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Fractional Analysis of Zinc in Rabbit Plasma

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An effective method for fractional analysis of zinc in rabbit plasma was established, using polyethylene glycol. Rabbit plasma zinc was separated into two fractions; the zinc profile in the precipitate showed two peaks, which were not albumin-bound zinc, on gel filtration, while the zinc in the supernatant proved to be albumin-bound zinc. The proposed method was used to investigate the effect of feeding on plasma zinc in rabbits. The decrease of total plasma zinc on feeding was significant, and was mainly due to that of albumin-bound zinc.

Keywords——fractional analysis; plasma zinc; polyethyleneglycol; gel filtration; atomic absorption spectrophotometry; states of zinc; feeding; fasting

Zinc plays an important role in the therapy of some diseases.[2,3] Although many reports have appeared on the total concentrations of zinc found in various tissues, there have been very few attempts to analyze fractionated zinc-containing materials.[4,5] However, it seems important to determine zinc contents accurately in fractionated samples in order to understand the functional role of zinc from physiological and pathological points of view.

Recently, Giroux tried to estimate human serum zinc by separating it into two fractions, namely α_2-macroglobulin-bound zinc and albumin-bound zinc, using polyethylene glycol.[6] In order to analyze rabbit plasma zinc, the authors established an easier and simpler method, again using polyethylene glycol. The state of plasma zinc separated by the proposed method was investigated by gel chromatography. Furthermore, the method was found to be advantageous to determine the effect of feeding on the concentration of rabbit plasma zinc.

Experimental

Materials——Polyethylene glycol solution (PEG solution) was prepared by dissolving 100 g of polyethylene glycol 6000 (Nakarai Chemicals Ltd.), 0.03 g of Tris and 0.1 g of sodium azide in 400 ml of water. The pH of the solution was adjusted to 7.1 with glacial acetic acid.[6] Plasma samples were taken from male rabbits (about 2 kg) and healthy adult men by treating the blood with heparin. The heparinated blood samples were subjected to centrifugation at $3000 \times g$ for 10 min, and the supernatants were used for this study.

Separation of Plasma with PEG——One milliliter of plasma was mixed with 1 ml of PEG solution, and the mixture was kept in ice-water for 10 min. The mixture was then centrifuged at $3000 \times g$ for 3 min and divided into a supernatant and a precipitate. The precipitate was redissolved in 0.95 ml of 1 mM EDTA–0.05 N NH_4OH.

Gel Chromatography——One milliliter of plasma and the PEG-separated precipitate redissolved in 0.15 M NaCl solution, were each subjected to gel chromatography on Sephadex G-150 (Pharmacia Fine Chemicals). The conditions of gel chromatography were as follows: gel bed; 1.5 cm (i.d.) × 38 cm, eluent; 0.3 M NaCl–0.05 M Tris-HCl (pH 7.8), flow rate; 3 ml/hr, fraction volume; 1 ml/tube.

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Determination of Zinc and Protein——Zinc concentration was measured by atomic absorption spectrophotometry (flame method) with a Hitachi 308 atomic absorption spectrophotometer, and UV absorption at 280 nm was measured with a Shimadzu UV 100-02 spectrophotometer.

Treatment of Rabbits——Eight rabbits were divided into two groups, namely a fasting group (four rabbits) and a feeding group (four rabbits). They were fasted from the night before the experiment. In the morning, every rabbit of the feeding group was given 100 g of pellet-type feed (RC4, Oriental Yeast Co., Ltd.) during 2 hr and then fasted for the next 4 hr. All the rabbits of the fasting group were fasted for the corresponding period. Just before feeding of the feeding group and 6 hr later, blood was sampled from the auricular vein of all rabbits in both groups.

Results and Discussion

A method has been reported to separate human zinc (Giroux's method) but none of the separation methods has been tested on rabbit plasma zinc. There were some differences in the state of zinc in rabbit plasma and in human serum, as described below. Therefore, the authors tried to establish a suitable separation method for rabbit plasma zinc.

The concentration of PEG, the time and the temperature of separation were examined in order to establish a simpler method. By modifying Giroux's method as regards the time and temperature of the experiment, good separation of zinc fractions was achieved with rabbit plasma, as shown in Fig. 1. Change of temperature and reduction of the operating time did not affect the separation profile of zinc.

Zinc in the precipitate was analyzed by atomic absorption spectrophotometry. Owing to the high viscosity of the supernatant containing PEG, the atomization rate of samples in the atomic absorption spectrophotometer was considerably reduced. However, this disadvantage was overcome in the proposed method, since the precipitate could be redissolved in 1 mM EDTA–0.05 M NH_4OH, and zinc could thus be analyzed precisely by atomic absorption spectrophotometry.

To examine the validity of the proposed method, gel chromatography was used. Three elution peaks of zinc were observed with control rabbit plasma. They were numbered I (fraction numbers 16—22), II (24—28) and III (28—36) from the highest molecular peak.

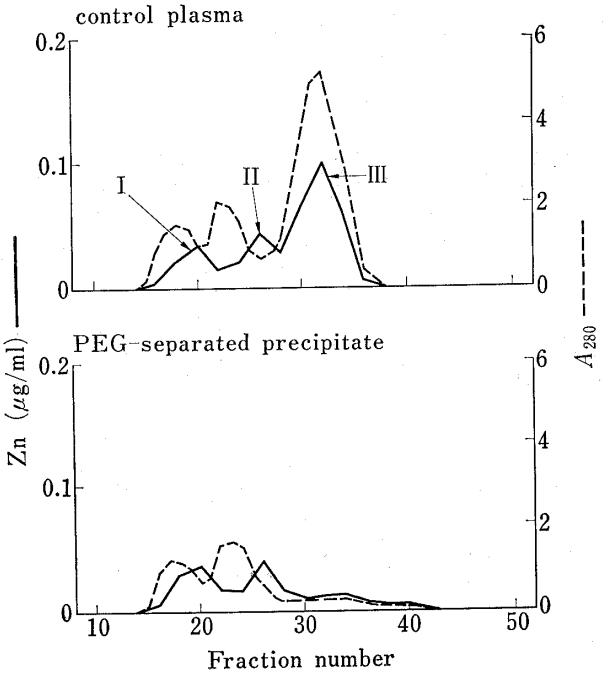

Fig. 1. Elution Profiles of Zinc and Protein of Rabbit Plasma on Gel Chromatography

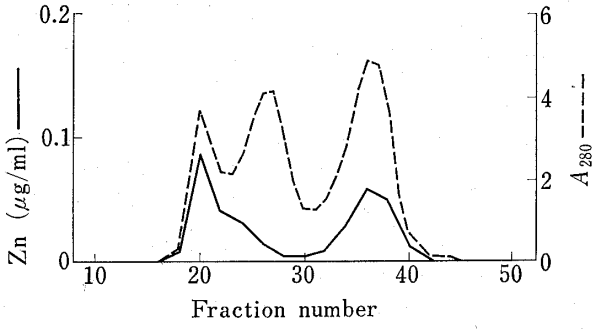

Fig. 2. Elution Profiles of Zinc and Protein of Human Plasma on Gel Chromatography

The solution containing the precipitate with PEG contained I and II, but did not contain albumin or albumin-bound zinc (Fig. 1). The recoveries of zinc from the column were almost quantitative. Thus, it appears that the amount of zinc in the precipitate corresponds to the sum of zinc found in I and II, and zinc in the supernatant is zinc in III. The profile of zinc in human plasma showed two peaks on gel filtration, as shown in Fig. 2. It was thus found that the number of zinc peaks in rabbit plasma was different from that in human plasma.

Comparison of the molecular sizes of the zinc peaks of rabbit plasma with those of human plasma suggested that zinc in III is albumin-bound zinc and zinc in I may be α_2-macroglobulin-bound zinc.[7] The state of zinc in II, which appears to have a molecular weight of 140000—160000 as measured by gel filtration, remains unknown.

Zinc concentrations in rabbit plasma analyzed by the present method before and after feeding are shown in Table I. In this table, plasma zinc is separated into three categories, namely $[Zn]_{total}$, $[Zn]_{precipitate}$ and $[Zn]_{total}-[Zn]_{precipitate}$. They are given as the concentrations in 1 ml of plasma before separation. The zinc concentration defined as $[Zn]_{total}-[Zn]_{precipitate}$ corresponds to that in the supernatant determined after electrodialysis.[8]

The values of $[Zn]_{total}$, $[Zn]_{precipitate}$ and $[Zn]_{total}-[Zn]_{precipitate}$ did not decrease during fasting. However in rabbits of the feeding group, the value of $[Zn]_{total}$ decreased from 1.63 $\pm$0.11 μg/ml to 0.66$\pm$0.06 μg/ml. Although the value of $[Zn]_{precipitate}$ decreased slightly, the value of $[Zn]_{total}-[Zn]_{precipitate}$ decreased significantly from 1.13$\pm$0.08 μg/ml to 0.28$\pm$0.02 μg/ml. This change of total plasma zinc after feeding therefore appeared to be mainly due to that of albumin-bound zinc.

Table I. Change of Zinc Distribution in Rabbit Plasma after Feeding

Group	Time[a] (hr)	Zn concentration[b] (μg/ml)		
		$[Zn]_{total}$	$[Zn]_{precipitate}$	$[Zn]_{total}-[Zn]_{precipitate}$
Feeding (n=4)	0	1.63$\pm$0.11	0.50$\pm$0.04	1.13$\pm$0.08
	6	0.66$\pm$0.06	0.37$\pm$0.04	0.28$\pm$0.02
Fasting (n=4)	0	1.60$\pm$0.19	0.44$\pm$0.04	1.16$\pm$0.19
	6	1.79$\pm$0.17	0.39$\pm$0.03	1.40$\pm$0.16

a) Blood sampling time,
0; just before feeding rabbits of the feeding group.
6; 6 hr after the above time.
b) Mean $\pm$ SD.

The above results show that the level of albumin-bound zinc in plasma may fluctuate significantly under physiological or pathological conditions in both rabbit and man. However, the total zinc in human plasma has not been reported to change significantly. One possible explanation is that the ratio of the concentration of albumin-bound zinc to that of total zinc is smaller in human plasma than in rabbit plasma.

The present method should be useful to differentiate the states of plasma zinc and to estimate albumin-bound zinc and other zinc compounds. It is likely that plasma zinc of other species can also be separated and analyzed by the present method under suitable conditions. Moreover, it has been reported that the concentration of serum zinc is reduced in various specific conditions (*e.g.*, infections, chronic disease of the liver, acute myocardial infarctions and pregnancy).[9] The present method may thus contribute to an understanding of the relationship between diseases and the behavior or state of plasma zinc. The results of further studies using the present method will be reported shortly.

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