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Glycogen Debranching Enzyme: Purification, Antibody Characterization, and Immunoblot Analyses of Type III Glycogen Storage Disease[1]

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SUMMARY

Type III glycogen storage disease is caused by a deficiency of glycogen debranching-enzyme activity. Many patients with this disease have both liver and muscle involvement, whereas others have only liver involvement without clinical or laboratory evidence of myopathy. To improve our understanding of the molecular basis of the disease, debranching enzyme was purified 238-fold from porcine skeletal muscle. In sodium dodecyl sulfate–polyacrylamide gel electrophoresis the purified enzyme gave a single band with a relative molecular weight of 160,000 that migrated to the same position as purified rabbit-muscle debranching enzyme. Antiserum against porcine debranching enzyme was prepared in rabbit. The antiserum reacted against porcine debranching enzyme with a single precipitin line and demonstrated a reaction having complete identity to those of both the enzyme present in crude muscle and the enzyme present in liver extracts. Incubation of antiserum with purified porcine debranching enzyme inhibited almost all enzyme activity, whereas such treatment with preimmune serum had little effect. The antiserum also inhibited debranching-enzyme activity in crude liver extracts from both pigs and humans to the same extent as was observed in muscle. Immunoblot analysis probed with anti–porcine-muscle debranching-enzyme antiserum showed that the antiserum can detect debranching enzyme in both human muscle and human liver. The bands detected in human

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samples by the antiserum were the same size as the one detected in porcine muscle. Five patients with Type III and six patients with other types of glycogen storage disease were subjected to immunoblot analysis. Although anti-porcine antiserum detected specific bands in all liver and muscle samples from patients with other types of glycogen storage disease (Types I, II, and IX), the antiserum detected no cross-reactive material in any of the liver or muscle samples from patients with Type III glycogen storage disease. These data indicate (1) immunochemical similarity of debranching enzyme in liver and muscle and (2) that deficiency of debranching-enzyme activity in Type III glycogen storage disease is due to absence of debrancher protein in the patients that we studied.

INTRODUCTION

Glycogen debranching enzyme is a eukaryotic enzyme that contains two catalytic activities on a single polypeptide chain. It has been shown by three independent groups that the enzyme is a large monomeric protein with a molecular weight of 160,000–170,000 and is without evidence of subunits (Gordon et al. 1972; White and Nelson 1974; Taylor et al. 1975). The two catalytic activities are oligo 1,4-1,4 glucantransferase (E.C.2.4.1.25) and amylo 1,6 glucosidase (E.C.3.2.1.33). These two activities occur at separate catalytic sites on a polypeptide chain and can function independently of each other (Bates et al. 1975; Gillard and Nelson 1977).

Deficiency of glycogen debranching-enzyme activity causes Type III glycogen storage disease (Cori disease or limit dextrinosis). In this form of glycogen storage disease, a polysaccharide accumulates in the fasting state, with a structure resembling that of the phosphorylase limit dextrin. Patients with Type III glycogen storage disease vary remarkably, both clinically and enzymatically (Van Hoof and Hers 1967; Brown and Brown 1968; Howell and Williams 1983). Some patients have liver involvement manifested by hepatomegaly and hypoglycemia; a very few patients have muscle weakness and wasting but no clinically apparent liver disease; and many patients have both liver and muscle problems. Enzymatically, patients with both myopathy and liver involvement have an enzyme defect in muscle and liver, whereas patients with only liver involvement lack enzyme activity in liver and have normal activity in muscle. In addition, some patients who lack the enzyme in muscle have neither myopathy nor storage of excessive amounts of polysaccharide, although the isolated polysaccharide resembles limit dextrin. The enzymatic variation in Type III glycogen storage disease suggests that expression of debranching enzyme in human liver and muscle could be under separate genetic control.

The molecular basis for Type III glycogen storage disease is not clearly understood; nor are the reasons for the variability in organ involvement and clinical severity. It is difficult to understand how a monomeric enzyme that is

normally present in virtually all tissues can genetically vary in its presence in specific tissues. The present investigation demonstrates enzyme purification, antibody characterization, and molecular analyses of Type III glycogen storage disease with anti–porcine debranching-enzyme antibody.

MATERIAL AND METHODS

Chemicals

The following chemicals were purchased from Sigma Chemical (St. Louis): phenylmethylsulfonyl fluoride (PMSF), dithiothreitol, glycogen (from rabbit liver), phosphorylase b, hexokinase, glucose-6-phosphate dehydrogenase, phosphoglucomutase, putrescine, cyanogen bromide–activated Sepharose 4B, and complete Freund's adjuvant. Nitrocellular paper was purchased from Schleicher and Schull (Keene, NH); ^{125}I-protein A was from Amersham Radiochemicals (Arlington Heights, IL); and DEAE-cellulose, Coomassie brilliant blue, protein-molecular-weight standards, and reagents for polyacrylamide gels were from Bio-Rad Laboratories (Richmond, CA).

Patients

The sources of human liver and muscle samples were diagnostic biopsies obtained over the past several years from patients clinically suspected to have glycogen storage disease or other liver or muscle disease. After enzymatic and/or pathological diagnosis, if there was any specimen remaining it was stored at −70 C. Some samples have been kept frozen for >2 years. All human liver and muscle samples used in the present investigation were from existing specimens already obtained as described above.

Five patients had Type III glycogen storage disease and ages ranging from 4 mo to 21 years at the time of biopsy; six patients had other types of glycogen storage disease (including one patient with exercise intolerance). Their ages ranged from 14 mo to 12 years. Although muscle samples were available for assay from only three of the five Type III patients and resulted in the demonstration of a debrancher deficiency in both liver and muscle, the clinical findings for the two other children were not inconsistent with muscle-debrancher deficiency in addition to the proven lack of the enzyme in hepatic tissue.

Purification of Debrancher Enzyme

Fresh porcine skeletal muscle (shoulder portion) was obtained from a local abattoir. The purification procedures were those described for rabbit-muscle debranching enzyme (Taylor et al. 1975; White et al. 1981) with slight modification. Two hundred fifty grams of muscle was minced and homogenized in a Waring blender with 4 vol 4 mM ethylenediaminetetraacetate (EDTA), 2 mM ethyleneglycoltetraacetate (EGTA), pH 7.2., and 0.34 mM PMSF. The homogenate was centrifuged at 6,000 *g* for 45 min, and the supernatant was decanted through six layers of gauze (Step 1; table 1). The supernatant was adjusted to pH 7.0 with 2 M Tris. Ammonium sulfate was then added to the

TABLE 1

PURIFICATION OF PORCINE-MUSCLE DEBRANCHING ENZYME

Step	Volume (ml)	Protein (mg)	Activity (U)[a]	Specific Activity U/mg	Purification (fold)	Yield (%)
1. Extract	930	21,071	885	0.042	1	100
2. 41% Ammonium sulfate precipitation and dialysis	83	2,316	364	0.16	3.8	41
3. DEAE-cellulose, pH 7.2	204	150	290	1.9	45.2	33
4. Sepharose $NH(CH_2)_4NH_2$	40	26	262	10.0	238	30

[a] Defined as 1 μmol glucose liberated/min from a phosphorylase limit dextrin (after subtraction of glucose released from glycogen).

supernatant to make the final saturation 41%. The solution was stored at 4 C for 6 h and centrifuged at 10,000 *g* for 20 min. The precipitate was dissolved in buffer A (5 mM Tris, pH 7.2, 1 mM EDTA, 14 mM mercaptoethanol), dialyzed for 36 h against buffer A, and centrifuged to remove insoluble material (Step 2; table 1). The solution was applied to a DEAE-cellulose column (3 × 30 cm) and equilibrated in buffer A, and the column was then washed with buffer A plus 5 mM NaCl until the 280 nM absorbance fell below 0.01. The column was then washed with buffer A plus 50 mM NaCl. Effluent having a 280-nM absorbance >0.1 was collected (Step 3; table 1). The solution was directly layered onto a column (3 × 30 cm) of Sepharose-$NH(CH_2)_4NH_2$ (aminobutyl Sepharose) equilibrated in buffer A plus 50 mM NaCl. The aminobutyl Sepharose was synthesized by coupling cyanogen bromide–activated Sepharose 4B to putrescine according to the method described by Shaltiel and Er-El (1973). The column was washed with buffer A plus 50 mM NaCl until the absorbance fell below 0.01, and this treatment was followed by a second washing with buffer A plus 250 mM NaCl until absorbance again fell below 0.01. The debranching enzyme was then eluted with buffer A plus 500 mM NaCl. Effluent showing an absorbance >0.1 was collected (Step 4; table 1), concentrated to 1 mg/ml by vacuum dialysis, and dialyzed against H_2O, then against a buffer containing 50 mM glycerol-1-phosphate, 2 mM EDTA, 1 mM dithiothreitol, pH 7.0.

Preparation of Phosphorylase Limit Dextrin

Rabbit-muscle phosphorylase b obtained from Sigma Chemical was first purified by passing it through a small column of aminobutyl Sepharose. The column was washed with buffer A plus 250 mM NaCl, and the eluant was collected and concentrated. This fraction contained purified phosphorylase, which is free of debranching enzyme. The rabbit debranching enzyme was subsequently eluted with buffer A plus 500 mM NaCl. This rabbit preparation was used for molecular-mass comparison vis-à-vis the purified porcine debranching enzyme.

Purified phosphorylase was added to glycogen (3% w/v) in 50 mM phosphate buffer plus 0.1 mM adenosine monophosphate (AMP), pH 6.8, and the solution

was dialyzed against phosphate-AMP buffer for 48 h at 20 C and then dialyzed against 10 mM phosphate buffer, pH 6.8, for 16 h at 20 C. The solution was boiled and centrifuged, and the aliquots were stored at −20 C.

Assay of Enzyme Activity

Debranching-enzyme activity was determined using limit dextrin as the substrate (Brown and Brown 1966), and the product glucose was measured at 340 nm by means of the reduction of nicotinamide adenine dinucleotide phosphate in a coupled assay involving hexokinase and glucose-6-phosphate dehydrogenase. The reaction mixture contained 0.3% limit dextrin, 10 mM citrate buffer, pH 6.0, and 5 mM mercaptoethanol in a total volume of 500 μl. After incubation at 30 C for 20 min, the reaction was stopped by boiling and was centrifuged, and the supernatant was assayed for glucose. In a separate reaction, 0.3% glycogen was used instead of limit dextrin and the glucose production was measured under the same experimental conditions.

One unit of debranching-enzyme activity was defined as 1 μmol glucose liberated/min from a phosphorylase limit dextrin (after subtraction of glucose released from glycogen). Specific enzyme activity was expressed in terms of units per milligram protein. Protein was determined by means of the Bradford method (Bradford 1976), with bovine serum albumin used as the standard.

Polyacrylamide Gel Electrophoresis (PAGE)

PAGE was performed on 7% acrylamide gel or 7%–15% gradient gel according to the procedures of Laemmli (1970). For gel electrophoreses in the presence of sodium dodecyl sulphate (SDS) or SDS plus urea, the sample was heated for 5 min at 100 C in 2% SDS (or 2% SDS plus 6 M urea) containing 100 mM dithiothreitol, 50 mM Tris, pH 6.8, 10% glycerol, and 0.05% bromophenol blue. For nondenaturing gel, SDS was omitted from both the gel and the electrophoresis buffer. The sample was dissolved in 50 mM Tris, pH 6.8, 10% glycerol, and 0.05% bromophenol blue and subjected to gel electrophoresis with omission of the boiling-water-bath treatment. Gels were stained in 0.1% Coomassie blue, 7% acetic acid, and 10% ethanol and destained by diffusion in a solution of 7% acetic acid and 20% ethanol.

Antiserum Preparation

The antiserum against porcine-muscle debranching enzyme was produced in New Zealand rabbits by subcutaneously injecting 0.3 mg enzyme in 1 ml 50-mM glycerol-1-phosphate, 2 mM EDTA, and 1 mM dithiothreitol at multiple sites on the rabbits' backs. The enzyme was emulsified with an equal volume of complete Freund's adjuvant. After two injections 1 mo apart, the animals were given booster injections (0.1 mg) and then bled 10 days later. The rabbits were repeatedly given booster injections at 1–2-mo intervals. The serum was then collected and checked by means of Ouchterlony double immunodiffusion performed in 0.75% agarose in phosphate-buffered saline with 0.02% sodium azide.

Inhibition of Debranching-Enzyme Activity by Antiserum

Purified porcine-muscle debranching enzyme (containing 1.2 μg protein) or crude liver (25 μg) and muscle (50 μg) extracts were incubated for 1 h at 37 C in a total volume of 0.1 ml containing 10 mM citrate buffer, pH 6.0, 100 mM KCl, and various amounts of serum. After incubation, the reaction was assayed for debranching-enzyme activity. For controls, the same procedure was performed with preimmune rabbit serum. Since glucose production is measured in the debranching-enzyme assay, both immune and preimmune serum were dialyzed before use.

Immunoblot Analysis

Human liver and muscle samples were homogenized with 5 vol buffer containing 4 mM EDTA, 2 mM EGTA, pH 7.2, and 0.34 mM PMSF. The homogenate was centrifuged at 10,000 *g* for 15 min, and the supernatant was subjected to immunoblot analysis. One hundred micrograms of protein from the supernatant was applied to 7% SDS-PAGE according to the method of Laemmli (1970). Electrophoresis was for 15 h at 30 V. The electrotransfer of protein and immunological detection of protein were as described elsewhere (Towbin et al. 1979), with the following modifications: The proteins were electroblotted onto nitrocellulose paper by means of transblotter equipment (Bio-Rad Laboratories). The nitrocellulose filter was washed in 5% milk (Carnation nonfat dry milk) in phosphate-buffered saline for 1 h. The antiserum (1/100 dilution in fresh 5% milk) was then added, and the solution was incubated for 2 h. The filter was washed for 1 h with three changes of 1% milk and incubated with 5×10^5 cpm/ml ^{125}I-protein A for 2 h. Then the filter was washed three times with 1% milk and once with 0.5% Triton X-100, each time for 15 min. The filter was then dried and autoradiographed.

RESULTS

Purification of Porcine-Muscle Debranching Enzyme

The porcine-muscle debranching enzyme was purified by means of a three-step procedure involving ammonium sulfate precipitation, DEAE ion-exchange chromatography, and hydrophobic chromatography. Figure 1 shows the gel electrophoresis pattern of various fractions obtained during the purification process. After passing over the aminobutyl Sepharose column, phosphorylase and other proteins were eluted at 0.05 M NaCl and 0.25 M NaCl. The debranching enzyme was eluted out with 0.5 M NaCl. The enzyme was obtained in 30% yield with an overall 238-fold purification. A summary of the purification procedure is given in table 1.

Homogeneity and Molecular Weight of Porcine-Muscle Debranching Enzyme

Purified debranching enzyme migrated as a single protein band on PAGE in the presence of SDS or SDS plus urea, as well as under nondenaturing condi-

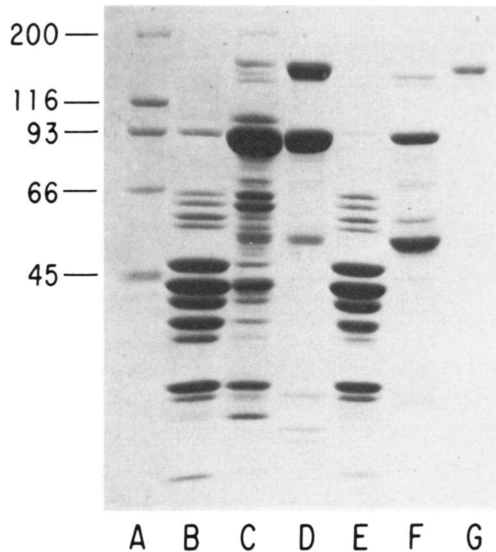

FIG. 1.—SDS-PAGE of fractions obtained during the purification of porcine-muscle debranching enzyme. The gel contained a 7%–15% gradient of acrylamide. Lane A, Molecular-weight marker proteins; lane B, supernatant after centrifugation of crude extract at 6,000 *g* for 45 min; lane C, precipitate from $(NH_4)_2\,SO_4$ fractionation; lane D, fraction from DEAE-cellulose chromotography; lanes E–G, fractions from Sepharose 4B–aminobutyl Sepharose that were eluted with 0.05 M NaCl (lane E), followed by elution with 0.25 M NaCl (lane F), and, finally, the purified debranching enzyme obtained from elution with 0.5 M NaCl (lane G). Lanes B–F contain ~30 μg of the protein from each fraction; lane G, 5 μg. Numbers on the left are the molecular masses, in kilodaltons, of the marker protein.

tions (fig. 2). The molecular weight of the porcine-muscle debrancher enzyme was estimated on the basis of SDS-PAGE by comparing it with both a molecular-weight standard and the rabbit enzyme (fig. 3). Both rabbit and porcine enzymes migrated identically in the SDS-polyacrylamide gel, and equal mixtures revealed only a single band. Thus, porcine debranching enzyme has a molecular weight of ~160,000.

Characterization of Anti–Debranching-Enzyme Antiserum

Immunization of rabbits with purified porcine-muscle debranching enzyme produced antisera. Figure 4 shows the antiserum reacting against purified porcine debranching enzyme with a single precipitin line. The antiserum also demonstrated a reaction having complete identity to those of both porcine muscle extracts and porcine liver extracts.

The antibody was tested for its ability to inhibit debranching-enzyme activity. Purified porcine debranching enzyme was incubated with increasing amounts of antiserum, and the debranching-enzyme activity was assayed. For controls, the same procedure was performed using preimmune rabbit serum. As demonstrated in figure 5, incubation of 5 μl antiserum with 1.2 μg purified enzyme at 37 C for 1 h inhibited almost all of the enzyme activity, whereas such treatment with preimmune serum had little effect.

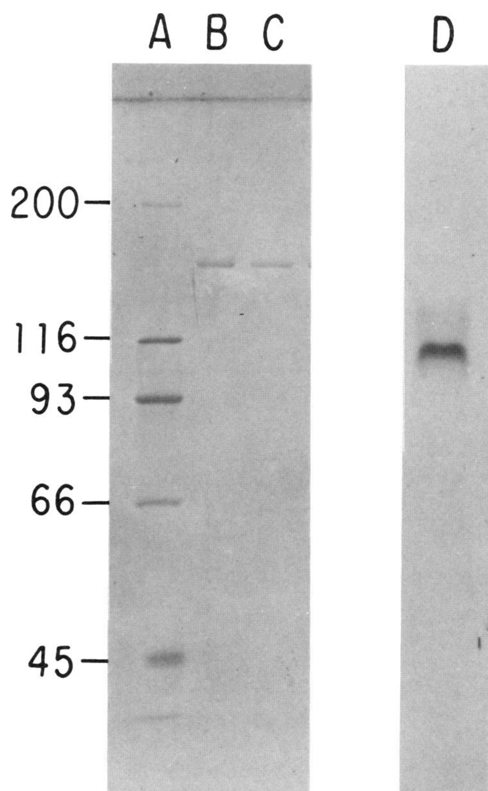

FIG. 2.—Seven-percent PAGEs of porcine debranching enzyme under denaturing (lanes A–C) and nondenaturing (D) conditions. Lane A, Marker proteins; lane B, in the presence of SDS; lane C, in the presence of SDS plus urea; and lane D, in the absence of SDS, urea, and dithiothreitol. Two micrograms of each protein was applied. Numbers on the left are the molecular masses, in kilodaltons, of the marker protein. Further experimental details are described in Material and Methods.

When crude liver and muscle extract from both human and porcine sources were incubated with antiserum, the antiserum inhibited porcine debranching enzyme to the same extent as it did the human enzyme (fig. 6). Enzyme activities from liver and muscle in either species were also similarly inhibited by the antiserum. These data, together with the immunoprecipitin identity of porcine liver and muscle in Ouchterlony double diffusion, indicate the immunochemical similarities of debranching enzyme in these two issues.

Immunoblot Analysis of Liver and Muscle Debranching Enzyme in Patients with Glycogen Storage Disease

Immunoblot analysis probed with anti–porcine-muscle debranching-enzyme antiserum showed that this antiserum can detect debranching enzyme in both human muscle and human liver (fig. 7). The bands detected in human samples by means of the antiserum were the same size as the one in porcine muscle; all

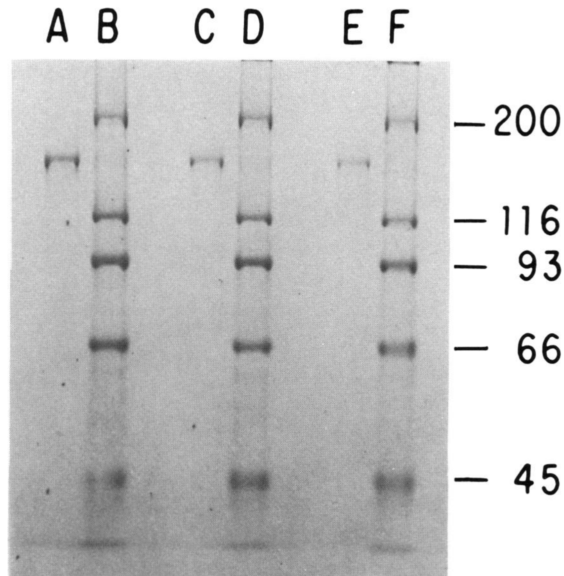

FIG. 3.—Seven-percent SDS-PAGE of porcine and rabbit debranching enzyme. Lanes B, D, and F, Marker proteins; lane C, purified porcine-muscle debranching enzyme; lane E, rabbit-muscle debranching enzyme; and lane A, mixture of both. Two micrograms of each protein were applied. Numbers on the right are the molecular masses, in kilodaltons, of the marker proteins.

were 160 kilodaltons in molecular mass as determined on the basis of SDS-PAGE. The antiserum also detected a same-size debranching-enzyme band in porcine liver (data not shown). Five patients with Type III and six patients with other types of glycogen storage disease were then subjected to immunoblot analysis (including one case with exercise intolerance of unknown etiology; fig. 7, lane 8). The immunoblot analyses were performed on coded samples without prior knowledge of the diagnoses. Whereas anti-porcine antiserum detected specific bands in all liver and muscle samples from both the patients with other types of glycogen storage disease (Types I, II, and IX) and the one case of exercise intolerance, the antibody detected virtually no debrancher protein in any of the liver and muscle samples from patients with Type III glycogen storage disease. It should be noted that sample 4 (liver) and sample 10 (muscle) were from the same patient with Type III glycogen storage disease. The debranching enzyme was deficient in both muscle and liver, and cross-reactive material for debranching enzyme was not detected. With prolonged exposure of the autoradiogram, a faint band was seen in sample 1 (liver) and sample 7 (muscle), indicating that a small amount of debranching enzyme may be present in some samples.

DISCUSSION

Debranching enzyme has been purified from and characterized in detail in many species, including rabbit (Gordon et al. 1972; White and Nelson 1974;

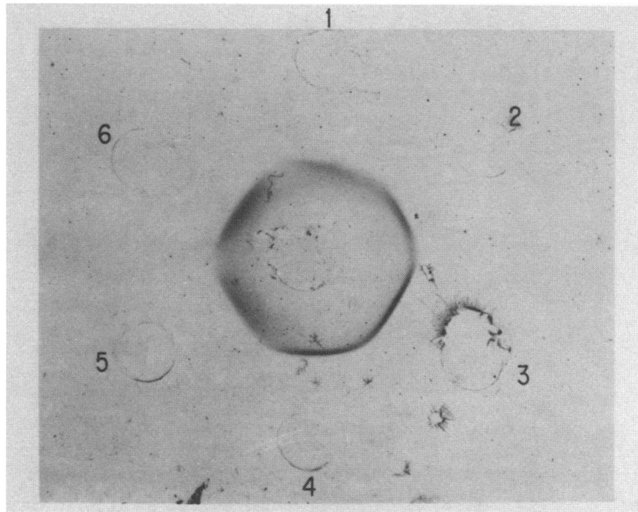

FIG. 4.—Ouchterlony double immunodiffusion. The center well contains antiserum raised against purified porcine-muscle debranching enzyme. Peripheral wells 1–4 contain purified porcine-muscle debranching enzyme in amounts of 0.1, 0.2, 0.4, and 0.6 μg, respectively. Well 5 contains 50 μg crude porcine-muscle extract, and well 6 contains 75 μg crude porcine-liver extract.

Taylor et al. 1975), dogfish (Becker et al. 1977), and baker's yeast (Lee et al. 1970). The present investigation selected porcine muscle as the source for enzyme purification because it is readily available and because the purified enzyme can be used to raise an antibody in rabbits. When detected by immunoblot analysis, the purified debranching enzyme from porcine muscle and the debranching enzyme in liver and muscle from both humans and porcines showed a molecular mass of ~160 kilodaltons, which is the same as that of the rabbit debranching enzyme. The liver debranching enzyme has been studied much less extensively than the muscle enzyme, and it appears that the debranching enzyme of liver and that of muscle are very similar proteins (Gordon et al. 1972). The present study provides, in addition to the molecular mass, the immunochemical evidence (by means of Ouchterlony and immunoinhibition study) of the similarity of the enzymes in liver and muscle. The mechanisms responsible for the control of tissue-specific expression of the debranching enzyme in these two tissues remain to be elucidated.

The antiserum prepared against porcine-muscle debranching enzyme inhibited the enzyme activity. The debranching-enzyme activity in the present study was measured in terms of production of glucose from phosphorylase limit dextrin. This method measures combined oligo 1,4-1,4 glucantransferase and amylo 1,6 glucosidase activities. Because these two activities have been shown to occur at separate sites and can function independently of each other, it will be of interest to examine whether antiserum would selectively inhibit one of the two activities or both of them. Preliminary experiments have suggested that the transferase component of the debranching enzyme is most readily inhibited.

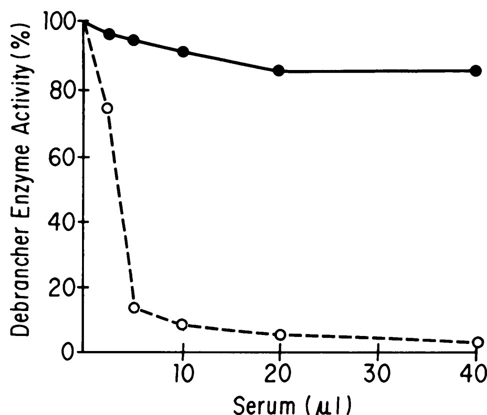

FIG. 5.—Inhibition of debranching-enzyme activity by antiserum against porcine-muscle debranching enzyme. A 1.2-μg amount of purified debranching enzyme was incubated with antiserum (○---○) at the indicated volume (abscissa) for 1 h at 37 C. The mixtures were then assayed for debranching-enzyme activity. As a control, the same incubations were performed with preimmune rabbit serum (●---●). The 100% point denotes specific debranching-enzyme activity of 7.0 U/mg protein.

Following preincubation of the purified enzyme with increasing amounts of antibody, aliquots were assayed with the phosphorylase limit dextrin or branched pentasaccharide (B_5) as substrate (Illingworth and Brown 1962). No inhibition of glucose formation from B_5 was found unless the preincubated sample had been centrifuged, in which case comparable extents of inhibition were found with the two substrates. For example, with one amount of antibody 65.4% and 63.4% remained, whereas doubling the amount of antibody reduced the remaining activities to 26.5% and 17.9% of the uninhibited rates (limit dextrin and B_5 used as substrates).

The anti–porcine debranching-enzyme antiserum was characterized by means of both Ouchterlony double immunodiffusion and inhibition of enzyme activity via immunoabsorption. The most rigorous proof of monospecificity and fidelity of the antiserum was provided by the results of immunoblot analysis of debrancher protein in glycogen storage disease. Although antiserum detected debranching enzyme in liver and muscle samples from patients with other types of glycogen storage disease, the antiserum detected virtually no debrancher protein in any of the samples from patients with Type III glycogen storage disease. In addition to the debranching-enzyme band, some smaller bands can be seen in control human liver samples. These bands were not seen with preimmune serum; nor were they seen in patients with Type III glycogen storage disease. Furthermore, when antiserum was incubated with purified debranching enzyme and specific antibody was eluted, the small bands as well as the debrancher-enzyme bands persisted along with purified antibody (Y.-T. Chen, J.-K. He, J.-H. Ding, and B. I. Brown, unpublished observation). All these data suggest that these smaller bands represent the degradation products of debrancher enzyme.

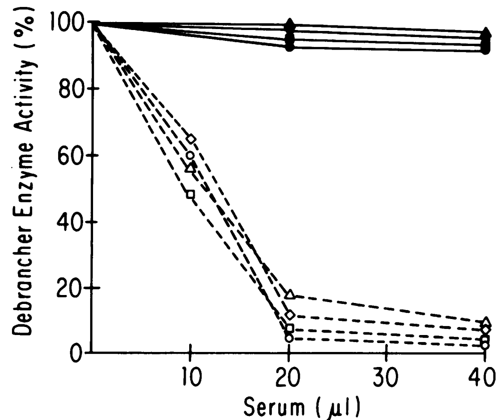

FIG. 6.—Inhibition of debranching-enzyme activity in the crude liver and muscle extract from both human and porcine sources by antiserum against porcine muscle-debranching enzyme. Amounts of crude extract protein are 25 μg each from porcine liver (△---△) and human liver (○---○) and 50 μg each from porcine muscle (□---□), and from human muscle (◇---◇). These extracts were incubated with antiserum at the indicated volume (abscissa) for 1 h at 37 C. The mixtures were then assayed for debranching-enzyme activity. Solid lines and solid symbols indicate corresponding samples incubated with preimmune serum. The 100% point denotes specific debranching-enzyme activity of 0.276, 0.147, 0.096, and 0.052 μ/mg for porcine liver, human liver, porcine muscle, and human muscle, respectively.

The present study detected no immunological reactive protein in Type III glycogen storage disease. Previous studies with antiserum against partially purified debranching enzyme, using antibody-consumption tests, have revealed a similar phenomenon (Dreyfus and Alexandre 1971). It would be interesting to examine those patients reported to have selective loss of either transferase activity or glucosidase activity. Immunoblot analysis might predict cross-reactive materials in such patients.

Preliminary studies using cultured fibroblasts and lymphocytes from normal controls indicate the presence of 160-kilodalton debranching-enzyme protein in these tissues. No debranching-enzyme band was detected in fibroblasts from four patients with Type III glycogen storage disease, a group including one patient whose disease is confined to the liver (Ding et al. 1986). The demonstration of the specific debranching-enzyme band in cultured fibroblasts and lymphocytes would make the molecular analysis of the disease much easier.

The results indicate that the anti–porcine debranching-enzyme antiserum that cross-reacts with human debranching enzyme can be used not only as a molecular tool for analysis of Type III glycogen storage disease at the protein level but also as the probe to screen the human liver or muscle cDNA library for clones encoding the debranching enzyme. Obtaining these molecular tools will allow us to (1) subtype the Type III glycogen storage disease patients in detail at the molecular level, (2) correlate the subtype and the clinical manifestation of the disease, and, finally, (3) answer on a molecular level questions about the function, structure, and regulation of this enzyme that contains two

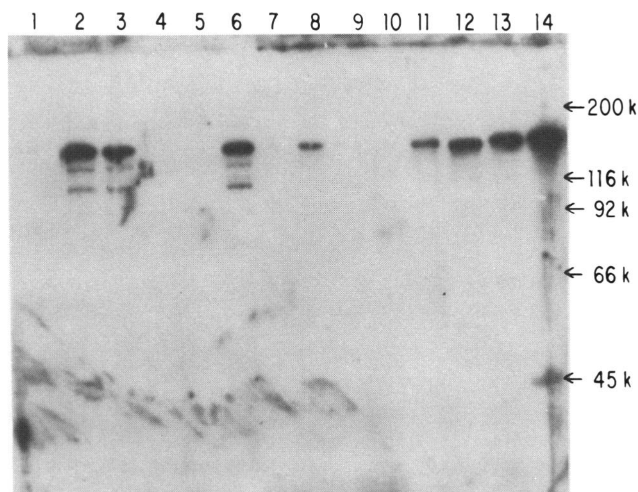

FIG. 7.—Immunoblot analysis of protein from liver and muscle of patients with glycogen storage diseases that was probed with antiserum against porcine debranching enzyme. In lanes 1–12 each well contains 100 μg of protein from crude extract of liver (wells 1–6) or muscle (wells 7–12) from patients with glycogen storage disease. The diagnoses are as follows: lanes 1, 4, 5, 7, 9, and 10, Type III glycogen storage diseases; lanes 2 and 3, Type I glycogen storage disease; lane 6, Type IX glycogen storage disease; lanes 11 and 12, Type II glycogen storage disease; and lane 8, patient with exercise intolerance and normal debranching-enzyme activity. Lanes 4 (liver) and 10 (muscle) are from the same patient with Type III glycogen storage disease. Lane 13 contains 0.5 μg of purified porcine-muscle debranching enzyme, and lane 14 contains 100 μg of crude porcine-muscle extract. Numbers on the right are the molecular masses, in kilodaltons, of the marker protein.

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