Purification and Properties of Rabbit Liver Phosphorylase Phosphatase*

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A procedure for the purification of rabbit liver phosphorylase phosphatase is described. The specific activity of the preparation is 2,100 units/mg of protein, representing a 25,000-fold purification. During the initial steps of the purification a large activation of enzyme activity was observed. The molecular weight of the purified enzyme was estimated by Sephadex G-75 chromatography to be 35,000, and by sucrose density ultracentrifugation to be 34,000 (2.9 S). On Na dodecyl-SO$_4$ polyacrylamide disc gel electrophoresis a single component with a molecular weight of 34,000 was observed. The pH optimum is 6.9 to 7.4, and the $K_{m}$ for rabbit muscle phosphorylase a is 2 $\mu$M. The same procedure is also applicable to the extensive purification of phosphorylase phosphatase from rabbit muscle.

There are three enzyme systems in mammalian glycogen metabolism which are controlled by phosphorylation-dephosphorylation reactions: phosphorylase, phosphorylase kinase, and glycogen synthase (1-5). Rabbit muscle phosphorylase was the first enzyme shown to exist in two interconvertible forms, phosphorylase a, the active form, and phosphorylase b, which is inactive except in the presence of its allosteric modulator, AMP. This interconversion of mammalian phosphorylase has become a classical example of the modulation of enzyme activity by the covalent modification of the enzyme protein and involves the addition or removal of a phosphate group at a specific serine residue (1-3). Extensive studies of the activation of phosphorylase have revealed an elegant system of phosphorylation reactions controlled by the hormonally directed formation of cAMP$^1$ (1-5). In comparison to our knowledge of the phosphorylating enzymes, phosphorylase kinase and cAMP-dependent protein kinase (6), much less is known of the properties of the corresponding phosphatases of glycogen metabolism, the enzymes that serve to reverse the effects of the kinases.

Phosphorylase phosphatase (EC 3.1.3.17) catalyzes the dephosphorylation of phosphorylase a and is the best known of these protein phosphatases. It was first described in 1943 by Cori and Green (7) in rabbit muscle, as an enzyme activity which converted phosphorylase a into phosphorylase b. The nature of this conversion was later defined by Krebs and Fischer (9) for the rabbit muscle enzyme, and by Wosilait and Sutherland (3) for the dog liver enzyme, as a phosphorylase reaction. Partial purification of the rabbit muscle enzyme (8, 9) and of the liver enzyme (3) have been reported. Some of the properties of the enzyme are obscure; for example, numerous molecular weights for the enzyme from various sources have been reported, as well as the presence of multiple molecular weight forms (1, 10, 11). In addition, the existence of active and inactive forms of the enzyme has been proposed (11-13). In this work we report the development of a procedure for the isolation of the enzyme from rabbit liver and an examination of some of the properties of the purified enzyme.

EXPERIMENTAL PROCEDURES

Materials

Frozen rabbit livers (type II) were obtained from Pel-Freez Biologicals, Inc. Other chemicals and biochemicals were obtained as follows: imidazole (Grade I), bovine serum albumin, ovalbumin, glucose 1-phosphate (Grade III), Azocasein, myoglobin (Grade I) from Sigma Chemical Co.; shellfish glycogen, Azocasein, urea (ultrapure), ammonium sulfate (Enzyme Grade), and cytochrome c (horse heart) from Schwarz/Mann; Azocoll from Calbiochem; DEAE-Sephadex A-50 and Sephadex G-75 from Pharmacia Fine Chemicals; and acid-washed charcoal (Norit A) from Pfanstiehl Laboratories. Imidazole was recrystallized from an acetone/toluene mixture. Glycogen was purified by trichloroacetic acid and ethanol precipitation. Homogeneous human erythrocyte carbonic anhydrase C was a gift of Dr. P. L. Whitney (14), and hexaminidine-Sepharose (15) was a gift of Drs. W. Awaad and K. Vosbeck (16), all from the Department of Biochemistry, University of Miami, Miami, Florida. Phosphorylase b was prepared from rabbit muscle as described by Fischer and Krebs (17). It was recrystallized three times and then converted into the a form as described by Krebs and Fischer (18) with the use of rabbit muscle phosphorylase kinase (19). The phosphorylase a was treated with Norit to remove AMP and recrystallized (20) three times (the crystals were dissolved in 50 mM Tris-HCl, pH 7.0, 30 mM mercaptoethanol, 0.4 M NaCl at room temperature, and then dialyzed against 50 mM Tris-HCl, pH 7.0, 30 mM mercaptoethanol, 0.4 M NaCl at room temperature, and then dialyzed against 50 mM Tris-HCl, 30 mM mercaptoethanol, pH 7.0, at 4°C). A second Norit treatment was carried out after the first recrystallization.
This was carried out with the use of a buffer system described previously (21), in gels of 8% acrylamide composition and 6 cm length. Gels were stained for protein with Coomassie blue. Na dodecyl-SO₄ gel electrophoresis was carried out as described by Weber and Osborn (22) with gels of 7.8% acrylamide composition and 10-cm length. The preparation of protein samples for Na dodecyl-SO₄ gel electrophoresis was carried out as follows. A 100-μl sample (4 to 5 μg of protein) was mixed with 100 μl of 2% Na dodecyl-SO₄, 8 M urea, 2% mercaptoethanol, and 0.1 M sodium phosphate, pH 7.1, and incubated at 30°C for 2 hours. Coomassie blue was used as an internal marker for the determination of molecular weight.

Assay of Phosphorylase Activity

This was carried out by the procedure of Hedrick and Fischer (23). Phosphorylase solutions (50 μl) were mixed with 50 μl of substrate solution (0.15 M glucose 1-phosphate, 2% glycogen, and 40 mM imidazole chloride, pH 6.5) and incubated at 30°C for 5 or 10 min. The reactions were stopped by the addition of 2 ml of 0.072 M H₂SO₄; determination of the inorganic phosphate released was carried out by addition of 2 ml of 1% ammonium molybdate/4% ferrous sulfate in 1 N H₂SO₄. The color developed was read at 700 nm after 2 min. Activities are expressed in units, where 1 unit is the amount of enzyme which releases 1 μmol of P₂/min.

Assay of Phosphorylase Phosphatase Activity

The assay system contained 0.2 mg/ml of rabbit muscle phosphorylase a, 50 mM imidazole chloride, 0.5 mM dithiothreitol, and 0.5 mM EDTA, pH 7.2. Theophylline was added to a concentration of 5 mM unless otherwise indicated. The reaction mixtures were incubated at 30°C, samples (0.1 ml) were removed at zero time and 5 min, and the reaction stopped (3) by addition of 0.4 ml of a buffer containing sodium fluoride (0.1 M NaF), 60 mM imidazole chloride, 0.5 mM dithiothreitol, 5 mM EDTA, and 1 mg/ml of bovine serum albumin, pH 6.5. These solutions were then assayed for phosphorylase a activity. The percentage of conversion of phosphorylase a activity was then determined and used to calculate the mass amount of phosphorylase a converted. One unit of activity was taken as the amount of enzyme which converted 0.2 mg (1 nmol of dimer) of phosphorylase a per min. Enzyme solutions were usually diluted so that the percentage of conversion under the above conditions fell between 20 to 50%. The time course of the reaction was approximately linear up to 50% conversion, as observed by Wosilait and Sutherland (3). Equivalent assay values were obtained if either rabbit liver phosphorylase a or ¹⁴C-labeled rabbit muscle phosphorylase a was used in the assay.

Protein Determinations

These were carried out by the method of Lowry et al. (24), following trichloroacetic acid precipitation. Bovine serum albumin was used as a standard.

Sucrose Density Ultracentrifugation of Phosphorylase Phosphatase

Phosphorylase phosphatase (1.5 units) was applied to the top of a 5 to 20% sucrose gradient in Buffer A (50 mM imidazole/5 mM EDTA/0.5 mM dithiothreitol, adjusted to pH 7.45 with 5 N HCl). Human erythrocyte carbonic anhydrase C was included in the sample as an internal marker (MW 27,900, 2.65 S, Ref. 25). Centrifugation was for 16 hours at 45,000 rpm in a SW 50L rotor in a Beckman L2-65B ultracentrifuge at 4°C. Fractions of 3 drops each were collected from the bottom of the tube. These were then assayed for phosphorylase phosphatase activity and for carbonic anhydrase activity (14).

Purification of Rabbit Liver Phosphorylase Phosphatase

Step 1. Acid Precipitation—Frozen rabbit livers (type II, Pel-Freez, 938 g) were homogenized with 3 volumes (2,800 ml) of cold Buffer A in a Waring Blender. All subsequent steps were carried out at 4°C unless otherwise stated. The pH of the homogenate was adjusted to 5.8 with 1 M acetic acid, and 1 g of Norit A per liter of homogenate was added. The homogenate was then centrifuged at 10,000 × g for 20 min to remove cell debris, precipitated protein, and the Norit A. The pH of the supernatant was then adjusted to 7.2 with 2 M Tris base.

Step 2. Ammonium Sulfate Precipitation—The supernatant was brought to 70% saturation with ammonium sulfate by addition of the solid salt. The pH of the solution was maintained at 7.2 during this process by the addition of 2 M Tris base. After 30 min, the precipitate was then dissolved in a minimum volume of Buffer (20 mM Tris-HCl, 1 mM MgCl₂, pH 8.0); final volume at this stage was 590 ml. Enzyme activity and protein assays were carried out on a sample of the solution which was dialyzed overnight against Buffer A.

Step 3. Ethanol Precipitation—The cold, redissolved ammonium sulfate pellet was then poured into 5 volumes of room temperature 95% ethanol (2,450 ml). The mixture was immediately centrifuged (5,000 × g, 5 min, 4°C). The supernatant was discarded, and the pellet was extracted by homogenization with 469 ml of Buffer A in a Waring Blender. The suspension was then centrifuged (16,000 × g, 15 min) and the pellet again extracted with 469 ml of Buffer A. The two extracts were combined and dialyzed overnight against Buffer A to remove ammonium sulfate and ethanol.

Step 4. Ammonium Sulfate Precipitation—Fractionation—The ethanol extract was brought to 40% saturation with solid ammonium sulfate. After 30 min, the precipitate was removed by centrifugation (10,000 × g, 15 min) and discarded. The supernatant was then brought to 75% saturation with solid ammonium sulfate. After 30 min, the precipitate was collected by centrifugation as before. During the centrifugation process the pH was maintained at 7.2 with 2 M Tris base. The pellet was dissolved in a minimum volume of 0.18 M NaCl in Buffer A (total volume, 52 ml) and dialyzed overnight against at least two changes of the same buffer.

Step 5. DEAE-Sephadex Chromatography—A DEAE-Sephadex A-50 column (30 × 2.0 cm) was equilibrated with 0.18 M NaCl in Buffer A. The enzyme solution from the previous step was loaded onto the column, which was then washed with 0.18 M NaCl in Buffer A until the absorbance of the fractions at 280 nm returned close to base-line. The column was then eluted with 0.24 M NaCl in Buffer A. Fraction volumes of 10 ml each were collected at a flow rate of 30 ml/hour. The enzyme activity was routinely eluted following a large peak of protein. A typical elution profile for this purification step is shown in Fig. 1. Purifications of 10 to 50 fold and recoveries of 70% of the applied activity were usually obtained. In this and in subsequent steps, plastic test tubes and containers were used for the collection and storage of enzyme solutions because of the low protein concentrations encountered.

Since a slight amount of tailing in the peak of inert protein (Fig. 1) could make a large difference in the degree of purification obtained, the enzyme at this stage was rechromatographed on DEAE-Sephadex. The enzyme in the pooled fractions was first directly absorbed to DEAE-Sephadex. This was done by diluting the pooled fractions with Buffer A so that the NaCl concentration was reduced to 0.1 M. DEAE-Sephadex (about 10 ml packed volume) equilibrated with 0.1 M NaCl in Buffer A was added and the suspension stirred for 20 min.

Additional amounts of DEAE-Sephadex were added (5-ml batches) until a test of a centrifuged sample showed that all of the activity was absorbed. The suspension was then filtered on a sintered glass funnel under suction, and the DEAE-Sephadex with the adsorbed enzyme was transferred to the top of a column of DEAE-Sephadex (20 × 2.5 cm)
Step 5. DEAE-Sephadex Chromatography—The concentrated enzyme solution from the previous step was then chromatographed on a Sephadex column (100 x 2.5 cm) equilibrated with Buffer A. The column was washed again adsorbed on DEAE-Sephadex in a batchwise manner. This time, the gel was poured directly into a column and eluted by upward flow with 0.4 M NaCl in Buffer A. This last step allowed for a 10-fold concentration of the enzyme to a volume of 6 ml.

Step 6. Sephadex G-75 Chromatography—The pooled fractions from the previous step were chromatographed on a Sephadex G-75 column (100 x 2.5 cm) equilibrated with Buffer A. The active fractions were then pooled.

Step 7. Hexanediamine-Sepharose 4B Chromatography—The pooled fractions from the previous step were chromatographed on a hexanediamine-Sepharose 4B column (30 x 0.8 cm) equilibrated with Buffer A. This was used as an ion exchange support, although it may be noted that hydrophobic adsorption effects may play a role in separations obtained on this type of support (26, 27). The pooled fractions were loaded onto the column, which was then washed with one column volume of Buffer A. The column was then eluted with a linear gradient of NaCl in Buffer A (100 ml of Buffer A against 100 ml of 1 M NaCl in Buffer A). Fractions of about 3 ml each were collected at a flow rate of 25 ml/hour. The enzyme activity was eluted at about 0.3 M NaCl. The active fractions (41 to 53) were pooled and dialyzed against 60% glycerol in Buffer A (a mixture of one part of Buffer A to one half parts of glycerol). After overnight dialysis the volume was reduced from 33 ml to 6.2 ml. The final step of purification involved rechromatography on the hexanediamine-Sepharose. The enzyme solution was diluted 4-fold with Buffer A to reduce the glycerol and salt concentration and rechromatographed on a hexanediamine-Sepharose column as described above. The active fractions were pooled and dialyzed overnight against 60% glycerol in Buffer A. The solution was then stored at -28°C.

RESULTS

Purification of Rabbit Liver Phosphorylase Phosphatase

The over-all purification of the enzyme is summarized in Table I for the preparation described under "Experimental Procedures." A specific activity of 2,100 units/mg of protein was obtained, equivalent to the conversion of 2.1 μmol of phosphorylase dimer/min/mg of protein, or the release of 4.2 μmol of P_i/min/mg of protein. The over-all purification was 25,000-fold, even when the large increase in activity after the ethanol precipitation step was taken into account.

The reproducibility of the procedure may be assessed from the following data collected over a series of experiments. Specific activities at the end of the ethanol precipitation step were 2.2 ± 0.8 units/mg of protein (14 experiments), at the end of the DEAE-Sephadex chromatography, 161 ± 39 units/mg of protein (6 preparations), while for several preparations carried to the Sephadex G-75 stage, specific activities in the range of 800 to 1200 units/mg of protein were obtained. Preparations carried to the latter stage were consistently found to contain only two protein bands by disc gel electrophoresis; the activity was associated with the slower migrating band in experiments in which gels were sliced and the enzyme activity determined in extracts of the gel slices. A similar pattern was observed after the first hexanediamine-Sepharose chromatography. The removal of the contaminant was achieved by the second hexanediamine-Sepharose chromatography, when a single band of protein was observed on disc gel electrophoresis and also on Na dodecyl-SO₄ disc gel electrophoresis. The preparation therefore appeared to be homogeneous.

The initial precipitation steps, in particular the ethanol precipitation step, proved to be the key to the purification of the enzyme. Our earlier attempts to purify the enzyme from tissue extracts by conventional procedures were otherwise largely unsuccessful. During the initial precipitation steps (Table I) about 97% of the inert protein is removed, and a large activation (6- to 10-fold) was reproducibly observed. It should be noted that the conditions used for the ethanol treatment (addition of 5 volumes of room temperature 95% ethanol) are very different from those originally described by Wosilait and Sutherland (3), which utilized fractionation with 25% ethanol at a more conventional temperature of -20°C. It would appear that phosphorylase phosphatase is unusually stable to the denaturing conditions of the ethanol precipitation, or is reversibly denatured during the procedure. We have found that the activation by ethanol treatment occurs with a concomitant conversion of phosphorylase phosphatase from less active, multiple molecular weight forms into a single form of lower molecular weight (28).

It was of some concern as to whether the activity obtained at the ethanol precipitation step was actually phosphorylase phosphatase. This caution arises because a proteolytic enzyme such as trypsin can mimic the action of phosphorylase phosphatase (29). However, no proteolytic activity in preparations carried to this stage could be demonstrated by incubation with commercial protease substrates such as Azocoll, Azocoll, or Azo-casein. In addition, inactive rabbit muscle phosphorylase prepared by the action of phosphatase preparations could be completely converted into phosphorylase a by rabbit muscle phosphorylase kinase.

Enzyme preparations after the DEAE-Sephadex stage were found to be extremely unstable, but could be stored for as long as

### Table I

<table>
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<th>PROCEDURE</th>
<th>ACTIVITY (units)</th>
<th>PROTEIN (mg)</th>
<th>SPECIFIC ACTIVITY (units/mg)</th>
<th>YIELD (%)</th>
<th>PURIFICATION (fold)</th>
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<td>-</td>
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<tr>
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<td>8</td>
<td>16,600</td>
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<td>0.18</td>
<td>2,086</td>
<td>4</td>
<td>24,700</td>
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*Calculated on the basis that the crude homogenate contained 9,900 units of phosphorylase phosphatase activity.
as 10 months without loss of activity in the presence of 60% glycerol at \(-28^\circ\).

Properties of Rabbit Liver Phosphorylase Phosphatase

Physical Properties—The molecular weight of purified phosphorylase phosphatase was estimated by Sephadex G-75 chromatography to be 35,000 (Fig. 2). On sucrose density ultracentrifugation with carbonic anhydrase C as a marker, values of 2.92 S and 2.83 S for the sedimentation coefficient were obtained in two runs by the method of Martin and Ames (30). An estimated molecular weight of 34,000 was obtained by the empirical relationship of Martin and Ames (30). On Na dodecyl-SO\(_4\) disc gel electrophoresis, a single protein band was observed with an apparent molecular weight of 34,000 (Fig. 3). These data indicate that rabbit liver phosphorylase phosphatase isolated by our procedure consists of a single polypeptide chain.

Enzymic Properties—The pH optimum with rabbit muscle phosphorylase a as the substrate was found to be pH 6.9 to 7.4 (Fig. 4). The \(K_m\) for rabbit muscle phosphorylase a was found to be 2 \(\mu\)M; data for three determinations were 2.0 (1.5 to 2.5), 2.2 (1.2 to 4.1), and 2.1 (1.7 to 2.6) \(\mu\)M, respectively. The values in parentheses represent the 95% confidence limits. The data were analyzed by the method of Bliss and James (31) with the use of the computer program of Hanson et al. (32) which was adapted for the Univac 1106 system by Dr. J. F. Woessner of the University of Miami. The turnover number of the enzyme, estimated from the \(V_{\text{max}}\) (Fig. 5), is 5.2 mol of phosphorylase a dimer converted per mol phosphorylase phosphatase per s. Metal ions were found to be inhibitory; at 3 \(\text{mM}\) concentrations, inhibitions of 25%, 37%, and 64% were noted for Ca\(^{2+}\), Mg\(^{2+}\), and Mn\(^{2+}\), respectively.

Factors Influencing Assay of Phosphorylase Phosphatase

Theophylline was routinely included in the assay system for phosphorylase phosphatase since methylxanthines were reported by Wosilait and Sutherland (3) to have an activating effect. In crude tissue extracts the effect of theophylline was very marked. The source of this activation was shown to be the reversal of the inhibition of the phosphatase reaction by theophylline.
The inhibition of the reaction by adenine nucleotides, particularly AMP, has been extensively documented, shown to be substrate-directed, and to be reversed by addition of glucose (33–38). The effects of the addition of theophylline or glucose (or both) to the assay of a rabbit liver extract are shown in Table III. Marked increases in activity are seen, even though the extract had been Norit-treated in an attempt to remove endogenous adenine nucleotides. When the same extract was partially purified to the ethanol precipitation step, much smaller effects of theophylline and glucose were seen (Table III). (The data also reflect the increase in the total activity after the ethanol treatment.)

The sensitivity of the assay to low concentrations of adenine nucleotides requires precautions to be taken during the preparation of the phosphorylase a substrate in the form of repeated treatments with Norit. This was done to remove traces of adenine nucleotides which might be carried over from the phosphorylase b into a conversion. The effects of the Norit treatment on the assay system are shown in Fig. 6. Enzyme purified to the ethanol precipitation stage was assayed with the use of phosphorylase a which had been given either one or two Norit treatments, respectively. The assays were carried out in the absence and presence of theophylline or glucose (or both). With phosphorylase a which had received a single Norit treatment, a much lower activity was obtained, and this was markedly increased in the presence of theophylline and glucose (Fig. 6). Following a second Norit treatment of the same phosphorylase preparation, it is seen that these effects largely disappeared, and the theophylline alone was sufficient to elicit the maximal activity.

**Application of Procedure for Purification of Phosphorylase Phosphatase from Rabbit Muscle**

At an early stage in the development of the methodology, an attempt was made to isolate rabbit muscle phosphorylase phosphatase, by means of essentially the same procedure as that described under “Experimental Procedures” with the following exceptions. The buffer pH was 7.2, and a single chromatography on DEAE-Sephadex, followed by chromatography on Sephadex G-50 and G-75 was used. The acid precipitation step was omitted since rabbit muscle phosphorylase phosphatase is precipitated by this procedure (7). The results of this experiment are shown in Table IV. A 5000-fold purified enzyme was obtained with a specific activity of 755 units/mg of protein. The preparation after the Sephadex G-75 step was found to be inhomogeneous by disc gel electrophoresis, although the major protein band coincided with the activity measured in extracts of a sliced gel. The preparation was very unstable, as shown by the loss in specific activity during the second Sephadex chromatography step; a possible explanation for this might be the presence of proteolytic activity, since the contaminating bands were all fast migrating, some material even being found in the dye front. The behavior of the rabbit muscle enzyme during the purification steps was very similar to that of the liver enzyme; in addition, the enzyme was eluted on Sephadex G-75 chromatography at a volume indistinguisha-

![Fig. 6. The effects of glucose and theophylline on the phosphorylase phosphatase reaction. The crosshatched bars show the activity obtained in the assay system when rabbit muscle phosphorylase a which had been given a single Norit treatment was used as a substrate. Shaded bars show the activity obtained when the same phosphorylase a was given a second Norit treatment. C, no additions; T, 1 mM theophylline added; G, 30 mM glucose added; TG, 1 mM theophylline and 30 mM glucose added.](image-url)
able from that of purified rabbit liver phosphorylase phosphatase on the same column, indicating a similarity of molecular weight.

**Discussion**

The procedure described in this work allows the isolation of rabbit liver phosphorylase phosphatase to apparent homogeneity, and may be useful for the preparation of highly purified phosphorylase phosphatase from other tissue sources. The only previous purification of liver phosphorylase phosphatase has been that described in the work of Wosilait and Sutherland (3) in which the properties of a 120-fold purified preparation of the dog liver enzyme were examined. Until recently the only procedure described for the preparation of highly purified rabbit muscle phosphorylase phosphatase was that of Hurd et al. (8, 9); work from the same laboratory has now resulted in the development of a method which allows a 6000-fold purification of the rabbit muscle enzyme (38). The other phosphoprotein phosphatases of glycogen metabolism have not been isolated, although rabbit muscle glycogen synthase phosphatase has been purified 1000-fold (39).

The procedure we have developed allows the isolation of rabbit liver phosphorylase phosphatase as a single protein species with a molecular weight of about 34,000. This is similar to the molecular weight of 32,000 reported by Gratecos et al. (38) for the rabbit muscle enzyme, although a marked tendency of their preparation to form aggregates was noted. We have not observed this with our enzyme preparations, which were made on a much smaller scale (about 1/30 the weight of starting material) than that reported by Gratecos et al. (38). The enzyme protein concentrations (less than 0.1 mg/ml at all steps of purification) that we dealt with may have been too low for aggregation to be observed.

There have been a number of other recent studies of less pure preparations of phosphoprotein phosphatases with activity toward either glycogen synthase, phosphorylase, or phosphorylase kinase (10, 40–45); these preparations have in common apparent molecular weights much greater than 34,000, and in some cases, the observation of multiple forms. We have also observed multiple molecular weight forms of phosphorylase phosphatase activity in crude rabbit liver extracts on gel permeation chromatography (46). Following the initial precipitation steps, however, the enzyme activity was always found to behave as a single form during column chromatography. In a separate study (28) of rat tissue extracts, we have shown that the activating effects of ethanol treatment are accompanied by the conversion of multiple molecular weight forms of the enzyme into a single form of lower molecular weight (about 32,000). The mechanism proposed for this effect was the selective removal of an inhibitor protein with which the enzyme forms a complex (28). The existence of inhibitor proteins of phosphorylase phosphatase has been demonstrated in rabbit liver (46).

The possibility that the enzyme we have isolated may represent only the catalytic portion of a larger complex remains a subject for further investigation, particularly in terms of the possible regulation of the enzyme. This possibility may also provide an explanation for the conflict between the behavior of the isolated enzyme as a protein of 34,000 and the behavior of the enzyme in cruder preparations as a material of much higher molecular weight.

Following the observation by Kato and Bishop (39) that rabbit muscle glycogen synthase phosphatase preparations also dephosphorylated histones, a number of other studies of partially purified preparations of phosphoprotein phosphatase activity from several tissue sources have provided support for the existence of enzyme entities which are capable of acting on two or more of the phosphoenzymes of glycogen metabolism (10, 39, 41–45), and have led to the concept that a single phosphoprotein phosphatase may fill the role for the dephosphorylation of glycogen synthase, phosphorylase, and phosphorylase kinase. Our preparation of rabbit liver phosphorylase phosphatase has been shown to dephosphorylate glycogen synthase from the same tissue source; liver glycogen synthase phosphatase activity has also been shown to co-purify with phosphorylase phosphatase during our purification procedure (47). The enzyme which has classically been regarded as phosphorylase phosphatase may thus have a wider specificity and role in mammalian glycogen metabolism.

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