A NEW GLYCOGEN PHOSPHORYLASE PRESENT IN THE RAT TISSUES CONTAINING THE BRAIN-TYPE ISOZYME

The active monomer of brain-type isozyme

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1. Introduction

It is now well established that there are at least three types of glycogen phosphorylase (EC 2.4.1.1) isozyme; muscle (MM), liver (LL) and brain (BB) types in mammalian tissues in common [1–6]. The brain-type isozyme is the predominant type in various fetal rat tissues and hepatomas as well as in the adult brain and so called the fetal type [5,6]. The type I phosphorylase of rabbit heart is also identical with the brain type. In continuing our studies on the brain or fetal type isozyme [5–8], we have found a new glycogen phosphorylase (B') in the rat tissues and hepatomas containing the brain-type isozyme on polyacrylamide disc-gel electrophoresis, at pH 7.3. Based on various observations, B' is suggested to be the enzymatically active monomer subunit of dimeric BB detected only by the electrophoresis so far.

2. Materials and methods

Sprague-Dawley rats were used and the Yoshida ascites hepatoma AH 130 [9] was harvested as in [8]. Glucose-1-P and AMP were purchased from Boehringer, Mannheim and purified on Dowex 1 × 2 columns as in [10]. Oyster glycogen was purchased from Nakarai Chemicals, Kyoto. Ten percent of the glycogen suspension was hydrolyzed in 0.05 N HCl at 100°C for 30 min, then cooled and neutralized with 2 M Tris.

Antibodies to the three phosphorylase isozyme types were prepared in rabbits as in [6,8].

Polyacrylamide disc-gel electrophoresis was performed as in [11] slightly modified as follows: Samples were directly applied on each column (5 × 70 mm) in 15% sucrose solution without making sample gels. Electrophoresis was carried out at constant 0.5 mA/column until bromophenol blue moved 1 cm into the small pore gels (for about 50 min); then raised to 1 mA/column (for about 60 min). For re-electrophoresis of the BB and B' separated in one gel, each segment of gel containing the respective activity was cut out and re-packed into two separate columns of > 2–3 mm large-pore gel, and electrophoresis carried out under the same conditions as above. After electrophoresis, phosphorylase activity was stained with iodine solution as in [8].

3. Results

The rat brain was homogenized with a Teflon homogenizer in 4 vol. 62.5 mM Tris–HCl (pH 7.4), containing 25 mM 2-mercaptoethanol and 6.25 mM EDTA, and AH 130 cells were sonicated in the same homogenizing medium as in [8]. The homogenates were incubated at 30°C for 45 min to convert a-form phosphorylases into b-forms, and centrifuged at 105 000 × g for 45 min. The supernatant of brain was diluted 4-fold with the homogenizing medium. HCl–imidazole solution, 1.5 vol., 6.5 mM (pH 6.8) containing 25% sucrose were added to the respective 105 000 × g supernatants, and a 0.1 ml sample was applied to each column.

As shown in fig.1, a new glycogen phosphorylase B′ was observed in brain as well as in AH 130 cells on the polyacrylamide disc-gel electrophoresis, at pH 7.3. B′ migrated faster than any of the known b-form phosphorylase isozymes (BB, MM and LL) and their hybrids (MB and LB). Not only AMP but also sodium sulfate at high concentrations was essential for its activity. Furthermore, B′ could synthesize glycogen even without added primer glycogen and therefore B′ was remarkably different from the known isozymes. None of the activities other than that of B′ could be detected without added primer glycogen even after a prolonged incubation of 2 or 3 days. Of the compounds tested only glucose-1-P was effective as substrate for B′ in the direction of glycogen synthesis on polyacrylamide gels. Glucose, maltose, glucose-6-P and UDP-glucose did not act as substrate for it at 40 mM. The glycogen synthesized by B′ on gels was completely degraded to form glucose-1-P when the substrate solution was replaced by 0.2 M P_i solution (pH 6.1), containing 1 mM AMP and 0.75 M sodium sulfate, and incubated at the same temperature. As can be seen in fig.2, B′ was absorbed together with BB and LB specifically by anti-BB antibody. Both anti-LL and anti-MM antibodies had no effect on B′ activity,

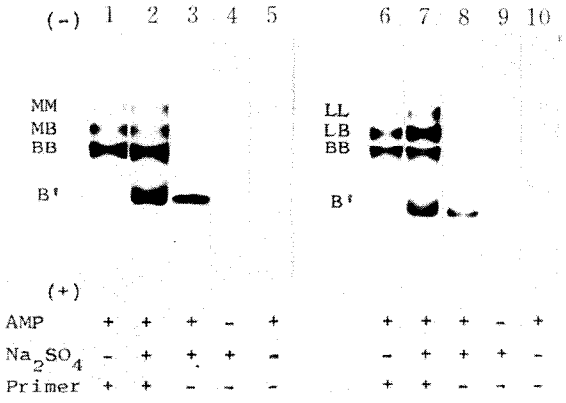

Fig.1. Polyacrylamide disc-gel electrophoretic patterns of brain and AH 130 phosphorylases. Electrophoresis was performed as in the text using 7.5% acrylamide small-pore gels. After electrophoresis, gels were incubated in the substrate solution at 30°C for 1 h in the presence and absence of 1 mM AMP, 0.75 M sodium sulfate, and 2% hydrolyzed glycogen, as indicated. Gels 1–5, brain phosphorylases; gels 6–10, AH 130 phosphorylases.

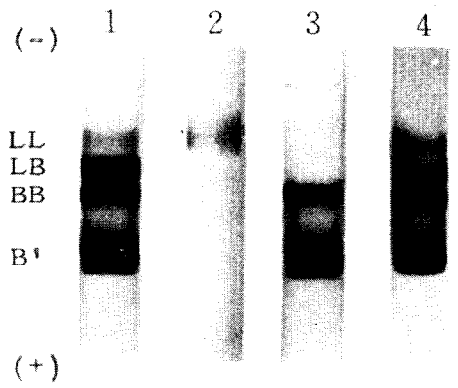

Fig.2. Immunoabsorption of AH 130 phosphorylases with antibodies to the three isozyme types. Aliquots of the 105 000 × *g* supernatant of AH 130 cells were contacted with appropriate amounts of antibodies to the three isozyme types, respectively, at 30°C for 10 min, then subjected to the electrophoretic analysis. Gel 1 is a control, and gels, 2, 3 and 4 show the patterns absorbed with anti-BB, anti-LL and anti-MM antibodies, respectively. Phosphorylase activities in all gels were detected in the presence of 1 mM AMP, 0.75 M sodium sulfate, and 2% hydrolyzed glycogen.

although LB was absorbed by anti-LL antibody. By changing polyacrylamide concentration under the similar electrophoretic conditions to those in fig.1 by the method in [12], B′ was suggested to be mainly different in size from BB (also from MB and MM) (fig.3A) and using several standard proteins was estimated to be mol. wt 113 000 and approximately half the molecular weight (200 000) of BB and other isozyme types (fig.3B). Furthermore, as shown in fig.4, it was found that both BB and B′ once separated in a gel, convert into each other to give similar patterns to the original pattern in gel 1, on the re-electrophoresis of BB and B′. This interconversion may occur in the solution above or within the large-pore gel in rapid equilibrium after elution from the original gel segment. Thus, it is strongly suggested that B′ is the active monomer dissociated from BB under the electrophoretic conditions.

4. Discussion

The newly found B′ in adult rat brain as well as in AH 130 cells is also detectable electrophoretically in

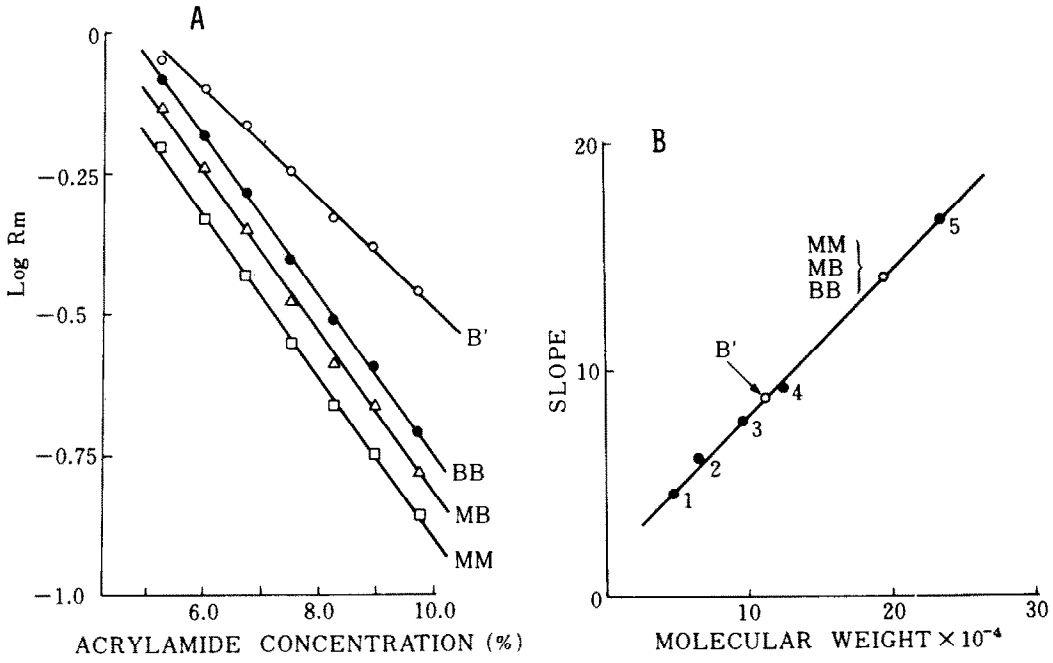

Fig.3A. Effect of small-pore gel acrylamide concentration on electrophoretic mobilities of brain phosphorylases. ~0.02 unit 105 000 × *g* supernatant was applied on each olumn in which small-pore gel acrylamide concentration was varied at regular intervals of 0.75% from 5.25%–9.75%. Electrophoresis was carried out under routine conditions and phosphorylase activity was stained as in fig.2. Relative mobility (Rm) of each phosphorylase was plotted against small-pore gel acrylamide concentration. Fig.3B. Molecular weight determination of brain phosphorylase by the method in [12]. Standard proteins used are: 1, ovalbumin; 2, bovine serum albumin; 3, yeast hexokinase; 4, glycerol 3-phosphate dehydrogenase; 5, pyruvate kinase.

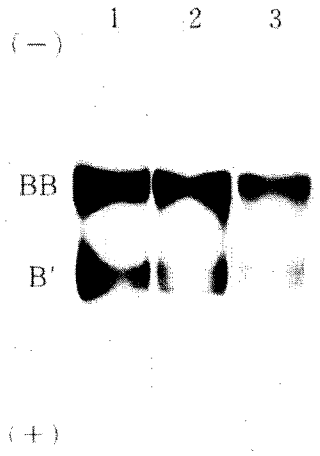

Fig.4. Re-electrophoretic patterns of BB and B′. Electrophoresis was as in the text. Gel 1 represents the electrophoretic pattern of purified BB; gel 2 and gel 3, re-electrophoretic patterns of BB and B′, respectively. Phosphorylase activity was stained as in fig.2, in which gel 1 was incubated for 1 h in the substrate solution, and gel 2 and gel 3 for 2 h.

the rat tissues containing the brain-type isozyme such as various fetal tissues, adult brain and heart, as well as various hepatomas as reported [5–8].

B′ shows a distinct phosphorylase activity to catalyze the synthesis and degradation of glycogen using glucose-1-P, glycogen and P_i as substrates and AMP and high concentrations of sodium sulfate as activators. B′ was immunochemically crossreactive with BB, its molecular weight was approximately half the molecular weight of BB, and BB and B′ are interconvertible with each other. Furthermore, the purified BB, which was obtained from the rat brain by ammonium sulfate fractionation, glycogen precipitation and DE-52 cellulose chromatography and finally contained only a single protein equivalent to monomeric subunit of BB on SDS-electrophoresis (not shown), released still the same amount of B′ as in the crude extract so far as detected by the above-mentioned electrophoresis (gel 1 in fig.4). Accordingly, B′ was denied to be such a degraded fragment of BB as b′ of muscle

phosphorylase [13] and concluded to be the active monomer subunit of BB. B′ differs greatly from BB in that its activity is dependent not only on AMP but also on high concentrations of sodium sulfate, and B′ has a unique property to synthesize glycogen without added primer glycogen, so that it differs greatly from other isozymes including BB. Since the primer-independent phosphorylases so far reported are of plant origin [14,15], B′ may be the first primer-independent one found in mammalian tissues. Although it is still disputable whether rabbit muscle phosphorylase is primer glycogen-independent or not [16,17], no crystalline rabbit muscle phosphorylase (b-form) activity was detectable on polyacrylamide gels without primer glycogen, even though > 10-fold the activity of brain or AH 130 phosphorylase was applied.

Enzymatically-active monomer subunits are important for the understanding of the quaternary structure and function of a multisubunit enzyme. However, monomers of rabbit muscle phosphorylase obtained by treatment with chemical reagents [18–21] or covalently-bound to Sepharose [22] showed little if any activity. Indeed, as far as investigated, we could separate B′ from BB only by polyacrylamide disc-gel electrophoresis at pH 7.3, and have been unable to separate them by Sephadex G-150 gel filtration, DE-cellulose chromatography or isoelectric focusing, or to detect the monomers of MM or LL like B′ from BB even by the electrophoretic method described here. Based on these and other data, we suppose that although B′ may be present in very small amounts in solution in rapid equilibrium with BB, it dissociates from BB under the electrophoretic conditions, migrates faster than BB and is entrapped within polyacrylamide gel pores, where B′ re-associates to BB with difficulty and shows the unique properties reported here.

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