taken for immunoprecipitation of transferrin and prothrombin. The results were calculated as cpm/mg protein, and expressed as % of maximum value. The values are mean of two experiments differing by less than 5%. The curves illustrating the time-course of [^{3}H]leucine-labelled transferrin and prothrombin (from Fig. 2) are included for comparison.

somes from warfarin-treated rats, where prothrombin precursor has accumulated, only 20% of the prothrombin was in the membrane fraction.

Discussion

Our results show that the intracellular transport of serum albumin, prothrombin and transferrin in rat are different. The half-times for secretion are approx. 30, 53 and 75 min for albumin, prothrombin and transferrin, respectively, whereas the minimal transit times are 15–20 min for albumin and transferrin and approx. 30 min for prothrombin. The observed secretion kinetics of serum albumin and transferrin are in agreement with Morgan and Peters [10,32]. They used diethyl ether anaesthesia during the intravenous injection of radioactive isotopes, and re-anaesthetized the rats before removal of blood. Only one blood sample was obtained from each rat. In the present work the rats were kept under pentobarbital anaesthesia for approx. 2 h, making it possible to obtain several blood samples from each rat. The similarity of the results obtained by the two different methods indicates that the treatment may have no significant effect on the secretion kinetics of plasma proteins. Similar differences between the intracellular transport of transferrin and serum albumin have been found in isolated rat hepatocytes [3], mouse hepatoma cells [39] and human hepatoma cells [2,4].

The posttranslational γ-carboxylation of glutamic acid residues in prothrombin seems to be a prerequisite for the transport from rough endoplasmic reticulum to the Golgi, since prothrombin accumulates in rough endoplasmic reticulum during vitamin K deficiency and the only known function of vitamin K in eucaryotes is as cofactor for the vitamin K-dependent carboxylase [18]. Upon injection of vitamin K, the accumulated prothrombin in warfarin-treated rats was secreted with a half-time of approx. 37 min, about 15 min shorter than that of newly synthesized prothrombin in normal rats (Fig. 3). This indicates that the retention of prothrombin as compared to albumin is mainly confined to rough endoplasmic reticulum, and that the carboxylation of prothrombin in rough endoplasmic reticulum is not the rate-limiting step. Our results suggest that prothrombin is retarded during the transport through rough endoplasmic reticulum, prior to a sorting of carboxylated prothrombin from the precursor. This rules out the possibility that the delay in the transport of prothrombin as compared to albumin is due to low affinity for receptors responsible for the transfer from rough endoplasmic reticulum to the Golgi, as suggested for transferrin [2,3]. There may be a retention in Golgi, too, as $t_{1/2}$ for accumulated prothrombin from rough endoplasmic reticulum is approx. 7 min longer than $t_{1/2}$ for albumin from synthesis to secretion.

Investigation of the incorporation of labelled

leucine and glucosamine into newly synthesized proteins in rough microsomes showed that glucosamine is incorporated in rough endoplasmic reticulum approx. 15 min later than leucine. The time-courses of [^{3}H]leucine- and [^{3}H]glucosamine-labelled plasma prothrombin reflected this difference, and indicated that newly synthesized glucosamine-labelled prothrombin is not mixed in rough endoplasmic reticulum with previously synthesized leucine-labelled prothrombin. The corresponding time-courses for plasmatransferrin coincided, suggesting that newly synthesized [^{3}H]glucosamine-labelled transferrin is mixed with previously synthesized [^{3}H]leucine-labelled transferrin in an intracellular pool, from which differently labelled molecules are secreted at random. This is consistent with a previous report showing that transferrin accumulates in rough endoplasmic reticulum and that the subsequent secretion follows first-order kinetics [10]. These results suggest that the transport mechanisms of the two proteins are different, and that prothrombin is transported into circulation in a more orderly sequence than transferrin. Moreover, a pattern of first-order kinetics is not easily adjusted to the secretion of prothrombin, suggesting that there is no pool of prothrombin in rough endoplasmic reticulum from which the molecules are secreted at random. Treatment of rough microsomes with 0.05% sodium deoxycholate released significantly more albumin and transferrin than prothrombin, indicating that prothrombin is more tightly associated with the membranes of rough endoplasmic reticulum than albumin and transferrin. Taken together, our results suggest that prothrombin is bound to some receptor protein in the membrane during the transport through rough endoplasmic reticulum. The number of binding sites in the membrane may, however, be limited, as the increase of prothrombin during warfarin treatment was found mainly in the luminal fraction of the rough microsomes.

The observed differences in intracellular transport between albumin, transferrin and prothrombin indicate that there is a specific transport apparatus for each of the three proteins. Parent et al. [4] reported that nine secretory proteins in a human hepatoma cell line could be divided into three classes with similar kinetics of secretion within each group. Whether corresponding classes exist in rat remains to be investigated.

Acknowledgement

We wish to thank Dr. Terje E. Michaelsen for advice in preparing the anti-(rat prothrombin) serum.

References

1 Palade, G. (1975) Science 189, 347–358.
2 Lodish, H.F., Kong, N., Snider, M. and Strous, G.J.A.M. (1983) Nature 304, 80–83.
3 Fries, E., Gustafsson, L. and Peterson, P.A. (1984) EMBO J. 3, 147–152.
4 Parent, J.B., Bauer, H.C. and Olden, K. (1985) Biochim. Biophys. Acta 846, 44–50.
5 Cardelli, J.A., Golumbeski, G.S. and Dimond, R.L. (1986) J. Cell Biol. 102, 1264–1270.
6 Fitting, T. and Kabat, D. (1982) J. Biol. Chem. 257, 14011–14017.
7 Schönholzer, F., Schweingruber, A.M., Trachsel, H. and Schweingruber, M.E. (1985) Eur. J. Biochem. 147, 273–279.
8 Scheele, G. and Tartakoff, A. (1985) J. Biol. Chem. 260, 926–931.
9 Yeo, K.-T., Parent, J.B., Yeo, T.-K. and Olden, K. (1985) J. Biol. Chem. 260, 7896–7902.
10 Morgan, E.H. and Peters, T., Jr. (1985) J. Biol. Chem. 260, 14793–14801.
11 Helgeland, L. (1977) Biochim. Biophys. Acta 499, 181–193.
12 Wu, G.E., Hozumi, N. and Murialdo, H. (1983) Cell 33, 77–83.
13 Verbanac, K.M. and Heath, E.C. (1986) J. Biol. Chem. 261, 9979–9989.
14 Farquhar, M.G. (1985) Annu. Rev. Cell Biol. 1, 447–488.
15 Bole, D.G., Hendershot, L.M. and Kearney, J.F. (1986) J. Cell Biol. 102, 1558–1566.
16 Grant, G.A. and Suttie, J.W. (1976) Arch. Biochem. Biophys. 176, 650–662.
17 MacGillivray, R.T.A. and Davie, E.W. (1984) Biochemistry 23, 1626–1634.
18 Suttie, J.W. (1985) Annu. Rev. Biochem. 54, 459–477.
19 Carlisle, T.L. and Suttie, J.W. (1980) Biochemistry 19, 1161–1167.
20 Bentley, A.K., Rees, D.J.G., Rizza, C. and Brownlee, G.G. (1986) Cell 45, 343–348.
21 Jorgensen, M.J., Cantor, A.B., Furie, B.C., Brown, C.L., Shoemaker, C.B. and Furie, B. (1987) Cell 48, 185–191.
22 Suttie, J.W., Hoskins, J.A., Engelke, J., Hopfgartner, A., Ehrlich, H., Bang, N.U., Belagaje, R.M., Schoner, B. and Long, G.L. (1987) Proc. Natl. Acad. Sci. USA 84, 634–637.
23 Shah, D.V., Swanson, J.C. and Suttie, J.W. (1984) Thromb. Res. 35, 451–458.
24 Helgeland, L., Christensen, T.B. and Janson, T.L. (1972) Biochim. Biophys. Acta 286, 62–71.

25 Kreibich, G. and Sabatini, D.D. (1974) Methods Enzymol. 31, 215–225.
26 Gogstad, G. and Helgeland, L. (1978) Biochim. Biophys. Acta 508, 551–564.
27 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
28 Laemmli, U.K. (1970) Nature 227, 680–685.
29 Mancini, G., Carbonara, A.O. and Heremans, J.F. (1965) Immunochemistry 2, 235–254.
30 Tollersrud, O.K. and Helgeland, L. (1986) Thromb. Res. 42, 737–747.
31 Swanson, J.C. and Suttie, J.W. (1985) Biochemistry 24, 3890–3897.
32 Morgan, E.H. and Peters, T., Jr. (1971) J. Biol. Chem. 246, 3508–3511.
33 Suttie, J.W. (1973) Science 179, 192–194.
34 Boer-van den Berg, M.A.G., Thijssen, H.H.W. and Vermeer, C. (1986) Biochim. Biophys. Acta 884, 150–157.
35 Harauchi, T., Takano, K., Matsuuera, M. and Yoshizaki, T. (1986) Jpn. J. Pharmacol. 40, 491–499.
36 Shah, D.V. and Suttie, J.W. (1972) Arch. Biochem. Biophys. 150, 91–95.
37 Suttie, J.W., Nelsestuen, G.L. and Shah, D.V. (1973) Thromb. Diathes. Suppl. 54, 37–49.
38 Jamieson, J.C. and Ashton, F.E. (1973) Can. J. Biochem. 51, 1281–1291.
39 Ledford, B.E. and Davis, D.F. (1983) J. Biol. Chem. 258, 3304–3308.