THE DISTRIBUTION OF ALBUMIN PRECURSOR PROTEIN AND ALBUMIN IN LIVER

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Received July 12,1976

SUMMARY: Two different proteins precipitable with antiserum to albumin exist in liver. One is albumin, the other is precursor albumin. Liver cells in suspension contain mainly precursor, but secrete only albumin. In subcellular fractions isolated from liver homogenate, 95.3% of anti-albumin precipitable protein in the rough endoplasmic reticulum, 51.4% in the smooth endoplasmic reticulum, 33.5% in the Golgi apparatus and 0% in the supernatant fraction was precursor albumin. The results suggest that albumin precursor is synthesized in the rough endoplasmic reticulum and converted into albumin in the smooth endoplasmic reticulum and the Golgi apparatus.

After intravenous injection of [^{14}C]leucine into rats, radioactivity precipitable with antiserum to albumin in subcellular preparations from liver increased first in the rough endoplasmic reticulum, then in the smooth endoplasmic reticulum and the Golgi apparatus, and finally in the blood (1). Protein isolated from liver or hepatoma by precipitation with anti-albumin consists of an albumin-like protein and albumin. The albumin-like protein differs from albumin by an oligopeptide extension at the N-terminus (2-5) and appears to be a precursor in the biosynthesis of albumin (5-10). The results reported in this paper suggest that the conversion of precursor albumin into albumin takes place mainly in the smooth endoplasmic reticulum and to a lesser extent in the Golgi apparatus.

MATERIALS AND METHODS

The preparation of the subcellular fractions and their enzymatic characterization have been described elsewhere (11-13). Cells were incubated under conditions optimized for protein synthesis (14). Albumin and its precursor protein were isolated as reported previously (10). Analytical procedures, animals and chemicals have been described earlier (5,10,15,16).

RESULTS AND DISCUSSION

Distribution of Albumin and Albumin Precursor Protein in Liver Cell Suspensions: Liver cell suspensions were incubated for 40 min with 1 μCi/ml L-[1-^{14}C]leucine, 60 Ci/mole. Thereafter, incorporation of amino acids into protein was terminated by rapid cooling to 2°. Cells and medium were separated by centrifugation at 30xg for 15 min and anti-albumin precipitable protein was isolated from each.

The elution pattern obtained for the DEAE-cellulose chromatography of anti-albumin precipitable protein from the cells is shown in Fig.1. Two peaks were observed for both the radioactivity and the anti-albumin precipitable protein curves. The first peak corresponded to precursor albumin, the second to albumin. The majority of newly synthesized anti-albumin precipitable protein in the cells was found in the precursor albumin peak. Only a small fraction was albumin.

The pattern obtained in the chromatography of anti-albumin precipitable protein from the medium is described in Fig.2. One broad peak only was observed for the curves representing anti-albumin precipitable protein and radioactivity. Furthermore, the radioactivity and anti-albumin precipitable protein peaks

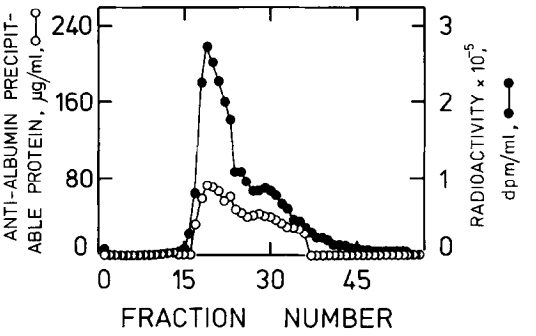

Fig.1. Chromatography on DEAE-cellulose of Anti-albumin Precipitable Protein Isolated from Rat Liver Cells Incubated with L-[1-^{14}C]leucine. Column size 10 x 200 mm. Elution with a gradient of 75 to 230 mM Tris/HCl buffer, pH 7.7. Fractions were collected beginning with the start of the gradient. Radioactivity and anti-albumin precipitable protein were measured in each of the fractions as described elsewhere (10).

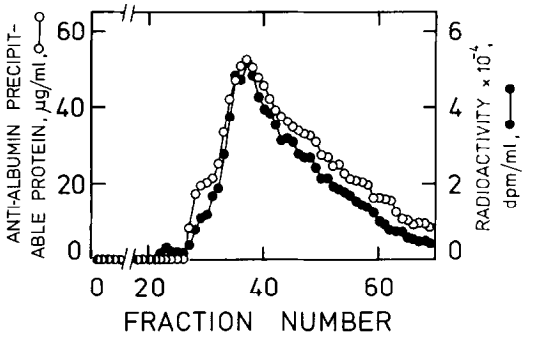

Fig.2. Chromatography on DEAE-cellulose of Anti-albumin Precipitable Protein Isolated from the Medium of Rat Liver Cells Incubated with L-[1-^{14}C]leucine. Chromatography conditions were similar to those described in the legend to Fig. 1.

coincided. The material in the combined peak fractions was identified as albumin by co-chromatography on DEAE-cellulose with albumin from serum (not shown). Thus, the medium from the cell suspensions contained albumin, but no albumin precursor.

The results depicted in Figs. 1 and 2 suggest that the conversion of precursor albumin into albumin occurs in the cells,

but not in the medium. The experiments described in the following section were designed to determine the intracellular location of the conversion of precursor albumin into albumin.

Ratio of Albumin Precursor Protein to Albumin in Subcellular Fractions from Liver: The amounts of albumin and its precursor in subcellular fractions from rat liver were estimated by measuring the dilution of added radioactively labelled precursor albumin or albumin.

Rough and smooth endoplasmic reticulum and the supernatant fraction were isolated from a homogenate prepared from 50 rat livers. Golgi apparatus was obtained separately from 20 other rat livers. The yields are given in the first columns of Table 1. All samples were stored at -20°.

Cell suspensions were prepared from 40 rat livers and incubated with 1 μCi/ml L-[U-^{14}C]phenylalanine, 495 Ci/mole, for 55 min in a total volume of 3.48 liter. Further incorporation of amino acids into protein was stopped by rapid cooling. Cells and supernatant were separated by centrifugation for 15 min at 30xg. Albumin precursor protein was isolated from the cells and albumin was prepared from the medium. 0.74 mg of precursor albumin, 2.2 mCi/g, and 3.9 mg albumin, 1.2 mCi/g, were obtained.

The specific radioactivities of this precursor albumin and albumin were adjusted by addition of non-labelled precursor albumin and albumin, respectively, to values appropriate for addition to the subcellular fractions. Then, 2.54 mg (3.43×10^5 dpm) of precursor albumin was added to the rough endoplasmic reticulum, 2.86 mg (3.12×10^5 dpm) to the smooth endoplasmic reticulum and 1.87 mg (6.02×10^5 dpm) to the Golgi

TABLE I

Proteins in Subcellular Fractions of Rat Liver

Subcellular Fraction	Protein	Protein Precipitable with Anti-albumin	Albumin Precursor	Albumin
	(mg/kg wet weight)		%	%
Rough Endoplasmic Reticulum	1 330	8.8	95.3	1.3
Smooth Endoplasmic Reticulum	320	4.6	51.4	47.9
Golgi Apparatus	380	10.6	33.5	65.7
Supernatant Fraction	16 400	400	0	103

Subcellular fractions were isolated as described in the text. Radioactive precursor albumin or albumin prepared from liver cell suspensions were added and isolated again from the mixture. Amounts of precursor albumin or albumin in subcellular fractions were determined from the dilution of the radioactive proteins by non-radioactive precursor albumin and albumin contained in the subcellular fractions. The albumin values given in the table, except that for the supernatant fraction, were corrected for contamination by absorbed albumin using the values previously estimated by addition of ^{131}I-albumin to liver before homogenization and isolation of the cell fractions (1). Therefore, the sum of precursor albumin plus albumin is not 100%.

apparatus, while 0.93 mg of albumin containing 2.37×10^6 dpm was added to 25% of the supernatant fraction. Precursor albumin and albumin were isolated again from the resulting mixtures.

From the decrease in the specific radioactivity of precursor albumin, the amounts of precursor albumin present in the rough endoplasmic reticulum, smooth endoplasmic reticulum and Golgi apparatus prior to addition of the labelled compound were calculated. The amount of albumin in the supernatant fraction was determined by measuring the decrease in the specific radioactivity of added radioactive albumin. The values obtained are summarized in Table 1. The amounts of albumin in the rough endoplasmic reticulum, smooth endoplasmic reticulum and Golgi apparatus and of precursor albumin in the supernatant fraction were calculated by subtracting the values for precursor albumin and albumin, respectively, from total anti-albumin precipitable protein in the subcellular preparations. No significant amount of albumin was found in the rough endoplasmic reticulum. Thereafter, the ratio of albumin to albumin precursor increased from smooth endoplasmic reticulum to Golgi apparatus to the supernatant fraction which contained albumin only. Albumin in the supernatant fraction reflected mainly blood albumin from the livers used to obtain the subcellular fractions (18). Contamination of the subcellular fractions other than the supernatant fraction by albumin of extracellular origin can probably be neglected (1,17).

Table II summarizes the enzymatic characteristics of the cell fractions used in this study. From the estimates of cross-contamination based on this data, the smooth microsome fraction contained less than 10% Golgi apparatus and the Golgi preparation was contaminated with endoplasmic reticulum to about 18%. Therefore, the amount of cross-contamination in the cell fractions could not account for the large differences in the

TABLE II

Enzymatic Characterizations of Liver Cell Fractions

SPECIFIC ACTIVITY

	Galactosyl Transferase	Glucose-6-phosphatase	5'-Nucleotidase	NADH-Cytochrome c reductase	Succinate-Cytochrome c reductase	% Contamination with Endoplasmic Reticulum*	% Contamination with Golgi**
Golgi Apparatus	641	0.061	0.235	0.399	0.043	18	-
Rough Endoplasmic Reticulum	12	0.305	0.046	2.27	0.012	-	1.9
Smooth Endoplasmic Reticulum	41	0.196	0.192	2.53	0.005	-	6.4

The specific activity is expressed as μmoles/min/mg protein of the original preparation (before mixing with albumin or its precursor), except for the specific activity of galactosyl transferase, which is given in nmoles/hr/mg protein. Assays were carried out at 32° except those for glucose-6-phosphatase and galactosyl transferase which were performed at 37°.

* Estimated from the amounts of glucose-6-phosphatase and NADH-cytochrome c reductase compared to the values for smooth endoplasmic reticulum.

** Estimated from the galactosyl transferase value.

ratio of precursor albumin to albumin observed in the subcellular fractions.

The results presented in this paper strongly support the concept that albumin is synthesized via a precursor protein and that precursor albumin is converted into albumin in the smooth endoplasmic reticulum and the Golgi apparatus. The presence in these two organelles of a proteolytic enzyme involved in the conversion is thus indicated. The steady state ratios of albumin precursor to albumin which we observed in the purified cell fractions are minimal values since some continuation of proteolysis may occur during the 6 to 12 hours required for isolation.

Acknowledgements: The able technical assistance of Robert Katt and Kathryn Dewey is acknowledged. This study was supported in part by NIH grants AM 14632 and AM 17223 and by a grant from the N.H. & M.R.C.

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