GLYCOGEN PHOSPHORYLASE KINASE DEFICIENCY: A SURVEY OF ENZYMES IN PHOSPHORYLASE ACTIVATING SYSTEM*

Yorihiko Morishita†, Kaoru Nishiyama†, Hirohei Yamamura†
Soichi Kodama§, Hirokuni Negishi§, Masafumi Matsuo§
Tamotsu Matsuo§ and Yasutomi Nishizuka†

From Department of Biochemistry† *and Department of Pediatrics*§
Kobe University School of Medicine
Kobe, Japan

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SUMMARY A four year-old Japanese boy with hepatomegaly and hypoglycemia has low activity of hepatic phosphorylase. A survey of enzymes involved in the phosphorylase activating system has revealed that liver phosphorylase kinase is deficient although adenosine 3',5'-monophosphate (cyclic AMP)-dependent protein kinase and total phosphorylase measured in a mixture supplemented by rabbit muscle phosphorylase kinase show normal activities. The hormone receptor as well as adenyl cyclase system appears to be normal since cyclic AMP increases immediately after intravenous injection of glucagon. His muscle phosphorylase activating system is normal.

In 1959 Hers (1) described hepatic glycogenosis with low activity of phosphorylase. In rapid expansion of knowledge in the mechanism of activation of phosphorylase in the muscle (2) and liver (3), deficiencies other than that of phosphorylase were postulated which, nevertheless, resulted in a low phosphorylase activity. In fact, in 1966 Hug et al. (4) described first a child with hepatic glycogenosis with low phosphorylase activity which was restored to normal value by the addition of phosphorylase kinase obtained from rabbit muscle. Subsequently, similar syndromes caused by deficiencies of phosphoryl-

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ase activating system were reported by the same authors (5), Huijing (6) and Yutaka and Yabuuchi (7). More recently, Hug et al. (8) proposed a glycogen storage disease which was characterized by deficiencies of both phosphorylase kinase and adenosine 3',5'-monophosphate (cyclic AMP)-dependent protein kinase in the liver and muscle. However, in none of these cases cyclic AMP-dependent protein kinase was directly assayed and a survey was made of the enzymes involved in the phosphorylase activating system. The complete cross-reactivity of muscle and liver phosphorylase activating systems was clarified only recently (9), and the detailed properties of hepatic cyclic AMP-dependent protein kinase were described in preceding reports from this laboratory (10-12). The present studies were undertaken, therefor, to examine the enzymes involved in the phosphorylase activating system of a child with hepatomegaly with low phosphorylase activity in the liver.

MATERIALS AND METHODS

The patient was a four year-old Japanese boy with hepatomegaly and hypoglycemia. The edge of the liver was palpable 5 cm below the right costal margin. Otherwise he was clinically healthy and blood sugar increased after administration of glucagon. Two liver specimens were obtained by open biopsy: one was excised before and the other was 5 min after intravenous injection of glucagon (Novo, 400 mg). A part of each specimen (about 200 mg) was quickly frozen in an acetone-dry ice mixture, and the cyclic nucleotide was assayed by the method of Takeda and Ohga (13) which modified the Gilman's method (14). Another part of specimen (about 800 mg) was weighed and homogenized with 5 volumes of 0.25 M sucrose containing 3.3 mM $CaCl_2$ and 6 mM 2-mercaptoethanol using a Potter-Elvehjem type glass-Teflon

TABLE I

Summary of experimental data

Experimental conditions for each assay are described under Materials and Methods Total (active plus inactive) phosphorylase was assayed as follows: the reaction mixture (0.1 ml) contained 2 µmoles of Tris-Cl at pH 8.5, 5 µmoles of NaF, 3 µmoles of 2-mercaptoethanol, 0.1 µmole of ATP, 1 µmole of magnesium acetate, 0.1 nmole of cyclic AMP, 0.9 µg of rabbit muscle phosphorylase kinase and 150-400 µg of liver or muscle supernatant under test. After incubation was carried out for 20 min at 30°, 0.03 ml of a solution containing 5 µmoles of ^{32}Pi (50 cpm/nmoles), 1 mg of glycogen was added. The mixture was incubated for additional 5 min at 30°. Then, ^{32}Pi was precipitated as a triethylamine-phosphomolybdate complex by the method of Sugino and Miyoshi (20) and the radioactive organic phosphate, namely glucose-1-phosphate, remaining in the supernatant was determined after centrifugation for 10 min at 4,000 x g using a Packard Tri-Carb liquid scintillation spectrometer, Model 3320. Phosphorylase activating system was assayed under the same conditions except that 100 µg of rabbit muscle phosphorylase b instead of phosphorylase kinase was added to the preincubation mixture. The number in parenthesis refers to the number of experiments.

		Liver		Muscle	
		Control	Patient	Control	Patient
Glycogen (% weight of wet tissue)		3.7 (2)	8.0	<0.5 (1)	<0.5
Cyclic AMP (pmoles/mg protein)	before glucagon administration	1.66 (2)	1.35	-	-
	after glucagon administration	9.14 (2)	7.48	-	-
Total phosphorylase (nmoles glucose-1-p produced/min/mg protein)		29 (2)	28	68 (1)	89
Phosphorylase activating system (nmoles glucose-1-P produced/mg protein)		250 (2)	2	264 (1)	266
Cyclic AMP-dependent protein kinase (nmoles Pi incorporated into histone/min/mg protein)	without cyclic AMP	0.12 (2)	0.09	0.27 (1)	0.22
	with cyclic AMP	0.77 (2)	0.51	1.81 (1)	1.31
Glycogen synthetase (nmoles glucose transfered/ min/mg protein)		0.23 (2)	0.30	0.26 (1)	0.31
Glucose 6-phosphatase (nmoles Pi produced/min/ mg protein)		15.7 (2)	10.1	-	-

TABLE II

Phosphorylase activity after preincubation with ATP and cyclic AMP

Experiment were carried out under the conditions given in Table I, except that exogenous muscle phosphorylase and muscle phosphorylase kinase were omitted from the preincubation mixture, and Tris-Cl at pH 8.5 was replaced by Tris-maleate at pH 6.8.

	Liver	Muscle
	(*nmoles glucose-1-P produced/mg protein*)	
Patient	< 1	83
Control	27	58

homogenizer. The homogenates were filtered through 2 layers of gauze to remove fibrous tissues. An aliquot of the filtrate was directly employed for glycogen determination and glucose 6-phosphatase assay. Glycogen was determined as glucose using the glucostat kit (15) after hydrolysis in 2.5 N HCl for one hour in a boiling water bath. Glucose 6-phosphatase was assayed under the conditions described by Cori and Cori (16) and inorganic phosphate was determined by the method of Chen et al. (17). Another aliquot of the filtrate described above was centrifuged for 20 min at 20,000 x g, and the supernatant was used for assay of other enzymes. Cyclic AMP-dependent protein kinase was assayed with calf thymus whole histone as substrate as described eariler (18). Glycogen synthetase and phosphorylase were assayed under the conditions described previously (9). Total (active and inactive) phosphorylase and phosphorylase activating system were assayed as described in Table I. Phosphorylase kinase was prepared from rabbit skeletal muscle by the method of Cohen (19) and the step 5 fraction (0.9 unit/min/mg protein) was employed. Crystalline rabbit muscle phosphorylase b was purchased from Boehringer Mannheim (lot 7232317). These enzyme preparations were free of each other and of cyclic AMP-dependent protein kinase under the conditions

employed. Cyclic AMP-dependent protein kinase was purified partially from rat liver as described previously (12) (3.5 nmoles of phosphate incorporated/min/mg protein). Muscle specimens were obtained from straight abdominal muscle and analyzed by the same procedures. Other chemicals were obtained from commercial sources. All manipulations were carried out at 0-4°.

RESULTS AND DISCUSSION

The patient liver was whitish and the concentration of glycogen was twice as much as that in the control liver (Table I). Cyclic AMP was found in a normal quantity and increased markedly upon intravenous injection of glucagon, indicating that the hormone receptor as well as adenyl cyclase in the patient liver was normal (Table I). The liver extract showed practically no activity of phosphorylase even after incubation with ATP and cyclic AMP (Table II); under these conditions inactive phosphorylase was shown to be converted to active phosphorylase (3). However, total phosphorylase measured after incubation with ATP, cyclic AMP and rabbit muscle phosphorylase kinase was found to be in the same order of magnitude with that found in the control normal liver (Table I). Experiments fortified by rabbit muscle phosphorylase b revealed that the patient liver showed practically no capacity of activating phosphorylase (Table I). Cyclic AMP-dependent protein kinase in the patient liver was similar to that found in the control liver and well responded to the cyclic nucleotide (Table I). These results indicated that the patient liver was deficient of phosphorylase kinase. Further evidence for this interpretation was provided by the experiments shown in Table III, where endogenous phosphorylase was shown to be activated in a preincubation mixture supplemented by muscle phosphorylase kinase but not by liver

TABLE III

Effects of rat liver cyclic AMP-dependent protein kinase and rabbit muscle phosphorylase kinase on activation of liver phosphorylase

Experiments were carried out under the conditions given in Table I, except that Tris-Cl at pH 8.5 was replaced by Tris-maleate at pH 6.8. Where indicated, 1.8 μg of rabbit muscle phosphorylase kinase and 15 μg of rat liver cyclic AMP-dependent protein kinase were added to the preincubation mixture, and 280 μg protein of either patient liver or normal liver supernatant was employed.

Test liver	Addition during preincubation	Phosphorylase activity
		(nmoles glucose-1-P produced/tube)
Patient	None	0.4
	Phosphorylase kinase	7.0
	Protein kinase	0.4
	Phosphorylase kinase + protein kinase	6.7
Control	None	7.7
	Phosphorylase kinase	7.2
	Protein kinase	6.2
	Phosphorylase kinase + protein kinase	7.1
None	Phosphorylase kinase	0.04
	Protein kinase	0.2
	Phosphorylase kinase + protein kinase	0.12

cyclic AMP-dependent protein kinase. Phosphorylase kinase alone could not degrade glycogen under these conditions. Glycogen synthetase and glucose 6-phosphatase were found to be normal (Table I). In contrast to the liver, the patient muscle phosphorylating system was normal and the glycogen content was also found to be in the normal range (Table I and II).

Several reports have appeared in the literature indicating that glycogenosis in the liver and muscle may be caused by deficiencies of phosphorylase activating system, and some of the patients have been suggested to be deficient of phosphorylase kinase (4-7). Hug et al. (8) have also suggested glycogenosis caused by simultaneous deficiencies of phosphorylase kinase and cyclic AMP-dependent protein kinase. The present case of the hepatic glycogenosis is

clearly shown to be caused by deficiency of phosphorylase kinase but not of other enzymes. Hepatic cyclic AMP-dependent protein kinase has been found by Langan (21) and detailed properties of the kinase as well as the mode of action of the cyclic nucleotide have been clarified only recently (9-12). Preceding reports from this laboratory (9, 11) have also shown that cyclic AMP-dependent protein kinases obtained from various mammalian tissues including liver and muscle apparently lack tissue- as well as species-specificities and phosphorylate same specific seryl and threonyl residues of several enzymes and proteins such as phosphorylase kinase, glycogen synthetase, lipase, histone, ribosomes and membrane-associated proteins. Thus, the deficiency of cyclic AMP-dependent protein kinase probably results in multiple disorders in the processes controlled by the cyclic nucleotide. However, so far no case has been equivocally established in which cyclic AMP-dependent protein kinase is missing. Another point to be noted here is that in deficiencies of phosphorylase or its activating system no increase in the blood sugar may be anticipated upon intravenous administration of glucagon. However, the present patient showed normal glucagon tolerance although phosphorylase kinase was almost completely deficient in the liver. Similar observations have been made by other workers for hepatic glycogenosis caused by deficiencies of phosphorylase (22, 23) as well as of its activating system (5, 7, 24). This discrepancy may be inevitable for further investigations.

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