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Effect of Ginseng Principle on Pyruvate Kinase Activity in Rat Liver

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In comparing the effect of ginseng principle on the activity of pyruvate kinase (EC 2.7.1.40), there was a marked difference among various diet groups. Administration of the extract (fraction 5) from the roots of *Panax ginseng* C.A. Meyer increased the activity of hepatic pyruvate kinase in rats fed on a laboratory pellet chow. Maximum increase in the enzyme activity was observed 1 hr after the administration of ginseng principle, and it was found that this response depended on the amount of fraction 5 administered to rats. However, rise of the enzyme activity due to ginseng treatment did not seem to involve *de novo* protein synthesis which requires mRNA production. In contrast, when the diet contained an excess of carbohydrate, ginseng treatment resulted in a decreased enzyme level, and the elimination of food resulted in the loss of the effect of ginseng principle.

Keywords——*Panax ginseng* C.A. Meyer; pyruvate kinase; rat liver; different nutritional states; enzyme activity; cycloheximide; actinomycin-D

Our previous report[2-4] showed that ginseng principle had an effect on lipid and sugar metabolism *in vivo* when administered to rats. A single intraperitoneal administration of the extract increased the incorporation of labeled precursor into total lipid in the rat liver, and the incorporation depended directly on the amount of ginseng principle administered, and a reduction of carbohydrate in the liver was observed by the administration of ginseng principle. From these results, it may be considered that the ginseng extract turns the metabolic flow towards the direction of lipogenesis by the conversion of sugar, and these experiments have given some insight into the functional significance of enzyme regulation.

The present paper reports a research concerning the effect of ginseng principle in the activity of pyruvate kinase (EC 2.7.1.40), the key glycolytic enzyme.

Materials and Methods

Animals and Diet——Male rats of the Wistar strain, initially weighing 70—85 g, were used. A group of 6 rats was kept in a wire-bottomed cage under a conventional lighting regimen with a dark night. Animals

Table I. Composition of the Synthetic Diet

	g/100 g diet		
Casein	10	40	70
α-Cornstarch	65.9	35.9	5.9
Sucrose	15	15	15
Soybean oil	2	2	2
Salt mixture[5]	4	4	4
Vitamin mixture[5]	1	1	1
Cellulose powder	2	2	2
Choline chloride	0.1	0.1	0.1

1) Location: *3190 Gofuku, Toyama 930, Japan.*
2) T. Yokozawa, H. Seno, and H. Oura, *Chem. Pharm. Bull.* (Tokyo), **23**, 3095 (1975).
3) T. Yokozawa and H. Oura, *Chem. Pharm. Bull.* (Tokyo), **24**, 987 (1976).
4) T. Yokozawa, K. Kanai, M. Takefuji, and H. Oura, *Chem. Pharm. Bull.* (Tokyo), **24**, 3202 (1976).
5) A.E. Harper, *J. Nutr.*, **68**, 405 (1959).

were fed either laboratory pellet chow (CLEA Japan Inc., Tokyo; protein 24.0%, lipid 3.5%, carbohydrate 60.5%) or synthetic diet freely. The synthetic diet contained 2 g of soybean oil, 4 g of salt mixture,[5] 1 g of vitamin mixture,[5] 2 g of cellulose powder, 0.1 g of choline chloride, and the indicated amount of casein. The weight was made up to 100 g by the addition of an appropriate amount of α-cornstarch (see Table I). The dry components of the diet and oil were mixed thoroughly in a mortar, and choline chloride (50% EtOH solution) and an appropriate amount of tap water were added for kneading the diet to a suitable consistency. The animals were fed freely on the experimental diet for 7 days before sacrifice.

Chemicals——The sodium salt of ADP, the barium salt of phosphoenolpyruvic acid, and cycloheximide were purchased from Sigma Chemical Co., U.S.A. The barium salt of the reagent was converted to the potassium salt before use. Actinomycin-D was obtained from Mann Research Laboratory, New York, U.S.A., and suspended in 1% EtOH solution at a concentration of 0.5 mg/ml. It was then dissolved by freezing and thawing, and diluted 5-fold with 1% EtOH solution. Other reagents used were of the highest purity available.

Extraction and Partial Purification of Ginseng Radix——Fraction 5 was prepared from the extract of root of *Panax ginseng* C.A. Meyer produced in Kumsan, Korea, as previously described.[6] Chemical examination of fraction 5 gave a positive Liebermann–Burchard reaction, and it was found to contain 69.6% of hexose (glucose equivalent) determined by the Tsugita–Akabori method.[7] Further study indicated that fraction 5 consisted of ginsenoside-Rb_1, -Rb_2, -Rc, -Rc_2, -Rd, -Re, and -Rg_1.[6]

Measurement of Enzyme Activity——Rats were sacrificed by a blow on the head and exsanguinated. Rats were killed between 2 and 3 pm to avoid the effect of circadian variation.[8] The liver was removed quickly, placed in liquid nitrogen, and weighed rapidly. Average time between laparotomy and deep-freezing of the tissue was about 20 sec. A 10% (w/v) homogenate was prepared in a medium with 10 up-and-down strokes of a motor-driven, Potter-type Teflon-glass homogenizer. The medium contained 0.15 M KCl, 0.005 M $MgSO_4$, and 0.001 M EDTA. The homogenate was centrifuged at 105000×*g* for 60 min in the cold, and the supernatant liquid was used for the enzyme assay.

Pyruvate kinase activity was assayed routinely by the 2,4-dinitrophenylhydrazone method, according to a slight modification of the method of Kimberg and Yielding.[9] The assay mixture contained 50 μmol of Tris-HCl buffer (pH 7.5), 2 μmol of phosphoenolpyruvate, 2 μmol of ADP, 5 μmol of $MgSO_4$, and 100 μmol of KCl in a total volume of 0.5 ml. The reaction was started by the addition of 0.05 ml of a suitably diluted enzyme solution to 0.5 ml of the assay mixture. After incubation at 37° for 3 min, the reaction was terminated by the addition of 1.25 ml of 2,4-dinitrophenylhydrazine (0.0125% in 2 N HCl), and the mixture was allowed to stand for 10 min at 37°. Then 3.0 ml of 2.4 N NaOH solution containing 0.001 M EDTA was added to the mixture and its optical density was measured at 510 nm, after a full color development (after about 10 min).

Results

Stimulating Effect of Ginseng Principle on Pyruvate Kinase Activity

Examination was made to determine the dose-response relationship of graded doses of ginseng principle (fraction 5) administered intraperitoneally to rats. Control animals were

Table II. Dose–Response Relationship of Fraction 5 on Pyruvate Kinase Activity in Rat Liver

Material	Dose (mg)	Enzyme activity (μmol/min/g liver)
Control	—	39.05±0.86 (100)
Fraction 5	2.5	41.92±2.04 (107)
Fraction 5	5	42.64±0.78 (109)
Fraction 5	10	49.88±2.39 (128)
Fraction 5	20	54.08±2.01 (138)

Graded dose of fraction 5 was administered intraperitoneally to rats. Control animals were treated with an equal volume of saline. At 1.5 hr after treatment, rats were sacrificed by cutting the carotid artery. Groups of 5 to 6 rats were used for each experiment. The data are presented as mean±S.E. Figures in parentheses indicate percentage to the control value.

6) H. Oura, S. Hiai, Y. Odaka, and T. Yokozawa, *J. Biochem.* (Tokyo), **77**, 1057 (1975).
7) A. Tsugita and S. Akabori, *J. Biochem.* (Tokyo), **46**, 695 (1959).
8) T. Yokozawa and H. Oura, *Agric. Biol. Chem.* (Tokyo), **41**, 2291 (1977).
9) D.V. Kimberg and K.L. Yielding, *J. Biol. Chem.*, **237**, 3233 (1962).

treated with an equal volume of saline. At 1.5 hr after the treatment, the animals were sacrificed by a blow on the head and exsanguinated. As shown in Table II, the activity of pyruvate kinase in the liver depended on the amount of fraction 5 administered to rats and the administration of 20 mg increased it 1.4-fold over that of the control level.

To obtain further evidence for the effect of fraction 5 on the enzyme activity, the time course experiment was followed. As shown in Fig. 1, maximum increase was 36% at 1 hr after the treatment. The increase was still 32% at 2 hr after the treatment, but was not significant after 4 hr.

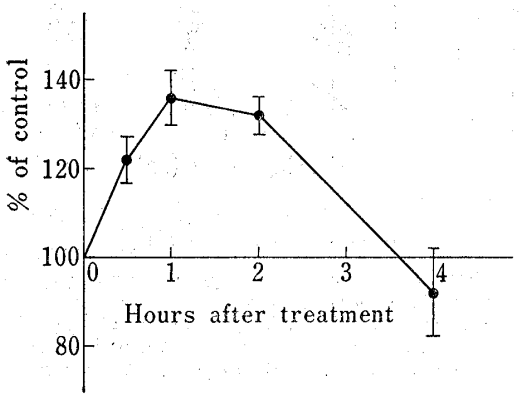

Fig. 1. Time Course of the Effect of Fraction 5 on Pyruvate Kinase Activity in Rat Liver

At the indicated time after intraperitoneal administration of fraction 5 (10 mg), rats were sacrificed by cutting the carotid artery. Groups of 5 to 6 rats were used for each experiment. Data are expressed as percentage of control value. Vertical bars show standard errors.

Table III. Effect of Cycloheximide Injection on the Rise of Pyruvate Kinase Activity due to Ginseng Treatment

Cycloheximide	Treatment	Enzyme activity (μmol/min/g liver)
−	Control	33.42±1.02
−	Fraction 5	43.29±0.99
+	Control	30.54±2.01
+	Fraction 5	39.19±0.72

Fraction 5 (10 mg) was administered intraperitoneally to rats 1.5 hr before sacrifice. Cycloheximide (50 μg/100 g body weight) was injected 34.5 hr before ginseng treatment.[10] Groups of 5 to 6 rats were used for each experiment. The data are presented as mean±S.E.

Effect of Cycloheximide and Actinomycin-D on the Rise of Pyruvate Kinase Activity due to Ginseng Principle Treatment

In examining the mode of action of ginseng principle, we followed the line of approach to see if the rise in enzyme activity could be blocked by the inhibitors of protein synthesis.

Table III shows the effect of cycloheximide on ginseng-induced elevation of hepatic pyruvate kinase activity. Cycloheximide caused a slight decrease in the enzyme activity. By treatment with ginseng, the activity in pyruvate kinase increased by about 30% over that of the control animals. When the rat was administered the inhibitor and then treated with ginseng principle, the enzyme level was still 1.28-fold higher. This phenomenon was quite similar to the effect in the case of actinomycin-D, though the data are not shown. Accordingly, rise of the enzyme activity due to treatment with ginseng principle does not seem to involve *de novo* protein synthesis which requires mRNA production.

Effect of Carbohydrate in Diet on Pyruvate Kinase Activity

Table IV shows the effect of fraction 5 treatment on the liver of rats fed on a synthetic diet containing various amounts of carbohydrate. From the formula of the synthetic diet (Table I), 80.9% carbohydrate diet contains 10% casein and 20.9% carbohydrate diet, 70% casein. As shown in Table IV, administration of ginseng principle decreased the level of pyruvate kinase in the liver of rats fed a 80.9% carbohydrate diet. Decrease of the enzyme level by ginseng treatment was also observed in rats fed a 50.9% carbohydrate diet, but there was no statistically significant difference between the control and fraction 5-treated group. The data in Table IV further indicate that the effect of ginseng principle was completely

10) G. Weber, *Adv. Enzyme Regul.*, **7**, 15 (1969).

Table IV. Effect of Fraction 5 on the Activity of Hepatic Pyruvate Kinase in Rats fed on Diets containing Various Carbohydrate Levels

Carbohydrate level (%)	Treatment	Enzyme activity (μmol/min/g liver)
80.9	Control	72.72±2.30 (100)
	Fraction 5	58.57±2.50 (81)
50.9	Control	63.81±2.80 (100)
	Fraction 5	60.18±1.43 (94)
20.9	Control	28.73±0.90 (100)
	Fraction 5	38.60±3.57 (134)

Rats kept on above diets were given an intraperitoneal administration of fraction 5 (10 mg). Control animals were treated with an equal volume of saline. At 1.5 hr after treatment, rats were sacrificed by cutting the carotid artery. Groups of 5 to 6 rats were used for each experiment. The data are presented as mean±S.E. Figures in parentheses indicate percentage to the control value.

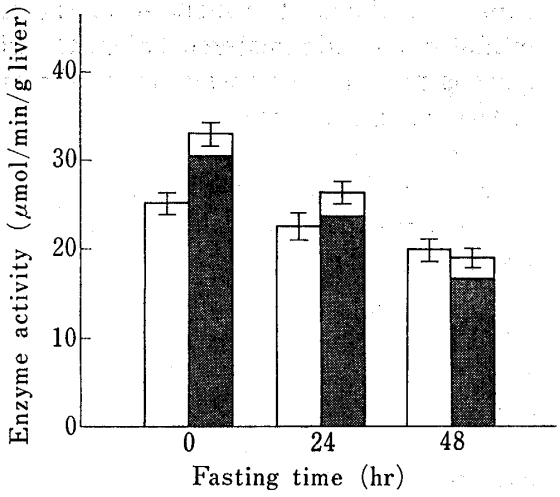

Fig. 2. Effect of Fraction 5 on Pyruvate Kinase Activity in Fasted Rat Liver

Rats were administered intraperitoneally 10 mg of fraction 5. Control animals were treated with an equal volume of saline. At 1.5 hr after treatment, rats were sacrificed by cutting the carotid artery. Groups of 5 to 6 rats were used for each experiment. Vertical bars show standard errors.

□ control group. ■ fraction 5-treated group.

reversed when carbohydrate in the diet was decreased from 50.9 to 20.9%. These facts indicate that the nutritional state of an animal may play an important role in regulating the effect of ginseng principle.

Effect of Fasting on Pyruvate Kinase Activity

The effect of fasting is shown in Fig. 2. Increase of the enzyme activity due to ginseng treatment was confirmed in the experiment carried out with the liver of non-fasted rats, but fasting gradually cancelled the effect of ginseng treatment in the liver of normal rats. It became clear that the elimination of food resulted in the loss of the effect of ginseng principle.

Discussion

Pyruvate kinase (EC 2.7.1.40) catalyzes the last step of glycolysis. A low pyruvate kinase activity favors gluconeogenesis from amino acids and other precursors, and a high pyruvate kinase activity favors the degradation of carbohydrate and its conversion into fat.[11] Thus, its activity is important in the regulation of the two opposing pathways of liver carbohydrate metabolism, gluconeogenesis *vs.* glycolysis.

In the present experiments, it is apparent that the level of pyruvate kinase in the liver is altered by treatment of animals with ginseng. As shown in Fig. 1, maximum increase in the enzyme activity was observed 1 hr after the administration of fraction 5. This response gradually decreased and approached the control level 4 hr after the treatment. Sensitivity of hepatic enzyme level was shown by determining the dose–response relationship of graded doses of the ginseng principle and the administration of 20 mg increased it 1.4-fold over that of the control level (see Table II).

However, increase in the pyruvate kinase activity due to ginseng treatment does not appear to involve *de novo* production of the enzyme protein, because the dose of cycloheximide or actinomycin-D, which inhibited the activity of pyruvate kinase slightly in control rats,

11) H.A. Krebs and L.V. Eggleston, *Biochem. J.*, **94**, 3C (1965).

could not abolish the increase in the enzyme activity due to ginseng principle when the inhibitor was administered before the ginseng treatment. Accordingly, it is considered that ginseng principle may regulate the direction of liver carbohydrate metabolism through control of the enzyme activity.

It was shown in our previous paper[2] that ginseng principle had an effect on lipid and sugar metabolism when administered to rats. A temporary increase in hepatic lipogenesis was observed 4 hr after the treatment, while a significant decrease in the glycogen content was found 6—8 hr after the administration of ginseng principle. Considering these observations, it may be that pyruvate kinase acts as a trigger for the initiation of the metabolic stimulation.

On the other hand, it has already reported that the activity of pyruvate kinase in the liver showed exceptionally large variation when the dietary regime was changed.[11–13] Krebs and Eggleston[11] demonstrated that the activity of this enzyme was greatly increased in the liver of rats fed on a high-carbohydrate diet, and the difference in enzyme activity between diets low and high in carbohydrate was about 10-fold. More recently, we reported that the level of the enzyme in the liver increased with the feeding of carbohydrate but was unaffected by the feeding of protein or fat.[14] Nutritional influence provides precursors for enzyme biosynthesis, supplies key metabolites, and contributes to the level of molecules such as free fatty acids and amino acids which may serve as a regulatory signal for pyruvate kinase.[10] From these reasons, it is necessary to examine the quantitative alteration in the enzyme activity due to ginseng treatment under different nutritional conditions.

In comparing the effect of ginseng treatment on the pyruvate kinase activity of rat liver in different nutritional states, there was a marked difference among different diet groups. When the diet contains an excess of carbohydrate, ginseng treatment results in a decreased enzyme level. In contrast, when the diet is low in carbohydrate, ginseng principle produces an increase of the enzyme activity, and the elimination of food results in the loss of the liver function (see Table IV and Fig. 2). Such alterations in the enzyme activity showed that ginseng principle provided a metabolic pattern compatible with the behavior of the overall metabolic pathways under various physiological circumstances.

The present discussion touched upon the functional significance of pyruvate kinase. However, preliminary examination has shown that the level of serine dehydratase (EC 4.2.1.13), which catalyzes the degradation of serine to pyruvate and ammonia, also provides a substantial contribution to the effect of ginseng principle.[15] Further studies will be needed for the elucidation of the effect produced by the ginseng treatment.

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12) G. Weber, R.L. Singhal, N.B. Stamm, and S.K. Strivastava, *Fed. Proc.*, **24**, 745 (1965).
13) T. Tanaka, Y. Harano, F. Sue, and H. Morimura, *J. Biochem.* (Tokyo), **62**, 71 (1967).
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