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Glycogen Debranching Enzyme in Bovine Brain

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Glycogen debranching enzyme was partially purified from bovine brain using a substrate for measuring the amylo-1,6-glucosidase activity. Bovine cerebrum was homogenized, followed by cell-fractionation of the resulting homogenate. The enzyme activity was found mainly in the cytosolic fraction. The enzyme was purified 5,000-fold by ammonium sulfate precipitation, anion-exchange chromatography, gel-filtration, anion-exchange HPLC, and gel-permeation HPLC. The enzyme preparation had no α-glucosidase or α-amylase activities and degraded phosphorylase limit dextrin of glycogen with phosphorylase. The molecular weight of the enzyme was 190,000 and the optimal pH was 6.0. The brain enzyme differed from glycogen debranching enzyme of liver or muscle in its mode of action on dextrins with an α-1,6-glucosyl branch, indicating an amino acid sequence different from those of the latter two enzymes. It is likely that the enzyme is involved in the breakdown of brain glycogen in concert with phosphorylase as in the cases of liver and muscle, but that this proceeds in a somewhat different manner. The enzyme activity decreased in the presence of ATP, suggesting that the degradation of brain glycogen is controlled by the modification of the debranching enzyme activity as well as the phosphorylase.

Key words: brain, glycogen debranching enzyme, enzyme purification, glycogen.

The glycogen debranching enzyme is involved in the liver and muscle in the breakdown of glycogen in concert with glycogen phosphorylase (*1, 2*). Phosphorylase catalyzes the removal of α-1,4-glucosyl residues from the outermost chains of the glycogen molecule until approximately four glucose residues remain on either side of the α-1,6-branch. The role of the debranching enzyme is to remove the branches of phosphorylase limit dextrin formed so as to allow phosphorylase degradation to continue. The enzyme has two distinct active sites on a single polypeptide chain, corresponding to maltooligosaccharide transferase (1,4-α-glucan: 1,4-α-glucan 4-α-glucosyltransferase, EC 2.4.1.25) and amylo-1,6-glucosidase (dextrin 6-α-glucosidase, EC 3.2.1.33) (*3–9*). It transfers the maltotriosyl residue from one short branch of phosphorylase limit dextrin to the non-reducing-end of another, and then removes the remaining α-1,6 branch point glucose residue to generate maltodextrin.

In the human genome, there is only one glycogen debranching enzyme gene, containing at least two promoter regions, and in liver and muscle, isoform mRNAs are expressed differentially in a tissue-specific manner (*10*). Although it is well known that glycogen exists in the brain as well as in liver and muscle, little is known about the degradation of brain glycogen. It could be broken down by the same enzymes and in the same manner as in liver or muscle. On the other hand, considering the physiological characteristics of the brain, it can be speculated that brain glycogen may be degraded by other enzyme proteins, and hence with different substrate specificities, in a different manner. It thus remains to be clarified whether the brain enzyme is identical to either the muscle or liver forms, or whether it is a third isozyme. There have been two reports indicating the existence of a glycogen debranching enzyme in the brain. Immunoblot analysis of various tissues with a polyclonal anti-porcine muscle enzyme anitibody has shown the presence of a glycogen debranching enzyme in porcine brain (*11*). With regard to enzyme activity, amylo-1,6-glucosidase activity has been detected in mouse brain (*12*). If brain amylo-1,6-glucosidase has maltooligosaccharide transferase activity, it will be a glycogen debranching enzyme involved in the breakdown of brain glycogen. As an initial step in the studying the molecular basis of the brain enzyme, purification is necessary. This paper reports the partial purification and characterization of glycogen debranching enzyme from bovine brain.

MATERIALS AND METHODS

Materials—Bovine brain was purchased from Matsubara Meat Plant (Matsubara). Glycogen was from Wako Pure Chemicals (Osaka), rabbit muscle phosphorylase a was from Sigma (St. Louis, Mo, USA), the Vivapore concentrator and Vivapore 5 membrane (30,000 cut-off) were from

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Abbreviations: FD4-3, Glcα1-4(Glcα1-6)Glcα1-4Glcα1-4GlcPA; FD5-3, Glcα1-4Glcα1-4(Glcα1-6)Glcα1-4Glcα1-4GlcPA; FD5-4, Glcα1-4(Glcα1-6)Glcα1-4Glcα1-4Glcα1-4GlcPA; FD6-4, Glcα1-4Glcα1-4(Glcα1-6)Glcα1-4Glcα1-4Glcα1-4GlcPA; FD7-4, Glcα1-4Glcα1-4Glcα1-4(Glcα1-6)Glcα1-4Glcα1-4Glcα1-4GlcPA; FD7-3, Glcα1-4Glcα1-4Glcα1-4Glcα1-4(Glcα1-6)Glcα1-4Glcα1-4GlcPA; FD7-6, Glcα1-4(Glcα1-6)Glcα1-4Glcα1-4Glcα1-4Glcα1-4Glcα1-4GlcPA; FD7-7, Glcα1-6Glcα1-4Glcα1-4Glcα1-4Glcα1-4Glcα1-4Glcα1-4GlcPA; GlcPA, 1-deoxy-1-[(2-pyridyl)amino]-D-glucitol residue; PA-maltohexaose, Glcα1-4Glcα1-4Glcα1-4Glcα1-4Glcα1-4GlcPA.

Vivascience (Gloucestershire, UK), and the Diaflo-membrane YM-30 (30,000 cut-off) was from Amicon Japan (Tokyo). Wakosil-II 5C18 HG and 5Diol 300 columns were from Wako, the Super Q column was from Tosoh (Tokyo), and DEAE-Sephacel and Sephacryl S-300 were from Amersham Pharmacia Biotech (Uppsala, Sweden). The glucose C-II Test kit was from Wako. Glcα1-4(Glcα1-6)Glcα1-4Glcα1-4Glcα1-4GlcPA (FD5-4) and Glcα1-4Glcα1-4(Glcα1-6)Glcα1-4Glcα1-4Glcα1-4GlcPA (FD6-4) were prepared as described previously (*12*). Glcα1-4(Glcα1-6)Glcα1-4Glcα1-4GlcPA (FD4-3), Glcα1-4Glcα1-4(Glcα1-6)Glcα1-4Glcα1-4GlcPA (FD5-3), Glcα1-4Glcα1-4Glcα1-4(Glcα1-6)Glcα1-4Glcα1-4Glcα1-4GlcPA (FD7-4), Glcα1-4Glcα1-4Glcα1-4Glcα1-4(Glcα1-6)Glcα1-4Glcα1-4GlcPA (FD7-3), Glcα1-4Glcα1-4Glcα1-4Glcα1-4Glcα1-4Glcα1-4GlcPA (FD7-6), and Glcα1-6Glcα1-4Glcα1-4Glcα1-4Glcα1-4Glcα1-4Glcα1-4GlcPA (FD7-7) were prepared by the partial acid hydrolysis of *O*-α-D-glucosyl-β-cyclodextrin, followed by reductive amination of the liberated reducing-ends with 2-aminopyridine (*13*) and separation by HPLC in a manner similar to that used for FD5-4 and FD6-4.

Cell Fractionation—Bovine cerebrum (4.1 g) was homogenized in 37 ml of 5 mM Tris-HCl buffer, pH 7.3, containing 0.32 M sucrose using a Potter-Elvehjem-type teflon homogenizer. The homogenate was centrifuged at 900 ×*g* for 10 min. The resulting precipitate (P1 fraction) was suspended in 20 mM phosphate buffer, pH 6.5, containing 0.05% gelatin, 5 mM EDTA, and 10 mM β-mercaptoethanol to make 15 ml, and the supernatant was centrifuged at 7,000 ×*g* for 10 min. The resulting precipitate (P2 fraction) was suspended in the same buffer to make 15 ml. The supernatant was centrifuged at 105,000 ×*g* for 1 h. The resulting supernatant (cytosolic fraction) was dialyzed against the same buffer, and the pellet (microsomal fraction) was suspended in the same buffer to make 15 ml.

Glycogen Debranching Enzyme Assay—Glycogen debranching enzyme was assayed by measuring the amylo-1,6-glucosidase activity using FD6-4 as the substrate as reported previously (*12*). A mixture (120 μl) containing 6.7 μM FD6-4, 50 mM maleic acid–NaOH buffer (pH 6.0), 0.02% gelatin, 2 mM EDTA, 4 mM β-mercaptoethanol, and the enzyme preparation was incubated at 37°C for an appropriate period. The enzymatic reaction was stopped by adding 75 μl of 1 M acetic acid and heating at 100°C for 3 min. The fluorogenic product in the digest, Glcα1-4Glcα1-4Glcα1-4Glcα1-4Glcα1-4GlcPA (PA-maltohexaose), was measured by HPLC as follows. A Wakosil-II 5C18 HG column (6.0 × 150 mm) was eluted with 50 mM ammonium acetate buffer, pH 4.5, containing 0.05% 1-butanol at a flow rate of 1.5 ml/min. The elution was monitored by observing the fluorescence at 400 nm (excitation at 320 nm). One unit of debranching enzyme was defined as the amount of enzyme that releases 1 nmol of PA-maltohexaose per h under the conditions employed.

Protein Assay—Protein was assayed by measuring the absorbance at 280 nm using bovine serum albumin as a standard.

Purification of Glycogen Debranching Enzyme—Step 1. Preparation of the crude enzyme solution: Bovine cerebrum (395 g) was homogenized in 3,550 ml of 5 mM Tris-HCl buffer, pH 7.3, containing 0.32 M sucrose using a Potter-Elvehjem homogenizer. After the homogenate was centrifuged at 10,000 ×*g* for 20 min, the resultant supernatant was filtered through a Hyflo Super-Cel and the filtrate was dialyzed against 20 mM phosphate buffer, pH 6.5, containing 0.05% gelatin, 5 mM EDTA, and 10 mM β-mercaptoethanol. The dialysate was used as the crude enzyme solution.

Step 2. Ammonium sulfate precipitation: Ammonium sulfate was added to the crude enzyme solution to give 15% saturation. The precipitate was removed by centrifugation, and ammonium sulfate was added to the supernatant to give 45% saturation. The resultant precipitate was collected by centrifugation and dialyzed against 7 mM phosphate buffer, pH 6.5, containing 10 mM β-mercaptoethanol.

Step 3. DEAE-Sephacel chromatography: The dialyzed solution (140 ml) was applied to a DEAE-Sephacel column (1.9 × 52 cm) equilibrated with 7 mM phosphate buffer, pH 6.5, containing 10 mM β-mercaptoethanol, and the column was washed with 100 ml of the same buffer. Glycogen debranching enzyme was eluted with a linear gradient of 7 mM to 0.65 M phosphate buffer (600 ml). The pooled solution containing glycogen debranching enzyme was concentrated by ultrafiltration using a Diaflo-membrane YM-30.

Step 4. Sephacryl S-300 gel filtration: The solution obtained in Step 3 was applied to a Sephacryl S-300 column (3.0 × 140 cm) equilibrated with 20 mM phosphate buffer, pH 6.5, containing 0.01% bovine serum albumin, 5 mM EDTA, and 10 mM β-mercaptoethanol, and then eluted with the same buffer. The debranching enzyme fractions were pooled and concentrated to a small volume by ultrafiltration using a Diaflo-membrane YM-30 and a Vivapore 5 membrane.

Step 5. Super Q HPLC: Anion-exchange HPLC was performed on a Super Q column (7.5 × 75 mm). The column was equilibrated with 20 mM phosphate buffer, pH 7.5, containing 2 mM β-mercaptoethanol, at a flow rate of 1.0 ml/min. The solution obtained in Step 4 was injected onto the column, which was washed with 5 ml of the same buffer. The column was then eluted with a linear gradient of 20 to 180 mM phosphate buffer in 35 min. The glycogen debranching enzyme fractions were pooled, and concentrated to a small volume using a Vivapore 5 membrane.

Step 6. Gel permeation HPLC: Gel permeation HPLC was performed on a Wakosil 5Diol 300 column (1.0 × 30 cm) at the flow rate of 0.5 ml/min. The elution buffer was 0.2 M phosphate buffer, pH 6.5, containing 2 mM β-mercaptoethanol

Molecular Weight Measurement of the Enzyme—The molecular weight of glycogen debranching enzyme was estimated by gel filtration on a Sephacryl S-300 column (1.6 × 140 cm) equilibrated with 20 mM phosphate buffer, pH 6.5, containing 0.01% bovine serum albumin, 5 mM EDTA, and 10 mM β-mercaptoethanol. Thyroglobulin (MW 669,000), ferritin (MW 443,000), alcohol dehydrogenase (MW 150,000), serum albumin (MW 66,000), and carbonic anhydrase (MW 29,000) were used as standards.

Partial Purification of Bovine Liver and Skeletal Muscle Enzyme—The bovine liver and skeletal muscle enzymes were partially purified from liver and skeletal muscle, respectively, by ammonium sulfate precipitation, DEAE-Sephacel anion-exchange chromatography, and Sephacryl S-300 gel filtration as in the case of the brain enzyme. Each enzyme preparation hydrolyzed FD6-4 to yield only PA-maltohexaose, and was free of α-glucosidase and α-amylase.

Preparation of Phosphorylase Limit Dextrin from Glycogen—Phosphorylase limit dextrin was prepared from glycogen basically according to the method of Hers *et al.* (*14*). A mixture of 200 mg glycogen and 60 units phosphorylase a in 13 ml of 0.1 M sodium phosphate buffer, pH 7.4, was incubated in a cellophane bag and dialyzed against 0.1 M sodium phosphate buffer, pH 7.4. After 24 h, 12 units of phosphorylase a were added to the internal solution and incubation and dialysis were continued for 24 h. The enzymatic reaction was stopped by heating at 100°C for 5 min, and the insoluble material produced was removed by centrifugation. The resultant solution was used as phosphorylase limit dextrin solution for assaying the activity of maltooligosaccharide transferase.

Measurement of Maltooligosaccharide Transferase Activity—The assay of the maltooligosaccharide transferase activity of glycogen debranching enzyme is based on the principle that when maltooligosaccharide transferase activity removes branches of limit dextrin in cooperation with the amylo-1,6-glucosidase activity, the coupled enzyme, phosphorylase, liberates non-reducing-end glucose residues as glucose 1-phosphate. The maltooligosaccharide transferase activity can therefore be assayed by determining the amount of glucose 1-phosphate. To a mixture of 200 μl of the phosphorylase limit dextrin solution described above and 25 μl phosphorylase a (6 units), 100 μl of the enzyme solution was added, and the mixture was incubated at 37°C for 16 h. The enzymatic reaction was stopped by heating at 100°C for 5 min, and the resulting denatured protein was removed by centrifugation. To 250 μl of the reaction mixture, 36 μl of 1 M hydrochloric acid was added to adjust the pH to 2.0. The mixture was heated at 90°C for 2 h. Under the conditions used, glucose 1-phosphate liberated by phosphorylase is hydrolyzed to glucose, while α-1,4- and α-1,6-glucosidic bonds are not hydrolyzed. The solution was neutralized with 50 μl of 0.1 M sodium hydroxide, and the amount of glucose produced was measured with a glucose C-II-Test kit.

PH Dependence of Amylo-1,6-Glucosidase Activity—Mixtures (120 μl) containing 6.7 μM FD6-4, 50 mM maleic acid-NaOH buffer at various pHs, 0.02% gelatin, 2 mM EDTA, 4 mM β-mercaptoethanol, and 0.15 units of the enzyme were incubated at 37°C for 10 min. The enzymatic reaction was stopped by adding 75 μl of 1 M acetic acid and heating at 100°C for 3 min. The amount of PA-maltohexaose produced was measured by HPLC.

Measurement of Amylo-1,6-Glucosidase Activity in the Presence of Metal Cations—The enzyme (0.13 units) was preincubated with 1 mM metal chloride in 98 μl of 20 mM phosphate buffer, pH 6.5, containing 0.01% bovine serum albumin and 10 mM β-mercaptoethanol, at 37°C for 30 min. To the solution, 2 μl of 0.40 mM FD6-4 was added and then the mixture was incubated at 37°C for 1 h. The enzymatic reaction was stopped by adding 75 μl of 1 M acetic acid and heating at 100°C for 3 min. The amount of PA-maltohexaose produced was measured by HPLC.

Measurement of Amylo-1,6-Glucosidase Activity in the Presence of the Allosteric Effectors for Glycogen Phosphory-

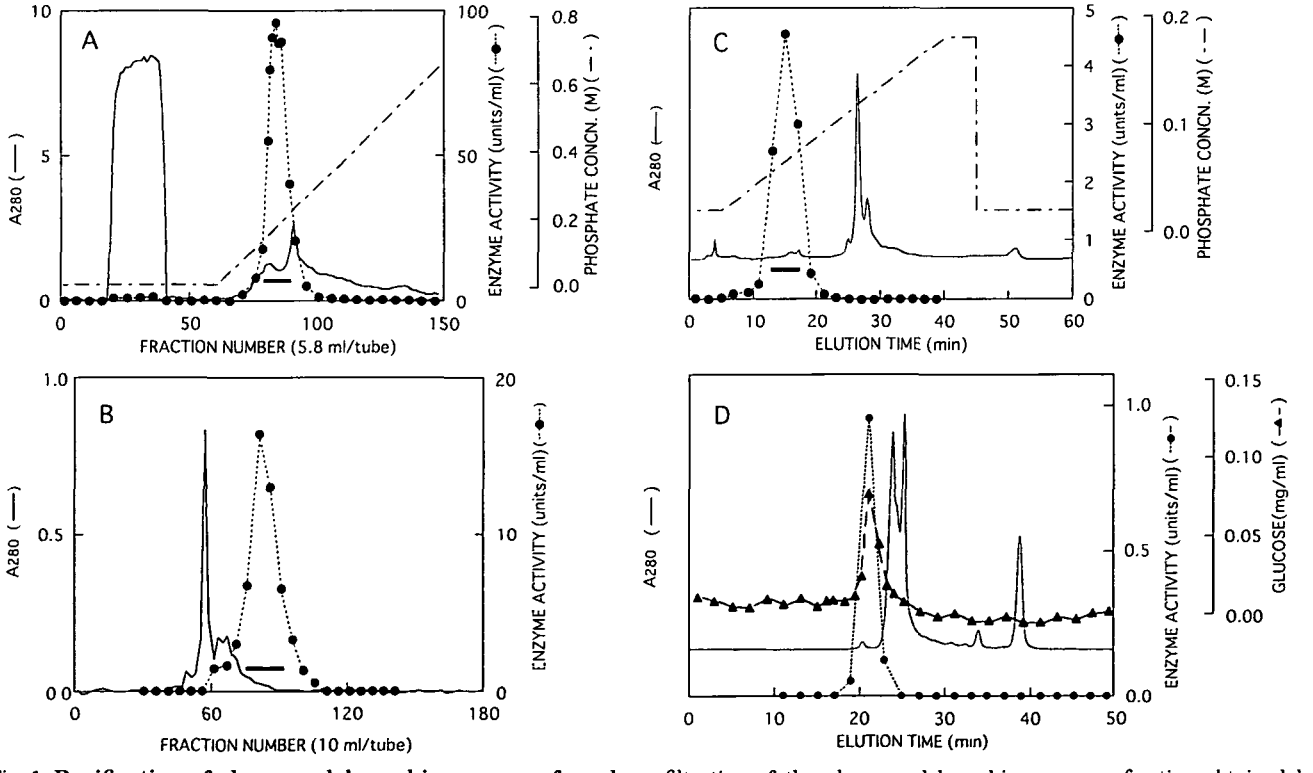

Fig. 1. **Purification of glycogen debranching enzyme from bovine brain.** Chromatographies were carried out as described in "MATERIALS AND METHODS"; (—) protein; (···) enzyme activity. The fractions indicated by the bars were collected for further purification. (A) DEAE-Sephacel chromatography of the precipitate obtained following 15–45% saturation with ammonium sulfate. (B) Sephacryl S-300 gel filtration of the glycogen debranching enzyme fraction obtained by DEAE-Sephacel chromatography. (C) Super Q HPLC of the glycogen debranching enzyme fraction obtained by Sephacryl S-300 gel filtration. (D) 5Diol 300 gel permeation HPLC of the glycogen debranching enzyme fraction obtained by Super Q HPLC.

lase—The enzyme (0.046 units) was preincubated with each allosteric effector for glycogen phosphorylase in 98 ml of 20 mM phosphate buffer, pH 6.5, containing 5 mM EDTA, 0.01% bovine serum albumin, and 10 mM β-mercaptoethanol, at 37°C for 30 min. To the solution, 2 μl of 0.40 mM FD6-4 was added and then the mixture was incubated at 37°C for 1 h. The enzymatic reaction was stopped by adding 75 μl of 1 M acetic acid and heating at 100°C for 3 min. The amount of PA-maltohexaose produced was measured by HPLC.

Measurement of the Rates of Hydrolysis for Fluorogenic Dextrins with a Glucosyl α-1,6 Branch—A mixture (120 μl) containing 6 μM fluorogenic dextrin, 50 mM maleic acid–NaOH buffer (pH 6.0), 0.02% gelatin, 2 mM EDTA, 4 mM β-mercaptoethanol, and 0.8 units of the enzyme was incubated at 37°C for an appropriate period. The enzymatic reaction was stopped by adding 75 μl of 1 M acetic acid and heating at 100°C for 3 min. The fluorogenic product in the digest was measured by HPLC.

RESULTS AND DISCUSSION

Cellular Distribution of Glycogen Debranching Enzyme—Following homogenization and cell-fractionation of bovine cerebrum, the glycogen debranching enzyme activity of each fraction was assayed by measuring the amylo-1,6-glucosidase activity using FD6-4 as a substrate. The substrate is hydrolyzed by the enzyme to yield fluorogenic PA-maltohexaose. α-Glucosidases or α-amylases also hydrolyze the substrate to give other fluorogenic products; however, this was not a problem because PA-maltohexaose can be separated from the other products by HPLC. The activities of the P1, P2, P3 (microsomal fraction), and cytosolic fractions were 40, 27, 10, and 50 units, respectively. The activity of the cytosolic fraction was thought to be attributable to glycogen debranching enzyme dissociated from protein-glycogen particles as in the case of the rabbit skeletal muscle enzyme (7). The cytosolic fraction was soluble and had the greatest activity, and was therefore considered suitable as a starting material for the purification of brain glycogen debranching enzyme.

Purification of Brain Glycogen Debranching Enzyme—Bovine brain glycogen debranching enzyme was partially purified from cerebrum as described in "MATERIALS AND METHODS" (Fig. 1). The α-glucosidase activity in the crude enzyme preparation was removed by DEAE-Sephacel chromatography. Bovine serum albumin was added to the elution buffer for Sephacryl S-300 chromatography in order to stabilize the enzyme, and was later separated easily from glycogen debranching enzyme by Super Q HPLC. The purification was carried out while monitoring the amylo-1,6-glucosidase activity, as glycogen debranching enzyme should have both maltooligosaccharide transferase and amylo-1,6-glucosidase activities. The maltooligosaccharide transferase activity did indeed coincide with the amylo-1,6-glucosidase activity (Fig. 1D). Considering that a glycogen debranching enzyme has been detected in porcine brain by immunoblot analysis (*11*), it is reasonable to think that the amylo-1,6-glucosidase obtained is a glycogen debranching enzyme with a maltooligosaccharide transferase activity. Since the enzyme becomes less stable as the purification proceeds, further purification will make it necessary to find a way to reduce the inactivation. Although the enzyme preparation depicted in Fig. 1D was not completely purified, it was considered sufficiently pure for examining the enzymatic properties. The process of purification is summarized in Table I.

Characterization of Purified Glycogen Debranching En-

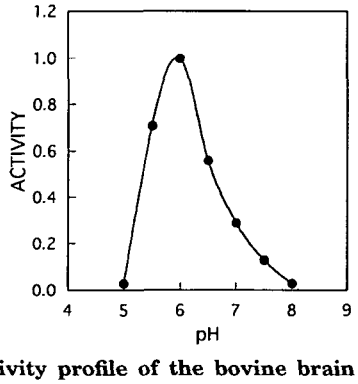

Fig. 2. **PH/activity profile of the bovine brain glycogen debranching enzyme.** The amylo-1,6-glucosidase activity of the enzyme was measured at various pH values as described in "MATERIALS AND METHODS." The activity at the optimal pH was taken as unity.

TABLE II. **Effects of metal cations on enzyme activity.** The amylo-1,6-glucosidase activity of the enzyme was measured in the presence of 1 mM metal chloride as described in "MATERIALS AND METHODS."

Ion added	Activity[a]
Control	1.00
Ca^{2+}	1.09
Mn^{2+}	1.05
Mg^{2+}	0.86
Ba^{2+}	1.54
Sr^{2+}	1.05
Fe^{2+}	0.07
Fe^{3+}	0.72
Co^{2+}	0.15
Ni^{2+}	0.25
Zn^{2+}	0.04
Cd^{2+}	0.00
Hg^{2+}	0.03
Cu^{2+}	0.06

[a]The activity without the addition of metal chloride was taken as unity.

TABLE I. **Summary of glycogen debranching enzyme purification from bovine brain.**

Purification step	Protein (mg)	Total activity (units)	Specific activity (units/mg)	Recovery (%)	Purification (-fold)
Crude enzyme	35,000	23,000	0.66	100	1.0
$(NH_4)_2SO_4$ precipitation	4,900	11,000	2.3	48	3.5
DEAE-Sephacel chromatography	100	8,400	84	37	130
Sephacryl S-300 gel filtration	7.8	4,400	560	19	850
Super Q HPLC	1.3	1,100	890	4.8	1,300
5Diol 300 gel permeation HPLC	0.049	150	3,100	0.65	4,700

zyme—The molecular weight of the enzyme was found to be 190,000 by gel filtration on Sephacryl S-300 (data not shown). A large molecular weight is one of the characteristics of a glycogen debranching enzyme with the two catalytic activities arising from gene duplication. The value is slightly larger than that of the rabbit muscle enzyme (160,000) (*6, 7*) or the human muscle enzyme (165,000) (*15*). The pH dependence of the amylo-1,6-glucosidase activity of the enzyme was also examined (Fig. 2), and the optimum pH was found to be 6.5. The effects of metal cations on the enzyme activity are shown in Table II: the amylo-1,6-glucosidase is inactivated by Fe^{2+}, Hg^{2+}, Zn^{2+}, Cd^{2+}, and Cu^{2+}.

Effects of Allosteric Effectors for Glycogen Phosphorylase on the Amylo-1,6-Glucosidase Activity of the Enzyme—The activity of rabbit muscle glycogen phosphorylase is allosterically influenced by glucose, AMP, ATP, and glucose 6-phosphate (*16*). Glycogen breakdown is believed to be controlled by modifications of the phosphorylase activity only, even though glycogen debranching enzyme is involved in degradation. Because it was thought that one or more these substances might also be an effector for glycogen debranching enzyme in the brain, their effects on the activity of the bovine brain enzyme were examined. The enzyme activity decreases in the presence of ATP (Fig. 3), while it is influenced only slightly by AMP, glucose 1-phosphate, glucose 6-phosphate, or glucose (Table III). These findings are consistent with depression in the activity of phosphorylase *b* by ATP. Considering that the ATP content in rat brain has been reported to be about 2 mmol/kg (*17*), the results shown in Fig. 3 suggest the possibility that the breakdown of brain glycogen is regulated not only through the control of glycogen phosphorylase activity by allosteric effectors, but also through the control of glycogen debranching enzyme activity by ATP.

Comparison of Substrate Specificities of the Brain, Liver, and Muscle Enzymes—The substrate specificity of an enzyme is determined by the three-dimensional structure of the active site. It was expected that the difference in the active sites of the two enzymes could be detected sensitively by comparing their substrate specificities for various substrates. The rates of hydrolysis of fluorogenic dextrins with a Glcα1-6 structure by the brain, liver, and muscle en-

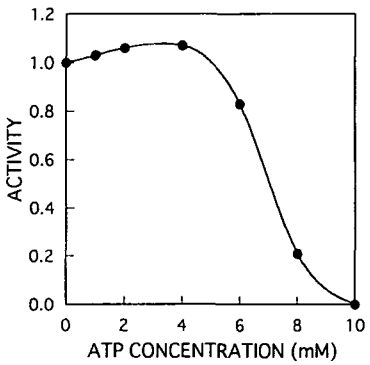

Fig. 3. **Effect of ATP on the amylo-1,6-glucosidase activity of bovine brain glycogen debranching enzyme.** The amylo-1,6-glucosidase activity of the enzyme was measured at various ATP concentrations as described in "MATERIALS AND METHODS." The activity without ATP was taken as unity.

TABLE III. **Effects of allosteric effectors for glycogen phosphorylase on the amylo-1,6-glucosidase activity of bovine brain glycogen debranching enzyme.** The amylo-1,6-glucosidase activity of the enzyme was measured in the presence of the allosteric effectors as described in "MATERIALS AND METHODS."

Effector (concentration)	Activity[a]
Control	1.00
Glucose (1 mM)	0.95
Glucose (10 mM)	0.89
Glucose 1-phosphate (1 mM)	0.93
Glucose 1-phosphate (10 mM)	0.82
Glucose 6-phosphate (1 mM)	0.92
Glucose 6-phosphate (10 mM)	0.89
AMP (1 mM)	1.01
AMP (10 mM)	0.86
ATP (1 mM)	1.03
ATP (10 mM)	0.00

[a]The activity without the addition of effector was taken as unity.

TABLE IV. **Rates of hydrolysis of fluorogenic dextrins by bovine brain and other glycogen debranching enzymes.**

	Fluorogenic dextrin	(Fluorogenic product)	Relative rate of hydrolysis[a]		
			Brain	Liver	Muscle
FD4-3	G | G-G-G-GPA	(G-G-G-GPA)	2.3	3.1	2.5
FD5-3	G | G-G-G-G-GPA	(G-G-G-G-GPA)	21	33	29
FD5-4	G | G-G-G-G-GPA	(G-G-G-G-GPA)	1.0	1.0	1.0
FD6-4	G | G-G-G-G-G-GPA	(G-G-G-G-G-GPA)	17	25	17
FD7-3	G | G-G-G-G-G-G-GPA	(G-G-G-G-G-G-GPA)	5.1	14	11
FD7-4	G | G-G-G-G-G-G-GPA	(G-G-G-G-G-G-GPA)	21	32	22
FD7-6	G | G-G-G-G-G-G-GPA	(G-G-G-G-G-G-GPA)	1.9	3.3	3.4
FD7-7	G | G-G-G-G-G-G-GPA	(G-G-G-G-G-G-GPA)	1.1	1.7	1.2

[a]The relative rate of hydrolysis is expressed by taking the initial velocity of hydrolysis of FD5-4 as unity G, D-glucopyranose residue; GPA, 1-deoxy-1-[(2-pyridyl)amino]-D-glucitol residue; -, α-1,4-glycosidic linkage; |, α-1,6-glycosidic linkage.

zymes were compared. The dextrins were hydrolyzed by the three enzymes to the corresponding PA-maltooligosaccharides and glucose, and no other fluorogenic products were formed; the rates of hydrolysis are shown in Table IV. The fact that the liver and muscle enzymes have different amino acid sequences is well demonstrated by the values of their relative rates of hydrolysis of FD6-4 and FD7-4, *i.e.*, the fluorogenic dextrins are effective probes for discriminating the activie sites of the two enzymes. If the brain enzyme is identical to the liver or muscle enzyme, it should exhibit equivalent relative rates of hydrolysis. The values for the brain enzyme are different from those of the liver enzyme for most fluorogenic dextrins examined, and also different from those of the muscle enzyme for FD5-3, FD7-3, and FD7-6. The results indicate that the brain enzyme differs from both the liver and muscle enzymes with regard to the active site and amino acid sequence.

Bao *et al.* reported the structural organization of the human chromosomal glycogen debranching enzyme gene, and showed that the presence of at least two promoter regions causes the differential expression of isoform mRNAs in a tissue-specific manner (*10*). It is likely that in bovine brain, an enzyme with a substrate specificity different from those of the liver or muscle enzyme is expressed in a brain-specific manner, and is involved in the breakdown of brain glycogen.

The physiological role of brain glycogen has not yet been clearly explained. The brain consumes much more glucose than other organs or tissues, and glucose is supplied from the liver through blood circulation. A fall in the blood glucose concentration can result in serious brain damage. The breakdown of brain glycogen to glucose and glucose 1-phosphate may be a significant physiological process, and may proceed somewhat differently from the pathway in the liver or muscle.

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