CHICKEN MICROSOMAL ALBUMIN: AMINO TERMINAL SEQUENCE OF CHICKEN PROALBUMIN

Arnold M. Rosen and David M. Geller*

Department of Pharmacology, Washington University
School of Medicine, St. Louis, Missouri 63110

Received August 23,1977

SUMMARY: Chicken liver microsomes contain an albumin having an isoelectric point approximately 0.2 pH unit in excess of that of chicken serum albumin. Although the serum protein is also present in microsomes, only the basic albumin there becomes labelled and undergoes turnover in vivo. Sequence analysis of the purified basic microsomal albumin indicates that the first twelve residues are: *Arg-Asn-Leu-Gln-Arg-Met-Ala-Arg-Asp-Ala-Glu-His*. The data suggest that the octapeptide (underlined) is attached to the amino terminus of chicken serum albumin (the last four residues). The amino terminal sequence of the serum albumin precursor in chicken liver is thus markedly different from that of the rat and bovine proalbumins.

Recent studies of serum albumin biosynthesis and secretion by rat and bovine liver indicate that a polypeptide precursor, proalbumin, is involved in this process (1-9). The present report arises from an effort to extend this work to more distant species to see whether the pattern found in mammalian serum albumin biosynthesis is a more general phenomenon. The chicken was chosen as a representative of the line of birds, which is considered to have diverged from mammalian lines some 300 million years ago (10).

Materials and Methods

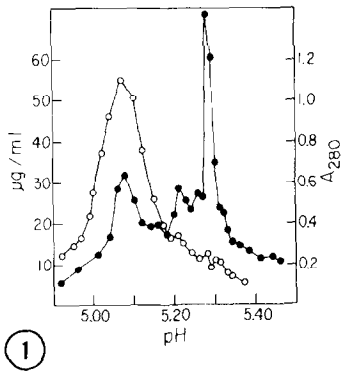
Biochemicals, including serum albumins unless indicated otherwise, were obtained from Sigma. Carrier Ampholytes for electrofocusing were from LKB. Chromatographic media were obtained from Pharmacia. Radiochemicals were products of Amersham.

Simultaneous counting of ^{14}C (35% efficiency) and ^{3}H (16.5% efficiency) was done using a Packard Model 3375 Scintillation Spectrometer.

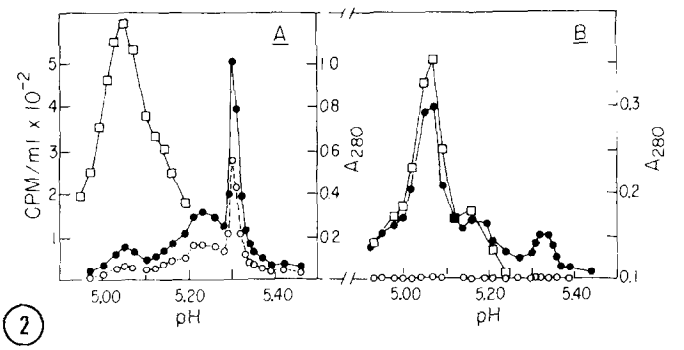
Monospecific goat antiserum against chicken serum albumin was prepared by Biotek Research, Inc., (St. Louis, Mo.) using antigen prepared by us. For this purpose crude chicken albumin was prepared by the procedure used for rat serum albumin (2); the protein was electrofocused in a pH4-6 sucrose density gradient column (as described previously (2))

*To whom all communications should be addressed.

ISSN 0006-291X



(1)



(2)

Figure 1. Comparison of chicken liver microsomal albumin with chicken serum albumin in separate electrofocusing runs (pH 4-6 gradient). Microsomal Albumin: *Closed Circles*. Using methods detailed previously (in Fig. 1 of reference 9) the albumin fraction was isolated by antibody precipitation (with goat anti-chicken albumin) from a deoxycholate extract obtained from one liver (4.2 g, from a 150 g chick) followed by acid dissociation. Electrofocusing was done with 10 mg bovine serum albumin and the chicken albumin content was determined by quantitative immunoelectrophoresis. Serum Albumin: *Open Circles*. Chicken serum albumin (18.6 mg) was electrofocused as previously described (2). Albumin content was determined by absorbance at 280 nm (A_{280}), uncorrected for Ampholytes (0.05-0.1 unit).

Figure 2. Characterization of albumin fractions labelled *in vivo*. A chicken (300 g) received 1 mCi L-[4,5-^{3}H] leucine (in 1 ml 0.9% NaCl, injected in a wing vein). At 70 min 25 μCi L-[1-^{14}C] leucine was given (in 0.5 ml 0.9% NaCl in the second wing vein). At 86 min. the animal was killed. Electrofocusing of microsomal and serum albumin fractions was done as in Fig. 1: ^{3}H, *Closed Circles*; ^{14}C, *Open Circles*; absorbance at 280 nm (as in Fig. 1), *Squares*. A. Microsomal Albumin (*Left panel*). Obtained from the liver using the procedure described in Fig. 1, albumin 31,910 cpm in ^{3}H and 15,120 cpm in ^{14}C was electrofocused with 29.3 mg chicken serum albumin. B. Serum Albumin (*Right panel*). The albumin fraction obtained from plasma (2 ml) using the procedure for preparation of rat serum albumin from serum (2):35,700 cpm in ^{3}H and 800 cpm in ^{14}C.

and the major protein band then desalted by filtration through Sephadex G-50 in 50mM ammonium bicarbonate followed by freeze-drying.

The chickens used were of the White Leghorn variety (Ken Roy, Berger, Mo.).

Results and Discussion

In the initial experiments with chicken liver, we characterized the chicken liver microsomal albumin fraction by electrofocusing. As shown in Fig. 1, this fraction consists of two major components. One is

apparently identical to the albumin found in serum (with an isoelectric point of pH 5.07). The second is a more basic protein, with an isoelectric point of pH 5.28.

Labelling experiments done in vivo indicate that this "basic albumin" behaves in a manner closely resembling that previously observed with rat proalbumin (2). The results of a typical experiment, using leucine labelled with ^{3}H and ^{14}C, are shown in Fig. 2. Injection of ^{3}H was followed 70 min. later by ^{14}C (ratio of injected cpm, $^{14}C/^{3}H$, of 0.05); 86 min following the administration of ^{3}H the animal was killed. Of the liver microsomal albumin fraction (Fig. 2a), the basic albumin (at pH 5.30 here) is the major component labelled, with essentially no incorporation at 16 min (^{14}C) in the serum protein (at pH 5.05). The latter protein may comprise as much as half of the albumin content in this preparation (Fig. 1). The ratio ($^{14}C/^{3}H$) found for the microsomal albumin fraction is 0.47. This indicates that approximately 90% of the ^{3}H label which had been present in this fraction at 16 min has left it by 86 min.

On the other hand the results found for the albumin fraction from plasma in this experiment (Fig. 2b) show that the serum protein is the major labelled component (at 86 min with ^{3}H). No labelled albumin has left the liver by 16 min (^{14}C, with a ratio of $^{14}C/^{3}H$ of 0.02). The significance of minor labelled components in plasma (at pH 5.15 and 5.33 in Fig. 2b) is not clear. Preparations of chicken serum albumin also display these minor bands on electrofocusing when assayed by immunochemical methods (as done in Fig. 1 for microsomal albumin). On sequence analysis of the protein fraction obtained at pH 5.3 by electrofocusing the serum protein in a separate experiment only the amino terminal sequence of chicken serum albumin was found. Thus the labelled component at pH 5.3 (in Fig. 2b) may be either a radiochemical contaminant, unrelated to albumin, or a derivative of newly synthesized albumin modified at some site other than that of the amino terminus.

Table I
Summary of Purification of
Chicken Proalbumin (ProCSA)

Fraction	Albumin	Protein	Purification (Yield)
I	73.0 mg	3110 mg	1X (100%)
II	66.0	558	5X (90%)
III	53.0	440	5.1X (72%)
IV	28.0	156	7.6X (38%)
V	19.0	22.4	36.1X (26%)
VI	12.0	14.1	36.2X (16%)
Pro CSA	2.8	2.8	42.6X (3.8%)

Starting with microsomal acetone powder (14.2 g, from 195 g fresh chicken liver using the procedure employed for bovine liver (9)), the purification procedure was identical to that detailed in Table 1, ref. 9. Fraction I, the initial extract, was treated with tricholoracetic acid, and a neutral extract of the acid protein precipitate was made (II). Fraction II was concentrated by acid ammonium sulfate precipitation (III) and applied to Sephadex G-200. The albumin fraction (IV) obtained in turn was chromatographed on Sephadex QAE A-50. The product (V) then was precipitated by trichloroacetic acid, dissolved in ethanol, and finally precipitated by ether to give VI. Fraction VI was electrofocused (with the result shown in Fig. 3); column fractions from pH 5.22 to 5.27 inclusive were desalted by filtration through Sephadex G-50 in 50mM ammonium bicarbonate followed by freeze-drying (pro CSA).

These results strongly suggest that the basic albumin of the microsomal fraction is a proalbumin. As is the case with the rat and bovine proalbumins, it might also possess a unique highly basic peptide at its amino terminus. Accordingly, suitable amounts of this protein were prepared for sequence analysis by chromatography (Table 1) and electrofocusing (Fig. 3). As shown in Table 2 analysis of the first twelve residues reveals only a single major amino terminal sequence: *Arg-Asn-Leu-Gln-Arg-Met-Ala-Arg-Asp-Ala-Glu-His*. The last four residues apparently represent the amino terminal sequence of chicken serum albumin (11). Only traces of a second sequence were found, apparently that of the amino terminal sequence of the serum protein (first residue, aspartic acid).

In any consideration of the significance of the structure of this albumin precursor it should be noted that additional structural differences

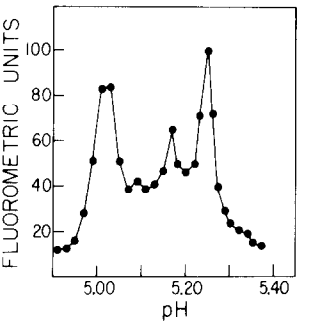


Figure 3. Final step in purification of chicken proalbumin. Fraction VI (Table 1) was electrofocused in a pH 4-6 gradient. Albumin content was determined by a fluorometric method (see Fig. 3 of reference 9) using 10 μL column fractions (with 10 μg bovine serum albumin giving a deflection of 90 units).

(from the final product, the serum protein) may also be present. This possibility is suggested by the fact that the chicken pro- and serum albumins differ in isoelectric point by only 0.2 pH unit; with the rat and bovine proteins this difference amounts to 0.3 (2, 9). Each of the unique amino terminal sequences of these precursors contains three arginine residues together with various neutral residues. It is possible that one of the positive charges contributed by the chicken pro sequence is cancelled by the addition of a single net negative charge to some other site on the protein. This might be by addition of one negatively charged residue or by elimination of one positive charge by formation of a neutral derivative.

In any case it is of interest to compare the structures of the three proalbumins. Both rat and bovine proteins have the same amino terminal hexapeptide, *Arg-Gly-Val-Phe-Arg-Arg* (6, 9). The chicken pro sequence by contrast is that of an octapeptide; although three arginine residues are present they are separated by at least two neutral residues. This situation differs from the marked homology evident when comparing chicken proparathyroid hormone with that of other species (13). Of

Table 2
Automated Edman Degradation
of Chicken Proalbumin

Cycle	Residue	Yield (nmol)
1	Arginine	6.8
2	Asparagine	7.0
3	Leucine	5.4
4	Glutamine	5.1
5	Arginine	5.2
6	Methionine	4.1
7	Alanine	3.9
8	Arginine	3.7
9	Aspartic Acid	4.2
10	Alanine	2.9
11	Glutamic Acid	2.8
12	Histidine	1.6

Chicken proalbumin (1.4 mg, 21.5 nmol) was sequenced with [^{35}S]-Phenylisothiocyanate (1.5 Ci/mole) as detailed elsewhere (12) using a Beckman 890C Sequencer. All sequencer reagents were purchased from Beckman.

course it is possible to consider that in chicken proalbumin residues 6 (methionine) and 7 (alanine) represent insertions between arginine residues 5 and 8. Indeed when compared to bovine, human and rat serum albumins, chicken serum albumin has an inserted glutamic acid as its third residue (11). At this point the amino terminal sequence of chicken proalbumin is remarkable in that it lacks the doublet of basic amino acid residues found at the junction of the pro peptide and polypeptide chains seen with the few pro proteins which have so far received extensive study (proparathyroid hormones, proalbumins and proinsulins).

ACKNOWLEDGEMENT

We are most grateful to Mr. John W. Jacobs for the sequence analysis. We also thank Mr. Charles Reed for assistance with intravenous injections. This work was supported by United States Public Health Service Grants AM-16226 and GM-21096.

REFERENCES

1. Judah, J.D., and Nicholls, M.R. (1971) Biochem. J. 123, 649-655.
2. Geller, D.M., Judah, J.D., and Nicholls, M.R. (1972) Biochem. J. 127, 865-874,

3. Russell, J.H. and Geller, D.M. (1973) Biochem Biophys. Res. Commun. 55, 239-245.
4. Judah, J.D., Gamble, M., and Steadman, J.H. (1973) Biochem. J. 134, 1083-1091.
5. Quinn, P.S., Gamble, M., and Judah, J.D. (1975) Biochem. J. 146, 389-393.
6. Russell, J.H. and Geller, D.M. (1975) J. Biol. Chem. 250, 3409-3413.
7. Dorling, P.R., Quinn, P.S., and Judah, J.D. (1975) Biochem. J. 152, 341-348.
8. Urban, J., Chelladurai, M., Millership, A., and Schreiber, G. (1976) Eur. J. Biochem. 67, 477-485.
9. Patterson, J.E., and Geller, D.M. (1977) Biochem. Biophys. Res. Commun. 74, 1220-1226.
10. Dayhoff, M.O. (1969) "Atlas of Protein Sequence and Structure", Natl. Biomed. Res. Fndn., Silver Springs, Md. 4, 40.
11. Blakeney, E.W., Jr. (1972) "Amino Acid Sequence of Chicken Serum Albumin: Tryptic Peptides." Ph.D. Thesis, University of Texas, Austin, Texas.
12. Jacobs, J.W. and Niall, H.D. (1975) J. Biol. Chem. 250 3629-3636.
13. Cohn, D.V. and Hamilton, J.W. (1976) Cornell Veterinarian 66, 278.