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Some Properties of 3-Phosphoglycerate Phosphatase from Developing Rice Grain[1]

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RUTH M. VILLAREAL AND BIENVENIDO O. JULIANO
Department of Chemistry, The International Rice Research Institute, Los Baños, Laguna, Philippines

ABSTRACT

Some properties of 3-P-glycerate phosphatase from developing caryopsis of rice (*Oryza sativa* L., variety IR26) were studied. The enzyme was found to be soluble and not bound to starch, and concentrated mainly in the pericarp-aleurone layer; its maximum activity was at 12 to 14 days after flowering. Contents of 3-P-glycerate and chlorophyll were highest in the grain at 7 to 8 days after flowering when starch synthesis was at a maximum. The enzyme was purified about 100-fold by precipitation with 50 to 80% ammonium sulfate, followed by chromatography through Sephadex G-200 and CM-Sephadex C-50. The pH optimum was from 5.7 to 6 and no cation was required for activity. The purified preparation had an apparent *Km* of 2.85 mM and was inhibited by Cu^{2+}, Hg^{2+}, Zn^{2+}, Fe^{3+}, molybdate, and F^-. The enzyme also exhibited high activity toward UTP, ATP, and *p*-nitrophenyl phosphate; moderate activity toward other phosphates; but no activity toward phytate. A molecular weight of about 23,000 was obtained for the 3-P-glycerate peak during gel filtration on Sephadex G-200, which corresponded to a value of 26,000 for the major protein fraction by thin layer gel filtration on Sephadex G-150. Zymograms of the whole extract and semipurified preparations showed two phosphatase bands with 3-P-glycerate as substrate.

3-P-glycerate phosphatase is present in plant species of varying photosynthetic systems (18). It catalyzes the cleavage o the phosphate group from 3-P-glycerate and is believed to provide an alternate route from glycerate to serine in place of photorespiration and the glycolate pathway in the leaves. A starch-bound enzyme is also present in spinach leaves that has properties very similar to those of the soluble form (19). The function of this enzyme was suggested to be related to the control of starch synthesis, since the pool size of 3-P-glycerate could regulate the activity of ADP-glucose pyrophosphorylase (7, 16) and, hence, could ultimately regulate starch synthesis.

This investigation was undertaken to characterize the 3-P-glycerate phosphatase in the developing rice grain. Attempts were also made to determine the probable physiological function of the enzyme by studying its developmental pattern and its location in the grain itself. Such knowledge could relate to our previous study of the factors that limit starch accumulation in the developing rice grain (14), and hence, grain yield.

MATERIALS AND METHODS

Developing grains of rice (*Oryza sativa* L., variety IR26) were obtained from the 1975 continuous cropping system trial of the Rice Production Training and Research department, IRRI. Grains were tagged at flowering and sampled in the mornings at specified intervals up to 21 days after flowering. These tagged grains were used as standards to classify periodic bulk samples used subsequently in the developmental study. The samples were dehulled and classified at 0 to 4 C. Duplicate batches of grains were analyzed at each developmental stages. For the study of enzyme distribution in the grain, samples at 9 to 10 days after flowering were dissected into their respective components: the hull, pericarp-aleurone layer, embryo, and endosperm. Statistical analyses were run on the data and LSD (5%) were calculated.

Chemical Analysis. 3-P-Glycerate was extracted from freshly dehulled grains with 20% $HClO_4$ at 0 to 4 C and immediately assayed by the method of Latzko and Gibbs (11). Samples were taken in the mornings. Grains were dehulled directly from intact plants and the caryopses (dehulled grains) were immediately frozen in Dry Ice. Chlorophyll was also extracted from fresh grains by boiling 80% (v/v) aqueous acetone and was determined after Bruinsma (2). Soluble protein of the redissolved trichloroacetic acid precipitate (for the crude extract) and of the enzyme solutions (for the more purified fractions) was measured using the method of Lowry *et al.* (12).

Enzyme Assay. 3-P-Glycerate phosphatase was assayed at 30 C with 10 μmol sodium cacodylate buffer (pH 5.5) in a total volume of 1 ml. The reaction time was 15 min for the crude enzyme and 12 min for the more purified preparations. The reaction was terminated by adding 0.25 ml 10% trichloroacetic acid and the precipitate was removed by centrifuging at 2,500*g* for 15 min. Phosphate released was determined in the supernatant as described by Anderson and Tolbert (1). The unit of enzyme activity corresponds to the number of μmol of Pi released/min of incubation period. Specific activity was expressed as units of enzyme activity/mg protein.

Enzyme Extraction and Purification. All steps in the extraction and purification procedures were done at 0 to 4 C. Fresh samples of dehulled grain, hull, pericarp-aleurone layer, endosperm, and embryo were macerated separately with mortar and pestle using 3 volumes of extraction medium containing 0.1 M cacodylate buffer, pH 6. The pericarps were previously washed three times with buffer to remove most of the starch granules adhering to the inner surfaces. The resulting slurry was filtered through four layers of cheesecloth and the supernatant was centrifuged at 25,000*g* for 15 min. The clarified crude extract was recovered for protein and enzyme assay and for further purification.

An initial fractional precipitation was made to locate the highest enzyme activity among the various $(NH_4)_2SO_4$ fractions of rice grain protein. This was found in the 50 to 80% $(NH_4)_2SO_4$ precipitate. This protein precipitate was dissolved in a minimal volume of 0.05 M cacodylate buffer (pH 6) and introduced to a Sephadex G-200 column (2.6 × 60 cm) previously equilibrated with 10 bed volumes of 0.05 M cacodylate buffer (pH 6). Upward elution was accomplished with a flow

[1] Part of this paper is from the thesis of R. M. Villareal, submitted to the University of the Philippines at Los Baños in partial fulfillment of the M.S. degree.

rate of 0.2 ml/min and the eluate was collected in 5-ml fractions. The active fractions were pooled and concentrated in dialysis bags with Aquacide at 0 C. The concentrated solution was then chromatographed on a CM-Sephadex C-50 column (1.2 × 27 cm) equilibrated with 0.05 M cacodylate buffer (pH 5.6). The sample was washed with 2 bed volumes of the cacodylate buffer and eluted with a 400-ml linear gradient from 0 to 4 M NaCl in the same buffer. The most active fractions were again pooled and concentrated in dialysis bags with Aquacide.

Enzyme Characterization. Purified enzyme preparations from both Sephadex G-200 and CM-Sephadex chromatography were assayed for pH optimum using three buffer systems ranging from pH 4 to 8. These buffer systems were 0.1 M acetate (pH 4 and 4.5), cacodylate (pH 5–6), and glycylglycine (pH 6.5–8). Purified enzyme from CM-Sephadex C-50 chromatography was assayed for substrate specificity using 11 other phosphate esters at 10 mM. This was also used to study the effect of 1 mM of nine cations and four anions on enzyme activity. These ions were incubated with the enzyme from CM-Sephadex C-50 at 30 C for 5 min prior to addition of 3-P-glycerate.

Purified enzyme from CM-Sephadex C-50 chromatography was also incubated with 1 to 20 mM of 3-P-glycerate to determine the apparent K*m*. Michaelis-Menten and Lineweaver-Burk plots were made on the replicated runs and apparent K*m* was calculated from the plot of $1/v$ (min/μmol) *versus* 1/S (M^{-1}).

Polyacrylamide Disc Gel Electrophoresis. Enzyme preparations (10–200 μg protein) were run on 7.5% polyacrylamide gels at pH 8.3 following the method of Davis (3). Protein was stained with 1% Amido black in 7% acetic acid while phosphatase activity was assayed with 3-P-glycerate or other phosphoester substrates at pH 5.5. Gels were incubated in 0.05 M cacodylate buffer (pH 5.5) with 25 mM substrate for 1 hr at 30 C. They 1were then washed with water and transferred to 0.01 M lead acetate in 0.05 M cacodylate buffer (pH 5.5). Some substrates formed white precipitate of lead salts which coated the gels. After shaking gently for 15 min, they were again washed with water (without removing the film of white precipitate) and transferred back to the initial incubating solution with only the substrate. Within 3 to 4 hr, the film of white precipitate dissolved and white bands began to appear as the more stable $Pb_3(PO_4)_2$ precipitate formed within the gel corresponding to the sites of phosphatase activity. Among the substrates tested were 3-P-glycerate, P-glycolate, glucose-1-P, fructose-6-P, and ATP. The usual simultaneous incubation of disc gels with both phosphate ester substrate and the precipitating reagent (Pb^{2+}) was not successful due to the very high insolubility of the lead salt of 3-P-glycerate. The substrates were precipitated by Pb^{2+} in the decreasing order of 3-P-glycerate > P-glycolate > ATP > glucose-1-P > fructose-6-P.

Thin Layer Gel Filtration. Semipurified enzyme from Sephadex G-200 column was co-chromatographed with several other standard proteins to obtain estimates of the mol wt of the major protein constituent. The gel plate was prepared following the method described by Pharmacia Fine Chemicals (15). Sephadex G-150 superfine was swollen and pre-equilibrated in the running buffer consisting of 0.05 M phosphate (pH 7) with 0.05 M NaCl. After development, the gel plate was blotted with a Whatman No. 3 paper. The protein absorbed by the paper was stained with 0.01% nigrosine in methanol-water-acetic acid (50:40:10, v/v/v). The mol wt of the sample was obtained from a plot of $1/R_{Cyt\ c}$ *versus* log of the mol wt of the standard proteins.

RESULTS

Changes in 3-P-Glycerate Phosphatase, 3-P-Glycerate, and Chlorophyll during Grain Development. The developmental pattern of 3-P-glycerate phosphatase in the rice grain is shown in Figure 1. Enzyme activity was present from anthesis to full ripening and was at a maximum at the dough stage, about 12 to 14 days after flowering. Soluble protein, on the other hand, was initially low, increased to an almost constant level at 10 to 13 days after flowering, and then decreased again (23). Hence, specific activity was highest at 1 to 3 days after flowering, and it decreased from 4 to 13 days followed by a slight increase again up to full maturity.

Chlorophyll was found exclusively in the pericarp layer and can be observed even in 2-day-old grains (Fig. 1). A maximum level of about 7 μg/10 grains was reached at 9 days after flowering; it tapered off up to maturity. The level of 3-P-glycerate of the whole caryopsis closely followed the Chl curve with 9.4 nmol/grain at 4 to 5 days after flowering, 13.6 nmol/grain at 7 to 8 days, and 6.5 nmol/grain at 12 to 14 days.

The disc gel electrophoresis run of the 0 to 80% $(NH_4)_2SO_4$ fractions of developing rice grains showed that two 3-P-glycerate phosphatase bands can be seen even in the early stages of development (Fig. 2). The band of faster mobility was initially less active than the band of slow mobility but its activity in-

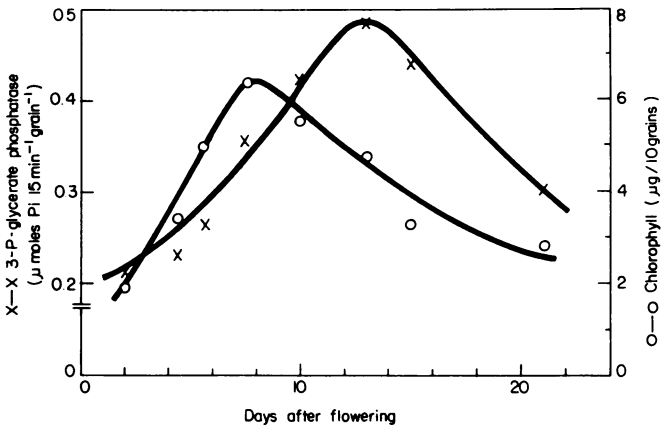

FIG. 1. Developmental pattern of 3-P-glycerate phosphatase and chlorophyll in IR26 rice caryopsis.

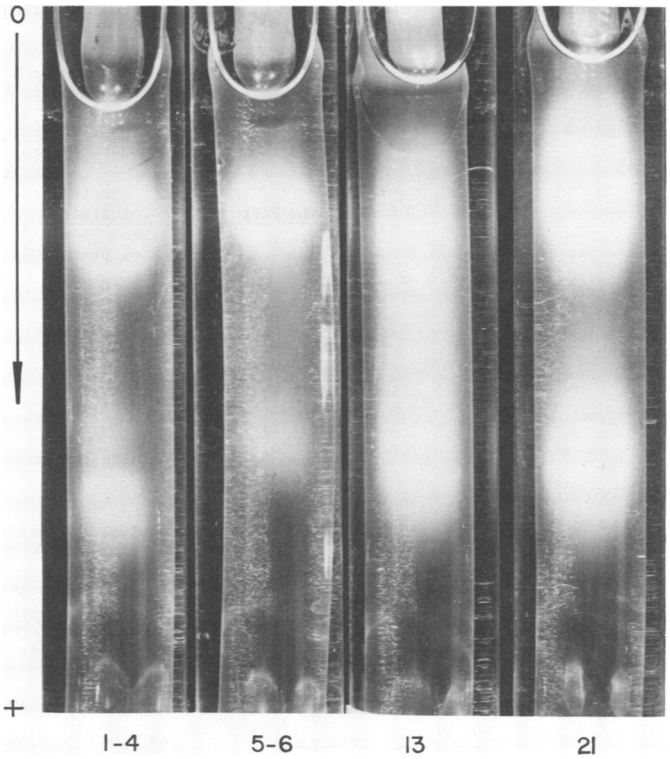

FIG. 2. Banding patterns of 3-P-glycerate phosphatase activity in the 0–80% $(NH_4)_2SO_4$ protein of extract of developing IR26 rice caryopsis. Gels were run at pH 8.3 with 200 μg protein/tube. Numbers below the tubes signify days after flowering.

creased toward maturity.

Distribution of 3-P-Glycerate Phosphatase in the Developing Rice Grain. The distribution of 3-P-glycerate phosphatase in developing rice grain at the late milky stage of development is shown in Table I. The pericarp-aleurone layer and the starchy endosperm had similar contributions to the weight and soluble protein of the caryopsis and together accounted for 80% of the soluble protein and 75% of the total weight of the grain. The enzyme was found to be concentrated mostly in the hull and in the pericarp-aleurone layer. Total enzyme activity in the component tissues of the caryopsis was much higher than that exhibited by the whole caryopsis alone. This was primarily due to the very high activity exhibited by the pericarp-aleurone layer; next to the hull enzyme, it also had the highest specific activity (Table I). The possible presence of any starch-bound activity in the endosperm was explored by assaying the residue (starch granules) after three further extractions with the buffer. However, only trace activity was obtained corresponding to only about 5% of the activity of the soluble enzymic preparation.

The hull extract exhibited comparable activities with both 3-P-glycerate and P-glycolate, unlike that of the pericarp-aleurone layer enzyme which was only 45% as active on P-glycolate as on 3-P-glycerate. Because of this difference in specificity, the enzyme was isolated only from dehulled grains. In addition, grain hull is a maternal tissue and is not really an integral part of the developing grain.

Further attempts to localize the enzyme within the pericarp-aleurone layer were made to determine whether it is in the cytosol or chloroplast. However, the procedure of Heber (8) for chloroplast isolation was not able to isolate whole, intact chloroplasts using the VirTis homogenizer because the pericarp cell walls were very tough. The enzyme present in whole intact pericarp-aleurone layer, on the other hand, was able to hydrolyze 3-P-glycerate at a rate of about 0.06 μmol/15 min.

Enzyme Isolation and Purification. The purification procedure employed during the study is shown in Table II. Preliminary studies conducted to establish the optimal conditions for enzyme extraction showed that the enzyme was extractable by buffers ranging from pH 4.5 to 8 (23). Specific activity of the crude extract was higher at extraction pH of 6 and below. We found no significant effect of adding sulfhydryl group preservatives (1 mM GSH or dithiothreitol), 1 mM EDTA, or 1 mM Mg^{2+} to the extraction buffer.

The enzyme was localized mainly in the 50 to 80% $(NH_4)_2SO_4$ precipitate; this fraction accounted for about 50% of the total activity and 20% of the total protein (23). It was stable in this form up to about 1 month at −20 C while partially purified enzyme from Sephadex G-200 elution was stable at −20 C for up to 2 months but lost 10% of its activity at 0 C within 1 week.

The Sephadex G-200 elution pattern consisted of one broad protein peak with 3-P-glycerate phosphatase coming out as a sharp peak at the tail end (23). This purification step further increased the specific activity of the enzyme by 25 times (Table II). The ratio of elution volume to void volume (Ve/Vo) was 1.71:1 for the protein peak and 2.377:1 for 3-P-glycerate phosphatase. The enzyme exhibited a mean mol wt of 22,700 ± 100 using the general formula of log mol wt = 6.698 − 0.987 Ve/Vo (4).

Chromatography through a CM-Sephadex C-50 column using a linear NaCl gradient further purified the enzyme, but the final yield was quite low, ranging from 3 to 8% (Table II). The enzyme eluted in a sharp peak at about 0.12 M NaCl (23). Activity of these enzyme preparations was linear with respect to incubation time and enzyme concentration used in the assays. Dialysis did not affect enzyme activity.

Characterization. The pH for maximum activity for 3-P-glycerate phosphatase was measured on concentrated active fractions from Sephadex G-200 and CM-Sephadex C-50 chromatography (Fig. 3). Optimum pH was observed between 5.7 and 6 in both cases. The enzyme exhibited normal hyperbolic saturation kinetics with the Michaelis plot for the CM-Sephadex C-50-purified enzyme giving an apparent *Km* for 3-P-glycerate of 2.85 mM (Fig. 4). An earlier trial on semipurified enzyme from Sephadex G-200 gave a value of about 1.6 mM.

No requirement for any of the cations tested was shown by the purified enzyme (Table III). Cu^{2+} was the most inhibitory,

Table I. Activity of Soluble 3-P-Glycerate Phosphatase, Soluble Protein and Weight Distribution in the Different Parts of IR26 Rice Grain at 9 to 10 Days after Flowering

Grain Part	3-P-Glycerate Phosphatase Activity		Distribution of	
	Specific Activity	Distribution[1]	Soluble Protein	Fresh Weight
	(μmol Pi min^{-1} mg protein^{-1})		%	
Hull	0.71	35	9.5	23
Pericarp-aleurone layer	0.47	95	39.5	37
Embryo	0.18	7	11.0	2
Starchy endosperm	0.06	9	39.2	38
Whole caryopsis (dehulled grain)	0.15	65	90.5	76
LSD (5%)	0.11	5	...	...

[1] Based on the sum of the enzyme activities in the hull and the whole caryopsis as 100%.

Table II. Summary of a Typical Purification of 3-P-Glycerate Phosphatase from Developing IR26 Rice Caryopsis at 12 to 14 Days after Flowering

Fraction	Total Protein	Total Units	Yield	Specific Activity	Enrichment
	mg	μmol Pi/min	%	μmol Pi min^{-1} mg protein^{-1}	
Crude extract	207	37.26	100	0.18	1
50-80% $(NH_4)_2SO_4$ fraction	45.0	14.85	40	0.33	2
Sephadex G-200 eluate	0.56	4.66	13	8.32	46
CM-Sephadex C-50 eluate	0.04	1.12	3	28.11	156

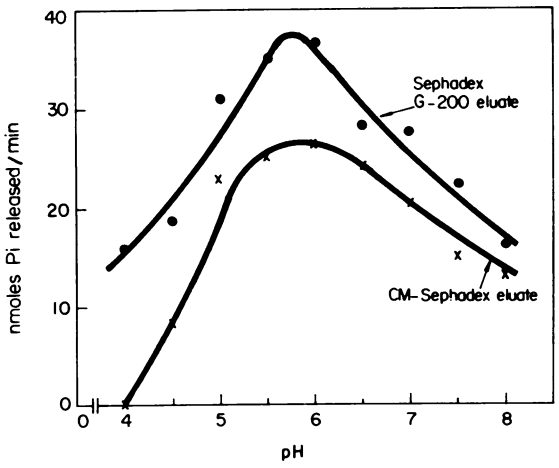

FIG. 3. pH activity profile of 3-P-glycerate phosphatase from developing IR26 rice caryopsis.

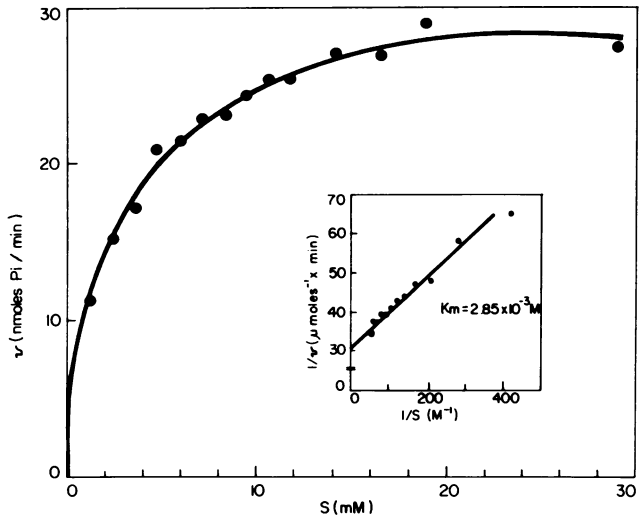

FIG. 4. Michaelis-Menten and Lineweaver-Burk plots of purified 3-P-glycerate phosphatase from IR26 rice caryopsis. Apparent *Km* was calculated from the plot of $1/v$ *versus* 1/S.

Table III. Effect of Metal Ions and Some Anions on 3-P-Glycerate Phosphatase Activity from Developing IR26 Rice Caryopsis

The enzyme from CM-Sephadex C-50 elution was incubated with 1 mM of the test ion at 30 C for 5 min prior to addition of the substrate.

Salt	Relative Activity	Salt	Relative Activity
Control	100	$FeCl_3$	39
$CuCl_2$	0	$MgCl_2$	93
$ZnCl_2$	32	$CaCl_2$	91
$CoCl_2$	80	Na molybdate	21
$MnCl_2$	97	Na fluoride	55
$PbCl_2$	59	Na tartrate	91
$HgCl_2$	16	Na ascorbate	88
		LSD (5%)	3

followed by Hg^{2+}, Zn^{2+}, and Fe^{3+}. Of the four anions tested that inhibit phosphatases, only molybdate and F^- exhibited significant inhibitory effects.

Among the 11 other substrates tested, only three of the naturally occurring compounds, UTP, ATP, and P-enol pyruvate, showed higher than 50% activity relative to 3-P-glycerate (Table IV). The enzyme seemed to show greater preference for the C_6 ester of the glucose-P; activity with the synthetic substrate *p*-nitrophenyl-P was also high. The enzyme was three times less active with P-glycolate, ribose-5-P, and ribulose-1,5-diP, while no activity was observed toward phytate.

The mol wt of the major protein spot obtained from thin layer gel filtration of a 50-fold purified enzyme from Sephadex G-200 was about 26,000 (23). This was comparable to the value of 22,700 obtained from the Ve/Vo of the enzyme on Sephadex G-200 column.

Two major 3-P-glycerate phosphatase bands were also observed on semipurified preparations similar to those noted in whole extracts as shown in Figure 2 (23). More intense bands were shown by the 50 to 80% $(NH_4)_2SO_4$ fraction confirming previous results which localized 50% of the total activity within this fraction. Further purification through Sephadex G-200 chromatography did not alter the number of bands although the faster moving bands tended to show higher activity. Phosphatase zymogram patterns obtained with the Sephadex G-200 eluate using other phosphate esters as substrate showed nonspecificity of the slower moving band (23). It manifested comparable activity with ATP, lesser activity with P-glycolate, and trace activities with G-1-P and F-6-P based on the intensity of the bands formed. These observations essentially agreed with the specificity of the purer enzyme prepared by CM-Sephadex C-50 chromatography (Table IV). The fast migrating band of 3-P-glycerate phosphatase coincided in mobility only with the fastest band with ATP as substrate. Additional bands were shown by gels incubated with ATP and P-glycolate which were not present in the gel incubated with 3-P-glycerate. This indicated the presence of contaminating phosphatases in the enzyme preparation. Protein staining showed the presence of other protein bands not coinciding with the bands of phosphatase activity.

The eluate from the CM-Sephadex C-50 column did not produce any phosphatase band on disc gels, although the quantitative assay in solution showed high activities. This is probably due to a number of factors, including the very low amounts of protein that were introduced in the gel (~10 μg) or to inactivation during the electrophoresis run. Amido black staining did not show any band at all, thus indicating the first possibility as more likely. Randall and Tolbert (17) also did not get any protein band from their most purified enzyme preparation.

DISCUSSION

The changes in the level of 3-P-glycerate closely followed the reported changes in the rate of starch accumulation in the rice grain with maximum value at 8 to 9 days after flowering (14). Since the activity of ADP-glucose pyrophosphorylase did not change significantly during the entire period of starch accumulation (14), the occurrence of the maximum level of 3-P-glycerate in the grain when starch accumulation was also highest suggests that 3-P-glycerate is a positive effector of ADP-glucose pyrophosphorylase. Dickinson and Preiss (5) reported that the conversion of glucose-1-P to ADP-glucose by the action of thi enzyme controls the rate of starch synthesis in the corn endosperm.

The changes in the levels of 3-P-glycerate and of 3-P-glycerate phosphatase in the developing grain of IR26 rice show an inter-

Table IV. Substrate Specificity of 3-P-Glycerate Phosphatase from Developing IR26 Rice Caryopsis

The enzyme was incubated with 10 mM of each substrate. A 100-fold purified enzyme from CM-Sephadex C-50 was used for the assay and activities were expressed relative to that of 3-P-glycerate at 100

Substrate	Relative Activity
3-P-glycerate	100
P-glycolate	30
Glucose-1-P	14
Glucose-6-P	45
Fructose-6-P	39
P-enol pyruvate	61
Ribose-5-P	35
Ribulose-1,5-diP	36
ATP	78
UTP	92
p-Nitrophenyl P	85
Phytate	0
LSD (5%)	2

esting trend. The apparent maximum level of 3-P-glycerate was at 7 to 8 days after flowering when the 3-P-glycerate phosphatase enzyme was only halfway to its peak activity (Fig. 1). Correspondingly, the level of 3-P-glycerate was only about one-half of the maximum level about 12 to 14 days after flowering when the phosphatase level was maximum. This suggests that the phosphatase enzyme may influence the net concentration of 3-P-glycerate in the developing rice grain and thus probably contributes to the regulation of the rate of starch accumulation in the grain.

Although the distribution of 3-P-glycerate in the developing rice grain was not ascertained, it is probably also concentrated in the pericarp as is the 3-P-glycerate phosphatase (Table I). 3-P-Glycerate phosphatase occurs mostly in the pericarp-aleurone layer, but it is probably more associated with the pericarp rather than the aleurone layer since it is present in Chl-containing tissues (18) and the mesocarp layer of the pericarp of developing rice grain has been shown to contain plastids (20). In addition, our phosphatase had no phytase activity, while the phosphatase of aleurone granules has phytase activity (24). Similarly, the level of 3-P-glycerate closely followed that of Chl which is exclusively found in the pericarp. Since leaf chloroplasts have been found to contain as much as five times higher concentration of 3-P-glycerate compared to nonchloroplastic tissues (9), 3-P-glycerate probably occurs mainly in the pericarp as the primary product of CO_2 fixation. Immature green barley pericarps have been shown capable of active photosynthesis and to contain ribulose-1,5-diP and P-enolpyruvate carboxylases (6).

A comparison of enzyme activity in the different parts of the grain showed a lower value for the whole grain (dehulled) compared to the combined activities of the component tissues (Table I). This may be explained by the possible occurrence of some inhibitors (allosteric effectors) in the extracts of the embryo or endosperm or both, which affect the pericarp-aleurone enzyme. Rice grain starch granules did not exhibit any significant activity in contrast to those found in spinach leaf (18).

Most of the properties of rice grain 3-P-glycerate phosphatase preparation are similar to the sugarcane leaf enzyme of Randall and Tolbert (17) and to the nonspecific acid phosphatases that have been isolated from cereals (10, 13, 22). The pH maximum of 5.7 to 6, the apparent lack of absolute specificity to 3-P-glycerate (Table IV), and the inhibition of its activity by Cu^{2+}, Hg^{2+}, Zn^{2+}, Fe^{3+}, molybdate, and F^- (Table III) all run parallel with previous results on partially purified phosphatases (10, 22). However, the absence of any phytase activity by rice grain 3-P-glycerate phosphatase (Table IV) distinguishes it from the phytase that was reported to be associated with the aleurone particles in the rice grain (24). The most purified enzyme preparation obtained after CM-Sephadex chromatography did not give any phosphatase or protein band on disc gels. Hence, the quantitative data on substrate specificity (Table IV) could not be verified electrophoretically. Phosphatases from the cereal grains almost always have high specificities toward ATP (10, 13, 21, 22).

The enzyme showed normal saturation kinetics with 3-P-glycerate exhibiting an apparent *Km* of 2.85 mM. This value is higher than those observed from the enzyme of sugarcane leaf (0.28 mM) and the starch-associated enzyme from spinach leaf (0.9 mM) (17). In the rice bran, the acid phosphatases isolated by Igaue and co-workers (10) had a *Km* range for *p*-nitrophenyl phosphatase of 0.2 to 0.5 mM, whereas Verjee's (22) wheat germ acid phosphatase had *Km* values ranging from 0.3 to 0.9 mM.

The mol wt of about 23,000 exhibited by the enzyme peak on Sephadex G-200 chromatography agreed well with the value of 26,000 obtained from thin layer gel filtration of the same preparation on Sephadex G-150. In contrast, the leaf enzyme from sugarcane exhibited a mol wt of about 160,000 on sucrose density gradient (17). Acid phosphatases from the rice bran also have higher mol wt ranging from 80,000 to 110,000 (10).

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