STUDIES ON INSULIN-LIKE SUBSTANCE IN *PANAX GINSENG*

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Abstract

It was found that water extract of *Panax ginseng* strongly inhibited adrenaline-induced lipolysis in isolated fat cells of rat epididymal adipose tissue. An antilipolytic action of the water extract was easily inactivated by treatment with pronase, suggesting that the active principle might be a protein or a peptide. Experiments were designed to purify the antilipolytic substance, or insulin-like substance, of the water extract. The water extract was dialyzed against distilled water.

The outer dialysate was subjected to DEAE-cellulose column chromatography, gelfiltration on sephadex G-50 column, avicel cellulose column chromatography and phospho-cellulose column chromatography, successively. The finally purified substance gave one spot on thin layer chromatography. The molecular weight was found to be around 1000. Experiments are now in progress to elucidate the structure of this insulin-like peptide.

Introduction

It is well known that *Panax ginseng* is efficacious against diabetes. However, the precise mechanism remains to be elucidated. In 1956, Sung *et al.*[1] reported that ginseng decreased high blood sugar level of rat induced by alloxan.

For 15 years, I have studied the mechanism of actions of fat mobilizing hormones, such as adrenaline, ACTH and growth hormone. In the course of this experiments, it was found that water extract of *Panax ginseng* contains antilipolytic substance just like insulin. The present investigation describes purification of this insulin-like substance from water extract of *Panax ginseng*.

Materials and Methods

Panax ginseng was supplied by Nikkan Korai-Ninjin, Co., Ltd. .

Isolated fat cells were prepared from rat (Wistar King strain) epididymal adipose tissue by the method of Rodbell[4].

A mixture of 0.5ml of fat cell suspension, equivalent to 200mg of adipose tissue, 0.5ml of Krebs-Ringer-phosphate buffer, pH 7.4, containing 5 % albumin and other additions was incubated at 37° C for 2 hr. Then Dole's extraction mixture was added and the free fatty acids (FFA) released were estimated by the method of Dole[5]. Lipolytic activity was expressed as the amount of FFA released from fat cells equivalent to 1 g of adipose tissue.

Results and Discussion

A mixture of *Panax ginseng* powder and 9 fold volume of distilled water was stirred at 4° C for 24 hr and centrifuged at 3000g for 10 min. It was found that the resultant supernatant fraction contained an antilipolytic substance. Fat cells were incubated with adrenaline and the supernatant fraction in the presence of 1 mg of trypsin inhibitor as described in "MATERIALS AND METHODS." Adrenaline-induced lipolysis was expressed as the difference of FFA released in the presence and absence of this hormone. As shown in Fig. 1, the supernatant fraction, or the crude extract, clearly inhibited adrenaline-induced lipolysis. In the following experiments, 10% inhibition by this ginseng extract was defined as one unit. It was found that the antilipolytic activity of the crude extract was easily inactivated by pronase but not by phospholipase C, amylase, hyaluronidase and trypsin.

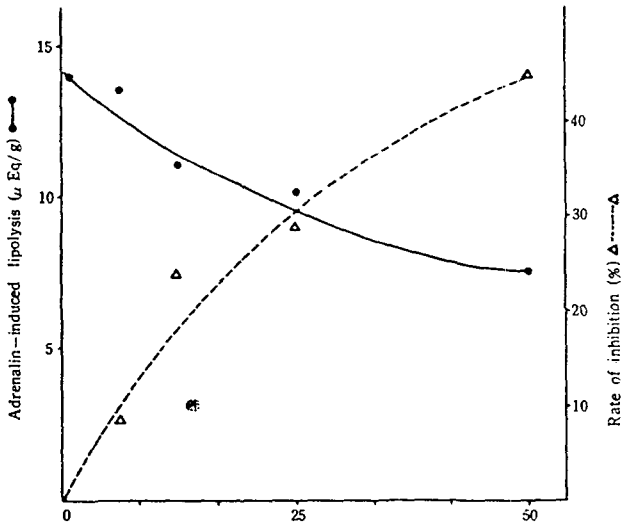

Fig. 1. Antilipolytic activity of crude ginseng extract.

Then, the supernatant fraction was dialyzed against distilled water, and the outer dialysate was applied to DEAE-cellulose column equilibrated with 10mM ammonium bicarbonate buffer (pH 8.0). An antilipolytic substance, or insulin-like substance, was eluted with 1M ammonium bicarbonate buffer (pH 8.0). The eluate was, then, applied to sephadex G-10 column (2.5×45 cm) and desalted. The eluate at the void fraction was subjected to gel filtration on sephadex G-50 column (2.2 × 76 cm) as shown in Fig. 2. The active fractions (tube No. 50–80) were collected, concentrated, adjusted to pH 5.0 and stood at 4° C overnight. The resultant precipitate was removed and the supernatant fraction was applied to Avicel cellulose column (3.7 × 90 cm). Elution was carried out with a mixture of N-butanol, acetic acid and water (3:1:1) as shown in Fig. 3. Insulin-like activity, or antilipolytic activity was found in both peak I and II. Peak II fraction was subjected to the same chromatography two times (Fig. 4), followed by desalting with sephadex G-10 column equilibrated with 0.02M ammonium acetate buffer (pH 3.54). The eluate at the void fraction was, then,

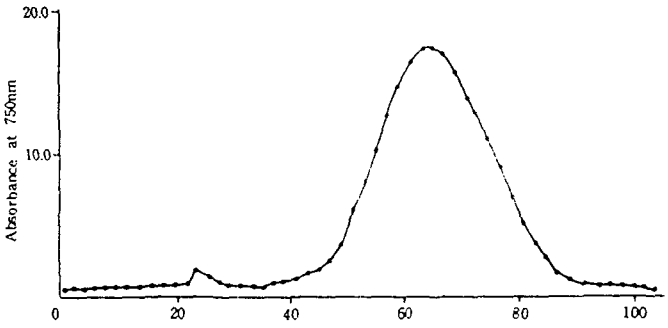

Fig. 2. Gel filtration of the insulin-like substance on sephadex G-50 column. Protein was estimated by the method described by Lowry *et al.*[6].

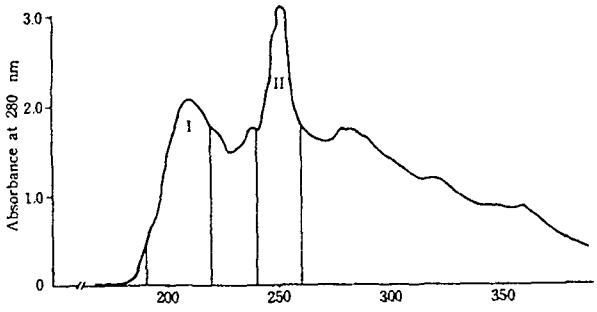

Fig. 3. Chromatography of the insulin-like substance on avicel cellulose column.

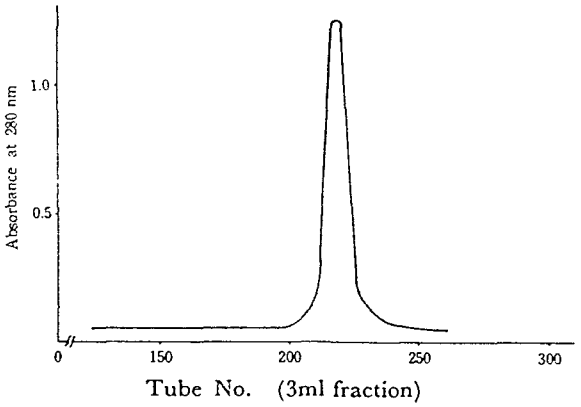

Fig. 4. Third chromatography of the peak II fraction on avicel cellulose column.

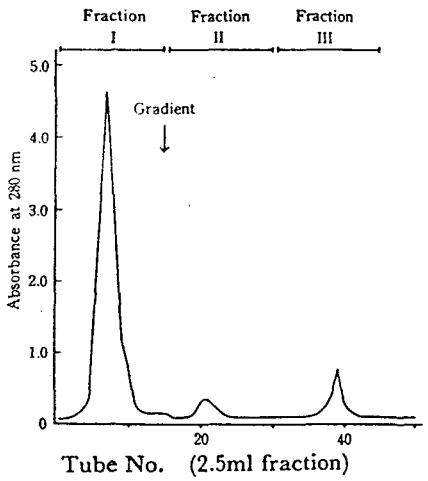

Fig. 5. Ion-exchange chromatography of the insulin-like substance on phospho-cellulose column.

applied to phospho-cellulose column equilibrated with 0.02M ammonium acetate buffer (pH 3.54). After washing with the same buffer, elution was carried out with a linear gradient to 0.3M ammonium acetate buffer (pH 9.0).

The insulin-like activity was found to be at fraction I and II, and the activity of fraction II was much higher than that of fraction I. Table 1 summarizes purification of the insulin-like substance. Specific activity was expressed as units per mg protein. The finally purified fraction (phospho-cellulose column fraction II) gave one spot on thin layer chromatography. The molecular weight was found to be around 1000 by ultracentrifugation method. Experiments are now in progress to elucidate the structure of this insulin-like substance.

Table 1. Purification of insulin-like substance from *Panax ginseng*

Purified step	Volume (ml)	Specific activity (U/mg)	Total activity (U)	Yield (%)
Panax ginseng extract	8850	6.02	340500	100.0
Outer dialysate	150	17.05	306700	90.1
DEAE-C column fraction	30	18.5	55000	16.2
Sephadex G-50 fraction	30	20.3	42000	12.3
Avicel cellulose column Peak II	5.0	412.2	3770	1.1
Phospho-cellulose column fraction	0.7	938.9	103	0.03

References

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