CHEMICAL EVIDENCE FOR THE DIFFERENCE BETWEEN ALBUMINS FROM MICROSOMES AND SERUM AND A POSSIBLE PRECURSOR-PRODUCT RELATIONSHIP.

J. Urban, A.S. Inglis, K. Edwards, and G. Schreiber

The Russell Grimwade School of Biochemistry (J.U., K.E., G.S.), Melbourne University, and Division of Protein Chemistry (A.S.I.), CSIRO, Parkville, Vic. 3052 Australia.

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SUMMARY: Immunologically isolated albumin from rat liver microsomes separates on DEAE-cellulose into almost equal proportions of an albumin-like protein and authentic albumin. Besides this similarity in immunological properties, both albumin species have almost the same molecular weight and amino acid composition. Furthermore, the amino acid sequences appear to be identical apart from an additional pentapeptide at the N-terminus of the albumin-like protein. It is suggested that the albumin-like protein represents a precursor which is converted to albumin by release of a pentapeptide from the N-terminus.

The following observations are simply explained by assuming a polypeptide precursor (a proalbumin) in the biosynthesis of serum albumin: (a) The difficulties in isolating albumin to radiochemical purity caused by the presence of a highly labelled albumin-like protein found only in liver and hepatoma (1-7). (b) The synthesis of an albumin-like protein, but not of albumin, in cell-free systems from liver (8). (c) The unlikely short half-life of 8 hours of total liver protein, calculated from net synthesis rate of albumin and the ratio of $[^{14}C]$albumin over total $[^{14}C]$protein in the liver (9,10). (d) The further increase of radioactivity in albumin after addition of cycloheximide or excess $[^{12}C]$leucine to liver slices incubated with $[^{14}C]$leucine (11).

Peptide maps of the albumin-like protein and of serum albumin are almost identical (12,13), and the same N-terminal amino acid was reported for both proteins (13). The results presented in this paper indicate that the amino acids at the N-

terminus differ. However, apart from an amino terminal pentapeptide extension of the albumin-like protein, the sequences of both molecules apear to be identical. Thus, this chemical evidence suggests that the albumin-like protein may be a proalbumin which is converted into albumin by release of a small peptide from the N-terminus.

MATERIALS AND METHODS

Maintenance of Buffalo rats, procedures for assays of protein, albumin and radioactivity, purification of authentic albumin from serum for use as antigen, and preparation of antiserum were as described previously (6). Radiochemically pure ^{14}C-labelled authentic albumin was obtained from the serum of one rat, which had received via portal vein 1.55 mCi of L-[U-^{14}C]-leucine (311 Ci/mole; Radiochemical Centre, Amersham, Great Britain), by fractionation with trichloroacetic acid, ethanol, ether and chromatography on DEAE-cellulose (6). Disc electrophoresis on sodium dodecyl sulfate polyacrylamide gels was performed according to the method of Laemmli (14) in 10% gels. Gels were stained with Coomassie blue and further analysed by autoradiography (15).

RESULTS AND DISCUSSION

Isolation and Identification of Albumin-Like Protein. Albumin was isolated from liver microsomes by immunoprecipitation with antiserum to authentic albumin from serum. Separation of the albumin from its antibody (16) yielded albumin preparations of 94 to 100% purity as regards other protein. This albumin could be separated into two fractions by chromatography on DEAE-cellulose (Fig. 1). The data of a typical isolation procedure are shown in Table 1. These experiments have been repeated five times with similar results, each time using freshly prepared microsomes. Assuming the same recovery for both of the albumins,

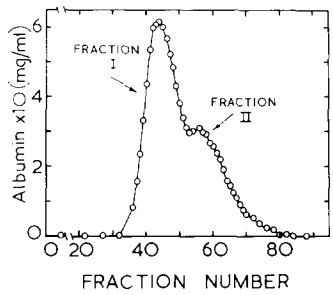

Fig. 1. Chromatography of Microsomal Albumin on DEAE-cellulose. Elution pattern from DEAE-cellulose (Table 1). The patterns of albumin elution and the absorbance at 280 nm (not shown in figure) coincided perfectly. Column: 1 x 31 cm. Elution: at a flow rate of 19 ml/hr with Tris/HCl, pH 7.7, of the following concentrations: 1) 60 ml 10 mM, 2) 35 ml 50 mM and 3) linear gradient of 100 ml 50 mM to 100 ml 300 mM. Fraction size 2.3 ml.

Table 1

Typical Protocol for Isolation of Two Albumin Fractions from Rat Liver Microsomes

Purification step		Protein (mg)	Albumin (mg)
Microsome homogenate + 0.7% deoxycholate		9,900	65
Immunoprecipitation, TCA, ethanol, ether		27	26
DEAE-cellulose*;	Fraction I	-	15
	Fraction II	-	10

* For physical and chemical studies Fraction I was further purified on a second DEAE-cellulose column.

Preparation of microsomes from 268 g of perfused liver. Perfusion with 60 ml of buffer (100 mM Tris-HCl, 250 mM sucrose, 25 mM KCl and 5 mM $MgCl_2$; pH 7.6). Homogenization in 2 volumes of the same buffer with 8 strokes in a Potter-Elvehjem (0.4 mm clearance; 1800 rpm). Combined homogenates centrifuged at 1,000 x g. Pellets homogenized and centrifuged a further 3 times. The 4 supernates centrifuged for 10 min at 12,500 x g and microsomes sedimented from this supernate for 30 min at 220,000 x g.

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the amount of Fraction I in liver microsomes can be calculated from their proportions on chromatography. We found values of 100 to 140 µg per g of liver wet weight. Co-chromatography of either Fraction I ("Proalbumin", Fig. 2) or Fraction II with

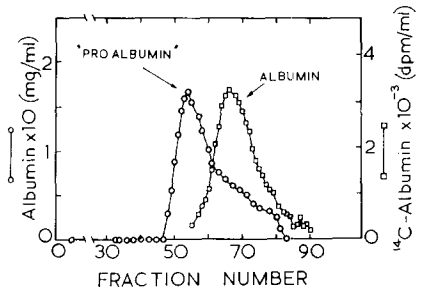

Fig. 2. Co-chromatography of Fraction I and ^{14}C-labelled Authentic Albumin from Serum on DEAE-Cellulose. An aliquot (3.6 mg) of the combined fractions 33 to 47 (Fig. 1) was mixed with 102,400 dpm authentic [^{14}C]albumin (1.25 mg) and chromatographed as described for Fig. 1. Column: 1 x 27 cm and 1.6 ml fractions. The albumin peak ("Proalbumin") corresponds to Fraction I albumin, the radioactivity peak (Albumin) corresponds to authentic albumin from serum. Again, absorbance peak (not shown in figure) and albumin peak coincided. Recovery of both total albumin and radioactivity was about 80%.

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^{14}C albumin from serum revealed that only the latter corresponds to authentic albumin. Thus, Fraction I is an albumin-like protein, which is precipitable with anti-albumin but is eluted prior to authentic albumin.

Close Similarity in Molecular Size of Albumin-Like Protein and Authentic Albumin. A mixture of Fraction I (4.5 mg) and ^{14}C-labelled authentic albumin (0.5 mg, 51,000 dpm) was chromatographed on a column of Sephadex G 100 (1 x 100 cm). Recovery of the sample was 97%. The specific radioactivity of albumin was constant throughout the fractions. Electrophoresis of another sample (2 µg Fraction I albumin + 5 µg [^{14}C]albumin) on sodium dodecyl sulfate polyacrylamide gels resulted in one single protein band at the position of albumin. This band coincided with the radioactivity band as shown by autoradiography.

Close Similarity in Amino Acid Compositions of the Two Proteins. Only the arginine and glycine values differed by more than 4% in both the 24 h and 48 h hyrolyses (Tab. 2). Assuming a value of

Table 2

Amino Acid Composition
of Albumin (A) and Albumin-Like Protein (P)

Amino Acid	Number of Residues			Mole % of Residues		
	A	P	Diff.	A	P	% Diff
ASP	53	53	-	9.2	9.0	-2
THR	31	32	1	5.4	5.5	+2
SER	24	24	-	4.1	4.2	+2
GLU	81	82	1	14.2	14.1	-1
PRO + ½CYS	60	60	-	10.4	10.2	-2
ALA	60	61	1	10.6	10.6	-
VAL	33	34	1	5.8	6.0	+3
MET	6	6	-	1.0	1.0	-
ILE	15	14	-1	2.5	2.4	-4
LEU	56	56	-	9.8	9.6	-2
TYR	21	22	1	3.7	3.8	+3
PHE	25	26	1	4.4	4.4	-
GLY	18	20	2	3.1	3.4	+10
TRP	1	1	-	0.2	0.2	-
LYS	53	53	-	9.2	9.2	-
HIS	15	15	-	2.5	2.6	+4
ARG	22	24	2	3.8	4.1	+8
TOTAL	574	583	9			

Proteins (0.5 mg of each) hydrolyzed under identical conditions in 4M methanesulfonic acid (17) for 24 h and 48 h periods and analysed with a Beckman 120B amino acid analyzer with a scale expander. Number of residues to nearest integer, assuming 56 residues of leucine present in each protein (approximates to residues per 68,000 g of protein). Proline and cystine values approximate and summed; proline peak contained cysteine.

56 for the number of leucyl residues in each protein, it is likely that there are a few more residues in the albumin-like protein than in the albumin molecule. The data for albumin are in good agreement with those reported by other authors (cf. Ref. 18).

Homologous Sequences for Albumin-Like Protein and Authentic

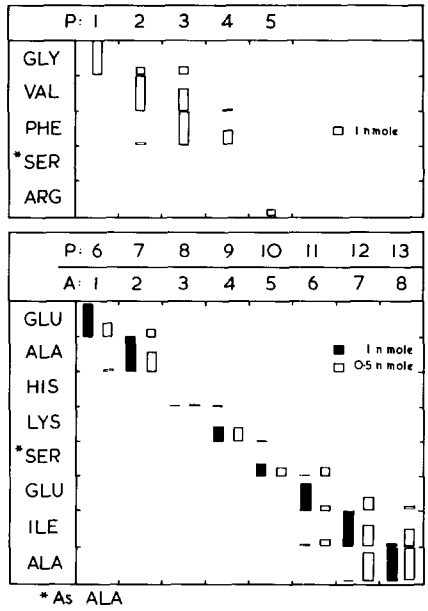

Fig. 3. Yield of amino acids after hydriodic acid hydrolysis (19) of phenylthiohydantoins (half of the sample). Sequences obtained with an Edman-Begg type (20) sequenator on 7.5 mg of albumin or 3.7 mg of albumin-like protein after an initial degradation cycle without addition of phenylisothiocyanate. Phenylthiohydantoin derivatives were also identified by thin layer chromatography (21). Serine at step 4 in the albumin-like protein was identified by the latter method only.

Albumin. The N-terminal amino acid sequences of the two proteins were determined (Fig. 3). They appear to be identical apart from a pentapeptide extension at the N-terminus of the albumin-like protein. The sequence of this pentapeptide was found to be glycine, valine, phenylalanine, serine, arginine, whereas in agreement with Bradshaw and Peters (22), the amino terminal sequence of albumin is glutamic acid, alanine, histidine, lysine, serine.

The sequence analysis of the albumin-like protein showed an indistinct first step on the thin layer chromatography plate and overlapping of residues occurred. However, amino acid analysis revealed that glycine was the only N-terminal amino acid present.

In contrast, Russell and Geller found with the dansyl chloride method a glutamyl residue (13) - the same N-terminus as for the albumin. A contamination of their preparation of albumin-like protein with albumin would be a possible explanation for the discrepancy.

The chemical evidence presented in this paper suggests a precursor (proalbumin)-product (albumin) relationship between the two albumin species in which the proalbumin is converted to albumin by release of a pentapeptide from the N-terminus.

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