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Regulation of pea seed pyrophosphate-dependent phosphofructokinase: Evidence for interconversion of two molecular forms as a glycolytic regulatory mechanism

(fructose 2,6-bisphosphate/glycolysis/gluconeogenesis)

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ABSTRACT **Two molecular forms of pyrophosphate-dependent phosphofructokinase (PP_i-PFK; pyrophosphate:D-fructose-6-phosphate 1-phosphotransferase; EC 2.7.1.90) have been found whose activity depends upon association and dissociation characteristics regulated by fructose 2,6-bisphosphate (Fru-2,6-P_2). PP_i-PFK was purified 200-fold from cotyledons of germinating pea seeds and found to exist in two interconvertible molecular forms. The two forms of PP_i-PFK have sedimentation coefficients of 6.3 and 12.7 S during ultracentrifugation in sucrose density gradients and also differ both in sensitivity to the activator Fru-2,6-P_2 and in affinity for the substrate fructose 6-phosphate. The major component of enzyme activity is in the large form (12.7 S), but the small, less-active, form (6.3 S) predominates when the enzyme preparation is extracted and stored in buffer without Fru-2,6-P_2 and glycerol. Urea (1 M) or pyrophosphate (20 mM) treatment results in at least a 50% loss of activity in the glycolytic direction, whereas these treatments had much less influence on the gluconeogenic direction activity. Concomitant with the loss of glycolytic activity the enzyme dissociates into the small form. Fru-2,6-P_2 stabilizes the large form of the enzyme against the dissociating effects of pyrophosphate and prevents the inactivation in the glycolytic direction during either urea or pyrophosphate treatment. The small molecular form of the enzyme is converted into the large form in the presence of Fru-2,6-P_2. We propose that glycolytic and gluconeogenic hexose metabolism in plants includes a regulatory mechanism induced by Fru-2,6-P_2 that involves the interconversion of two molecular forms of PP_i-PFK.**

The interconversion of fructose 6-phosphate (Fru-6-*P*) and fructose 1,6-bisphosphate (Fru-1,6-P_2) is a rate-limiting step in glycolysis and gluconeogenesis, and the regulation of the enzymes involved is subject to modulation by a variety of metabolites and inorganic ions. In higher animals, the direction of carbon flow through fructose phosphates is controlled by the relative activities of ATP-dependent phosphofructokinase (ATP-PFK, EC 2.7.1.11) and fructose-1,6-bisphosphatase (Fru-1,6-P_2ase, EC 3.1.3.11) (1). Fructose 2,6-bisphosphate (Fru-2,6-P_2), a recently discovered regulator of these two enzymes (2, 3), acts both to stimulate ATP-PFK activity and to inhibit Fru-1,6-P_2ase activity (4, 5); thus, increased Fru-2,6-P_2 concentration in an animal tissue increases glycolytic flux (6).

The discovery of a pyrophosphate-dependent phosphofructokinase (PP_i-PFK; pyrophosphate:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.90) in a parasitic amoeba (7), a bacterium (8), pineapple leaves (9), and a number of other plants (10–18) established that in these organisms the interconversions of Fru-6-*P* and Fru-1,6-P_2 involve an additional enzyme. In plants, PP_i-PFK is a cytoplasmic enzyme (11, 13, 14, 16–18) that reversibly interconverts Fru-6-*P*/Fru-1,6-P_2 and pyrophosphate/phosphate during *in vitro* experiments. The total activity of PP_i-PFK may be equal to or greater than the ATP-PFK activity, depending on the plant tissue (9, 13, 14, 16, 17); thus the contribution of PP_i-PFK to sugar metabolism in plants is probably significant. Fru-2,6-P_2 is a powerful activator of plant PP_i-PFK (10) at physiological concentrations (nM) (10–18) while inhibiting Fru-1,6-P_2ase activity (11, 18) and having no effect on cytoplasmic ATP-PFK (11). Plant glycolysis, therefore, depends on the regulation of both ATP-PFK and PP_i-PFK in a manner that remains to be resolved.

Recently we found that the activity of PP_i-PFK increased and the enzyme's sensitivity to Fru-2,6-P_2 changed during pea seed germination. On the basis of the behavior of PP_i-PFK after gel filtration, we suggested that the enzyme exists in two interconvertible forms that could be regulated by binding with Fru-2,6-P_2 and other ligands (15). These results prompted us to investigate these properties of PP_i-PFK further, using a partially purified enzyme preparation from germinating pea seeds. Primarily using sucrose density gradient centrifugation analysis, we now present evidence for the reversible interconversion of two molecular forms of PP_i-PFK. These results support the proposal that the interconversion of two molecular forms of PP_i-PFK is another mechanism for regulating glycolysis and gluconeogenesis in plants.

MATERIALS AND METHODS

Materials. Pea seeds (*Pisum sativum* cv. Alaska) were soaked overnight in running tap water and germinated for 2 days in vermiculite at room temperature in darkness.

Fru-6-*P*, Fru-1,6-P_2, Fru-2,6-P_2, *N*,*N*-bis(2-hydroxyethyl)glycine (Bicine), EGTA, 2-mercaptoethanol, and the auxiliary enzymes for the assay of PP_i-PFK were purchased from Sigma. Sucrose was obtained from J. T. Baker Chemical (Phillipsburg, NJ). Sephadex G-200 (fine, 40–120 μm) was from Pharmacia and DEAE-cellulose (DE-52) was obtained from Whatman.

Enzyme Assays. PP_i-PFK activity was assayed spectrophotometrically as described (9, 15). The assay was started by the addition of pyrophosphate (for the glycolytic direction) or potassium phosphate (for the reverse direction). Af-

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Abbreviations: Fru-6-*P*, fructose 6-phosphate; Fru-1,6-P_2, fructose 1,6-bisphosphate; Fru-2,6-P_2, fructose 2,6-bisphosphate; PP_i-PFK, pyrophosphate-dependent phosphofructokinase; ATP-PFK, ATP-dependent phosphofructokinase; Fru-1,6-P_2ase, fructose-1,6-bisphosphatase; Bicine, *N*,*N*-bis(2-hydroxyethyl)glycine.

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ter a short lag period, the reaction rates were constant during measurement. Catalase activity was assayed at 25°C by following the disappearance of hydrogen peroxide by absorbance at 240 nm (19).

Aldolase activity was assayed spectrophotometrically by measuring the oxidation of NADH at 340 nm in a system containing 0.1 M Hepes/NaOH at pH 8.0, 2.5 mM $MgCl_2$, 10 mM Fru-1,6-P_2, 0.2 mM NaOH, 2 μg of triose-phosphate isomerase, and 10 μg of α-glycerol-phosphate dehydrogenase in 1 ml.

Sucrose Density Gradient Centrifugation. Ultracentrifugation of PP_i-PFK was carried out at 40,000 rpm with a Beckman SW 41 rotor in a Beckman model 65B ultracentrifuge for 16 hr at 5°C with a 12-ml linear gradient from 5% to 25% (wt/vol) sucrose in 100 mM Bicine/NaOH buffer at pH 8.2, 1 mM EGTA, and 7 mM 2-mercaptoethanol. The samples were layered on top with 500 μl of the sample containing catalase (11.6 S) and aldolase (7.3 S) as internal markers. At termination of the centrifugation, 400-μl fractions were collected from the top with an ISCO density gradient fractionator (model 640). The sedimentation coefficients and molecular weights of the two forms of PP_i-PFK were determined by the method of Martin and Ames (20). Protein was measured with Coomassie blue stain according to Bradford (21).

Purification of PP_i-PFK. All purification steps were carried out at 0–4°C. *Step 1*: *Crude Extract*. Cotyledons (200 g) were washed and separated from pea seeds after two days of germination and homogenized in a Waring Blendor with 500 ml of 100 mM Bicine/NaOH, pH 8.2, containing 1 mM disodium EGTA, 7 mM 2-mercaptoethanol, and 5% (vol/vol) glycerol (buffer A). The homogenate was filtered through eight layers of cheesecloth and centrifuged at 16,000 × *g* for 30 min. The supernatant was defatted by passing through glass wool.

Step 2: *Ammonium sulfate fractionation*. Solid $(NH_4)_2SO_4$ (116 g) was added to 600 ml of supernatant with stirring for 30 min (35% saturation). After centrifugation at 25,000 × *g* for 20 min the precipitate was discarded and enzyme was precipitated by addition of 71 g of solid $(NH_4)_2SO_4$ (55% saturation). The precipitate (35–55% saturation fraction) was collected after centrifugation and dissolved in 40 ml of buffer A containing 100 μM Fru-2,6-P_2.

Step 3: *DEAE-cellulose (DE-52) chromatography*. The fraction from the $(NH_4)_2SO_4$ fractionation step was dialyzed overnight against buffer A and then applied to a DEAE-cellulose column (2.5 × 30 cm) that had been equilibrated with buffer A. The DEAE-cellulose column was eluted by a linear salt gradient from 0 to 0.6 M sodium chloride (in buffer A). PP_i-PFK eluted at 0.24 M salt. The active fractions were collected, pooled, and then concentrated by adding solid $(NH_4)_2SO_4$ to 55% saturation; after centrifugation, the precipitate was resuspended in buffer A containing 1 mM Fru-2,6-P_2.

Step 4: *Preparative sucrose density gradient centrifugation*. The enzyme preparation (3.5 ml) containing 1 mM Fru-2,6-P_2, obtained from the DEAE-cellulose chromatography step described above, was layered onto 5–25% linear sucrose gradients (prepared in buffer A) (30 ml) and then centrifuged for 5.5 hr (at 5°C) at 26,000 rpm in a Beckman SW 27 rotor on a Beckman 65B ultracentrifuge; 1.2-ml fractions were collected, starting at the top of the gradient. The sucrose gradient was measured by refractometry.

Step 5: *Gel filtration chromatography*. Active fractions were collected and pooled from step 4 and applied onto a Sephadex G-200 (2.5 × 38 cm) column, preequilibrated and eluted with buffer A. Under these conditions, PP_i-PFK activity was stable for at least 1 month when stored at low temperature (−20°C). The enzyme was purified over 200-fold by this procedure and was free of detectable activities of ATP-PFK and Fru-1,6-P_2ase.

RESULTS

Isolation and Kinetic Properties of the Two Forms of PP_i-PFK. Molecular heterogeneity of the enzyme was observed by assaying PP_i-PFK activity along sucrose density gradient sedimentation profiles (Fig. 1). When an enzyme preparation in the standard purification procedure (after step 3 with an enzyme stored in buffer A with Fru-2,6-P_2) was further purified by centrifugation in preparative sucrose density gradients, a broad activity peak was observed with an obvious shoulder at a lower density (Fig. 1*A*). But, when the enzyme was purified according to the same procedure described above in the buffer without glycerol (buffer B) and Fru-2,6-P_2, we observed only one symmetrical enzyme activity peak with a lower sedimentation velocity and a high sensitivity to Fru-2,6-P_2 activation (Fig. 1*A*).

Greater resolution of two enzyme forms was obtained by taking the peak fractions (fractions 11 and 15 from Fig. 1*A*) and analyzing their sedimentation properties on 12-ml sucrose gradients (Fig. 1*B*). The two forms had sedimentation coefficients of 6.3 and 12.7 S. Buffer A (with Fru-2,6-P_2) stabilized the enzyme in the large form, whereas the small form predominated in buffer B (without Fru-2,6-P_2). The results agreed with our previous finding that PP_i-PFK in developing pea seeds exists in two forms having different sensitivity to Fru-2,6-P_2 after gel filtration chromatography. The large and small form of the enzyme also showed kinetic differences after gel filtration (15).

The saturation curves of substrate affinity for Fru-6-*P* were different for the two molecular forms (Fig. 2). Lineweaver–Burk plots were linear, yielding K_m values of 7.7 mM and 2.6 mM for the small form and the large form, respectively. In the presence of activator, Fru-2,6-P_2 (1

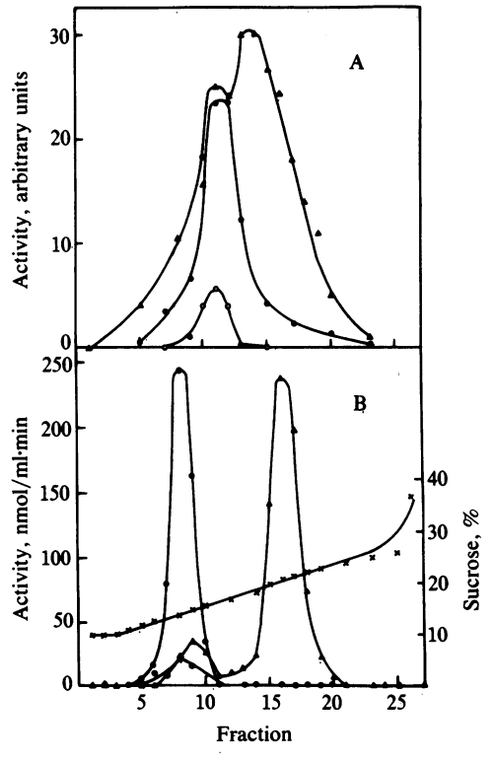

FIG. 1. Sucrose density gradient sedimentation profiles of PP_i-PFK activity. (*A*) PP_i-PFK activity profile of the enzyme isolated in the presence of Fru-2,6-P_2 and glycerol (▲); and isolated in the absence of Fru-2,6-P_2 and glycerol (○), then assayed upon the addition of Fru-2,6-P_2 (●). (*B*) Isolation of the small and large molecular forms of PP_i-PFK by recentrifugation of the peak fractions 11 and 15, respectively, from *A*. Symbols indicate the same experimental conditions as in *A*; ×, percent sucrose.

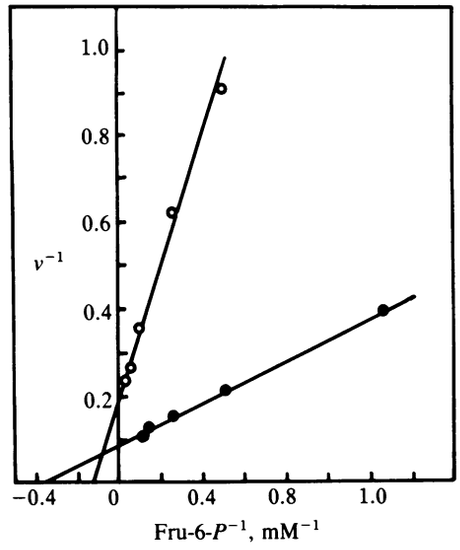

FIG. 2. Double-reciprocal plots of Fru-6-*P* saturation curves for the small (○) and large (●) molecular forms of PP_i-PFK. Velocity units are nmol/min per mg of protein.

μM), the K_m values of the small form decreased to 1.7 mM (data not shown). The two molecular forms of PP_i-PFK also had different Fru-2,6-P_2 activation sensitivities (Fig. 3). The activity of the small form (in the glycolytic direction) was greatly increased by Fru-2,6-P_2 (3) at physiological concentrations (K_a = 0.015 μM), while almost no effect of Fru-2,6-P_2 on the reverse reaction was observed, even at high Fru-2,6-P_2 concentrations. The large form showed less sensitivity to Fru-2,6-P_2 in both directions (reverse direction data are not shown).

Effects of Fru-2,6-P_2 on a Urea-Induced Dissociation of PP_i-PFK. When PP_i-PFK in the large molecular form was incubated with 1 M urea at 4°C for 10 min, more than 65% of the enzyme's activity in the glycolytic direction was lost (Fig. 4*A*). Most of the activity lost by urea treatment was regained in the presence of 1 μM Fru-2,6-P_2. Under the same conditions, the enzyme retained nearly 75% of its activity in the reverse direction. When the small molecular form of PP_i-PFK was treated similarly with 1 M urea (Fig. 4*B*), over 65% of its activity in the reverse direction was retained, and 1 μM Fru-2,6-P_2 added during the enzyme assay had no influence

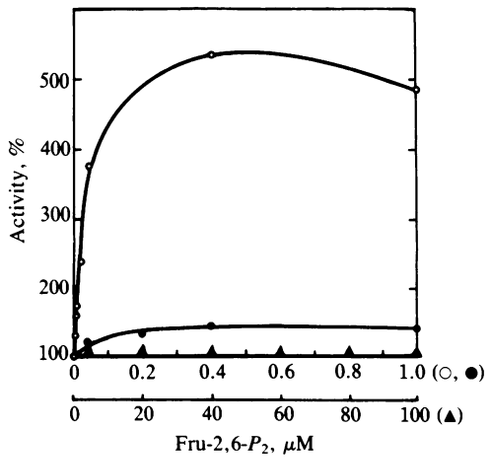

FIG. 3. Fru-2,6-P_2 concentration effects on the two molecular forms of PP_i-PFK. ○, Small form in the glycolytic direction; ▲, small form in the gluconeogenic direction; and ●, large form in the glycolytic direction.

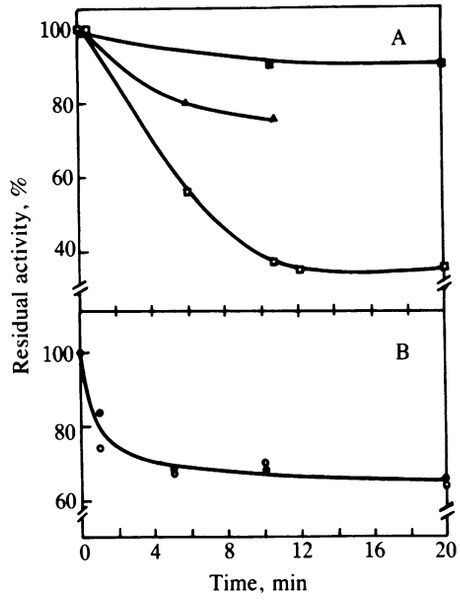

FIG. 4. Effects of urea and Fru-2,6-P_2 on the activity of PP_i-PFK. (*A*) PP_i-PFK was obtained in the large form by gel filtration and treated with 1 M urea at 4°C. PP_i-PFK activity was measured at timed intervals in the glycolytic direction in the absence (□) and presence (■) of 1 μM Fru-2,6-P_2 and in the gluconeogenic direction (▲) without Fru-2,6-P_2. (*B*) PP_i-PFK also was obtained in the small form by gel filtration and treated similarly with urea. Activity was measured in the gluconeogenic direction in the presence (●) of 1 μM Fru-2,6-P_2 and its absence (○).

on the reverse reaction activity during the time course of the incubation with 1 M urea.

The effects of urea treatment were analyzed both by gel chromatography and by sedimentation (Fig. 5). Gel chromatography indicated that treatment of the enzyme mainly in the large form by incubation with 1 M urea for 30 min at 4°C, followed by application onto a Sephadex G-200 column, caused the activity peak to shift to the elution position of the small form (Fig. 5*A*).

Ultracentrifuge sedimentation studies show that, after urea treatment, the activity of the large molecular form decreases and the small form sensitivity to Fru-2,6-P_2 increases slightly (Fig. 5 *B* and *C*). The proportion of two forms also changed, with the percentage of small form in the total activity increasing from 24% to 50% from the beginning of treatment (5 min, Fig. 5*B*) to incubation for 1.5 hr (Fig. 5*C*). These results reveal that there is an association/dissociation relationship between the two molecular forms of PP_i-PFK. Urea treatment, and also incubation with high concentration of pyrophosphate (results described later), changes the promoter/oligomer equilibrium, thereby inducing a reversible dissociation of the enzyme.

Fru-2,6-P_2-Induced Association. Results from kinetic studies and disassociation experiments (Figs. 2–5) raised the possibility that Fru-2,6-P_2 binding induced the formation of the large form of the enzyme and stabilized the enzyme in this form. Documentation of the Fru-2,6-P_2-induced association of PP_i-PFK is shown in Fig. 6. When the small form of the enzyme was incubated with Fru-2,6-P_2 (1 mM) for 24 hr at 4°C and then centrifuged in a sucrose gradient, the large form was present and contained about 80% of the enzyme activity (compare Fig. 6 *A* and *B*). Incubation of the small form with 1 mM Fru-1,6-P_2 also induced enzyme association into the large form (data not shown).

It also is known that the activity of plant PP_i-PFK is inhibited by NaCl. van Schaftingen *et al.* (12) reported that some anions, including NaCl, diminished the sensitivity of the en-

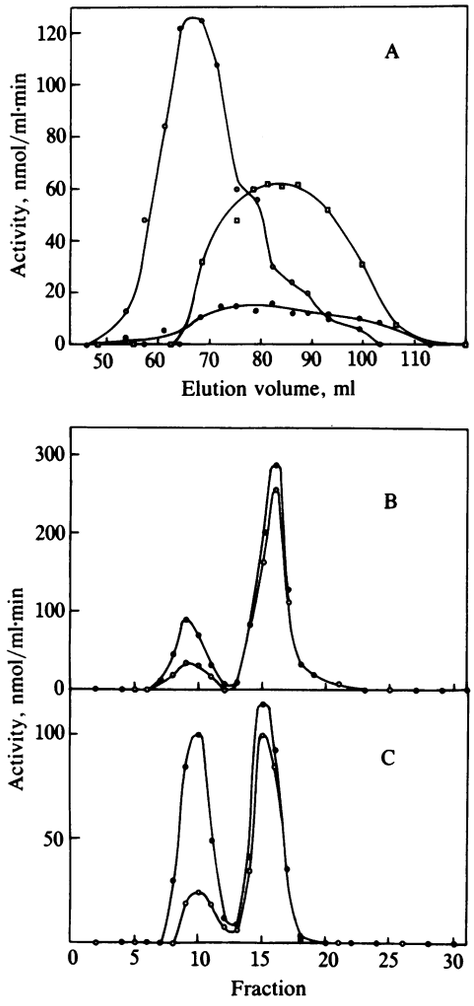

FIG. 5. Dissociation effects of urea treatment on PP_i-PFK activity in the glycolytic direction. (*A*) Distribution of PP_i-PFK activity during Sephadex G-200 gel filtration. Initially the enzyme was mostly in the large molecular form (○). Then a sample of the enzyme was treated with 1 M urea at 4°C for 30 min, chromatographed, and assayed in the presence (□) of 1 μM Fru-2,6-P_2 or its absence (●). Clearly, urea treatment induced the small molecular form of the enzyme. (*B*) Distribution of PP_i-PFK activity after sucrose density gradient centrifugation to separate the large and small molecular forms. Initially the enzyme was mostly in the large form, then it was treated for 5 min with 1 M urea at 5°C, centrifuged, and assayed in the absence (○) and in the presence (●) of 1 μM Fru-2,6-P_2. (*C*) Experimental conditions identical to *B* except the urea treatment lasted for 90 min. The large molecular form of PP_i-PFK is losing activity with extended urea treatment time.

zyme to Fru-2,6-P_2 at millimolar concentration. In experiments similar to those in Fig. 6, 0.25 M NaCl retarded the association of PP_i-PFK small form into the large form (data not shown).

Pyrophosphate-Induced Dissociation. Early kinetic studies demonstrated that PP_i-PFK activity in the glycolytic direction is inhibited at high concentrations of pyrophosphate (9). This pyrophosphate inhibition is relieved by Fru-2,6-P_2 (data not shown; see also ref. 11). As shown in Fig. 7, the sedimentation profile of PP_i-PFK activity was changed markedly by treatment with 20 mM pyrophosphate. When a PP_i-PFK preparation containing mostly the large molecular form (Fig. 7*A*) was incubated with 20 mM sodium pyrophosphate at 4°C for 3 hr and then centrifuged in a sucrose gradient, the small form appeared, with sedimentation coefficients showing a corresponding decrease from 12.7 S to 6.3 S (Fig. 7*B*). When the same enzyme preparation was incubated with the same

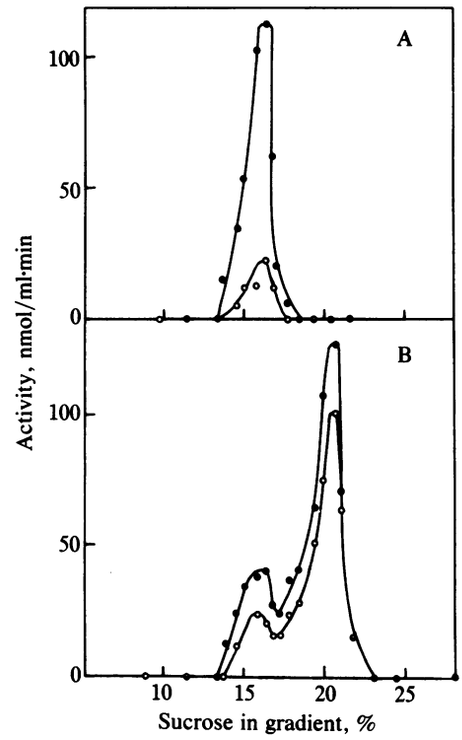

FIG. 6. Fru-2,6-P_2-induced association of PP_i-PFK. (*A*) The enzyme in the small molecular form was incubated 24 hr at 4°C without Fru-2,6-P_2, layered on a sucrose gradient, centrifuged, and assayed down the gradient in the absence (○) and in the presence (●) of 1 μM Fru-2,6-P_2. (*B*) The enzyme in the small molecular form (as in *A*) was incubated 24 hr at 4°C in the presence of 1 mM Fru-2,6-P_2, then centrifuged and assayed as in *A*. Clearly, the enzyme is mostly converted to the large molecular form.

amount of pyrophosphate in the presence of Fru-2,6-P_2 (1 mM), the dissociation was prevented and the sedimentation profile of PP_i-PFK activity showed that most of the activity was still present as the large form (Fig. 7*B*). These results

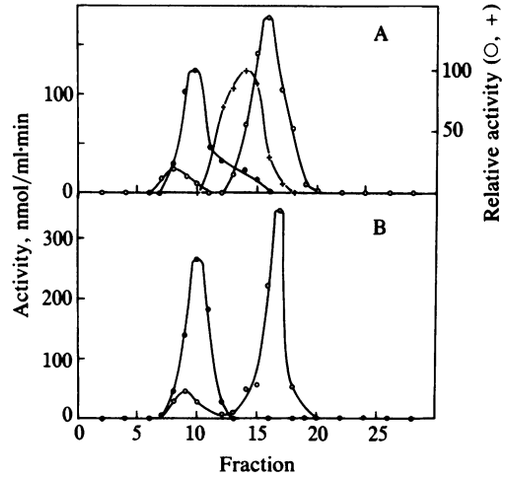

FIG. 7. Dissociation and association effect of pyrophosphate and Fru-2,6-P_2, respectively, on PP_i-PFK in sucrose density gradient profiles. (*A*) Initially the PP_i-PFK was mainly in the large form (○) as shown by its activity in the presence of 1 μM Fru-2,6-P_2. Also shown in the same profile are aldolase (●) and catalase (+) as standards. (*B*) The same PP_i-PFK preparation as in *A* was sampled and incubated for 3 hr at 4°C in the presence of 20 mM pyrophosphate (●) or in the presence of 20 mM pyrophosphate plus 1 mM Fru-2,6-P_2 (○), centrifuged, and assayed down the gradient in the presence of 1 μM Fru-2,6-P_2. Clearly, Fru-2,6-P_2 stabilized PP_i-PFK in the large molecular form.

clearly show that high concentrations of pyrophosphate cause the large molecular form to dissociate, and Fru-2,6-P_2 stabilizes the large form of PP_i-PFK against dissociation by pyrophosphate.

DISCUSSION

This demonstration of two molecular forms of PP_i-PFK furnishes a mechanistic explanation for the activation of plant PP_i-PFK by Fru-2,6-P_2 which was demonstrated in a mung bean preparation (10), and subsequently, with homogenates from a number of different higher plants (11–15, 17). In the original plant work PP_i-PFK was quite active in pineapple leaves (9) without added activators, but in some higher plants, PP_i-PFK was detected only after the addition of Fru-2,6-P_2 to the assay mixture (13). Generally Fru-2,6-P_2 activation of PP_i-PFK increases its maximal catalytic activity and decreases the K_m for Fru-6-P, while apparently changing the pattern of enzyme kinetics from sigmoidal to hyperbolic (10, 11, 12). With PP_i-PFK preparations from pineapple leaves, the maximal velocity is not stimulated much (20%) by Fru-2,6-P_2, although K_m values are lowered severalfold while the kinetics remain hyperbolic. We believe examples of Fru-2,6-P_2-insensitive enzymes from higher plants represent protein preparations that have been protected by Fru-2,6-P_2 against the dissociation phenomenon described in this work. Little is known about the effects of Fru-2,6-P_2 on PP_i-PFK from bacteria or lower plants other than the findings that Fru-2,6-P_2 did not stimulate PP_i-PFK activity of homogenates from *Rhodospirillum rubrum* (a photosynthetic bacterium), *Chara corallina* (an alga), or *Thuidium* sp. (a bryophyte) (13). The two other well-described examples of lower organisms (a bacterium and an amoeba) (7, 8) with high PP_i-PFK activity also have a protein that is not influenced by Fru-2,6-P_2 (6). These results suggest that Fru-2,6-P_2 modulation of PP_i-PFK activity evolved with higher plants.

The hexose bisphosphates, Fru-1,6-P_2 and glucose 1,6-bisphosphate, also activate PP_i-PFK from potato (12), mung bean (22), and pineapple (unpublished data) but at much higher (100- to 1000-fold) concentrations. Because Fru-2,6-P_2 stimulates the activity of PP_i-PFK at nanomolar concentrations, the possibility that other hexose bisphosphate activations are caused by Fru-2,6-P_2 as a reagent contaminant cannot be ignored. The properties and effects between Fru-1,6-P_2 and Fru-2,6-P_2 on plant PP_i-PFK remains to be compared. It seems unlikely that the regulation of PP_i-PFK is entirely analogous to liver ATP-PFK, in which Fru-2,6-P_2 binds at the same allosteric site as Fru-1,6-P_2 (23).

The results we present here provide evidence that pea seed PP_i-PFK exists in two interconvertible molecular forms having different sensitivities to Fru-2,6-P_2 activation. With the pineapple leaf PP_i-PFK we also have observed an increase in sensitivity to Fru-2,6-P_2 during acid treatment (4-morpholineethanesulfonic acid/Bicine buffer, pH 5) and urea treatment (1 M) (unpublished results), suggesting that the association-dissociation phenomenon may occur in other plants as well. Changes in the sensitivity of pea seed PP_i-PFK to Fru-2,6-P_2 during early seedling development (15) also show that developmental or physiological conditions of the plant tissue affect the molecular size distribution of the PP_i-PFK isolated from a plant tissue.

The association-dissociation process of proteins involving conformational changes is an important regulatory mechanism in a number of enzymes. Higher aggregation states, which are promoted by physiological concentrations of Fru-2,6-P_2, have been noted with rat liver and rabbit muscle ATP-PFK; and such protein aggregation leads to changes in the catalytic properties of the enzyme (24, 25). In the case of higher plant PP_i-PFK, the binding of ligands such as pyrophosphate induces conformational changes, thereby causing the enzyme to dissociate into the small form after treatment (this form is inactive in the glycolytic direction), whereas treatment with Fru-2,6-P_2 causes the enzyme to aggregate into the large form (active glycolytic form). We reported earlier that the large form of PP_i-PFK has a greater ratio of glycolytic activity to gluconeogenic activity than the small form (15). Control of its aggregation state is important because PP_i-PFK catalyzes glycolytic reactions in a freely reversible manner. Although PP_i-PFK clearly is a glycolytic enzyme in lower organisms such as an amoeba (7) or a bacterium (8), many higher plants also have abundant quantities of ATP-PFK (9, 11–17), an enzyme catalyzing an essentially irreversible reaction, which also produces Fru-1,6-P_2 during glycolysis. The suggestion has been made for developing castor beans (14) that PP_i-PFK could play an important role in gluconeogenesis in the cytoplasm. In plants it is not possible to assign PP_i-PFK an exclusive function in either glycolysis or gluconeogenesis at present. However, Fru-2,6-P_2 clearly stimulates the catalytic activity of PP_i-PFK in the glycolytic direction by promoting aggregation of enzyme protein, thereby regulating both glycolysis and gluconeogenesis in plants.

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