Unusual, Metabolite-dependent Solubility Properties of Phosphofructokinase

THE BASIS FOR A NEW AND RAPID PURIFICATION FROM LIVER, KIDNEY, AND OTHER TISSUES*

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THOMAS H. MASSEY‡ AND WILLIAM C. DEAL, JR.

From the Department of Biochemistry, Michigan State University, East Lansing, Michigan 48823

SUMMARY

In the presence of 5 mM MgCl₂, fructose 6-phosphate kinases from porcine liver and kidney and a variety of other sources: (a) precipitate at low ammonium sulfate concentrations (0 to 30 mM); (b) are completely soluble at slightly higher concentrations (>100 mM); and (c) do not precipitate at low (0 to 30 mM) ammonium sulfate concentrations if fructose-6-P is present or if Mg²⁺ is absent and 1 mM ATP is present. Although substrate effects on enzymes are well known, the precipitation-solubilization effects just described are, in many respects, novel. These properties also form the basis for a new, rapid and highly specific method for complete purification of porcine liver and kidney phosphofructokinases, as well as substantial purification of other phosphofructokinases. Porcine liver phosphofructokinase was purified 16,000-fold to homogeneity (specific activity = 100) with a 25% yield in only 8 hours. No conventional salt fractionation or column chromatography is required. The key step is repeated selective precipitation and dissolution of the enzyme by simply changing the concentration of ammonium sulfate in the solution. In contrast to conventional ammonium sulfate fractionation, the enzyme is precipitated and dissolved at lower and higher ionic strengths, respectively. The enzyme has a low isoelectric point (pI = 5). Metabolite-induced structural transitions are also detectable by polyacrylamide gel electrophoresis. In the absence of metabolites, the enzyme does not penetrate 4.2% polyacrylamide gels but in the presence of fructose-6-P or ATP or ADP, it does.

Phosphofructokinase (EC 2.7.1.11) has been partially or totally purified from several sources including Escherichia coli (2).

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‡ National Institutes of Health Postdoctoral Fellow (AM 40051) and Postdoctoral Trainee (Training Grant No. GM 1001). Present address, Norwich Pharmaceutical, Research and Development Labs, Norwich, New York 13815.
was varied. In order to eliminate the extraneous ammonium sulfate in these assays, the ammonium sulfate suspensions of the auxiliary enzymes were centrifuged and the pellets dissolved in the original volume of assay buffer. The specific activity of phosphofructokinase is expressed as units per mg of protein where 1 unit is defined as the conversion of 0.5 μmole of NADH to NAD in 1 min; this is equivalent to the phosphorylation of 1 μmole of fructose-6-P in 1 min.

Protein Determination—Protein concentrations were determined by a slightly modified version of the tannic acid turbidimetric method of Katzenellenbogen and Dobryszycka (13), with crystalline bovine serum albumin (Sigma) as a protein standard; the incubation was at 23° C instead of 30° C and was for 20 min instead of 10 min. The absorption at 660 nm was measured.

Purification Buffers—Homogenization buffer consisted of 50 mm Tris-Cl, 50 mm β-mercaptoethanol, and 5 mm EDTA at pH 8.0. Buffer A contained 50 mm Tris-Cl, 50 mm β-mercaptoethanol, 5 mm MgCl₂, 0.1 mm ATP, and 0.1 mm fructose-1,6-P₂ at pH 8.0. Buffer B contained Buffer A plus 110 mm ammonium sulfate. All pH measurements were made at room temperature.

Standard Polyacrylamide Gel Electrophoresis—Disc polyacrylamide gel electrophoresis of the purified enzyme was performed in 3-mm (inner diameter) glass tubes according to the method of Davis (14) with some modifications. The acrylamide concentration for the lower gel was 4.2% (w/v) and the methylenebisacrylamide was 0.11% (w/v). The spacer gel contained 3.5% acrylamide and 0.9% methylenebisacrylamide (both w/v).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis and subunit molecular weight analysis were performed by standard methods (15, 16) except that the gels contained 5% acrylamide and 0.18% methylenebisacrylamide.

A Special Subreservoir Modification for Polyacrylamide Gel Electrophoresis in the Presence of Various Metabolites—In order to study the effect of different metabolites in one experiment, Plexiglas tubes (19 mm inner diameter) were cut into 38-mm lengths and glued to the upper reservoir container, over the holes for the gel tubes. This provided an isolation cylinder, called a "subreservoir." Six milliliters of the upper reservoir buffer, containing 10% sucrose and the desired metabolite, were added to each of the subreservoirs. They were then carefully filled to the top with standard upper reservoir buffer. The entire upper reservoir was then filled with normal buffer to a level about ½ inch above the top of the subreservoirs. The sample (1 to 20 μl) containing bromphenol blue and about 20% sucrose was injected above the spacer gel, on which it settled. After focusing for about 2½ hours at 4° C, 0.2-ml fractions were taken from the top with a spring-loaded syringe fitted with a thin piece of polyethylene tubing.

Results

Purification Scheme

Unless indicated otherwise, centrifugation was carried out at 4° C in the Sorvall GSA rotor at 9,000 rpm for 15 min or in the Sorvall SS-34 rotor at 18,000 rpm for 5 min.

1. Homogenization Step—One partially frozen pig liver, usually about 1200 g, or six to eight pig kidneys, about 900 to 1200 g, were homogenized in a Waring Blender in 2 volumes of homogenization buffer (see "Materials and Methods"). The extract was centrifuged in a GSA rotor and the supernatant fluid was removed by aspiration. The supernatant liquid had a pH of 7.1 to 7.3 and contained some of the fluffy layer from the homogenate pellet.

2. Heat and Alcohol Step—First, β-mercaptoethanol (1 ml/100 ml of homogenate supernatant) and then 95% ethanol (20 ml/100 ml of homogenate supernatant) were added and the solution was incubated at 41 to 42° C for 45 min. After centrifugation in the GSA rotor, the clear red supernatant was filtered (Whatman No. 4).

3. Magnesium Alcohol Precipitation Step—After cooling Fraction 2 to less than 3° C, 1 mm MgCl₂ was added slowly (5 ml/100 ml). For incubation for about 30 min at 0° C, which allowed complete precipitation of phosphofructokinase, and centrifugation in the GSA rotor, the brown pelleted residues were combined and resuspended in 2 ml of Buffer A per 100 g of original wet tissue.

4. Washing—Fraction 3 was further dispersed in a 50-ml polycarbonate centrifuge tube with a Teflon pestle and centrifuged in an SS-34 rotor. The supernatant fluid was gently swirled to resuspend the upper fluffy layer of the pellet, transferred to a separate centrifuge tube, and centrifuged again. The second supernatant fluid was again gently swirled to resuspend the upper fluffy layer of the second pellet. The first and second hard brown pellets were combined and resuspended in a volume of Buffer A equal to 2% of the original wet weight of the tissue. The suspension was then dispersed with a Teflon pestle and the steps were repeated four times or until the supernatants after centrifugation were colorless and clear.

5. Extraction and Reprecipitation of Phosphofructokinase by Dilution—After centrifugation of the washed enzyme suspension, the pellet was suspended in Buffer B (0.2 ml per 100 g of original wet tissue), gently solubilized, and centrifuged at room temperature in a Sorvall SS-1 rotor at 10,000 rpm for 5 min. The
pellet was then re-extracted by the same procedure. The two supernatant fluids were combined, diluted with 4 volumes of Buffer A, and incubated at 0° for 15 to 30 min to allow precipitation of the enzyme.

The enzyme suspension was centrifuged in a 12-ml glass centrifuge tube in the Sorvall SS-34 rotor at 4° and the solubilization-precipitation steps were repeated until a white pellet was obtained upon centrifugation.

The enzyme, stored in Buffer A at 4°, was stable for at least a month if the mercaptoethanol was replenished. The stored enzyme appeared to be indistinguishable from freshly prepared enzyme if resolubilized and reprecipitated with Buffers B and A, respectively. Table I contains data for a typical purification experiment.

An Alternate Method for the Extraction of Liver Phosphofructokinase from the Washed Enzyme

After learning (see later section) that fructose 6-phosphate is a potent solubilizer of the enzyme in the presence of magnesium and at low ionic strength, we attempted to selectively extract the washed enzyme using fructose 6-phosphate. For this study, the pellet of washed enzyme, obtained from a single pig liver of 1.7 kg using the standard procedure, was resuspended in 17 ml of Buffer A and made 2 mm in fructose-6-P to dissolve the enzyme. After centrifugation at room temperature, the supernatant liquid was removed and ATP was added to a final concentration of 2.2 mm. This initiated conversion of the solubilizer, fructose-6-P, to the nonsolubilizer, fructose-1,6-P2. Within 10 to 15 s, the solution became cloudy, a heavy precipitate resulted after incubation at 0° for 30 min. The steps of solubilization by fructose-6-P and precipitation by addition of ATP were repeated. Although a substantial purification was achieved, the maximal specific activity was only 53. The activity recovery was quite good (45%). However, this modification does not seem desirable because of the low specific activity.

Kidney Phosphofructokinase

Porcine kidney phosphofructokinase was also purified by the standard procedure described previously. The Mg2+-alcohol suspension from kidney usually contained a large amount of insoluble, sandy-like material, possibly magnesium phosphate. This made centrifugal fractionation more difficult in the washing steps and probably partially accounted for the somewhat lower yields. Although the liver enzyme was quantitatively precipitated from the heat-alcohol filtrate fraction with Mg2+, usually only about 50% of the kidney enzyme could be precipitated from this fraction.

Table I allows a comparison of purification data for two typical preparations of the enzyme from pig liver and kidney. Preparations of kidney phosphofructokinase usually had slightly higher specific activities than the liver enzyme.

| Fraction                  | Total volume | Total protein | Total units | Specific activity units/mg | Total protein | Total units | Specific activity units/mg |
|---------------------------|--------------|---------------|-------------|---------------------------|---------------|-------------|---------------------------|
| Homogenate supernatant    | ml           | 180 g         | 1100        | 0.0063                    | 1230          | 87 g        | 0.014                     |
| Heat-alcohol filtrate     | 2750         | 62 g          | 468         | 0.0074                    | 2000          | 16 g        | 0.057                     |
| Magnesium-alcohol suspen-| 83           | 3.5 g         | 428         | 0.123                     | 30            | 0.64 g      | 0.79                      |
| Final suspension          | 8            | 2.1 mg        | 210         | 100                       | 230           | 1.8 mg      | 128                       |

The best physical evidence for homogeneity of the liver enzyme is that it yields a single band upon sodium dodecyl sulfate gel electrophoresis (Fig. 1). Further evidence for purity is the observation of constant specific activity through numerous experiments on solubilization of the precipitated enzyme, under a wide variety of conditions; in certain key experiments (Figs. 2, 5, and 6) described in the following sections of the paper, the curves for solubilization of phosphofructokinase activity and for solubilization of protein were both measured and found to be superimposable. Although the native phosphofructokinase migrates as a broad band in conventional polyacrylamide gel electrophoresis carried out under proper conditions (see next section), the absence of other bands and the absolute dependence of the migration of the single broad band upon the presence of specific metabolites also argues for homogeneity.

Requirement for Metabolites for Migration on Polyacrylamide Gels

Kemp (10) has previously reported that rabbit liver phosphofructokinase did not penetrate conventional polyacrylamide gels and we found similar results with pig liver phosphofructokinase. Studies described later suggested that ATP or fructose-6-P might depolymerize the enzyme and allow migration. In order to perform electrophoresis in the presence of these metabolite anions, a modified apparatus (see "Materials and Methods") was used to provide continuous replenishing of the desired metabolite anions. Kemp (10) has previously reported that rabbit liver phosphofructokinase did not penetrate conventional polyacrylamide gels and we found similar results with pig liver phosphofructokinase.

Comparison of Liver and Kidney
Phosphofructokinase by Disc Electrophoresis
in 1 mm ATP

In tests with the enzymes in separate gel tubes, the polyacrylamide gel patterns for the kidney phosphofructokinase were similar to those for the liver enzyme. In order to provide a more definite test for identity, samples of the individual enzymes were also run in different sectors of the same gel. The liver

Purification of phosphofructokinase from pig liver and kidney

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|---------------------------|--------------|---------------|-------------|---------------------------|---------------|-------------|---------------------------|
| Homogenate supernatant    | 3150         | 180 g         | 1100        | 0.0063                    | 1230          | 87 g        | 0.014                     |
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| Magnesium-alcohol suspen-| 83           | 3.5 g         | 428         | 0.123                     | 30            | 0.64 g      | 0.79                      |
| Washed enzyme             | 29           | 14 mg         | 197         | 14.1                      | 22            | 83 mg       | 494                      |
| Final suspension          | 10           | 2.1 mg        | 210         | 100                       | 8             | 1.8 mg      | 128                      |

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| Final suspension          | 10           | 2.1 mg        | 210         | 100                       | 8             | 1.8 mg      | 128                      |
FIG. 1. Polyacrylamide gel electrophoresis of pig liver enzyme in sodium dodecyl sulfate (SDS) gels and in conventional gels in the presence of metabolites. The pig liver phosphofructokinase was dissolved in a solution containing 1% sodium dodecyl sulfate, 1% $\beta$-mercaptoethanol, 0.1 M sodium phosphate, pH 7.1, and 10% glycerol, with a final protein concentration of about 0.5 mg per ml. The solution was heated at 100° for 15 min. Approximately 50 $\mu$g of the sodium dodecyl sulfate-denatured enzyme was layered on the gel. The experiment was performed at room temperature.

For the conventional gels, the enzyme was dissolved in a solution containing Buffer B with about 15% sucrose, yielding a final protein concentration of about 0.5 mg per ml. The gel mixture contained 0.4 M Tris (HCl) (pH 8.9), 4.2% acrylamide (w/v), and 0.11% bisacrylamide (w/v). The spacer gel contained 3.5% acrylamide, 0.9% bisacrylamide, and 70 mM Tris (HCl) at pH 6.9. Twenty-six micrograms of enzyme were layered on each gel. A special "subreservoir" apparatus was used (see "Materials and Methods") to maintain the metabolites' concentration in the gels.

The main reservoir buffer contained 0.19 M glycine and 25 mM Tris (pH 8.3). F6P, fructose-6-P.

Preparation showed, in addition to the main band, a small band of protein at the top of the spacer gel and another one at the interface of the spacer and lower gels. The kidney protein was essentially all in the main large patch, which migrated well into the lower gel.

**Kinetic and Physical Properties of Native Liver Phosphofructokinase**

**Allosteric Inhibition by ATP**—Since preliminary kinetic analysis of the pig liver phosphofructokinase showed an activity maximum near pH 8, this pH was used routinely for assays. However, allosteric inhibition by ATP was studied at pH 7.0 using a low fructose-6-P concentration (0.1 mM). Inhibition was observed at ATP concentrations above 50 $\mu$M; 1 mM ATP yielded approximately 90% inhibition.

**50 S and 80 S Peaks and a Rapidly Equilibrating System**—Upon ultracentrifugation, the enzyme exhibits two partially resolved maxima with sedimentation coefficients of 50 S and 80 S. The two maxima resolve much better at 4° than at 20°. The broadness of the 80 S peak cannot be due to diffusion, since a molecule of this size would not diffuse nearly so rapidly. Hence, this pattern reflects an associating-dissociating system. Although such unresolved peaks cannot be correlated with discrete species, these two S values correspond to molecular weights in the range of 3.7 and 7.4 million, respectively. The basic subunit molecular weight estimated from the sodium dodecyl sulfate gels (15, 16) was 80,000 (Fig. 1).

**Isoelectric Point**—Isoelectric focusing experiments were conducted in a custom modification of a polyacrylamide gel-electrophoresis apparatus (see "Materials and Methods"). The isoelectric point of purified pig liver phosphofructokinase was found to be 5.0.

**Characterization of the Precipitation Process**—After the initial discovery and preliminary optimization of the precipitation process, a systematic study was performed under a fixed set of standard conditions (see "Materials and Methods" and legend for Fig. 2).

**Effect of Protein Concentration on the Solubilization of Pig Liver Phosphofructokinase with Increasing Ammonium Sulfate Concentration**—The ammonium sulfate concentration at which protein solubilization became evident was about 40 mM with all protein concentrations (Fig. 2); this is predicted from the equations governing solubility products, since the total amount of protein in the solid phase should have no effect on equilibria in the solution phase. On the other hand, the ammonium sulfate...
Effect of Temperature—Fig. 3 demonstrates that as the temperature was increased the concentration of ammonium sulfate required to solubilize the enzyme was decreased. The effect of temperature on the solubility of the enzyme was taken into account in the purification procedure; the solubilization of the enzyme in Buffer B was carried out at room temperature while the precipitation process, dilution in Buffer A, was carried out at 0°. The reason for the slightly lower recovery of activity at 0° compared to 22.5° is not known.

Effect of pH—Fig. 4 shows the solubility curves of liver phosphofructokinase in ammonium sulfate at various values of pH. The solubility of the enzyme in 50 mM ammonium sulfate was chosen as an index. This value was plotted against the pH of the various buffers (not shown). The minimum in the curve occurred at a pH of about 7.5. The fact that the isoelectric point is about 5.0 makes it unlikely that isoelectric precipitation is the basis for the precipitation properties described in this paper.

Effect of Various Salts—Sulfate salts with ammonium and sodium cations yield essentially the same enzyme solubility pattern, whereas the chloride salts of the same cations dissolve the enzyme at a much lower ionic strength (Fig. 5). Moreover, there is a loss of enzyme activity at higher concentrations of chloride salts but not in sulfate salts. To assure that the enzyme in the chloride salts was dissolved, the centrifuge tube containing the sample for the last centrifugation in the ammonium chloride experiment (74 mM) was vigorously mixed to resuspend any potentially insoluble enzyme. This sample was then assayed by the usual dilution procedure (see "Materials and Methods"). The activity was slightly less than the value for the sample which had not been mixed to resuspend the potential pellet; hence, there was no insoluble enzyme present in the solutions containing high concentrations of chloride salts. Therefore, the protein had definitely been solubilized in the chloride solutions, but it had lost much of its activity.

Effect of Mg²⁺ and Other Divalent Cations—For the initial pre-
Fig. 6. Effect of magnesium chloride concentration on the solubilization of pig liver phosphofructokinase (PFK) by ammonium sulfate. Designed as in Fig. 5, except that no monovalent cations were added and the MgCl₂ concentration was varied. The buffer solution contained Buffer A plus 10 mM ammonium sulfate.

Fig. 7. Effect of various divalent cations on the solubilization of pig liver phosphofructokinase (PFK) in ammonium sulfate. Designed as in Fig. 6, except that the divalent cation species was varied using a constant concentration (5 mM).

Precipitation at the heat-alcohol step, 50 mM MgCl₂ was used, but with purified enzyme, much lower concentrations were adequate. Fig. 6 shows the effect of the concentration of magnesium chloride on the solubility of purified phosphofructokinase. At a concentration of 5 mM, various other divalent cation chlorides also decrease the solubility of the enzyme in ammonium sulfate (Fig. 7). As shown in Fig. 7, the order of precipitating effectiveness is: Mn²⁺ > Mg²⁺ > Ca²⁺ > Zn²⁺. However, as explained earlier, the best purification was always achieved with Mg²⁺, rather than with Mn²⁺.

Fig. 8. Effect of metabolites in the presence of MgCl₂ on the solubilization of pig liver phosphofructokinase (PFK) in ammonium sulfate. The buffer contained 50 mM Tris-Cl, 50 mM mercaptoethanol, and 5 mM MgCl₂, with the various indicated combinations of the metabolites of phosphofructokinase. All of the buffer solutions initially contained 20 mM ammonium sulfate except those involving fructose-6-P (F6P).

Effect of Various Metabolites on the Solubility of Phosphofructokinase

With Added Mg²⁺—In the presence of 5 mM Mg²⁺, 0.1 mM fructose-6-P is the only potent solubilizer of phosphofructokinase at low concentrations of ammonium sulfate (Fig. 8). Under these conditions, 0.01 mM ATP shows only a very slight solubilization effect while 0.01 mM fructose-1,6-P₂ has virtually no effect; the analogous curves for 0.1 mM concentrations (not shown) of these metabolites are indistinguishable from those for 0.01 mM.

Separate experiments (not shown) in the presence of 5 mM Mg²⁺ revealed only slight differences in solubilizing ability of a number of adenine nucleotides, including ATP, ADP, AMP, and cyclic adenosine 3',5'-monophosphate.

With EDTA and No Added Magnesium—When these studies were repeated in the presence of EDTA and with no added Mg²⁺, ATP proved to be a potent solubilizer (Fig. 9). With 1 mM ATP, the enzyme was essentially completely solubilized without added salt. The ATP solubilization effect must require free ATP, since it is observable only in the presence of sufficient chelating agent to yield a maximal concentration of free ATP. Fructose-1,6-P₂ had little or no effect on the solubilization curves, regardless of the concentrations of divalent cation and ATP. These solubilization effects are consistent with the depolymerization effects observed in gel electrophoresis.

The kidney enzyme yielded essentially the same ammonium sulfate solubility curve (not shown) as that for the liver enzyme.

DISCUSSION

Unusual Solubility Properties—The precipitation-solubilization phenomena described here are unusual. The phosphofructokinases described in this work precipitate at low ionic strength in the presence of Mg²⁺ at 0°C, if fructose-6-P and ATP are not present. In contrast, most nonparticulate enzymes are readily

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soluble in water alone. These properties form the basis for the simple and rapid purification procedure developed in this work.

**Metabolite Counteraction of Precipitation**—The counteracting effect of the metabolites, fructose-6-P and ATP, on the precipitation is similar to each previously reported effects of substrates on enzymes as selective substrate extraction (18, 19) and substrate elution (20, 21). The characteristically high specificity of such effects of metabolites is exhibited in the phosphofructokinase system; fructose-6-P completely solubilizes the enzyme but fructose-1,6-P₂ does not.

Except for qualitative differences, fructose-6-P and ATP appear to produce similar effects. One difference is that to achieve maximal effects, considerably higher (10 X) concentrations of ATP than fructose-6-P are required. This suggests that fructose-6-P is more tightly bound to the enzyme than is ATP, so that the particular binding site involved is saturated at lower concentrations. A second difference is that to achieve the ATP effect, Mg²⁺ (or certain other divalent metal ions) must not be present in appreciable concentration. This seems to indicate that only free ATP, and not MgATP, is capable of producing the effects.

**Role of Divalent Metal Ions**—Although it does not rule out the possibility of a direct effect of Mg²⁺ on the enzyme, all of our evidence is consistent with a model in which Mg²⁺ has only an indirect effect upon precipitation, namely by removing the solubilizing factor ATP (or ADP). The data of Bock (22) allow quantitative estimates of the ability of Mg²⁺ to sequester various phosphate-containing metabolites; there is good correlation between Mg²⁺ sequestering ability and precipitating ability with those metabolites (ATP and fructose-6-P) which solubilize phosphofructokinase in the absence of Mg²⁺. The stability constant for the magnesium-fructose-6-P complex is estimated to be about 100 M⁻¹, while that for MgATP is 10,000 M⁻¹ (22). Therefore, at the levels used, Mg²⁺ can sequester most of the free ATP but virtually none of the fructose-6-P. This could account for the fact that in the presence of Mg²⁺, fructose-6-P solubilizes phosphofructokinase but ATP does not.

The results with other divalent cations also support this model. The stability constants (22) for the combination of ATP with the indicated divalent cations are 56,000 M⁻¹ for Mn-ATP, 10,000 M⁻¹ for MgATP, and 5,900 M⁻¹ for Ca-ATP. The trend in the Mg²⁺-ATP stability constants, Mn >> Mg > Ca, parallels the ability of the divalent cations to keep the enzyme in a precipitated state, i.e. Mn²⁺ > Mg²⁺ = Ca²⁺ > Zn²⁺.

Although the purified enzyme is precipitated by 5 mM Mg²⁺, the enzyme in the heat-alcohol filtrate requires 50 mM Mg²⁺, presumably to sequester endogenous free ATP. Also, the purified enzyme does not require alcohol for precipitation, but alcohol must be present in the initial Mg²⁺ precipitation of the enzyme from the heat-alcohol filtrate fraction.

**Comparative Specific Activities**—The only previous purification studies on mammalian liver fructose-6-P kinases have utilized rabbit liver (10, 11). Specific activity values of 3 (11), 48 (10), and 68 (10) were reported, based on various procedures; the specific activity of 65 was obtained at the expense of poor yields but the value of 48 was obtained in 45% yield (10). The specific activities obtained in this study were 100 for pig liver phosphofructokinases and 128 for the pig kidney enzyme.

The maximum specific activities reported for phosphofructokinases from other sources range from 125 for the yeast enzyme (3) to 150 to 157 for the rabbit skeletal muscle (7) and sheep heart enzymes (6).

**REFERENCES**

1. Constantinedes, S. M., and Deal, W. C., Jr. (1970) J. Biol. Chem. 245, 246
2. Griffith, C. C., Houck, B. N., and Brand, L. (1967) Biochem. Biophys. Res. Commun. 27, 287
3. Atpodien, W., and Bodé, H. (1970) Eur. J. Biochem. 12, 136
4. Uveda, K., and Kuboka, S. (1970) J. Biol. Chem. 245, 3315
5. Mansour, T. E. (1963) J. Biol. Chem. 238, 2825
6. Mansour, T. E., Wakid, N., and Sphrouse, H. M. (1966) J. Biol. Chem. 241, 1512
7. Long, K. H., Marcus, P., and Larde, H. A. (1965) J. Biol. Chem. 240, 1893
8. Parmegiani, A., Luft, J. H., Love, D. S., and Krebs, E. G. (1966) J. Biol. Chem. 241, 4025
9. Odele, R., Guilloton, M., Dupuis, B., Rayon, D., and Rosenberg, A. J. (1968) Bull. Soc. Chim. Biol. 50, 2023
10. Kemp, R. G. (1971) J. Biol. Chem. 246, 245
11. Ramaiya, A., and Tejwani, G. A. (1970) Biochem. Biophys. Res. Commun. 39, 1149
12. Wakid, N., and Mansour, T. E. (1965) Mol. Pharmacol. 1, 53
13. Katzenellenbogen, W. M., and Dobryszczyk, W. M. (1969) Clin. Chim Acta 4, 515
14. Davis, B. J. (1964) Ann. N. Y. Acad. Sci. 121, 404
15. Webber, K., and Osborn, M. (1968) J. Biol. Chem. 244, 4406
16. Ershoff, A. L., Wechsler, D., and Maizel, J. V., Jr. (1967) Biochem. Biophys. Res. Commun. 28, 815
17. Dixon, M., and Webb, E. C. (1961) Advan. Protein Chem. 16, 197
18. Rose, I. A., and Warm, J. U. (1967) J. Biol. Chem. 242, 1635
19. Wilson, J. E. (1968) J. Biol. Chem. 243, 3640
20. Colowick, S. P. (1955) Methods Enzymol. 1, 90
21. Pogell, B. M. (1966) Methods Enzymol. 9, 9
22. Bock, R. M. (1960) in The Enzymes (Boyer, P. D., Larde, H. A., and Myers, K., eds) Vol. 2, p. 16, Academic Press, New York

*This procedure has been tested with phosphofructokinases from 15 different sources; it was successful with many, but not all, sources tested (T. H. Massey and W. C. Deal, Jr., unpublished results).
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