CONVERSION OF PROALBUMIN INTO SERUM ALBUMIN IN THE SECRETORY VESICLES OF RAT LIVER

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SUMMARY: The conversion site of proalbumin into serum albumin was investigated in the subcellular fractions of rat liver labeled with [^{3}H]leucine *in vivo*. In the cisternae-rich fraction of the Golgi complex as well as in the microsomal fraction most of the labeled albumin was detected as proalbumin, while in the secretory vesicles, which were obtained in increased amount by oral administration of ethanol, more than 70% of the labeled albumin was found as serum type, indicating that conversion of proalbumin into serum albumin occurs within the secretory vesicles in rat liver. Little accumulation of albumin was observed in colchicine-treated rats.

Recently evidence has been presented to establish the existence of a protein precursor, 'proalbumin', of serum albumin in rat liver (1-9). Although serum albumin and proalbumin are not distingushable in their immunological properties, a structural difference has been suggested from experiments with ion-exchange chromatography, electrofocusing, and peptide mapping (1,3,9). A precursor-product relationship, which was suggested from incorporation experiments with labeled amino acids (2,3,8), is supported by evidence that proalbumin is converted into serum albumin by limited tryptic hydrolysis *in vitro* (4) and the primary structure of proalbumin includes a penta- or hexapeptide attached to the NH_2-terminus of serum albumin (6,7).

This evidence for the existence of proalbumin in liver led us to an investigation on the conversion site of proalbumin into serum albumin *in vivo*. Judah and his co-workers reported previously that neither subfractions of microsomes nor the Golgi-rich fraction could be identified as the conversion site (3). Failure to identify the site may be ascribed to the rapid secretion and slight accumulation of albumin in liver after conversion of proalbumin (3).

Another possibility is that conversion occurs as a very rapid step just before secretion in some organelle that was not examined by Judah's group. This let us focus our interest on the secretory vesicles. A procedure for isolation of the secretory vesicles from rat liver in good purity and sufficient quantity for biochemical analysis has been developed by Ehrenreich et al.(10), using pretreatment of rats with ethanol or anti-secretory agents (colchicine, vinblastin etc.) (11,12) to increase the secretory vesicles and make possible their separation from other components.

In this paper we present an evidence showing that the conversion of proalbumin occurs within the secretory vesicles in livers of rats pretreated with ethanol.

MATERIALS AND METHODS

Preparation of rat serum albumin and antibody to serum albumin. These were prepared as described previously (13).

Preparation of the subcellular fractions of rat liver. In order to obtain large amounts of the secretory vesicles, rats were pretreated with either ethanol or colchicine (10,12). After 16 hr starvation, rats (Sprague-Dawley, weighing 300 to 350 g) were given 0.6 g ethanol/100 g body weight in 50% (w/v) solution by stomach tube 1.5 hr before sacrifice. In case of colchicine treatment, rats were given two injections intraperitoneally of 0.05 mg colchicine per 100 g body weight at 3 hr and 1.5 hr before sacrifice. Rats which were pretreated with either ethanol or colchicine, were injected intraportally with 200 μCi of [^{3}H]leucine after laparotomy under anesthesia with Nembutal (sodium pentobarbital solution, 50 mg/ml, 0.8 - 0.9 ml/100 g body weight). Rats were decapitated 20 min after injection of [^{3}H]leucine and livers were immediately perfused through portal vein with cold 0.25M sucrose. Livers were removed and the subcellular fractionation was carried out according to the method of Ehrenreich et al. (10). Of fractions thus obtained, a microsomal fraction and three subfractions of the Golgi complex were used for analysis of albumin. Three subfractions of the Golgi complex were electronmicroscopically characterized as described by Ehrenreich et al. (10,12); the light fraction, GF-1, consisted essentially of VLDL*)-loaded secretory vesicles, and the heavy fraction (GF-3) was enriched in the Golgi cisternae, while the intermediate fraction of GF-2 was a mixture of GF-1 and -3. The cisternae-rich Golgi fraction was also isolated from rat liver without any pretreatment with colchicine or ethanol, according to the modified method originally developed by Fleischer et al. (14) and Morré et al. (15), after injection of [^{3}H]leucine into the rats. The fraction obtained by this procedure was further subjected to the final sucrose density gradient centrifugation described in the method of Ehrenreich et al.(10) to remove any possible contamination of the secretory vesicles. The final fraction was designated as the control GF-3.

Preparation of the labeled albumin from the subcellular fractions. Each fraction of the microsomes and of the Golgi complex, GF-1, -2, and -3, was suspended in 0.04M veronal buffer, pH 8.6, and sonicated three times with an

*) Abbreviation used; VLDL, very low density lipoprotein

Umeda probe sonicator (Umeda Electric Co., Tokyo, Japan) at maximum power output for 30 sec, followed by the centrifugation at 105,000 g for 60 min. The resulting supernatant was used for the isolation of the labeled albumin, using the procedure of Judah and Nicholls (1).

Separation of proalbumin from albumin. Albumins were separated into proalbumin and albumin by DEAE-cellulose chromatography (1 x 40 cm) according to the method of Judah and Nicholls (1). Other details were described in the figure legends.

Determinations of radioactivity and protein. To fractions eluted from the DEAE-cellulose column trichloroacetic acid was added to final concentration of 5% after addition of one drop of bovine serum albumin (10 mg/ml) as carrier, if necessary. Each precipitate obtained by centrifugation at 2,500 rpm for 5 min was solubilized in NCS solubilizer and assayed for radioactivity in toluene scintillator in a Packard liquid scintillation spectrometer. Protein was determined by the method of Lowry et al. (16), with bovine serum albumin as standard, or by reading absorbance at 280 nm.

Materials. L-[4,5-^{3}H]leucine(5 Ci/mmole) was obtained from New England Nuclear, Boston, Mass., U.S.A.; NCS solubilizer, from Amersham/Searle, Arlington Heights, Ill., U.S.A.; DEAE-cellulose (DE-32), from Whatman Biochemicals, Maidstone, Kent, U.K.; Colchicine, from E. Merck, Darmstadt, Germany; and Nembutal (sodium pentobarbital), from Abbott Laboratories, North Chicago, Ill., U.S.A. All other chemicals were reagent grade and of commercial origin.

RESULTS AND DISCUSSION

In attempts to find an intracellular site of conversion of proalbumin we first applied the subfractionation method of Ehrenreich et al.(10) to rat liver which was labeled with [^{3}H]leucine after pretreatment with colchicine, one of anti-secretory agents (17). The labeled albumin, isolated from the subcellular fractions of liver by immunoprecipitation with anti-serum albumin, were applied to the DEAE-cellulose column after addition of carrier serum albumin.

As shown in Fig.1a, almost all of the labeled albumin isolated from the microsomes was eluted as proalbumin and little radioactivity was found in the peak of carrier serum albumin, as reported previously (1). Furthermore, an elution profile of the labeled albumin from the secretory vesicles (GF-1) was essentially the same as that from the microsomes, although a slight increase of radioactivity was found in the serum albumin region (Fig.1b). Separation of the labeled albumin from other subfractions (GF-2 and -3) showed similar profiles to those of the microsomes and GF-1. Ratios of newly synthesized albumin to proalbumin in each subfraction from the colchicine-treated rat liver were summarized in Table 1. These results indicate that little conversion of proalbumin into serum albumin, if any, occurs in the secretory vesicles that

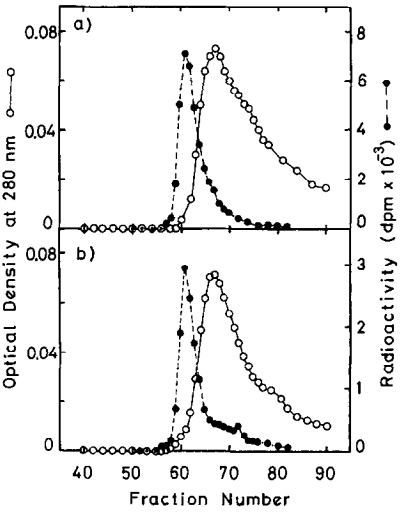

Fig. 1 DEAE-cellulose chromatography of albumin isolated from colchicine-treated rat liver. The labeled albumin in the subcellular fractions was prepared from colchicine-treated rat liver as described in Methods. After addition of carrier serum albumin (10 - 15 mg), each sample in about 2 ml was dialyzed overnight against 0.1M Tris-HCl buffer, pH 7.8, and applied to the DEAE-cellulose column (1 x 40 cm). The elution was done at room temperature with a 400 ml linear gradient of 0.1 - 0.3M Tris-HCl buffer, pH 7.8. Fraction size was 3 ml. a) microsomes, 62,000 dpm; and b) GF-1 (secretory vesicles), 26,000 dpm.

Table 1. Ratio of albumin to proalbumin in the subcellular fractions isolated from colchicine-treated rat liver

Subcellular fraction		Radioactivity (dpm)*)		Ratio
		Proalbumin	Albumin	(Alb./Proalb.)
GF-1	1)**)	9,120 (70.5)	3,820 (29.5)	0.42
	2)	5,630 (71.4)	2,260 (28.6)	0.40
GF-2	1)	29,180 (78.9)	7,810 (21.1)	0.27
	2)	8,590 (76.4)	2,660 (23.6)	0.31
GF-3		8,270 (79.2)	2,170 (20.8)	0.26
Microsomes		30,900 (84.8)	5,530 (15.2)	0.18

*) Values for proalbumin and albumin represent the total radioactivity (dpm) in the pooled fractions corresponding to proalbumin and albumin, respectively, separated by DEAE-cellulose chromatography as described in Fig.1 and 2. Values in parentheses represent the percentages of the total radioactivity recovered from the column chromatography.
**) Number of experiment.

accumulated in the liver after colchicine treatment. While this study was in progress, Dorling et al.(18) reported that colchicine did not cause accumulation of newly synthesized albumin in the liver despite its marked inhibition of secretion. Our results confirmed their evidence, revealing that conchicine is not a pertinent tool for our experimental purpose.

Secretory vesicles were also obtained in large quantity from ethanol-treated rat liver as a result of increase in lipoprotein synthesis (10). As shown in Fig.2a, DEAE-cellulose chromatography of the labeled albumin from the microsomes gave the similar elution profile as that of the microsomal fraction from colchicine-treated rats (Fig.1a). Fig.2b shows an elution profile of the albumin from the cisternae-rich fraction of the Golgi complex, indicating that prominent conversion did not occur in this organelle, although a slight increase of radioactivity was found in the fractions of serum albumin as compared with that of the microsomes (Fig.2a).

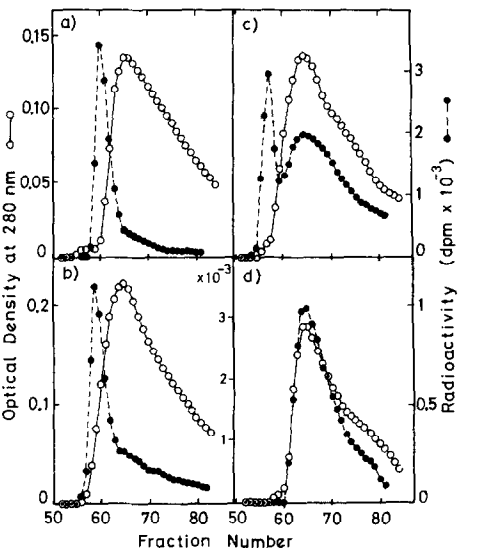

Fig. 2 DEAE-cellulose chromatography of albumin isolated from liver and serum of ethanol-treated rats. Chromatography was performed as described in Fig.1. Serum was collected from the same rats, and treated in a similar way for isolation of the labeled albumin but no carrier albumin was added in the sample for the chromatography. a) microsomes, 22,000 dpm; b) GF-3 (cisternal fraction), 24,000 dpm; c) GF-1 (secretory vesicles), 62,000 dpm; and d) serum, 11,000 dpm.

In contrast to the above results, the labeled albumin from the secretory vesicles separated into two peaks (Fig.2c). More than 70% of the radioactivity was found in the second peak which was eluted together with carrier serum albumin, indicating that the conversion of proalbumin into albumin occurred in the secretory vesicles. Table 2 shows the summary of proportions of albumin to proalbumin found in each subcellular fraction isolated from ethanol-treated rat liver. The values in the GF-2 would be reasonable because it is an intermediate fraction consisting of cisternae and the secretory vesicles. Although the ratio of albumin to proalbumin in the GF-3 shows a significant increase as compared with that in the microsomal fraction, it is not certain whether this increase indicates the onset of conversion at the cisternae of the Golgi complex or the contamination of the fraction with the secretory vesicles in which the majority of proalbumin was converted to albumin. In any event, the cisternae of the Golgi complex is not considered as the principal conversion site of proalbumin, which was confirmed by analyzing the labeled albumin in the cisternae-rich Golgi fraction isolated from rat liver free of effects by ethanol or colchicine (Table 2, control GF-3). Since no proalbumin was detected in the serum collected from the same rats (Fig.2d), conversion of proalbumin was

Table 2. Ratio of albumin to proalbumin in the subcellular fractions isolated from ethanol-treated rat liver

Subcellular fraction		Radioactivity (dpm)		Ratio
		Proalbumin	Albumin	(Alb./Proalb.)
GF-1	1)	11,060 (23.7)	35,650 (76.3)	3.22
	2)	4,800 (27.3)	12,920 (72.7)	2.66
GF-2	1)	14,670 (53.5)	12,730 (46.5)	0.87
	2)	8,340 (51.8)	7,750 (48.2)	0.93
GF-3		14,770 (63.8)	8,380 (36.2)	0.57
Microsomes		12,080 (81.6)	2,730 (18.4)	0.23
Control GF-3*)		9,900 (73.3)	3,600 (26.7)	0.36

*) Isolated from the intact rat liver, not pretreated with ethanol. See the Materials and Methods and Table 1 for further details.

complete before its secretion into the serum. Presence of proalbumin (about 25%) in the GF-1 might suggest that this fraction is still composed of heterogeneous vesicles in terms of the conversion process, although they appear to be homogeneous by electronmicroscopy (10-12).

In the present investigation on the conversion site of proalbumin we obtained two different results. In experiments using colchicine no prominent accumulation of the newly converted albumin was observed even in the fraction of the secretory vesicles. Although this effect of colchicine on the albumin production was also pointed out by Dorling et al. (18), our results present more direct evidence for the effect of the drug affecting the conversion process in the secretory vesicles. On the other hand, the use of ethanol made possible a detection of the conversion of proalbumin, indicating that the increase of the secretory vesicles induced by ethanol administration allowed the conversion process to proceed, resulting in apparent accumulation of the newly converted albumin.

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