ABSENCE OF A SPECIFIC COPPER(II) BINDING SITE IN DOG ALBUMIN IS DUE TO AMINO ACID MUTATION IN POSITION 3

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The elucidation of the primary sequence at the NH_2-terminal of dog serum albumin was undertaken by the dansyl-Edman technique on the intact protein molecule. This study was initiated because dog albumin failed to exhibit the characteristic albumin specificity for Cu(II) binding. The results show that a mutation in the primary sequence has replaced the important histidine-3 residue, critical to the binding of Cu(II), by a tyrosine residue. The primary sequence of the first eight residues was shown to be:

Glx-Ala-Tyr(?)Ser-Glx-Ile-Ala.

Albumin has long been known as the major Cu(II) transport protein in human serum (1,2). BSA* (3,4), HSA (5,6,7) and RSA (7) show a characteristic preferential binding of one Cu(II) ion. The Cu(II) binding site in these proteins is known to involve the NH_2-terminal amino group, two intervening peptide nitrogens and the imidazole nitrogen of the histidine-3 residue (7).

It has recently been observed that DSA bound very little Cu(II) and failed to exhibit any characteristics of a specific first binding site for Cu(II) (8). It was proposed that the lack of this specificity in DSA may arise from the absence of a histidine residue in position 3. Since the primary structure at the NH_2-terminal of DSA had not been established, sequence analysis by the dansyl-Edman method was undertaken on the intact protein. The results show that a mutation in the amino acid sequence of DSA has replaced the important histidine residue, in position 3, by a tyrosine residue.

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* Abbreviations: BSA, Bovine serum albumin; HSA, human serum albumin; RSA, rat serum albumin; DSA, dog serum albumin; DNS-, dimethylaminonaphthalene-5-sulphonyl.

MATERIALS AND METHODS

Lyophilized DSA, Fraction V, was purchased from Pentex Inc., Kankakee, Illinois, and was further purified as indicated below. All chemicals involved in the dansyl-Edman reactions were sequanal grade, Pierce Chemical Co., Rockford, Illinois. All other chemicals used were of the highest purity. Polyamide plates were the product of Cheng Chin Trading Co., Taipei, Taiwan, and solutions of Ultra Pure urea (Schwarz-Mann, Orangeburg, New Jersey), were deionized before use with Rexyn 300, obtained from Fisher Scientific Co., Toronto.

DSA was purified by passing it through a Sephadex G-150 column (2.6 cm x 90 cm) equilibrated with bicarbonate buffer (1.94×10^{-2} M $NaHCO_3$, 0.5 M NaCl, $1^{\circ} \times 10^{-3}$ M NaN_3, + 5% CO_2), pH 7.5, at 4°, and eluted with the same buffer. The albumin was pooled and freeze-dried. The lyophilized protein was then dissolved in 0.5 M Tris-HCl buffer, pH 8.2, to a concentration of 10 mg/ml. Thereafter, the solution was made 8 M in urea by adding solid reagent. Reduction was carried out with 0.04 M dithiothreitol for 3 h, at room temperature. Alkylation was performed for 1.5 h at room temperature with 0.09 M iodoacetic acid. The reaction was stopped by exhaustive dialysis and the reduced and alkylated material was freeze-dried.

The dansyl-Edman degradation was performed on 400 nmoles of protein, both normal, and reduced and alkylated, according to the method of Percy and Buchwald (9). As this material represented 20 mg of protein the reaction volumes used during degradation were scaled up two-fold. The dansyl amino acids were identified on (5 cm x 5 cm) polyamide plates (10) using essentially the same chromatographic procedures as the above authors, with the addition of the following solvent system: 1 part 95% ethanol and 3 parts pyridine:acetic acid: water 1:1.1:250 (v/v/v), run at 90° to the first solvent. The majority of the hydrolyzates for the TLC plates were extracted with water-saturated ethyl acetate, except when there was an indication of a basic residue being amino-

terminal. These residues were extracted with pyridine:acetone 1:1 (v/v).

RESULTS AND DISCUSSION

The manual method of Edman degradation followed by dansylation permitted positive identification of seven of the first eight residues in both normal DSA and the reduced and alkylated DSA. The sequence was:

Glx-Ala-Tyr(?)Ser-Glx-Ile-Ala

Because of the use of whole protein material, no assignment of amides was possible. In addition, the thin layer chromatography was unable to give a positive confirmation of the fourth residue.

The only fluorescent spots observed on the TLC plate for the dansyl derivative of the third residue were a strong spot of yellow-green α-DNS-tyrosine (10), a moderate spot of yellow bis-DNS-tyrosine, and O-DNS-tyrosine. O-DNS-tyrosine seen here and on all other plates was the result of dansylation of other intrachain tyrosine residues.

This method of sequential degradation was not as efficient as automatic sequence analysis; however, its simplicity made it possible to find the critical residues of the NH_2-terminal sequence. It is interesting to note the high degree of homology which exists between the NH_2-terminal regions of BSA, RSA, HSA and DSA (11,12).

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Bovine:	Asp-Thr-His-Lys-Ser-Glu-Ile-Ala
Rat :	Glu-Ala-His-Lys-Ser-Glu-Ile-Ala
Human :	Asp-Ala-His-Lys-Ser-Glu-Val-Ala
Dog :	Glx-Ala-Tyr(?)Ser-Glx-Ile-Ala

The above primary structure had been determined on three samples of DSA, once using normal DSA and twice on reduced and alkylated DSA. The substitution of a tyrosine for a histidine in position 3 is a significant alteration. This difference involves a single nucleotide change in the codons for these amino

acids. The other six residues of DSA are identical or chemically similar with residues occurring in the above species. The results became ambiguous after eight residues and we were no longer able to positively identify the amino terminal residue.

The results indicate that a mutation in the amino acid sequence in the NH_2-terminal region of DSA has altered the favorable coordination geometry required for the specific binding of the Cu(II) ion. Further study of the sequence of an NH_2-terminal peptide, released by mild, limited digestion of DSA by pepsin, is presently being undertaken. It will be important to compare this sequence with peptide 1-24 of BSA, RSA and HSA (11,12), and to examine the extent of homology and its influence on the Cu(II) binding specificity.

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