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Purification and Properties of a Monofunctional Imidazoleglycerol-Phosphate Dehydratase from Wheat

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Imidazoleglycerol-phosphate dehydratase (EC 4.2.1.19) activity was detected in extracts of several monocotyledonous and dicotyledonous plants using a newly developed assay method. The enzyme was purified 114,000-fold (to apparent homogeneity) from wheat germ by five chromatographic steps. Its native relative molecular weight (M_r) was determined to be 600,000 to 670,000, and it consists of identical subunits of M_r 25,500. In wheat germ, the dehydratase, unlike those of prokaryotic origin, is not associated with histidinol phosphatase activity. The reaction product was identified as imidazoleacetol phosphate (IAP) by comparing it with synthetic IAP as an authentic reference. The K_m value for imidazoleglycerol phosphate was 0.36 mM at the optimal pH of 6.6. The enzyme required a reducing agent, such as 2-mercaptoethanol or dithiothreitol, and Mn^{2+} for maximal activity. 3-Amino-1,2,4-triazole competitively inhibited the activity with a K_i value of 46 μM. The purification of imidazoleglycerol-phosphate dehydratase from wheat germ and histidinol dehydrogenase from cabbage (A. Nagai, A. Scheidegger [1991] Arch Biochem Biophys 284: 127–132) suggests that at least the second half of the histidine biosynthesis in plants is identical to that in microorganisms.

His biosynthesis has been extensively studied in microorganisms, both biochemically and genetically (Loper et al., 1964; Martin et al., 1971; Artz and Holzschu, 1983). Four of the total of 10 enzymic steps in the pathway have been shown also to be present in higher plants (Wiater et al., 1971b, Wong and Mazelis, 1981; Nagai and Scheidegger, 1991). The activities of ATP phosphoribosyl transferase (EC 2.4.2.17), imidazoleglycerol-phosphate dehydratase (IGP dehydratase) (EC 4.2.1.19), and histidinol phosphatase (EC 3.1.3.15) were detected in crude extracts from shoots of barley, oat, and pea (Wiater et al., 1971b). Histidinol dehydrogenase (EC 1.1.1.23) was purified from cabbage (Nagai and Scheidegger, 1991) and its cDNA cloned (Nagai et al., 1991).

IGP dehydratase catalyzes the dehydration of IGP to IAP, the seventh step in the His biosynthetic pathway (Ames and Mitchell, 1955; Ames, 1957). In *Salmonella typhimurium*, both the dehydratase and histidinol phosphatase activities are embodied in a single protein (Staples and Houston, 1979). This bifunctionality is encoded in the *hisB* gene of *S. typhimurium* (Loper, 1961), *Escherichia coli* (Chiariotti et al., 1986), and *Azospirillum brasilense* (Bazzicalupo et al., 1987). IGP dehydratase in *Saccharomyces cerevisiae* is a protein of M_r 290,000 consisting of identical subunits of M_r 23,850 (Struhl, 1985). The yeast enzyme, encoded in the *his3* gene (Fink, 1964), is monofunctional and does not harbor the histidinol phosphatase activity (Millay and Houston, 1973; Glaser and Houston, 1974), which is encoded in the *his2* gene (Fink, 1964). The compilation of available data indicates that the two activities are found on a single gene only in prokaryotes and have apparently been split over evolutionary time into two genes in single-celled eukaryotes. In plants, IGP dehydratase activity has not been purified, nor has the corresponding gene been identified.

This paper reports the isolation and characterization of IGP dehydratase from wheat germ. The purified protein, with a native M_r of 600,000 to 670,000, catalyzes the dehydratase reaction but does not possess histidinol phosphatase activity.

MATERIALS AND METHODS

Plant Material

Hordeum vulgare L. (barley), *Brassica oleracea* L. (cabbage), *Cucumis sativus* L. (cucumber), *Lactuca sativa* L. (lettuce), *Zea mays* L. (maize), *Avena sativa* L. (oat), and *Pisum sativum* L. (pea) were cultivated in a growth chamber as previously described (Nagai and Scheidegger, 1991). Shoots were collected after 7 to 10 d of cultivation, frozen in liquid nitrogen, and stored at −80°C until use. The cell culture of *Rosa* Paul's Scarlet was a gift of Dr. André Strauss (Ciba-Geigy Ltd., Switzerland), and was cultured in suspension as previously described (Strauss et al., 1985). Wheat germ (*Triticum aestivum*) was purchased from Sigma, and rice germ (*Oryza sativa*) was from a local miller.

Chemicals

IGP was synthesized according to Ames (1957) and purified as described below. IAP was synthesized by Dr. Kenji Hayakawa and Dr. Sohail Mirza (Ciba-Geigy Ltd., unpublished data). Butyl-Toyopearl 650M and DEAE-Toyopearl

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Abbreviations: aminotriazole, 3-amino-1,2,4-triazole; Bis-Tris-propane, 1,3-bis[tris(hydoxymethyl)methylamino]propane; IAP, imidazoleacetol phosphate; IGP, D-*erythro*-imidazoleglycerol phosphate; PTH, phenylthiohydantoin; TEA, triethanolamine.

650M were purchased from Tosoh Co. (Tokyo, Japan). Formamidine hydrochloride was from Fluka and aminotriazole, D-ribose-5′-phosphate (barium salt), L-histidinol phosphate, alkaline phosphatase (from bovine intestine mucosa), and the M_r calibration kit for gel filtration were from Sigma. The M_r calibration kit for SDS-PAGE was from Bio-Rad, and the one for native PAGE was from Dai-ichi Chemicals (Tokyo, Japan). All other chemicals used were of reagent grade.

Purification of IGP

IGP synthesized from D-ribose-5′-phosphate and formamidine was adsorbed to and eluted by dilute HCl from Dowex 50 as described previously (Ames, 1957). Charcoal was added to the acidic elute (pH 1.0) to remove colored materials. The clear solution was neutralized with NaOH and loaded on a dry, charcoal column. Adsorbed IGP was eluted with 50% (v/v) methanol in 0.1 N HCl and lyophilized, and the resulting HCl salt was dissolved in methanol. Free IGP was precipitated by adding propyleneoxide and lyophilized. IGP was obtained as a white powder containing an equimolar amount of water.

Preparation of Plant Extracts for IGP Dehydratase Assay

Wheat germ and rice germ were processed into acetone powder. Frozen shoots were ground in a mortar under liquid nitrogen. All of the following steps were carried out at 4°C. Processed plant material or freshly harvested cultured tissues were homogenized with a Polytron blender with 200 mM TEA-HCl buffer (pH 7.2) containing 10% (w/v) Polyclar AT (G.A.F. Corp.). The homogenate was filtered through Miracloth (Calbiochem) and the insoluble material of the filtrate was removed by centrifugation. The protein in the supernatant was precipitated with ammonium sulfate (80% saturation) and dissolved in 50 mM TEA-HCl (pH 7.5) containing 1 mM $MnCl_2$ and 100 mM 2-mercaptoethanol. The preparation was desalted on Sephadex G-25 (Pharmacia-LKB) using the same buffer and tested for IGP dehydratase activity.

Purification of IGP Dehydratase from Wheat Germ

All procedures were carried out at 4°C except for the chromatographic steps on MonoQ and Superdex 200. In a typical purification, 1 kg of the acetone powder from wheat germ was suspended in 7 L of 200 mM TEA-HCl (pH 7.2). The suspension was centrifuged, and the supernatant was subjected to ammonium sulfate precipitation at 30% saturation. The precipitate was removed by centrifugation and the supernatant was loaded onto a Butyl-Toyopearl 650M column (30 cm in diameter × 18 cm in height) equilibrated with 50 mM TEA-HCl (pH 7.5) containing 1 mM $MnCl_2$, 100 mM 2-mercaptoethanol, and ammonium sulfate (20% saturation). The column was washed with 6 L of the equilibration buffer, and the enzyme activity was eluted with a descending gradient of ammonium sulfate from 20 to 15% saturation made in 1.5 L and a subsequent gradient from 15 to 5% in 7.5 L of the same buffer. The flow rate was kept at 17.5 mL/min during this chromatography. Active fractions between 13 and 11% ammonium sulfate were collected and the protein was precipitated with ammonium sulfate (80% saturation) and dissolved in 20 mM TEA-HCl (pH 7.5) containing 1 mM $MnCl_2$ and 100 mM 2-mercaptoethanol (purification buffer) and stored at −80°C.

After being thawed and centrifuged to remove insoluble materials, the solution was desalted on Sephadex G-25 and the resulting protein solution (250 mL) was loaded onto a DEAE-Toyopearl 650M column (5 cm in diameter × 50 cm in height) equilibrated with purification buffer. After the column was washed with 960 mL of purification buffer, the enzyme was eluted with a successive gradient of NaCl from 0 to 50 mM made in 240 mL of purification buffer and from 50 to 200 mM in 1.44 L. The flow rate was 4 mL/min for this chromatography step. The active fractions were collected, the protein was precipitated with ammonium sulfate added to 50% saturation, and the precipitate was dissolved in purification buffer containing ammonium sulfate at 20% saturation and stored at −80°C.

The above steps from acetone powder to DEAE Toyopearl were performed for another 1 kg of acetone powder, and the resulting active fractions of two runs were collected together. The active fractions were desalted on Sephadex G-25 and the resulting protein solution (142 mL) was subjected to MonoQ fast protein liquid chromatography (1.6 cm diameter × 10 cm height, Pharmacia-LKB) equilibrated with purification buffer. The chromatography was done as follows: at the flow rate of 8.0 mL/min, the column was washed for 10 min and eluted with the successive NaCl gradients (in the buffer) from 0 to 62.5 mM in 5 min, 62.5 to 125 mM in 15 min, and 125 to 150 mM in 20 min. The activity appeared in two peaks: one at 60 mM NaCl with the major protein peak was discarded, and the later, broad peak (110–140 mM NaCl) with higher specific activity was pooled and concentrated by ultrafiltration (Amicon YM30 membrane). This preparation was chromatographed twice on a Superdex 200 fast protein liquid chromatograph (2.6 cm diameter × 60 cm height, Pharmacia-LKB) using purification buffer containing 150 mM NaCl at a flow rate of 2.5 mL/min in the first run and 1.0 mL/min in the second. The enzyme preparation obtained was stored at −80°C until use.

Determination of IGP Dehydratase Activity

IGP dehydratase activity was determined by measuring imidazoleacetol obtained by hydrolyzing IAP. The dehydratase reaction mixture contained 50 mM Bis-Tris-propane-HCl buffer (pH 6.6), 100 mM 2-mercaptoethanol, 1 mM $MnCl_2$, 1 mM IGP, and 2 to 5 milliunits of enzyme in a volume of 0.25 mL. The reaction was started by the addition of substrate, incubated at 30°C, and stopped after 40 min by adding 1 N perchloric acid up to 10% volume of the reaction mixture. After centrifugation, the supernatant was adjusted to pH 10 by 1 M 2-ethylaminoethanol. Alkaline phosphatase and $MgCl_2$ were added to the mixture to final concentrations of 12 units/mL and 0.5 mM, respectively. After incubation at 45°C for 20 min, the reaction mixture was chilled in salt-ice. Five volumes of 5 N NaOH were added to the solution, and after 2 min the concentration of enolized imidazoleacetol was determined from the A_{370} using the absorbance coefficient of 10,400 (Ames and Mitchell, 1983). One unit of enzyme

activity was defined as the amount of enzyme catalyzing the formation of 1 μmol of IAP/imidazoleacetol per min under the assay conditions.

Determination of Histidinol Phosphatase Activity

Histidinol phosphatase activity was determined by measuring the formation of inorganic phosphate according to the method described earlier (Martin et al., 1971) with some modifications. The assay mixture contained 200 mM TEA-HCl (pH 8.2), 5 mM L-histidinol phosphate, and enzyme in a final volume of 180 μL. The reaction was started by the addition of substrate, run at 37°C for 180 min, and stopped by adding 1 N perchloric acid up to 10% volume of the reaction mixture. The mixture was centrifuged at 10,000 rpm for 3 min. A 180-μL aliquot of the supernatant was mixed with 420 μL of the ascorbate-molybdate reagent and incubated at 45°C for 20 min. The absorption was read at 820 nm against a control without substrate or enzyme. One unit of enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 μmol of phosphate per min under the assay conditions.

Protein Determination

Protein was determined by the method of Bradford (1976) using BSA as a standard.

Electrophoresis

SDS-PAGE was carried out according to Laemmli (1970) using a gradient gel (Phastgel 8–25, Pharmacia-LKB). Native PAGE was done as described previously (Davis, 1964) using a slab gradient gel (PAG plate 4/15, Dai-ichi Chemicals, Tokyo, Japan). IEF was carried out using a polyacrylamide gel (4%) with Selvalyt pH 3 to 7 as the carrier ampholyte. The isoelectric point was calculated using a calibration kit (Pharmacia-LKB).

RESULTS

Alkaline Phosphatase-Coupled IGP Dehydratase Assay

Direct determination of the enolized IAP in strong alkali at 280 nm (Ames, 1957), as done in all previously reported IGP dehydratase studies, was not applicable in crude plant extracts and slightly enriched enzyme preparations due to high background absorbances inherent to such plant preparations. Therefore, the alternative method of hydrolyzing IAP to imidazoleacetol and determining the absorption of its enolized form in alkali at 370 nm (Ames and Mitchell, 1955) was employed. Synthetic IAP was used for the development and optimization of this assay. Using alkaline phosphatase instead of HCl as described previously (Ames and Mitchell, 1955), the authentic IAP was completely hydrolyzed to imidazoleacetol. The completion of the hydrolysis was judged from the disappearance of the absorption peak of IAP at 280 nm and the appearance of the imidazoleacetol peak at 370 nm. The same treatment applied to pure IGP did not produce any absorption above 350 nm.

IGP Dehydratase Activity in Plants

Various monocotyledonous and dicotyledonous plants were assayed for IGP dehydratase activity using crude enzyme extracts concentrated by ammonium sulfate precipitation. Barley shoots and cultivated rose cells clearly showed higher specific enzyme activities than all the other plants tested (Table I). Some plant materials showed high background absorption around 370 nm in alkali, which interfered with precise activity determination even after ammonium sulfate precipitation and gel filtration. The failure to detect any activity in shoots of cucumber, lettuce, and oat might be attributed to this rather than to the absence of IGP dehydratase. Wheat germ exceeded all the other plants tested in the amount of extractable activity.

Purification and Some Properties of IGP Dehydratase from Wheat Germ

IGP dehydratase was isolated from wheat germ using five chromatographic steps (Table II). The initial extraction of

Table I. *Distribution of IGP dehydratase in different plant species and preparations*

For the calculation of extractable activity, the wet weight of plant material was used. The weight of cells from submersed tissue cultures was determined after collection by filtration with a cloth. Each value was the average of duplicate assays from one harvest.

Plant Source	Specific Activity	Extractable Activity
	milliunits/mg[a]	*milliunits/g*[b]
Barley shoots (*Hordeum vulgare* L.)	0.25	0.95
Cabbage shoots (*Brassica oleracea* L.)	0.13	0.22
Cucumber shoots (*Cucumis sativus* L.)	0	0
Lettuce shoots (*Lactuca sativa* L.)	0	0
Maize shoots (*Zea mays* L.)	0.06	0.27
Oat shoots (*Avena sativa* L.)	0	0
Rice germ	0.03	0.73
Wheat germ	0.05	3.20
Rose cell culture (*Rosa* Paul's Scarlet)	0.23	0.04

[a] Milliunits of activity per milligram of protein. [b] Milliunits of activity per gram of plant material.

Table II. *Purification of IGP dehydratase from wheat germ*

Data obtained in a typical purification are presented. In this purification, 2 kg of wheat germ was processed. All activities were measured after desalting with either Sephadex G-25 or dialysis. Protein and activity were determined from the average of duplicate assays. The activity in crude extract before $(NH_4)_2SO_4$ precipitation could not be detected. FPLC, Fast protein liquid chromatography.

Purification Step	Total Protein	Total Activity	Percent Recovery	Specific Activity	Purification
	mg	*milliunits*		*milliunits/mg*	*-fold*
$(NH_4)_2SO_4$ fractionation	128,000	6,400	100	0.05	1
Butyl-Toyopearl	5,600	4,870	76	0.87	17
DEAE-Toyopearl	565	1,040	16	1.8	36
Mono Q FPLC	25.3	652	10	25.8	516
Superdex 200 FPLC (run twice)	0.035	199	3	5,700	114,000

activity could be increased 10-fold by preparing an acetone powder instead of homogenizing wheat germ directly into the buffer. The recovery after the 114,000-fold purification was below 3%. The final enzyme preparation with a specific activity of 5.7 units/mg of protein was homogeneous as judged from SDS- and native-PAGE (Fig. 1) and IEF. The isoelectric point was 5.65. The enzyme activity was stable up to 30°C, but was decreased by 50% at 60°C when incubated for 40 min. Purified enzyme was stable at −80°C for at least 1 month.

The M_r of IGP Dehydratase

Purified IGP dehydratase eluted at an M_r of 600,000 on Superdex 200 gel filtration (Fig. 2). This apparent M_r was not altered in the absence of either Mn^{2+} (unlike what has been reported for IGP dehydratase from *S. typhimurium*; Brady and Houston, 1973) or 2-mercaptoethanol. An M_r of 670,000 was calculated from native-PAGE (Fig. 1). On SDS-PAGE, a single protein band was observed having an M_r of 25,500 under both reducing and nonreducing conditions (Fig. 1). Therefore, the subunits are not linked by disulfide bridges, but likely are associated by noncovalent interactions.

Separation of Histidinol Phosphatase from IGP Dehydratase

Histidinol phosphatase activity (0.45 milliunits/mg of protein) was detected in the crude extract of wheat germ after concentration by ammonium sulfate precipitation. Most of the phosphatase activity was separated from IGP dehydratase activity after DEAE-Toyopearl ion-exchange chromatography. The homogeneous IGP dehydratase preparation, ob-

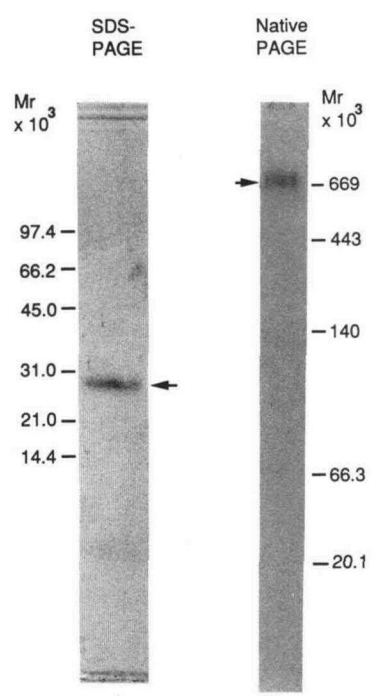

Figure 1. PAGE of purified wheat germ IGP dehydratase. For SDS-PAGE, samples were denatured in 0.1% (w/v) SDS and 2% (v/v) 2-mercaptoethanol at 95°C for 15 min. Protein was stained with Coomassie brilliant blue R-250.

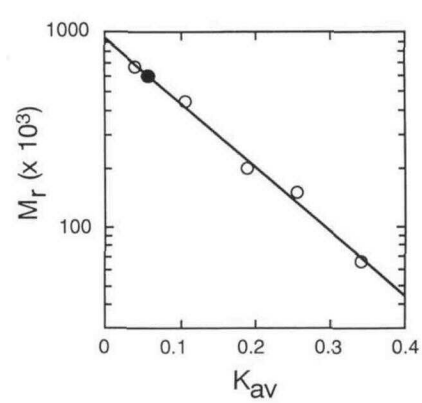

Figure 2. Calculation of the M_r of IGP dehydratase by plotting K_{av} values of Superdex 200 (HiLoad 26/60) gel filtration versus the logarithm of the M_r of the standard proteins. $K_{av} = (V_e - V_o)/(V_t - V_o)$ represents the fraction of the stationary gel volume that is available for diffusion of a solute, where V_e is the elution volume, V_o the void volume of the column, and V_t the total volume of the packed bed. The proteins were eluted at a flow rate of 1 mL/min at room temperature. The K_{av} value for IGP dehydratase was 0.056. ○, M_r standard proteins (M_r) as follow; thyroglobulin (669,000), apoferritin (443,000), β-amylase (200,000), alcohol dehydrogenase (150,000), and albumin (66,000). ●, IGP dehydratase.

tained after the second Superdex 200 gel filtration, showed no measurable phosphatase activity.

Enzymic Conversion of IGP to IAP

Based on the following observations, the enzymically formed reaction product from IGP using pure IGP dehydratase was identified as IAP: (a) The absorption spectra of synthetic IAP and of the reaction product, both of which were first hydrolyzed completely with alkaline phosphatase, were identical in strong alkali. (b) The reaction product eluted at the same retention volume as synthetic IAP (2.88 mL) on an Ultrasphere ODS column (4.6 mm × 25 cm, Beckman, 50 mM sodium phosphate [pH 5.8] as the eluent). The spectra (recorded with a photodiode array detector, Hewlett-Packard) of this compound and of synthetic IAP were identical and did not show any absorption maximum above 235 nm. (c) Neither IAP nor the reaction product formed a hydrazone derivative with either 2,4-dinitrophenylhydrazine or with *p*-nitrophenylhydrazine, even if incubated overnight at 95°C.

Kinetic Properties

The K_m value for IGP was determined as 0.4 mM. The enzyme showed a broad pH optimum ranging 6.5 to 7.0 (Fig. 3). Aminotriazole, one of the known competitive inhibitors for IGP dehydratase from *S. typhimurium* (Brady and Houston, 1973), yeast (Wiater et al., 1971a; Glaser and Houston, 1974), barley, and oat (Wiater et al., 1971b), also inhibited the purified wheat germ enzyme competitively, with a K_i = 46 μM at pH 6.6.

Requirement for Mn

To examine the effects of metals, various metal ions were added to the enzyme preparation from which Mn ion had been removed by passing through a Sephadex G-25 column using 50 mM TEA (pH 7.5) containing 100 mM 2-mercaptoethanol. The reaction was done with or without a metal ion species as chloride salt, except for Fe^{2+}, which was given as

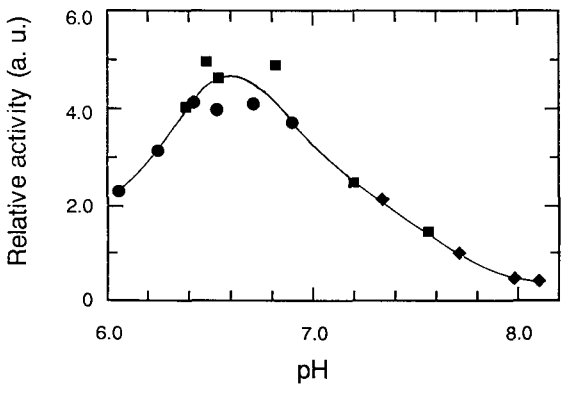

Figure 3. pH profile of the activity of purified wheat germ IGP dehydratase. Activity was determined in 50 mM buffer, 1 mM IGP, 100 mM 2-mercaptoethanol, and 1 mM $MnCl_2$. ●, Bis[2-hydroxymethyl]imino-tris[hydroxymethyl]methane-HCl. ■, Bis-Tris-propane-HCl. ♦, TEA-HCl.

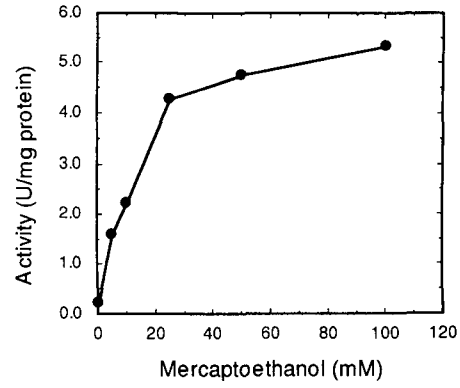

Figure 4. Dependency of IGP dehydratase activity on 2-mercaptoethanol. Purified IGP dehydratase was passed through a Sephadex G-25 column to remove 2-mercaptoethanol contained in the purification buffer. The reaction was started by the simultaneous addition of 0.5 mM IGP and 2-mercaptoethanol at various concentrations and was run at 37°C for 30 min.

$Fe(SO_4)(NH_4)_2$, at 1.5 mM in the presence of 1 mM EDTA to chelate the effect of trace amounts of Mn^{2+} that might have remained after the Sephadex G-25 chromatography. The working concentration of each metal ion was accordingly assumed to be 0.5 mM. Mn^{2+} enhanced the activity 7-fold at a concentration of 0.5 mM compared with the control that lacked any metal ions added. Mg^{2+}, Cu^{2+}, Ni^{2+}, Ca^{2+}, Fe^{2+}, and Fe^{3+} at 0.5 mM did not stimulate the activity. The half-maximal effect of Mn^{2+} was achieved at 0.11 mM.

Effect of Reducing Agents on the Activity

The enzyme was inactive in the absence of 2-mercaptoethanol. Activity was regained instantly by the addition of 100 mM 2-mercaptoethanol to the reaction mixture (Fig. 4). The same effect was obtained by other reducing agents such as DTT or DTE at 100 mM. The requirement for a reducing agent has been reported for the enzyme of *Neurospora crassa* (Ames, 1957), *S. typhimurium* (Ames et al., 1960), and *S. cerevisiae* (Glaser and Houston, 1974).

DISCUSSION

In this study IGP dehydratase was purified to apparent homogeneity from wheat germ as a protein having an M_r of 600,000 to 670,000. The enzyme is composed of at least 24 identical subunits, each possessing an M_r of 25,500. That the enzyme actually catalyzes the dehydration of IGP to IAP was confirmed by comparing the reaction product with synthetic IAP as authentic reference. The wheat enzyme, like the yeast enzyme (Millay and Houston, 1973; Glaser and Houston, 1974), is devoid of the phosphatase activity, although histidinol phosphatase activity is associated with IGP dehydratases of prokaryotic origin (Staples and Houston, 1979).

A comparison of the properties of IGP dehydratase from various organisms reveals several similarities among them (Table III): (a) Their K_m values for IGP fall in the submillimolar range; (b) aminotriazole acts as a competitive inhibitor of all of them with similar inhibition constants; (c) a reducing agent,

Table III. *IGP dehydratase from different organisms*

Property	*S. tryphimurium*	*S. cerevisiae*[a]	Wheat Germ
Subunit mol wt	46,000[b]	35,000	25,500
	40,500[c]	23,850[d]	
Number of subunits	6[b]	8 or 12	24?
Histidinol phosphatase activity	Yes	No	No
pH optimum	7.5[e]	7.2	6.6
K_m for IGP (mM)	0.71[b]	0.54	0.36
Mn^{2+} concentration for half-maximal activity	0.007[e]	0.007	0.11
K_i for aminotriazole (μM)	3.2[e]	23	46

[a] Glaser and Houston, 1974. [b] Staples and Houston, 1979. [c] Chiariotti et al., 1986. [d] Struhl, 1985. [e] Brady and Houston, 1973.

e.g. 2-mercaptoethanol, and Mn^{2+} are required for their activities; (d) they are all oligomeric proteins consisting of identical subunits, although the number of subunits and the monomeric M_r differ among organisms. However, the implication of these similarities is still unclear.

As far as we have examined, dissociation of wheat IGP dehydratase into its subunits occurred only in the presence of SDS. No dissociation took place in the presence of 1 M NaCl, 6 M urea, or 3 M guanidine hydrochloride (data not shown). Therefore, it is highly likely that the formation of the oligomeric structure is effected mainly by hydrophobic interactions.

The role of the reducing agent, e.g. 2-mercaptoethanol, in the activation of the enzyme is not yet fully understood. It is already stated for the *Neurospora* enzyme that with the addition of 0.14 mM 2-mercaptoethanol, the running of the reaction under anaerobic conditions becomes obsolete (Ames, 1957). The wheat germ enzyme requires 100 mM 2-mercaptoethanol for maximal activity. In principle, three functions of the reducing agent are conceivable. (a) It activates the enzyme by reducing disulfide bond(s) or other groups. (b) It protects the enzyme from inactivation by active oxygen or other reactive species generated during the reaction. (c) It is involved in the catalytic activity as a sort of cofactor. Our observation that even in the presence of 2-mercaptoethanol at a concentration lower than 100 mM maximal activity could be reached after a certain time lag (data not shown) strongly supports the first possibility.

IGP dehydratase from wheat germ is the second biosynthetic enzyme in the His pathway that has been purified and characterized from higher plants. The results presented, combined with the previously reported characterization of histidinol dehydrogenase from cabbage (Nagai and Scheidegger, 1991), strongly suggest that at least the second half of the His biosynthesis in plants is identical with that operating in microorganisms.

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