Polysaccharides in Fungi. XXXVI.¹⁾ Hypoglycemic Activity of a Polysaccharide (CS-F30) from the Cultural Mycelium of *Cordyceps sinensis* and Its Effect on Glucose Metabolism in Mouse Liver

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A polysaccharide (CS-F30) obtained from the cultural mycelium of *Cordyceps sinensis* showed potent hypoglycemic activity in genetic diabetic mice after intraperitoneal administration, and the plasma glucose level was quickly reduced in normal and streptozotocin-induced diabetic mice after intravenous administration. Administration of CS-F30 to normal mice significantly increased the activities of hepatic glucokinase, hexokinase and glucose-6-phosphate dehydrogenase, although the glycogen content in the liver was reduced. Furthermore, CS-F30 lowered the plasma triglyceride level and cholesterol level in mice.

Key words hypoglycemic activity; *Cordyceps sinensis*; polysaccharide; non-insulin dependent diabetes mellitus (NIDDM) model; glucose metabolism; cholesterol

A crude drug of *Cordyceps sinensis* (Ascomycetes) has been used as a super-effective nutritional traditional medicine in China. We previously reported that a polysaccharide (CS-F30) consisting of galactose, glucose and mannose (molar percent, 62 : 28 : 10) was isolated from an alkaline extract of the cultural mycelium of the fungus and showed significant hypoglycemic activity in normal and streptozotocin (STZ)-induced diabetic mice by intraperitoneal (i.p.) injection, and that the plasma glucose level was slightly lowered in normal mice by oral administration.²⁾ The present studies were undertaken to test the hypoglycemic activity of CS-F30 in genetic diabetic mice as a model of non-insulin dependent diabetes mellitus (NIDDM) by i.p. administration, and the activity in normal and STZ-induced diabetic mice by intravenous (i.v.) administration. Since CS-F30 hardly affected the plasma insulin level in mice as described previously,²⁾ we studied the effect of CS-F30 on the activity of key hepatic enzymes in glucose metabolism. Furthermore, the plasma cholesterol level and the plasma triglyceride level were analyzed.

MATERIALS AND METHODS

Materials Glucose-6-phosphate dehydrogenase was obtained from Sigma. Glucose B-test kit, Cholesterol E-test kit, and Triglyceride E-test kit were purchased from Wako Pure Chemical. All other reagents were of special reagent grade.

Animals Male normal mice (ddY strain, 4 weeks old) and male genetic mice (KK-Ay-TA strain, 5 weeks old) were obtained from Japan SLC and Clea Japan, respectively. A standard laboratory diet for normal mice or a high-calorie diet (CL-2, Japan Clea) for the genetic mice and water were given *ad libitum*. They were housed individually under temperature (about 21 °C)- and humidity (about 60%)-controlled conventional clean condition.

Hypoglycemic Activity Mice as normal subjects were used at 5 weeks of age, and streptozotocin-induced (i.p. injection to normal mice before 3 weeks of sample

administration), and genetic mice were used when their plasma glucose level was over 400 mg/dl. After i.p. or i.v. administration of a sample solution of CS-F30 (control: physiological saline), the plasma glucose level was measured by a glucose B-test kit based on the glucose-oxidase method, as described previously.²⁾

Measurement of Plasma Cholesterol and Triglyceride in Mice Plasma cholesterol level and plasma triglyceride level were measured using the cholesterol E-test kit based on the cholesterol oxidase-DAOS method³⁾ and the triglyceride E-test kit based on glycerol-3-phosphate oxidase-DAOS method,⁴⁾ respectively.

Preparation of Hepatic Enzymes Normal mice at 5 weeks of age were decapitated, and the livers were excised by the double hatched method.⁵⁾ Each liver was homogenized with ice-cold buffer, and the supernatant obtained by centrifugation of the homogenate was used for the measurement of enzyme activity.

Measurements of Activities of Hepatic Glucokinase, Hexokinase and Glucose-6-phosphate Dehydrogenase The liver was perfused with ice-cold 0.15 M KCl and 1 mM EDTA solution, and excised. The liver was homogenized with ice-cold buffer (2 mg/g liver)[10 mM cysteine, 1 mM EDTA, 0.1 M Tris-HCl buffer (pH 7.4)], and the supernatant was collected by centrifugation (20000 × *g*, 20 min, 4 °C) and used for measurements of glucokinase and hexokinase activities. The activities were assayed spectrophotometrically using glucose-6-phosphate dehydrogenase.⁶⁾

The liver was perfused, excised, and homogenized with the buffer (4 ml/g liver) as described above. The supernatant obtained by centrifugation (30000 × *g*, 20 min, 4 °C) was used for the measurement of glucose-6-phosphate dehydrogenase activity. The activity was assayed spectrophotometrically.⁷⁾

Measurement of Glycogen Content in Mouse Liver To the liver, 30% KOH solution (100 mg/2 ml) was added and the mixture was heated in a boiling water bath for 20 min. Ethanol (10 ml) was added to the mixture (5 ml), which was then kept at 4 °C for overnight. The mixture was centrifuged (3000 × *g*, 15 min, 4 °C), fractionated again

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twice with ethanol. The combined precipitate was dried in a desiccator, dissolved in water (3 ml), and measured for glucose content using the anthrone-sulfuric acid method.⁸⁾

Statistical Analysis All data are expressed as means $\pm$ S.E. The significance of difference between the means was evaluated using Student's *t*-test.

RESULTS

Effect of CS-F30 on Plasma Glucose Level In genetic diabetic mice of the NIDDM model, the plasma glucose level was remarkably reduced at 3–24 h after the i.p. administration of CS-F30 at a dose of 50 mg/kg, and its level at 6 h corresponded to that of normal mice (Table 1). By i.v. administration of a smaller amount of CS-F30 than that for i.p. administration, the plasma glucose level was significantly lowered at 3–6 h in both normal mice and STZ-induced diabetic mice (Table 2).

Effect of CS-F30 on Plasma Triglyceride Level and Cholesterol Level CS-F30 significantly reduced the plasma triglyceride level in normal mice at 3–6 h after i.p. administration (50 mg/kg) (Table 3). The plasma cholesterol level was significantly reduced in normal mice and STZ-induced diabetic mice at 3 h after i.p. administration (50 mg/kg), although the reduced levels returned to the original level 6 h later (Table 4).

Effect of CS-F30 on Glycogen Content As shown in Table 5, CS-F30 significantly decreased the glycogen content in normal mouse liver at 6–24 h and in STZ-induced diabetic mouse liver at 24 h after i.p. administration (50 mg/kg).

Effect of CS-F30 on Activities of Hepatic Glucokinase,

Table 1. Effect of CS-F30 on Plasma Glucose Level in NIDDM Model by i.p. Administration

Group (n=5)	Dose (mg/kg)	Relative plasma glucose level				
		0	3	6	24	48 (h)
Control	—	100	81 $\pm$ 5	90 $\pm$ 4	99 $\pm$ 1	95 $\pm$ 2
CS-F30	50	100	42 $\pm$ 3***	28 $\pm$ 2***	49 $\pm$ 9***	82 $\pm$ 4**

Plasma glucose level at 0 h (510–740 mg/dl) was adjusted to 100. Significantly different from control: ** $p < 0.01$, *** $p < 0.001$.

Table 2. Effect of CS-F30 on Plasma Glucose Level in Normal Mice and STZ-Induced Diabetic Mice by i.v. Administration

Group (<i>n</i> = 5)	Dose (mg/kg)	Relative plasma glucose level			
		0	3	6	24 (h)
Normal mice					
Control	—	100	88 ± 6	93 ± 2	93 ± 1
CS-F30	1	100	66 ± 3**	67 ± 4***	88 ± 4
CS-F30	5	100	67 ± 2**	65 ± 2***	86 ± 3*
CS-F30	10	100	68 ± 3*	65 ± 4***	80 ± 1**
Diabetic mice					
Control	—	100	97 ± 2	97 ± 4	100 ± 4
CS-F30	1	100	54 ± 4***	80 ± 7*	95 ± 6
CS-F30	10	100	53 ± 7***	52 ± 1***	100 ± 4

Plasma glucose level at 0 h (normal mice 170–210 mg/dl, diabetic mice 400–700 mg/dl) was adjusted to 100. Significantly different from control: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Hexokinase and Glucose-6-phosphate Dehydrogenase As shown in Table 6, CS-F30 significantly enhanced the glucokinase and hexokinase activities at 3–24 h and the glucose-6-phosphate dehydrogenase activity at 3 h, in normal mouse liver after i.p. administration at a dose of 50 mg/kg.

Table 3. Effect of CS-F30 on Plasma Triglyceride Level in Normal Mice

Group (n=5)	Dose (mg/kg, i.p.)	Relative plasma triglyceride level			
		0	3	6	24 (h)
Control	—	100	114 $\pm$ 7	115 $\pm$ 8	117 $\pm$ 7
CS-F30	50	100	76 $\pm$ 2**	78 $\pm$ 6**	100 $\pm$ 14

Plasma triglyceride level at 0 h (100–190 mg/dl) was adjusted to 100. Significantly different from control: ** $p < 0.01$.

Table 4. Effect of CS-F30 on Plasma Cholesterol Level in Normal Mice and STZ-Induced Diabetic Mice

Group (n=5)	Dose (mg/kg, i.p.)	Relative plasma cholesterol level			
		0	3	6	24 (h)
Normal mice					
Control	—	100	97±3	91±7	113±5
CS-F30	50	100	84±1**	89±5	102±6
Diabetic mice					
Control	—	100	87±2	85±3	92±1
CS-F30	50	100	77±1**	81±4	88±5

Each plasma cholesterol level at 0 h (normal mice 90–130 mg/dl, diabetic mice 130–210 mg/dl) was adjusted to 100. Significantly different from control: ** $p < 0.01$.

Table 5. Effect of CS-F30 on Glycogen Content in Normal and STZ-Induced Mice Liver

Group (<i>n</i> = 5)	Dose (mg/kg, i.p.)	Glycogen content (mg/g liver)			
		0	3	6	24 (h)
Normal mice					
Control	—	24.6 ± 0.6	30.3 ± 6.8	20.0 ± 2.1	30.4 ± 2.1
CS-F30	50		18.2 ± 4.2	11.3 ± 2.7*	11.3 ± 1.0***
Diabetic mice					
Control	—	30.5 ± 5.2	22.8 ± 2.4	18.9 ± 1.8	26.2 ± 1.9
CS-F30	50		21.7 ± 1.1	11.6 ± 3.0*	12.1 ± 1.9***

Significantly different from the control: * $p < 0.05$, ** $p < 0.01$.

Table 6. Effect of CS-F30 on Activities of Enzymes in Normal Mouse Liver

Group (n = 5)	Dose (50 mg/kg, i.p.)	Enzyme activity (%)		
		3	6	24 (h)
Glucokinase				
Control	—	100 ± 11	100 ± 12	100 ± 15
CS-F30	50	290 ± 17***	161 ± 13**	250 ± 19***
Hexokinase				
Control	—	100 ± 5	100 ± 15	100 ± 6
CS-F30	50	138 ± 15*	119 ± 17	132 ± 10*
Glucose-6-phosphate dehydrogenase				
Control	—	100 ± 13	100 ± 13	100 ± 28
CS-F30	50	259 ± 39**	123 ± 19	99 ± 9

Significantly different from the control: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

DISCUSSION

A water-soluble polysaccharide (CS-F30) was obtained from an alkaline extract of the cultural mycelium of *C. sinensis*. It is composed of galactose, glucose and mannose in a molar ratio of 62:28:10 and its molecular weight is about 45000.²⁾

Since we previously revealed that the plasma glucose level was significantly lowered in normal mice and STZ-induced diabetic model (IDDM) mice by i.p. administration (25—100 mg/kg) of CS-F30,²⁾ the hypoglycemic effect was tested using diabetic mice (KK-Ay-TA) of NIDDM model. In this model, the mice grow fat, and there is an increase in plasma insulin and insulin resistance in the peripheral tissues. The results of genetic diabetic mice indicate that CS-F30 is effective on the plasma glucose level in the NIDDM model of diabetes, in addition to the IDDM model, by i.p. administration. The potent hypoglycemic effect early after administration may be responsible for the incorporation of glucose into the cells.

We investigated the effect of CS-F30 following a different route of administration in normal mice and in the IDDM model. By i.v. administration of CS-F30, the plasma glucose level was lowered in normal mice and STZ-induced diabetic mice at a smaller amount and at an earlier time, compared with that following i.p. administration, described in a previous paper,²⁾ although the lowered level returned to the original level 24 h later, except for a dose of 10 mg/kg in normal mice. The hypoglycemic effect at an earlier time after i.v. administration has been also reported in tormentic acid obtained from *Poterium ancistroides*.⁹⁾ These results indicate that CS-F30 has strong hypoglycemic activity by i.v. administration. The fact provides a useful information on the metabolism and hypoglycemic mechanism of a polysaccharide (CS-F30).

The glycogen content was reduced in normal and STZ-induced diabetic mouse liver at later time than the plasma glucose level, and it has been reported that other hypoglycemic polysaccharides also reduced the glycogen content.^{10,11)} These results suggest that the hypoglycemic activity is not due to the conversion of glucose into glycogen. The reason for the reduction of glycogen at a later time, compared with plasma glucose level, remains obscure. The plasma triglyceride level in normal mice and the cholesterol level in normal mice and STZ-induced diabetic mice were significantly lowered. The data are of interest in the prevention of hyperlipemia.

Since CS-F30 was shown to have little effect on the plasma insulin level in normal mice in our previous studies,²⁾ we studied the polysaccharide's effect on the activities of key hepatic enzymes participating in glucose metabolism in normal mouse to consider the mechanism of hypoglycemic activity. CS-F30 remarkably potentiated the activities of glucokinase and hexokinase participating the intake of glucose in the liver, and it significantly raised the activity of hepatic key enzyme in the pentose phosphate cycle, glucose-6-phosphate dehydrogenase, at an early time, 3 h after the administration. The simultaneous activation of three hepatic enzymes at 3 h after i.p. administration may be induced by one signal through a mediator, e.g., cAMP in the cell. It has been reported that a glycan (ganoderan B) obtained from the fruiting bodies of *Ganoderma lucidum* significantly elicited the key hepatic enzymes participating in glucose metabolism, and it increased the plasma insulin level in normal mice.¹⁰⁾ In comparison with the data of ganoderan B, CS-F30 was suggested to more greatly enhance the enzyme activities. The results also indicate that an acceleration of glucose metabolism is one mechanism of the hypoglycemic activity of CS-F30, but the increase in insulin secretion does not contribute to the activity which differentiates it from ganoderan B.

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