BOVINE MICROSOMAL ALBUMIN: AMINO TERMINAL SEQUENCE OF BOVINE PROALBUMIN

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Received December 24,1976

SUMMARY: Bovine liver microsomes contain an albumin having an apparent isoelectric point approximately 0.3 pH unit in excess of bovine serum albumin. Sequence analysis of the purified protein shows that the first ten residues at the amino terminus are: Arg-Gly-Val-Phe-Arg-Arg-Asp-Thr-His-Lys. The data suggest that the hexapeptide (underlined), identical to that found in proalbumin from rat liver, is attached to the amino terminus of bovine serum albumin (the last four residues). By analogy with the rat liver system, this protein therefore is bovine proalbumin, a precursor of bovine serum albumin.

Recent studies of serum albumin biosynthesis and secretion by rat liver indicate that a polypeptide precursor, proalbumin, is involved in this process (1-7). These results have been duplicated by others interested in this problem (8). Only a minor disagreement has remained, regarding the structural details of rat proalbumin (9).

In an extension of a search for serum albumin precursors in other species we have examined bovine liver. We find that bovine liver microsomes contain an albumin having an amino terminal sequence identical to that found by us in rat liver. We suggest that this is bovine proalbumin.

Materials and Methods

Chemicals and Antisera - Biochemicals were the highest grade from Sigma. Carrier ampholytes for electrofocusing were from LKB. Chromatographic media were obtained from Pharmacia. Radiochemicals were products of Amersham/Searle. Rat serum albumin was made as previously described (2). Monospecific antisera directed against rat and bovine serum albumins (antigens purified by ion exchange and electrofocusing) were goat and rabbit sera, respectively.

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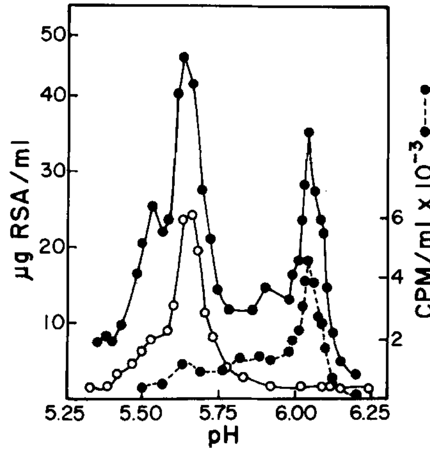

Figure 1. Rat liver: electrofocusing of albumin from microsomal and soluble fractions. A female Wistar rat (200 g) was given 25 μCi of [^{14}C]-valine i.p. After 14 min the animal was killed. The 6000 xg supernatant of the liver homogenate (2) was layered over 1.3 volumes of 0.5 M Sucrose-30 mM Tris pH 8 and centrifuged 90 min at 360,000 xg. The pellets were treated with deoxycholate to obtain microsomal albumin by antibody precipitation as before (2) except that bovine serum albumin (15 mg) was added as carrier. The albumins were electrofocused as previously described (2) (closed circles). Albumin from the soluble fraction was obtained by antibody precipitation of an aliquot (10 % of the total) of the top layer from the 360,000 xg centrifugation and electrofocused with 15 mg bovine serum albumin as above (2) (open circles). Rat albumin content of fractions was determined by quantitative immunoelectrophoresis (10).

Purification of bovine albumins - Beef liver, freshly obtained from the slaughter house, was chilled in ice. (all operations were done at 0-2° unless otherwise stated) and run through a meat grinder. Batches (300 g) were blended with 1.5 l 0.25 M sucrose for 1 min at high speed in a gallon Waring blendor. The homogenate was centrifuged 10 min at 2000 xg.

A crude microsomal preparation was made by calcium precipitation. Calcium chloride (1 M, added dropwise with stirring) was added to 10 mM, followed by centrifugation (13,000 xg, 20 min). The supernatant fluid, referred to as the *soluble fraction*, was stored at -30° for later use. The precipitate was suspended in 0.15 M NaCl and centrifuged (13,000 xg, 20 min). The washed pellet, suspended in a minimum volume of water, was added dropwise to 12 volumes of acetone and the precipitate obtained

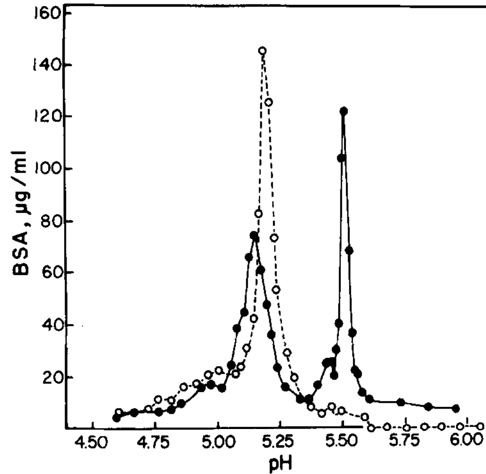

Figure 2. Bovine liver: electrofocusing of albumin from microsomal and soluble fractions. Bovine liver, freshly obtained from the slaughter house, was cut into small pieces with scissors. Microsomal and soluble fractions were obtained from 10 g, following the protocol of Fig. 1, except that a Polytron homogenizer (PT-10-ST, operated 10 sec. at high speed) was used. The albumins were obtained as in Fig. 1 using anti-bovine serum albumin and adding human serum albumin (15 mg) as carrier. Separate electrofocusing runs with microsomal albumin (closed circles) and albumin from an aliquot (7 %) of the total soluble fraction (open circles) were done as in Fig. 1 except that pH 4-6 Ampholytes were used. Bovine albumin content of fractions was determined by quantitative immunoelectrophoresis (10).

(suction filtration over Whatman #54 paper, washing with acetone and dry ether) taken to dryness (oil pump vacuum at 20°) and then stored at -30°.

Typical results obtained in the purification of *microsomal* albumin obtained by extraction of such acetone powders are given in Table 1.

Purification of albumin from the *soluble fraction* started with the addition of trichloroacetic acid (5 %) and centrifugation. The pellet then was subjected to extraction at pH 6 and purified following the protocol outlined for microsomal albumin in Table 1.

Results and Discussion

In a preliminary search for bovine proalbumin we were guided by our experience with the rat liver system. On electrofocusing (Fig. 1), rat

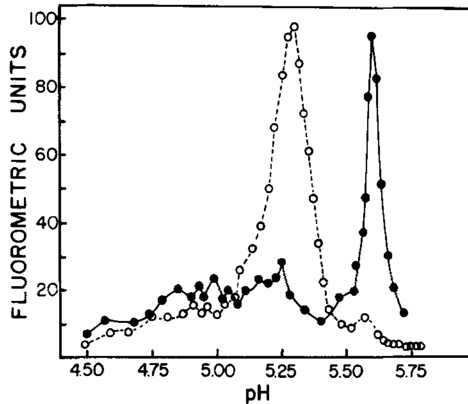

Figure 3. Isolation and characterization of bovine microsomal and soluble albumins for sequencing. Bovine albumin fractions from the same liver, purified to stage VI (Table 1) were electrofocused separately as in Fig. 2. Closed circles: microsomal albumin, 18.5 mg of protein from 180 g of liver. Open circles: albumin from soluble fraction, 25.5 mg of protein, from 30 g of liver. Albumin was determined by a fluorometric method (13). Aliquots (50 μl) of column fractions were added to 1.1 ml of 0.9 μM 8-anilinonaphthalene sulfonic acid in 90 mM potassium phosphate pH 6.8. Fluorescence was measured in a Farrand fluorometer with Corning filters 5860 (excitation) and 3385 (emission). A bovine serum albumin standard (25 μg) gave deflections of 80 (microsomal albumin) and 50 units (soluble albumin). Fluorescence was directly proportional to albumin concentration (<45 μg/ml) with the reagent blank equivalent to 0.9 μg/ml.

microsomal albumin is found to consist of rat serum albumin (unlabelled, at pH 5.70, the only component found in a sample of the soluble fraction from the same liver) and proalbumin (labelled, at pH 6.05).

An analogous experiment done with bovine liver is shown in Fig. 2. The microsomal albumin consists of two major components (apparent isoelectric points of pH 5.15 and 5.50). The acidic component is also found in the soluble fraction (pH 5.18). The isoelectric point of bovine serum albumin under these conditions is pH 5.2 - 5.3[1]. These results suggest that bovine liver microsomes, as in the case of the rat, may contain a mixture of serum albumin and proalbumin.

This suggestion is supported by analyses of milligram quantities of

[1]D. Geller, unpublished work.

Table 1
Summary of Typical Purification of
Bovine Microsomal Albumin

Fraction	Albumin	Protein	Purification (Yield)
I	40 mg	2450 mg	1x (100 %)
II	31 mg	902 mg	2.1x (78 %)
III	22.5 mg	413 mg	3.3x (56 %)
IV	18.5 mg	142 mg	8.0x (46 %)
V	10.3 mg	21.6 mg	29.3x (26 %)
VI	8.6 mg	16.7 mg	31.6x (22 %)

Acetone powder derived from 138 g calf liver was used here to prepare fraction I. Albumin was determined by quantitative immunoelectrophoresis (10). Protein was measured by colorimetric analysis, using bovine albumin standards (11). Acetone powder (12-14 gm, obtained from 140-120 gm liver) was stirred in 0.1 M Tris pH 7.8 (300 ml) for 90 min, followed by centrifugation (13,000 xg, 20 min). The extract (Fration I) was then made 5 % in trichloroacetic acid and centrifuged. The pellet, suspended in water, was brought to pH 6 by addition of Tris base and centrifuged again. The supernatant fluid (II) then was adjusted to pH 5 with acetic acid, and solid ammonium sulfate added to 75 % of saturation. The precipitate obtained by centrifugation was extracted with 50 ml 0.1 M Tris pH 7.8, and the supernatant fluid (III) applied to a G-200 Sephadex column (5 x 44 cm) equilibrated with 50 mM Tris pH 7.8. The albumin fraction from this column (IV) was applied directly to a QAE-A-50 Sephadex column (2.7 x 13 cm, 2.5 gm gel, dry weight) and chromatographed in 50 mM Tris pH 7.8 in a gradient of NaCl. The albumin fractions (eluted by 180-200 mM NaCl) were combined (V), and the protein obtained by addition of trichloroacetic acid (to 5 %) and centrifugation. The precipitate then was suspended in trichloroacetic acid (1 %), extracted with ethanolic trichloroacetic acid (1 %) and precipitated by ether, following the procedure used for dissociation of albumin-antibody complexes (12). The ether precipitate finally was extracted with water, centrifuged, and the supernatant fluid was freeze-dried (VI).

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purified bovine microsomal albumin. An example of the results of separate electrofocusing runs of purified albumins (obtained from the same liver) is shown in Fig. 3. In this case, the microsomal albumin (*closed circles*) consists of only one component (at pH 5.59), whereas the soluble fraction (*open circles*) albumin is found at pH 5.28. Preparations from other bovine livers have yielded essentially the same results: only one albumin in the soluble fraction (at pH 5.3), with the unique albumin (at pH 5.6) evident only in microsomes. However, unlike the microsomal preparation displayed in Fig. 3, all other microsomal preparations contain a mixture of the two albumins. It should also be noted that all attempts to modify our purification procedure so far have failed to improve upon it. We have not been able to obtain albumin

Table 2
Automated Edman Degradation of
Bovine Proalbumin

Cycle	Residue	Yield (nmol)
1	Arginine	8.0
2	Glycine	7.4
3	Valine	7.0
4	Phenylalanine	7.7
5	Arginine	5.2
6	Arginine	3.7
7	Aspartic acid	3.2
8	Threonine	1.3*
9	Histidine	2.6
10	Lysine	2.7

Bovine proalbumin (1 mg, 15 nmoles) was obtained by electrofocusing a preparation of microsomal albumin purified to stage VI (Table 1). The pooled presumptive proalbumin fractions (see Fig. 3) then were desalted by filtration through a G-50 Sephadex column equilibrated with 50 mM NH_4HCO_3 followed by freeze-drying. Sequencing was done with [^{35}S]-phenylisothiocyanate (1.5 Ci/mole) as detailed elsewhere (14), using a Beckman 890C Sequencer. All Sequencer reagents were purchased from Beckman.

*The threonine derivative undergoes partial decomposition during the conversion step in the Edman degradation (15).

from acetone powders prepared from frozen liver. Conventional antibody precipitation of albumins from stage I extracts (Table 1) also met with failure. Furthermore, inclusion of high concentrations (1 mM) of Phenylmethylsulfonyl fluoride at various stages in purification did not significantly modify the results.

It was noted that the difference in isoelectric points between the bovine albumins, 0.3 pH unit, is the same as that found for rat serum albumin and proalbumin (2). Sequence analysis of the alkaline bovine albumin therefore was done. The results (Table 2) show that the amino terminal sequence for the first ten residues is Arg-Gly-Val-Phe-Arg-Arg-Asp-Thr-His-Lys. The last four residues of this sequence are found in the amino terminal sequence (the first 4 residues) of bovine serum albumin (16). The first six residues are identical to the amino terminal hexapeptide of our rat proalbumin sequence (6). Other than these residues the first three cycles only gave evidence of a faint trace of the sequence Asp-Thr-His, indicating the presence of small amounts of bovine serum albumin.

It should be noted here that another laboratory has found the pentapeptide sequence Gly-Val-Phe-Ser-Arg for the amino terminal sequence of their rat proalbumin (9). We find no evidence of glycine in cycle 1. Furthermore there is no indication of serine residues in our bovine sequence.

Our finding of the same amino terminal sequence for two unique microsomal proteins from two species casts some doubt upon their results. In any event, the structural similarity to rat proalbumin strongly suggests that the alkaline albumin present in bovine liver microsomes is a bovine proalbumin.

ACKNOWLEDGEMENT

We are most grateful to Mr. John W. Jacobs for the sequence analysis. We also thank Ms. Carol Marvel for the development of the fluorometric analysis for albumin. This work was supported by United States Public Health Service grants AM-16226 and GM-21096.

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