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Studies on the Ginseng Polypeptide—Decreasing Blood Sugar and Hapatic Glycogen

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Ando *et al.*[1] found the root of Ginseng contained an active peptide which had anti-lipolysis function when they studied effective components of Ginseng to cure diabetes mellitus in 1980. In recent years we were making lots of the researches on the Ginseng polypeptide. We obtained a 14 peptide which could decrease levels of blood sugar and hepatic glycogen. However, amino acid composition of the peptide differs from one reported by Ando *et al.* The results of its purification, structure and functure and function were reported in this paper.

Purification and Structure Analysis of *Panax Ginseng* Peptide

1. Purification of *Panax Ginseng* polypeptide

The Ginseng root powder (1.0 Kg) and 65% ethanol were mixed (1:18 W/V). The solution was adjusted to pH 9 with ammonia, stirred at room temperature for 24h and centrifugalized at 7000 rpm. The supernatant was lyophilized. The producing powder was solvated in distilled water, heated at 100 °C for 1 min. The precipitate was removed by centrifugation at 10000 rpm. The supernatant was applied to Sephadex G-25 column (4 × 9 cm). Elution was carried out with distilled water. The peptide fraction was collected at 10 m*l*/tube and detected by UV-detector at 230 nm. The lyophilized pure fraction gave 10 mg of Ginseng peptide powder, which was tested by HPLC (Fig. 1).

2. Amino acid composition and primary sequence of Ginseng peptide

Hydrolysis of the peptide was carried out with 6N HCl at 110 °C for 24h[2,3]. Its amino acid composition was analysed by HITACHI 835-50.

From Table 1, the result differs from that given by Ando. The former contained Leu, the latter contained Ile. The amino acid sequence of Ginseng peptide was determined by DABITC/PITC method[3,4]. The sequence is E-T-V-E-I-I-D-S-E-G-G-G-D-A.

3. Secondary structure of Ginseng peptide

In order to obtain some information on the three-dimensional structure of Ginseng peptide and its possible action model, the study of its secondary structure was fulfilled. According to Chou-Fasman[5] and our own method that based on "BROAD SENSE WOBBLE HYPOTHESES"[6], Some prediction rules of secondary structure was brought up and the prediction of secondary structure of the peptide was made based on the sequence data. The result was that conformation of Ginseng peptide molecule behaved a segment of α-helicity consisted of the 1-9 residues and a random coil region consisted of the 10-14 residues.

Circular dichroism (CD) spectra of the peptide were showed in Fig. 2[7,8]. The negative Cotton effect around 200-220 nm indicated its some helical content, which was not affected by 1% SDS and not change from pH 4.0 to 8.0. Based on mean residue

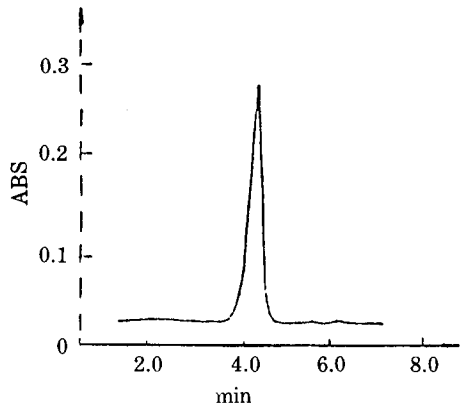

Fig. 1. Ginseng peptide mapping by HPLC. Column: μ-Bondapak; Mobile phase: 30% Methanol; Flow rate: 1.0 ml/min.

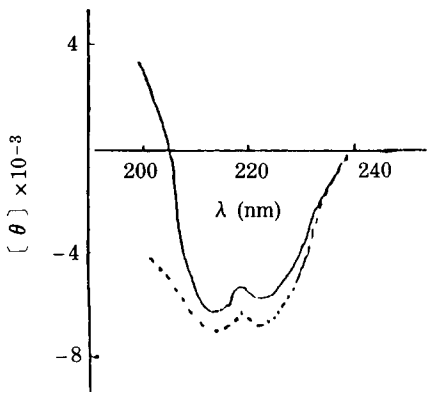

Fig. 2. CD spectra of Ginseng peptide solution at the different conditions.—, aqueous; ---- ,1% SDS solution.

Table 1. Amino acid composition of ginseng peptide

Amino acid	Amino acid analysis	Sequence determine
Asp	2.1	2
Thr	1.1	1
Ser	1.0	1
Glu	3.3	3
Gly	3.2	3
Ala	1.1	1
Val	1.2	1
Ile	2.2	2

ellipticity at this wavelength the helical content was found to be 60% in water and SDS respectively. The percentage of α-helix was found to be 64%. Both were very close to eath other. These results showed the peptide α-helix-formed potentiality.

4. Three-dimensional structure of Ginseng peptide

Based on the primary structure, the secondary structure in solution was determined in detail by CD, Chou-Fasman methods. We did some theoretical researches on the molecular conformation such as calculation of the relative conformation energy and atomic coordinate of molecule by ECEPP/2 (empirical conformation energy program for peptide) combined with the data from crystalographic database, simulation of the possible energy-mini-

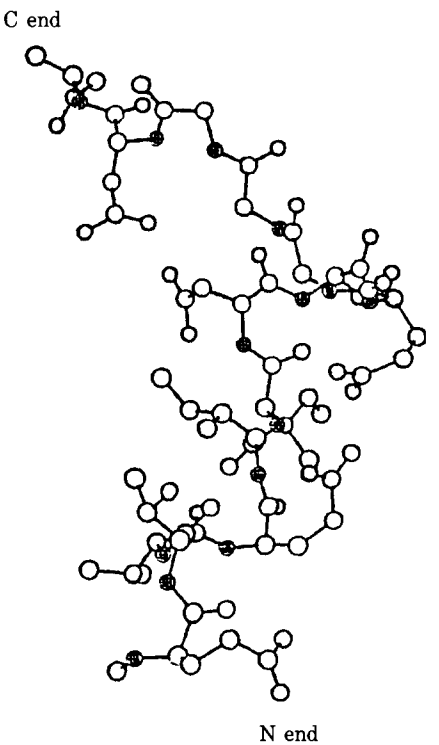

Fig. 3. Molecular model of Ginseng lpeptide ●-----N.

mized conformation by energy-optimized method and build of the three-dimension model of the polypeptide molecule by computer molecule-graphic system[9]. The spatial structure of peptide was obtained and its three-dimension model was built

Fig. 4. Photography of Ginseng peptide crystals.

(see Fig. 3). The shape of the model showed that its structure was a α-helix linked with a random coil segment. It corresponded with the conclusion of its secondary structure.

Having calculated conformation energies, we found dihedral angles of the 5th, 6th and 7th residues of the α-helix affected total conformation energy enormously (especially the 6th). It indicated that the residues might be concerned in molecular function and provided an important scientific basis for further study on the active site, action mechanism and the relationship between structure and function of it.

We used the vapor diffusion method[10] to grow crystal of the peptide. Droplets (0.5 μl), containing concentrations of precipitant being belower than that needed to rander the peptide insoluble, were placed over reservoirs containing higher precipitant concentration. The droplet then slowly equilibrated with the cell through a vapor phase. The photography of Ginseng peptide crystals showed in Fig. 4.

Ginseng Peptide Chemical Synthesis and Its Gene Clone

We finished the peptide chemical synthesis with a polypeptide synthesizer and its gene synthesis with a DNA synthesizer. The gene was cloned with *E. coli* JM101 as a receptor and pUC19 as a vector. And then the cloned strain was gotten and identified by in situ colony hybridization, Southern blot and electrophoresis.

Table 2. Effect of ginseng peptide on contents of blood sugar, blood total lipid and liver glycogen.

Group mg/kg	Blood sugar mg%	Blood total lipid mg%	Liver glycogen mg/100 mg L.W.T.
Control	146±18	4.01±0.637	1.36±0.844
Ginseng peptide 50	120±15**	3.72±0.891	0.68±0.598
100	114±23**	3.64±1.060	0.79±0.647
200	110±24**	3.91±0.993	0.50±0.469*

* $p<0.05$, ** $p<0.01$ as compared with control group. L.W.T. liver wet tissue.

Table 3. Effect of multiple injection of ginseng peptide on contents of blood sugar, total lipid and liver glycogen.

Group mg/kg	Blood sugar 4[a]	Blood sugar 7[a]	B.T.L. mg%	Liver glycogen mg/100 mg L.W.T.
Control	135±10.7	135±27.6	464±101.2	5.72±2.427
G.P. 50	116±20.2*	130±19.6	462±64.9	3.62±1.584*
100	120±13.8*	108±17.8*	449±72.9	2.25±1.211***

* $p<0.05$, *** $p<0.001$; [a] Days after administration: B.T.L. = blood total lipid.
G.P. = ginseng peptide.

The Effect of Ginseng Peptide on the Carbohydrate Metabolism

1. Effect of Ginseng peptide on levels of blood sugar, blood total lipid and liver glycogen in normal mice[12,13]

32 male mice divided equally into four groups according to dosages set in Table 2. Experimental groups received an intravenous injection. The control group was injected the same amount physiological saline. After 2 hour, mouse heads were cut off and blood was taken to be determined blood sugar and blood total lipid and the liver tissues were taken to be determined hapatic glycogen. The results indicated that Ginseng peptide obviously decreased blood sugar and hepatic glycogen (especially large dosages) but had no effect on blood total lipid (see Table 2).

Table 4. Effect of ginseng peptide on the on the hyperglycemia induced by adrenaline

Group	Blood sugar (mg%)		Blood total lipid (mg%)		Liver glycogen (mg/100 mg)	
	2[a]	30	2	30	2	330
Control	117±17	118±24	698±89	619±126	3.58±0.575	1.93±0.86
Adr. 50 μg/kg	116±31	134±27	592±96	621±129	2.41±0.508***	1.07±0.579*
G.P. 100 mg/kg + Adr. 50 μg/kg	102±29	92±16##	525±47	649±219	1.49±0.812***#	0.58±0.264***#

* $p<0.05$, *** $p<0.001$ as compared with control group; # $p<0.05$, ## $p<0.01$ as compared with Adr. group; [a] = times after injection of adrenaiie (minute); Adr.= adrenaline.
G.P. =ginseng peptide.

2. Effect of multiple injection of Ginseng peptide on blood sugar, blood total lipid and liver glycogen[12,13]

According to dosages set in Table 3, 30 mice were divided equally into three groups. Experimental groups were given hypodermic injection once a day for 7 days. The control group was injected physiological saline (10 ml/kg). By 1h after the 4th administration, blood from eyesocket vein was taken to be determined blood sugar. By 1h after final administration, mouse heads were cut off, blood and liver tissue were taken to be determined blood sugar, blood total lipid and hapatic glycogen. The results were shown in Table 3.

As shown in Table 3, Ginseng peptide injected into mice for many times greatly decreased blood sugar and glycogen, but had no effect on blood total lipd.

3. Effect of Ginseng peptide on high blood sugar led by adrenaline

48 mice were divided equally into three groups. The 1st and 2nd ones received physiological saline via hypodermic injection once a day for 7 days. The 3rd group was injected Ginseng peptide (100 mg/kg). By 20 min after final administration, the 2nd and 3rd groups were injected adrenaline (50 μg/kg) via abdominal cavity. After 2 or 3 minutes, half of mouse heads of above three groups were cut off and the same treatments were made as above. The result was that the peptide could decrease high blood sugar and promote decrease of hapatic glycogen led by adrenaline. But they had no effect on blood total lipid.

4. Effect of Ginseng peptide on high blood sugar led by glucose[13,14]

120 male mice were divided equally into three groups. The 1st and 2nd ones were injected physiological saline (10 ml/kg) via hypoderm once a day for 6 days. The 3rd group was given Ginseng peptide as the same process. After final administration, the 2nd and 3rd groups were injected glucose (2g/kg) via vein, the first group was injected physiological saline (5 mg/kg) in the same amount. After 2, 60, 120, 240 and 480 min, 8 mouse heads of each group were cut off to be determined blood sugar and hepatic glycogen. We found that high blood sugar and hepatic glycogen increase led by glucose were inhibited by successive injection via hypoderm, Ginseng peptide evidently decreased blood sugar after injecting glucose 1 and 2h and decreased hapatic glycogen in whole observations (Fig. 6,7).

5. Effect of Ginseng peptide on the blood sugar, blood total lipid and hapatic glycogen in mice intoxicated with alloxan[13,14]

According to the dosages set in Table 5, 48 male mice divided equally into four groups. Two of those were injected Ginseng peptide via hypoderm once a day, the other groups were injected physiological saline in same amount (5 ml/kg). By 1h after the 2nd injection of the peptide, the control group was injected physiological saline (5 ml/kg) via tail vein, the other three groups were injected alloxan (100 mg/kg). After intoxication at intervals of 5 days, blood sugar was determined from eyesocket vein

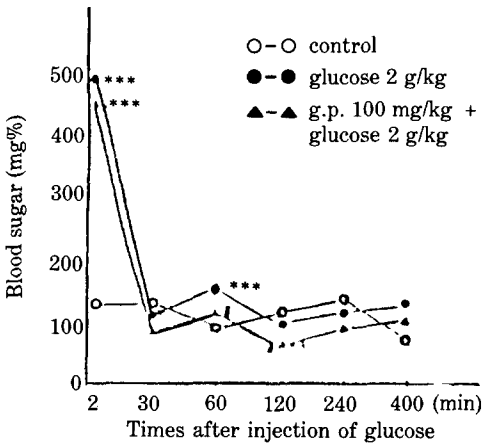

Fig. 6. Effect of ginseng polypeptide on the hyperglycemia induced by injection of glucose. g.p. = ginseng polypeptide.

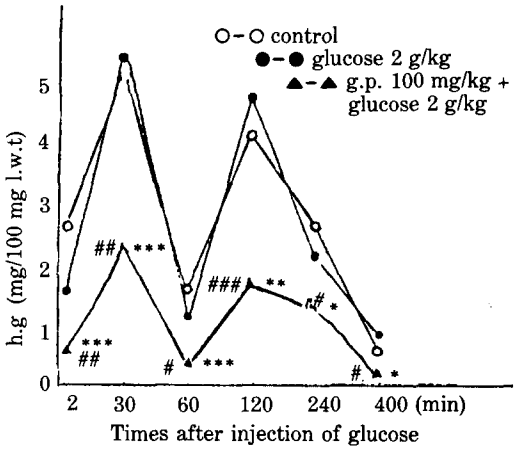

Fig. 7. Effect of ginseng polypeptide on the content of hepatic glycogen after injection of glucose. g.p. = ginseng polypeptide. h.g. = hepatic glycogen.

Table 5. Effect of ginseng peptide on the contents of blood sugar, total lipid and liver glycogen in mice intoxicated with alloxan

Group mg/kg	Blood sugar (mg%) 5[a]	10	15	20	25	B.T.P. mg%	Liver glycogen (mg/100 mg)
Control	159±22	121±36	134±11	114±14	115±23	505± 82	5.96±3.071
Alloxan 100	178±23*	179±47**	177±58*	157±68	158±47	646± 92**	8.34±4.723
G.P. 50+alloxan 100	151±23#	167±22	159±27	133±41	103±25##	598±121	2.16±1.731*##
G.P. 100+ailxan 100	150±25#	160±29**	136±23#	120±18	121±31#	553± 92#	1.91±1.638***###

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ as compared with control group; # $p<0.05$, ## $p<0.01$ ### $p<0.001$ as compared with allxan group; [a] = time (days) after injection of allxan; b = liver wet tissue; G.P. = ginseng peptide.

blood. By 2h after final administration, the mouse heads were cut off and blood sugar, blood total lipid and liver hapatic glycogen were determined. We found that blood sugar and blood total lipid increased greatly. Also hapatic glycogen increased in some extent. But both of them had no significance statistically.

Discussion

From ancient times, *Panax Ginseng* has been used for treatment of various diseases including diabetes mellitus, as an important folk drug in Asian countries such as China, Korea and Japan. Ando *et al.* and our studies has all shown that Ginseng root contained an acidic peptide. But the peptide component from two reports was slightly different.

Based on the primary and secondary structures of Ginseng peptide, we did some theoretical researches on the molecular conformation. We found that the dihedral angles of the 5th, 6th and 7th residues within the α-helix affected the total conformational energy enormously (especially the 6-Ile). These indicated that these residues might be concerned in molecular biological function, which provided an important basis for further studies on the active site, action mechanism and the relationship between the structure and function of Ginseng peptide.

The studies on physiological function of Ginseng peptide showed that it could decrease the levels of blood sugar and hepatic glycogen under various experimental conditions. In general, blood total lipid has no significant change. But Ginseng peptide

could also decreased blood total lipid when mice intoxicated with alloxan. Its effect on high blood sugar led by glucose via intravenous injection showed that it promoted glucose utilization of organizm, i.e. hapatic glycogen decrease with blood sugar decrease. This showed that the effect of Ginseng peptide on carbohydrate metabolism differs from that of insulin which could promote synthesis of glycogen besides promoting glucose utilization.

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