RAT SERUM ALBUMIN BIOSYNTHESIS: EVIDENCE FOR A PRECURSOR

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SUMMARY: An albumin-like protein (pro-albumin) has been isolated from rat liver microsomal albumin by isoelectric focusing. Unlike intracellular serum albumin, it becomes highly radioactive in the course of albumin biosynthesis at the expense of labeled amino acids in vivo. The albumin-like protein is immunologically indistinguishable from rat serum albumin and has the same (or similar) N-terminus: glutamate (or glutamine). Maps of tryptic peptides suggest that the albumin-like protein may be a peptide derivative of serum albumin.

Recent studies of serum albumin biosynthesis and secretion by rat liver suggest that a polypeptide precursor may be involved in this process (1, 2). In vivo, secretion of labeled serum albumin begins only after a delay of about 10-15 minutes following injection of labeled amino acids. During this lag period the level of radioactivity in microsomal albumin rises to a maximum value (2, 3). Fractionation of microsomal albumin isolated at this stage indicates that the bulk of the label is found in an "albumin-like" protein (or proteins) having a more alkaline isoelectric point than serum albumin (2).

In support of the precursor hypothesis we find that serum albumin and albumin-like proteins are immunologically indistinguishable, have the same (or similar) N-termini, and yield (with one exception) the same tryptic peptides. This and previous evidence (2) provide strong support for the proposal that the albumin-like protein from the liver microsomal albumin fraction is indeed pro-albumin (4).

METHODS AND MATERIALS

Preparation of labeled microsomal albumin. Female Wistar

rats (180-200 g) obtained from National Laboratory Animal Co. (Creve Coeur, Mo.) were injected i.p. under light ether with radioactive amino acids in 150 mM NaCl ([^{14}C]-protein hydrolysate, or L-leucine-1-[^{14}C], Amersham/Searle Corp.). After 15 min (unless otherwise indicated), the animals were killed and deoxycholate extracts of liver microsomes prepared as previously described (2). Microsomal albumin was obtained by precipitation with excess rabbit or goat antiserum (antisera prepared by immunization with rat serum albumin purified by isoelectric focusing), followed by dissociation of the antibody precipitate. Then carrier albumin, prepared from rat serum, was added and the radioactive proteins precipitated by ether (5).

Isoelectric focusing. This was carried out as previously described (2), using pH 5-7 carrier ampholyte (LKB), except all solutions other than that for the cathode were 6 M in urea. The urea (ultrapure, Schwarz/Mann) was dissolved just before use. Columns were run at 1000 V. for 40 hr at 2°. Fractions were collected with an LKB 7000 Ultrorac. Each measured pH (at 20°) was corrected for the effect of urea upon the electrodes by subtraction of 0.42, according to Ui (6).

Electrophoresis in SDS[1] polyacrylamide gel was performed according to Weber and Osborn (7) using an 8 % gel with 2.7 % crosslinking.

Trypsin. Trypsin Sigma Type III was treated with TPCK according to Carpenter (8).

RESULTS AND DISCUSSION

Isolation and characterization of the albumin-like protein. In order to study the highly radioactive component in the micro-

[1] Abbreviations: SDS, sodium dodecyl sulfate; TPCK, L-(1-tosylamido-2-phenyl) ethyl chloromethyl ketone; Tris, tris (hydroxymethyl)amino methane.

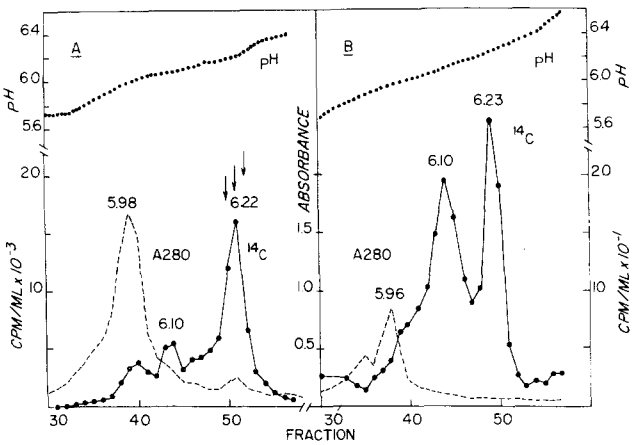

Figure 1. Microsomal albumin was made from 8 rat livers (54.3 g, wet wt.), as described in Methods, using rabbit antiserum. One rat had been labeled with ^{14}C-leucine (25 µCi). A: The pooled microsomal albumin fraction was electrofocused with 30 mg carrier serum albumin. The protein obtained from combined fractions 50, 51, and 52 (arrows) is designated albumin-like protein. B: An aliquot (10,000 cpm) of albumin-like protein from A (see text), combined with 17 mg carrier serum albumin, was electrofocused. For A and B, 32 drop fractions were collected. Dashed line, absorbance at 280 nm; closed circles, ^{14}C. Numbers above the curves are the apparent isoelectric points.

somal albumin fraction, milligram amounts of this labeled protein were required. We failed to do this with procedures which did not use antibody (e.g., solvent and salt fractionation, gel chromatography, isoelectric focusing). A variety of labeled proteins invariably were obtained which had more acidic isoelectric points than that derived with antibody. Added marker serum albumin suffered the same fate. These artifacts may have been generated by partial proteolysis (lysosomal proteases?).

We finally resorted to antibody precipitation of microsomal albumin from deoxycholate extracts, followed by electrofocusing of the dissociated albumin in 6 M urea (Fig. 1A). Urea had to be used here; otherwise several more acidic species again were obtained. Although the profile of radioactivity in Fig. 1A is identical to that obtained in labeling experiments with one rat liver, a significant protein band (280 nm absorbance) is now

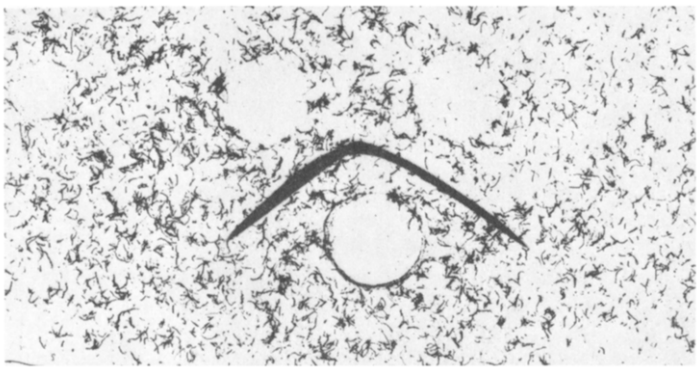

Figure 2. Double immunodiffusion on a microscope slide was carried out in 2.5 % Difco agar - 0.15 M NaCl (by modification of the method of Clausen (11)). The center well contained 10 µL rabbit antiserum, with (upper left) 8 µg albumin-like protein, compared to (upper right) 11 µg rat serum albumin. Insoluble protein was stained with coomassie brilliant blue.

evident which coincides with the major radioactive species (at pH 6.22), superimposed upon the background absorbance due to carrier ampholyte. From the run shown in Fig. 1A, fractions 50, 51, and 52 (arrows) were combined, yielding 650 µg albumin-like protein (after dialysis and gel filtration to remove urea, sucrose, and ampholyte).

After storage for a month in water at -20° C, an aliquot of albumin-like protein was combined with carrier serum albumin and electrofocused (Fig. 1B). Much of the original radiochemical species (focused at pH 6.23 in Fig. 2A) is now found at pH 6.10. No radioactivity is associated with the serum albumin marker. The same results (Fig. 1A and B) were obtained with a second preparation. These observations suggest that the secondary band of radioactivity at pH 6.1 which we routinely observe in preparations of labeled microsomal albumin electrofocused in 6 M urea may be derived from the more alkaline major radioactive species.

Preparations of albumin-like protein gave a single band on electrophoresis in SDS-polyacrylamide gels which could not be

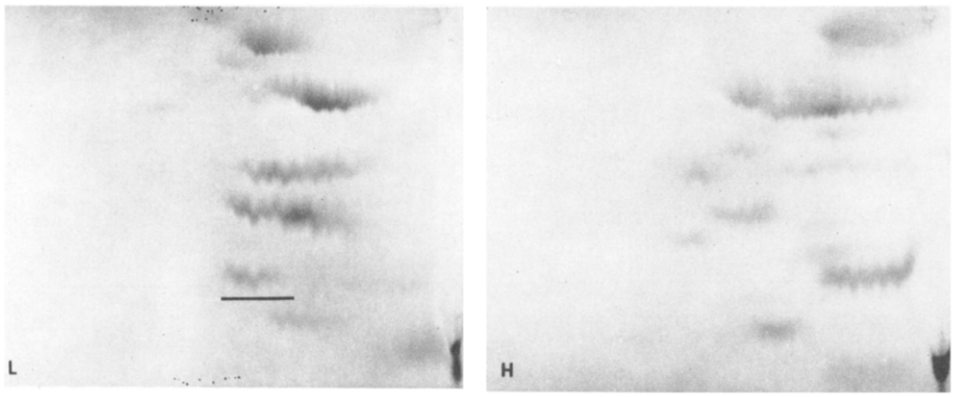

Figure 3. Radioautograms (L and H) of two dimensional tryptic peptide maps of microsomal albumin labeled with [^{14}C]-protein hydrolysate. Labeled microsomal albumin (77,600 cpm, from one rat given 50 µCi, mixed with carrier serum albumin, 12.8 mg total protein) was reduced and cyanoethylated by a modification of the method of Seibles and Weil (13). The protein (6 mg/ml, in 6 M guanidine HCl-0.2 M Tris pH 9.4) was reduced by dithiothreitol (ten fold molar excess over protein disulfide) over nitrogen for 4 hr at 20°. Then the pH was lowered to 8.7 with 1 M Tris HCl, and acrylonitrile (eight fold molar excess over dithiothreitol) added. After 1 hr the reaction was stopped with excess mercaptoethanol (1 vol/vol acrylonitrile). After exhaustive dialysis against water (2°), the product was freeze dried. (Carboxymethylation of this with iodoacetamide and subsequent amino acid analysis showed < 0.3 out of the original 35 µmoles -SH/55 µmoles leucine available for carboxymethylation; no alkylation of lysine could be detected). The cyanoethylated protein then was acylated by a modification of the method of Atassi and Habeeb (14). Citraconic anhydride (100 x excess over free amino groups) was added to the protein in 6 M guanidine HCl-0.1 M pyrophosphate pH 8.1, maintaining the pH at 8.0-8.4 by addition of solid Na_2CO_3 (stirring). The mixture then was dialyzed briefly (against 50 mM pyrophosphate pH 9.3) to remove excess salt. Acylation was repeated as above after first readjusting the pH to 8.1 (with solid Na_2HPO_4). Less than 5-10 % of the free amino groups of the starting material remained in the product. The acylated protein was transferred to 50 mM NH_4HCO_3 by gel filtration, then digested by trypsin: to 1 mg/ml 50 mM NH_4HCO_3 was added trypsin (10 µg/mg). After 4 hr at 37°, an equal amount of trypsin was added. After a total of 8 hr, excess soybean trypsin inhibitor was added and the digest fractionated on G-50 Sephadex (0.9 x 56 cm) in 50 mM NH_4HCO_3: a high molecular weight fraction (H), emerging in the region of the void volume, and a low molecular weight fraction (L), the remainder of the column. H & L were deacylated (adjusted to pH 4 with acetic acid, then incubated 4 hr at 40°); tryptic digestion of H then was repeated (as above).

The peptide maps (L, 2.6 mg protein and 19,000 cpm, and H, 3.8 mg protein and 33,000 cpm, spotted in upper right corner) were generated by electrophoresis (to left) in pyridine acetate pH 3.6, followed by chromatography in butanol-acetic acid-water for 19.5 hr at 20°. After exposure to film (Kodak No Screen, NS54T) for two months, papers were sprayed with ninhydrin and Sakaguchi reagents (15). The only radioactive area not giving a color reaction with both reagents is underlined (in panel L).

distinguished from rat serum albumin. A single N-terminal amino acid was found by dansylation and electrophoresis (9): glutamate (or glutamine). The N-terminus of rat serum albumin is glutamate (10). On double immunodiffusion with rabbit antiserum directed against rat serum albumin, the albumin-like protein appeared to be immunologically identical to rat serum albumin (Fig. 2).

Peptide maps of serum albumin and albumin-like protein. These data suggest that serum albumin and albumin-like proteins differ only by additional charged residues. Our peptide maps also are consistent with this view (Fig. 3).

In order to avoid the large number of peptides anticipated in a complete tryptic digest (4, 10, 12), we used a two-step digestion (detailed in Fig. 3). A preparation of labeled microsomal albumin (separate experiments showed 70-80 % of the label was associated with the albumin-like protein, as in Fig. 1) was subjected to reductive alkylation (cyanoethylation); the product then was acylated to block all (lysine) amino groups. Exhaustive tryptic digestion of this derivative gave a mixture of arginine peptides, which were split into low (L) and high (H) molecular weight fractions by gel filtration. L was deacylated and mapped directly; H was deacylated, then subjected to trypsin again (to give a mixture of arginine and lysine peptides) before mapping. All of the radioactive spots (Fig. 3L and 3H) coincided exactly with carrier peptides (ninhydrin stain), except for one spot (underlined, in L). This spot, unlike all of the other labeled peptides in L, also did not stain for arginine. Furthermore, it was not found on analogous peptide maps prepared from labeled serum albumin (which appeared to be otherwise identical to L and H). In a recent study of more complex tryptic peptide maps prepared by conventional methods, Judah, et al., have made similar observations (4).

The albumin-like protein thus may contain a unique peptide (released by trypsin) in addition to (or substituting for a portion of) the amino acid sequence of serum albumin. In any event, the great degree of homology is consistent with the proposed precursor-product relationship between albumin-like protein (pro-albumin (4)) and serum albumin.

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