BIOLOGICAL EVIDENCE FOR A PRECURSOR PROTEIN OF SERUM ALBUMIN

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Received April 8, 1975

SUMMARY: Radioactive leucine was injected into the portal vein of rats followed after 15 seconds by a 180 fold excess of non-radioactive leucine. An albumin-like protein in the liver became highly labelled within 15 minutes after injection. After 150 minutes, the radioactivity in the albumin-like protein had decreased to one tenth. In the serum, radioactively labelled albumin started to appear after 15 minutes and increased thereafter up to 150 minutes after injection. Radioactivity in albumin within the liver remained constant at a low level. These results suggest that the albumin-like protein is a biological precursor protein of serum albumin, i.e. a proalbumin.

Albumin isolated from liver or hepatoma homogenates by immunoprecipitation was contaminated with a protein of a high turnover rate. Further purification including repeated ion-exchange chromatography and/or disc electrophoreses in polyacrylamide gels of various pH is necessary to yield pure albumin (1-6). Incorporation of [^{14}C]leucine into albumin continued after addition of cycloheximide or excess [^{12}C]leucine to liver slices, suggesting the existence of a precursor protein of albumin (7). Isolation, amino acid composition and N-terminal amino acid sequence of an albumin-like protein from rat liver microsomes were reported recently (8). The albumin-like protein differed from albumin by an N-terminal pentapeptide extension, suggesting a possible biological conversion into albumin by removal of the five amino acids from the N-terminus (8).

In this paper, studies on the time course of [^{14}C]leucine incorporation into the albumin-like protein, albumin and total protein in liver and serum are described. The results strongly

support the hypothesis that the albumin-like protein is indeed a proalbumin, i.e. a biological precursor protein of serum albumin.

MATERIALS AND METHODS

Affinity Chromatography: Sepharose 4B was activated with 25 g cyanogen bromide per 100 g of Sepharose (9). Albumin (50 mg) purified from rat serum (6) was covalently linked to 20 g of activated Sepharose in 0.1 M sodium carbonate-bicarbonate, pH 9, plus 1 M sodium chloride (9). Antibodies to albumin from rat serum were raised in rabbits (6) and isolated by chromatography on albumin-Sepharose. One gram of these antibodies was coupled to 200 g activated Sepharose in 0.1 M sodium citrate, pH 6.5, plus 1 M sodium chloride (9).

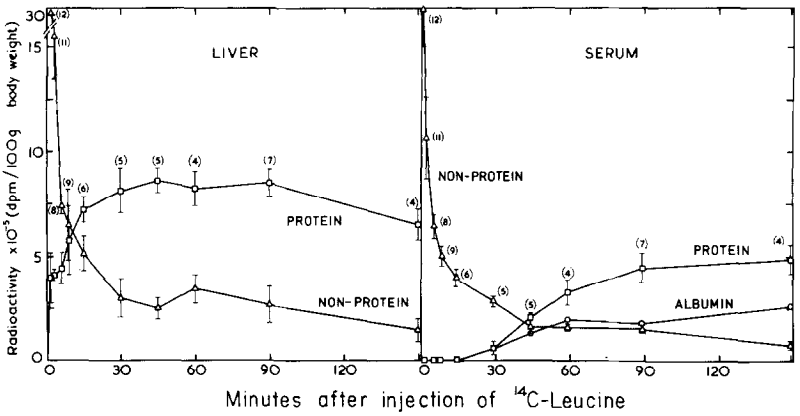

Fig. 1. Radioactivity in Protein and Non-Protein in Liver and Serum after Intraportal Injection of [^{14}C]Leucine. Male buffalo rats starved overnight received 5 µCi L-[1-^{14}C]leucine (59 Ci/mole) per 100 g body weight followed 15 seconds later by 15 µmoles L-[^{12}C]leucine. At indicated times 2 ml of blood were taken from the caval vein and livers were clamp-frozen and homogenized in ice-cold buffer. The number of animals for each time point is given in parentheses. Standard deviations are indicated. Albumin was isolated to radiochemical purity (6) from the combined sera of each time group. The average liver weight of the starved rats was 3.1 g per 100 g body weight. The average serum volume per 100 g body weight was assumed to be 3.3 ml (10).

Liver homogenates were treated with 0.7% deoxycholate followed by precipitation with 5% trichloroacetic acid, two washes of the precipitate with ethanol/ether (1:2, v/v) and two washes with ether. The precipitate was then extracted with 0.5 M Tris/HCl, pH 7.7. The extract was diluted 1:5 with water and all protein binding to anti-albumin was separated by affinity chromatography on the anti-albumin-Sepharose.

All other methods were as described previously (6).

RESULTS AND DISCUSSION

Radioactive leucine was injected into the portal vein of rats followed after 15 seconds by a chase with non-labelled leucine.

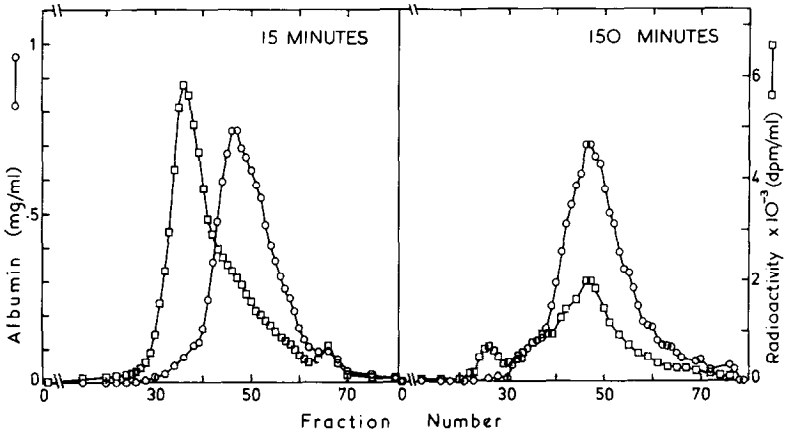

Fig. 2. DEAE-Cellulose Chromatography of Anti-Albumin Binding Protein from Livers 15 Minutes and 150 Minutes after Injection of [^{14}C]Leucine (see Fig. 1). Protein binding to anti-albumin was separated from the liver homogenates by chromatography on anti-albumin-Sepharose 4B (see Materials and Methods). In the two ion-exchange chromatographies, the specific radioactivity of albumin was constant throughout fractions 42 to 62 (= albumin peak). The curves of the absorbance at 280 nm (not shown in figure) and albumin concentration in the eluate ran parallel. Column: 1 x 40 cm. Elution: at 19 ml/hr with Tris/HCl, pH 7.7, in the following order: 1) 300 ml 10 mM, 2) 40 ml 50 mM and 3) 250 ml of a linear gradient of 50 mM to 250 mM buffer. Fractions of 2.1 ml were collected after 70 ml of the gradient had passed the column.

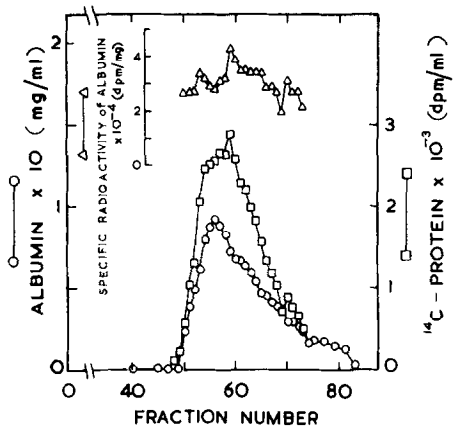

Fig. 3. Co-Chromatography on DEAE-Cellulose of GlyValPheSerArg-Albumin (8) from Liver Microsomes and the Highly Labelled Albumin-Like Protein from Total Liver Homogenates. The non-labelled GlyValPheSerArg-albumin was isolated from microsomes as described recently (8) and 1.3 mg were mixed with 0.5 mg (55,000 dpm) highly labelled albumin-like protein eluted from DEAE-cellulose prior to albumin. The highly labelled protein was isolated from total liver homogenates 12 minutes after intracaval injection of 100 µCi L-[1-^{14}C]leucine without using immunoprecipitation (6). Using anti-albumin, both proteins were measured in a single radial immunodiffusion technique (11) with rat serum albumin as a standard. Curves of absorbance at 280 nm (not shown in Figure), radioactivity and albumin coincided. Column: 1 x 24 cm. Elution: as described for Figure 2, except, 3) 160 ml of 50 mM to 300 mM buffer, collected in fractions of 1.3 ml.

The radioactivity in total liver protein increased for about 30 minutes after injection and remained constant thereafter for a further 60 minutes (Fig. 1). In the serum, radioactivity in total protein and in albumin appeared after 15 minutes and increased thereafter throughout the whole period of observation. In the liver, an albumin-like protein became highly labelled to about 90,000 dpm/mg within 15 minutes (Fig. 2). After 150 minutes, the radioactivity in the albumin-like protein had decreased to a tenth of the 15 minute value. In contrast, the specific radioactivity of liver albumin was the same at both times, being only 2,600 dpm/mg (Fig. 2). No albumin-like protein

can be found in serum (6,8). Co-chromatography on DEAE-cellulose (Fig. 3) suggests that the highly labelled albumin-like protein eluted prior to albumin (see Fig. 2) is identical with a recently described protein different from albumin by a pentapeptide extension at the N-terminus (8). In conclusion, chemical structures and kinetics of labelling support strongly the hypothesis that the albumin-like protein is an *in vivo* precursor protein to albumin, i.e. a proalbumin, which is converted into albumin during or shortly after secretion.

ACKNOWLEDGEMENT. We are very grateful to Miss P. Heyma for excellent technical assistance. This work was supported by NHMRC Grant No. 162.

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