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DISTRIBUTION OF PROTEIN-BOUND ZINC IN SERUM OF ANALBUMINEMIC RAT

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The distribution of protein-bound zinc in serum of rat with analbuminemia was analyzed with gel filtration and affinity chromatography. From the profiles of chromatography, the zinc present in analbuminemic rat serum is composed of two principal species in similar to that of Sprague-Dawley rat: one fraction is firmly bound to α_2-macroglobulin, and a second fraction is more loosely bound to various proteins.

In the serum of most species approximately two thirds of the zinc present is bound to albumin, and one third to α_2-macroglobulin(1-3). Albumin has been suggested as carrier of zinc in serum(1,4,5). However, various reports have suggested that transferrin rather than albumin might be the physiologically-important zinc carrier(6,7). But, it has been seemed as a fact that albumin is the major zinc carrier in serum.

Recently, Nagase and colleagues established a strain of rats which is completely deficient in serum albumin from a stock of Sprague-Dawley rats(8). Since, this rats are seemed to be a very good model for studies on the zinc-binding proteins of serum other than albumin, we took an interest in the distribution of protein-bound zinc in serum of this rats.

This paper reports studies on the distribution of protein-bound zinc in serum of rats with analbuminemia.

Materials and Methods

Animals: Nagase analbuminemic rats were a gift from Dr. S. Nagase (Sasaki Institute, Tokyo Japan). The controls used were Sprague-Dawley rats supplied by CLEA Japan(Kanagawa, Japan). Wistar rats were

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obtained from Shizuoka Agricultural Cooperative Association(Shizuoka, Japan). Rats were used at 6-7 weeks old in the experiment.
Serum zinc content: Serum was diluted with 0.1 N HCl, and the zinc content was measured by the atomic absorption spectrophotometer (SHIMAZU AA-630).
Column chromatography: Serum(10 ml) was applied to a Sephacryl S-300 column(2.5 x 90 cm). The column was eluted with 0.1 M NaCl-25 mM Tris/HCl buffer(pH 8.0) at 4°C at a flow rate of 20 ml/hr. 6 ml fractions were collected, and zinc content in each fraction was measured by the atomic absorption spectrophotometer.

Serum(25 ml) was applied to a Blue Sepharose CL-6B column(2.5 x 20 cm). The column was eluted with 50 mM NaCl-50 mM Tris/HCl buffer(pH 8.0) at 4°C at a flow rate of 10 ml/hr, the bound material was eluted with 360 ml of 0.2 M NaSCN-50 mM Tris/HCl buffer(pH 8.0) and then the bound zinc was elluted with 240 ml of 50 mM NaCl-0.4 mM EDTA-50 mM Tris/HCl buffer(pH 8.0) at 4°C at a flow rate of 20 ml/hr. 6 ml fractions were collected, and zinc concentration was measured by the colorimetric method(9) and by the atomic absorption spectrophotometer.

The proteins, which were separated into two fractions by the chromatography on Blue Sepharose CL-6B, futher were fractionated with Zn-Sepharose 6B column(1.5 x 15 cm). The gel columns were washed with several volumes of water. The gel was converted to the zinc chelate form by equlibrating with aqueous $ZnSO_4 \cdot 7H_2O$(5 mg/ml). The column was then washed with water, and equilibrated with 1 M NaCl-20 mM Tris/HCl buffer(pH 8.0). The proteins were applied to a column. The column was washed with 1 M NaCl-20 mM Tris/HCl buffer(pH 8.0). The proteins, which were absorbed to a column, were eluted with 1 M NaCl-50 mM EDTA-20 mM Tris/HCl buffer(pH 8.0). 3 ml fractions were collected and A_{280} of each fraction was measured.
Sodium dodecyl sulfate-polyacrylamide gel electrophresis: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed on a 7.5% acrylamide(10).
Protein content: Protein content was measured by the colorimetric method(11),using bovine serum albumin as the standard.

Results and Discussion

Serum zinc level: Fig. 1 shows the serum zinc level of Sprague-Dawley rat, analbuminemic rat and Wistar rat. The low level in

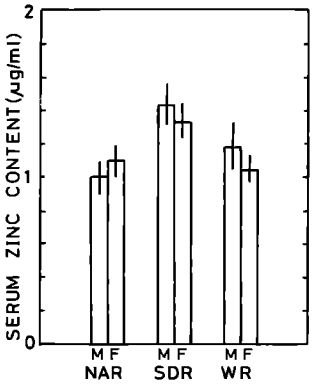

Fig. 1. Serum zinc content of analbuminemic rat. NAR; Nagase analbuminemic rat, SD; Sprague-Dawley rat, WR; Wistar rat. All values are means ± S.D.(n=7-12)

serum zinc content was observed in the serum of analbuminemic rat(male:0.997 ug/ml. female:1.097 ug/ml) as compared to the serum of Sprague-Dawley rat(male:1.433 ug/ml. female:1.332 ug/ml). But as compared to Wistar rat(male:1.181 ug/ml. female:1.031 ug/ml), the significant difference was not observed. The serum zinc level of analbuminemic rat was seemed to be the physiologically-normal level.

Distribution of serum zinc on Sephacryl S-300 column: In order to obtain the information about the distribution of serum zinc in analbuminemic rat, the serum was analysed by the chromatography on Sephacryl S-300. About 70% of total serum zinc of Sprague-Dawley rat was detected in the fraction contained albumin(fraction nos. 50-65, Fig.2-A). The pattern of distribution of the serum zinc in analbuminemic rat was almost the same as that in Sprague-Dawley rat(Fig. 2-B). About 70% of total serum zinc of analbuminmic rat was detected in fraction nos. 50-65.

Distribution of serum zinc on Blue Sepharose CL-6B column: In order to obtain the information about zinc binding form the serum was analysed by the affinity chromatography on Blue Sepharose CL-6B. Affinity chromatography on Blue Sepharose CL-6B has been used for the selective removal of albumin from serum(3,12,13). Serum was applied to a column, equiliblated low-NaCl buffer. Albumin binds to the

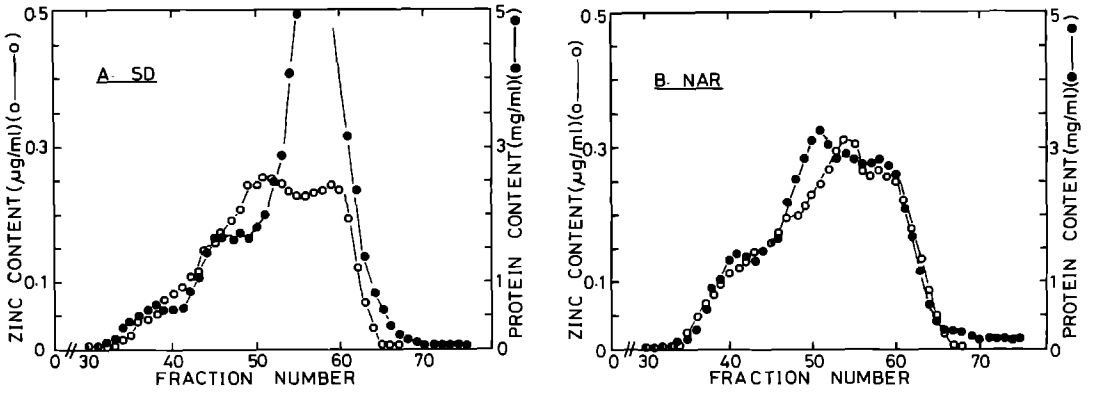

Fig. 2. Chromatography of serum on Sephacryl S-300. SD; Sprague-Dawley rat, NAR; Nagase analbuminemic rat.

immobilzed dye on the gel matrix. The column was subsequently eluted with NaSCN buffer and EDTA buffer, which remove albumin and zinc from binding sites on the gel(Fig. 3). Zinc was located in three fractions. The first fraction was firmly associated with α_2-macroglobulin. The second fraction was associated with albumin. The third fraction seemed to be loosely associated with albumin or other proteins. However, a major part of the third fraction seemed to be associated with albumin. As compared to the serum of Sprague-Dawley rat(Fig. 3-A), there was not a significant changes in the distribution of zinc in the serum of

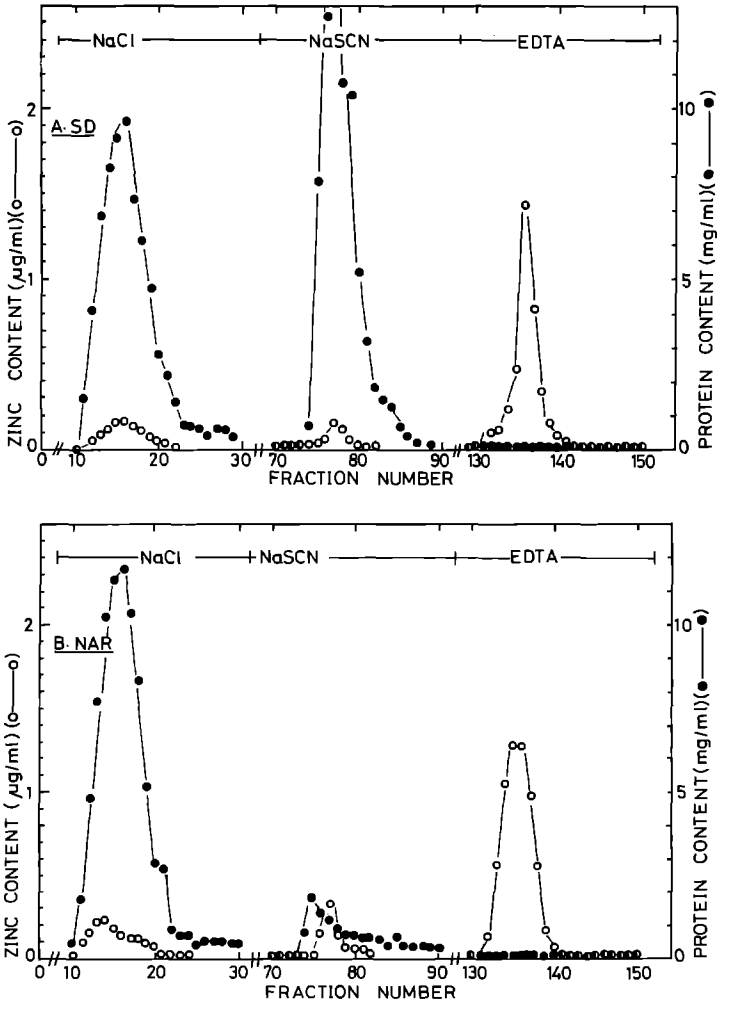

Fig. 3. Chromatography of serum on Blue Sephrose CL-6B.

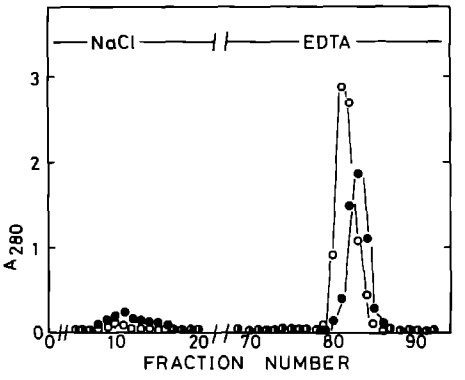

Fig. 4. Chromatography of serum protein of analbuminemic rats on a Zn-chelate Sepharose 6B. The first and second fractions, obtained from Blue Sepharose chromatography of whole serum, were applied to Zn-chelate Sepharose column(first; 16 mg, second; 7 mg). (o); the first fraction. (●); the second fraction.

analbuminemic rat. 70% of the total serum zinc was detected in the fraction(second and third fractions) absorbed on the column(SD:22.63 ug. NAR:20.77 ug). 30% of the total serum zinc was detected in the first fraction contained α_2-macroglobulin(SD:7.69 ug. NAR:7.63 ug). The result demonstrates that 70% of serum zinc of analbuminemic rat is loosely bound form in similar to the serum zinc of Sprague-Dawley rats.

Zinc binding capacity of serum proteins of analbuminemic rats: The proteins contained in the first and second fraction, which were prepared by the chromatography on Blue-Sepharose CL-6B, were almost absorbed to Zn-Sepharose 6B column(Fig 4)(first:90%. second:80%). Then it has been shown that the zinc bound to these proteins in vitro is loose binding form from the profiles of chromatography on Blue Sepharose CL-6B(data not shown). The results suggests that the zinc binding proteins in place of albumin are composed of a number of protein.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of zinc-binding proteins : Fig. 5 shows electrophoretic patterns of proteins which were fractionated with Blue-Sepharose CL-6B column and Zn-Sepharose 6B column. In analbuminemic rat, the proteins contained

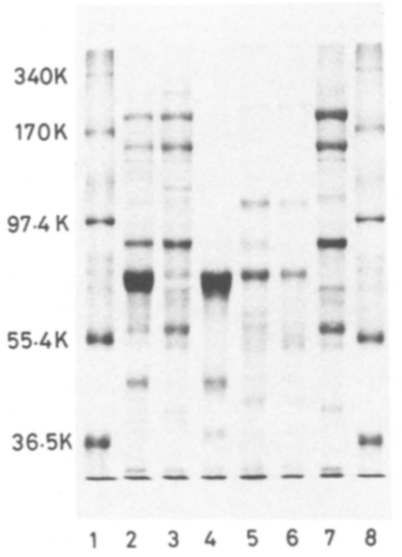

Fig. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the proteins absorbed on Blue Sepharose CL-6B and Zn chelat Sephrose 6B. (1),(8); calibration proteins, (2); whole serum of SD, (3); whole serum of NAR, (4); absorbed serum proteins of SD from Blue Sepharose CL-6B , (5); absobed serum proteins of NAR from Blue Sepharose CL-6B, (6); serum proteins of NAR, absorbed on Blue Sepharose CL-6B and absorbed Zn-chelate Sepharose 6B, (7); serum proteins of NAR, passed through on Blue Sepharose CL-6B and absorbed on Zn-chelate Sepharose 6B. Approximately 5 μg of protein was applied to each column except whole serum(10 μg).

in the fraction from Blue Sepharose CL-6B column and Zn-chelate column were resolved many components by gel electrophoresis.

The present study demonstrates that the serum zinc of analbuminemic rat is the physiologically-normal level in spite of deficient serum albumin, and that the zinc present in analbuminemic rat serum is composed of two principal species in similar to the serum of Sprague-Dawley rat. One fraction is firmly bound to proteins(α_2 -macroglobulin), and a second fraction is more loosely bound to various proteins. we have hypothesized that the proteins contained in the second fraction on Blue Sepharose CL-6B chromatography take the place of albumin. However, we haven't sufficient evidence to support this hypothesis. The zinc binding protein in serum of analbuminemic rat requires further investigation.

Acknowledgement

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