Bot. Bull. Academia Sinica **23**: 45-61 (1982)

STUDIES ON THE α-AMYLASE FROM THE GERMINATED RICE SEEDS*

JEI-FU SHAW

Institute of Botany, Academia Sinica, Nankang, Taipei Taiwan 115, Republic of China

LEA-YEA CHUANG

Department of Chemistry, National Kaohsiung Teacher's College Kaohsiung, Taiwan 115, Republic of China

(Received November 23, 1981; Accepted December 19, 1981)

Abstract

We have purified a quite heat-stable α-amylase from germinated rice seeds. The purification procedures involved ammonium sulfate fractionation, selective heat treatment, acetone fractionation and glycogen complex formation. The α-amylase is quite active between pH 4 to 10 with optimum pH at 4.5. The optimum temperature is 60°C. The purified α-amylase was determined by polyacrylamide gel electrophoresis to be a monomer with estimated molecular weight 50,500, and each mole was found to firmly bind approximately 1.3 gram-atom of Ca^{++} by atomic absorption spectrometer. The extinction coefficient $E^{1\%}_{1cm}$ at 280 nm was determinated to be 13.7. The enzyme requires Ca^{++} for its activity since it was inactivated by EDTA and reactivated by the addition of calcium salts. The enzyme activity was increased by the addition of 2.5 mM calcium salts.

The calcium ion has stabilizing effect on the enzyme against denaturation. The enzyme in the presence of 0.02 M calcium acetate still retained 65% of original activity while the enzyme in the absence of calcium acetate completely lost the activity after ten week storage. In thermal denaturation experiments, the α-amylase with added calcium salt showed much better stability against heat. The activation energies for the thermal inactivation reaction of α-amylase in the presence and absence of 0.02 M calcium acetate were estimated by Arrhenius plot to be 46.3 Kcal/mol and 21.0 Kcal/mol respectively.

The Michaelis constant of the enzyme is 2.4×10^{-2} M (moles of glucosidic bonds/l), the maximum velocity is 1.55 mg glucose equivalent formed per minute per mg enzyme and the activation energy of the enzyme reaction is 14.1 Kcal/mol.

Introduction

Rice is a major crop in Taiwan, especially in the southern area. The

* This work was supported by National Science Council Grant NSC 70-0204-M017-02. A portion of this work has been presented in 1st Asian & Pacific Chemistry Congress (April 26, 1981, at Singapore) and 12th Annual Meeting of Chinese Biochemical Society (Oct. 3, 1981. at National Chinhua University).

harvest time is usually in the rainy season, a substantial amount of rice germinated as a result of high humidity.

The economic values of germinated rice was greatly decreased and sometimes it was even wasted. As part of a general study for the utilization of the germinated rice seeds, we found a quite heat-stable α-amylase present in significant amount.

Amylases have been widely used in industry such as paper making, alcoholic beverage, syrup, crystalline glucose and bread.

There are many different sources of α-amylases that have been purified, including animal and plant sources: hog pancreas, human pancreas and saliva, rat pancreas, pigeon pancreas, shore crabs, malted barley, malted sorghum, malted wheat, soy beans, broad beans (Thoma *et al.*, 1971; Mutzbauer and Schulz, 1965; Heatley, 1958; Greenwood *et al.*, 1965); bacterial and mold sources: *B. subtilis*, *Aspergillus oryzae*, *B. coagulans*, *A. candidus*, *Pseudomonas saccharophila*, *B. polymyxa*, *B. macerans*, *A. niger*, *B. amyloliquefaciens*, and *B. stearothermophilus* (Takagi *et al.*, 1971; Robyt and French, 1964; Minoda and Yamada, 1963; Welker and Campbell, 1967; Manning and Campbell, 1961). Most of the enzymes from these sources lost most or all of their activities before reaching the gelatinization temperature of starch, therefore, their application values are greatly limited.

In the present report, we have purified α-amylase from germinated rice seeds and studied its properties in some detail. We found the thermal stability of this α-amylase is better than most of the α-amylases from other sources, therefore it could be useful for industrial applications.

Materials and Methods

Materials

Rice seeds (Kaohsiung No. 1) were purchased from local grocery store. Soluble starch (Wako pure chemical industries). Bovine serum albumin (Neutritional Biochemicals Corporation). Glucose, maltose, 3,5-dinitrosalicylic acid, sodium dodecyl sulfate, acrylamide, N′,N-methylenebisacrylamide, glycogen, Ovalbumin, chymotrypsinogen A and lysozyme were obtained from Sigma Chemical Company. Ammonium sulfate and Folin-Ciocalteu's phenol reagent were from Merck Company. All other chemicals were of reagent grade.

Enzyme assay

α-Amylase activities were determined by modified dinitrosalicylic acid method (Fischer and Stein, 1961). The reaction system consists of 2.5 ml of 1% soluble starch in 0.01 M phosphate buffer (pH 7.6), 0.15 ml H_2O and 0.05 ml of the enzyme solution. The solution was incubated at 30°C for 5 minutes. After the incubation, the reaction was terminated by adding 1.5 ml of a color

reagent (The 100 ml color reagent contained 1 g 3,5-dinitrosalicylic acid, 30 g potassium sodium tartrate, 20 ml of 2 N NaOH). The mixture was then incubated in a boiling water bath for 5 minutes. After cooling to the room temperature, the absorbance of the solution was measured at 540 nm using a Hitachi 100-20 Spectrophotometer. One mg of glucose solution reacting with the color reagent will produce an absorbance of 1.0 under the same condition. The unit of the enzyme was defined as the amount of enzyme which produced one mg equivalent of glucose per minute.

Measurement of protein

The protein content was determined by the method of Lowry *et al.*, (1951) using bovine serum albumin as a standard.

Germination procedure of the rice seeds

Rice seeds were pretreated with 0.3% H_2O_2 for 30 minutes and then soaked in distilled water for 3 days. The swollen seeds were transferred to distilled water-wet cheesecloth. Germination proceeded at 30°C in the dark for 4 days.

Purification of the enzyme

Step 1. Extraction of the enzyme. The germinated rice seeds were homogenized in 0.01 M phosphate buffer (pH 7.6) by polytron homogenizer for 5 minutes. The solution was centrifuged at 25,500 g for 15 minutes. The supernatant was taken as the crude extract (Fraction I). All manipulations were performed at 4°C unless otherwise indicated.

Step 2. Ammonium sulfate fractionation. Solid ammonium sulfate was slowly added to fraction I to 20% saturation over a 30 minutes period. The solution was mantained at pH 7 throughout by the addition of 1 M potassium hydroxide. Protein precipitating in this fraction contained little enzyme activity and was removed by centrifugation (25,500 g, 20 min). Additional ammonium sulfate was added to the supernatant to yield a 50% saturated solution. After centrifugation, the precipitate, which contained most of the enzyme activity, was dissolved in approximately 5 volumes of 0.01 M phosphate buffer (pH 7.6) (Fraction II).

Step 3. Selective heat treatment. The fraction II was heated in a water bath for 10 minutes at 65°C. After cooling to 4°C, the denatured protein was removed by centrifugation. The supernatants were pooled (Fraction III).

Step 4. Fractionation with acetone. The fraction III was kept at 2°C in an ethanol-ice bath. Acetone (−10°C) was added, dropwise with stirring (45 minutes) to the enzyme solution to give a final concentration of 30%. After removing the precipitate by centrifugation, the supernatant was brought to 70% acetone. The precipitate was collected after centrifugation, and dissolved in 0.01 M phosphate buffer, pH 7.6 (Fraction IV).

Step 5. Glycogen complex formation. 40% (v/v) alcohol was added to the fraction IV. One mg glycogen per 160 amylase units were then added to the supernatant after removing the precipitate by centrifugation. The suspension was stirred for 5 minutes and then centrifuged at 10,600 g. The precipitated complex was dissoved in phosphate buffer, and the resultant solution was maintained at 18°C for 6 hours to hydyolyze the glycogen. It was then dialyzd against 0.01 M phosphate buffer, pH 7.6, containing 0.02 M calcium acetate at 2°C for 12 hours (Fraction V). The dialysate was stored in a refrigerator and used for the subsequent studies.

Assessment of purity

Disc gel electrophoresis on polyacrylamide gel (10%) at pH 8.3 was carried out as described by Gabriel (1971) to monitor the extent of enzyme purification.

Molecular weight determination

The molecular weight of the purified enzyme (Fraction V) was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under fully dissociating conditions according to the method of Webber & Osborn (1969) on 10% SDS-polyacrylamide gels. Ten μg of the purified enzyme or reference marker protein in phosphate buffer (pH 7.1) containing 0.25% SDS and 2 μl β-mercaptoethanol were boiled for 5 minutes before electrophoresis to ensure complete dissociation of subunits. The marker proteins are bovine serum albumin (M.W. = 68,000), Ovalbumin (M.W. = 43,000), Chymotrypsinogen A (M.W.=25,700) and Lysozme (M.W.=13,900).

Analysis of the enzyme catalyzed reaction product

Nine tenth ml of 1% starch solution was mixed with 0.1 ml enzyme of fraction I to V respectively and incubated at 30°C for 1 hour. The reaction was terminated by boiling for 5 minutes. After cooling, the reaction products were analyzed by paper chromatography using Whatman 3 mm paper with solvent system butanol/pyridine/water (10:3:3 by volume). After the development, the paper was dried and sprayed with color reagent (prepared by dissolving 0.93 g of aniline and 1.66 g phthalic acid in 100 ml of water saturated *n*-butanol).

Results

Change in amylase activity during germination

As shown in Fig. 1, the amylase activity increased as a result of germination. At eighth day, both total activity and specific activity reached the maximum.

Purification of α-amylase from germinated rice seeds

Table 1 summarized the purification of α-amylase from 400 g rice seeds germinated eight days in the dark. After glycogen complex formation step, the enzyme is essentially pure since the disc gel electrophoresis according to the method of Gabriel (1971) revealed only one single band (Fig. 2). The absorbance of the purified α-amylase at 280 nm $E^{1\%}_{1\,cm}$ is 13.7.

Table 1. *Summary of purification procedure of α-amylase from germinated rice seeds**

Step of purification	Volume (ml)	Amylase activity (units/ml)	Total activity (units)	Protein (mg/ml)	Specific activity (units/mg)	Recovery (%)
1. Fraction I: Crude extract	754	16.7	12,590	1.76	9.5	100
2. Fraction II: Ammonium sulfate fractionation	73.4	101	7,410	4.07	24.8	58.8
3. Fraction III: Selective heat treatment	92.2	64.5	5,950	1.5	43.0	47.3
4. Fraction IV: Acetone fractionation	35	88	3,080	0.5	176	24.5
5. Fraction V: Glycogen complex formation	15	174	2,610	0.52	335	20.7

* The amount of rice seeds is 400 g.

The identification of α-amylase

The products of starch hydrolysis catalyzed by amylase were examined by paper chromatography. As shown in Fig. 3, the products are largely glucose and a small amount of maltose. This suggests that the purified enzyme is α-amylase.

Molecuar weignt determination

The molecular weight of rice α-amylase was determined by SDS polyacrylamide gel electrophoresis according to the method of Webber & Osborn (1969). The purified α-amylase either in the presence or absence of β-mercaptoethanol revealed only one single band with the same mobility on the gel suggested that this enzyme is a monomer. As shown in Fig. 4, the mobility of this enzyme is between that of bovine serum albumin (M. W.=68,000) and ovalbumin (M. W.=43,000). The estimated molecular weight is 50,500.

Calcium analysis

The calcium contents of the rice α-amylase in various purification steps were determined by atomic absorption spectrometer. Table 2 shows that the calcium content of purified enzyme (Fraction V) is a constant value 1.046 μg

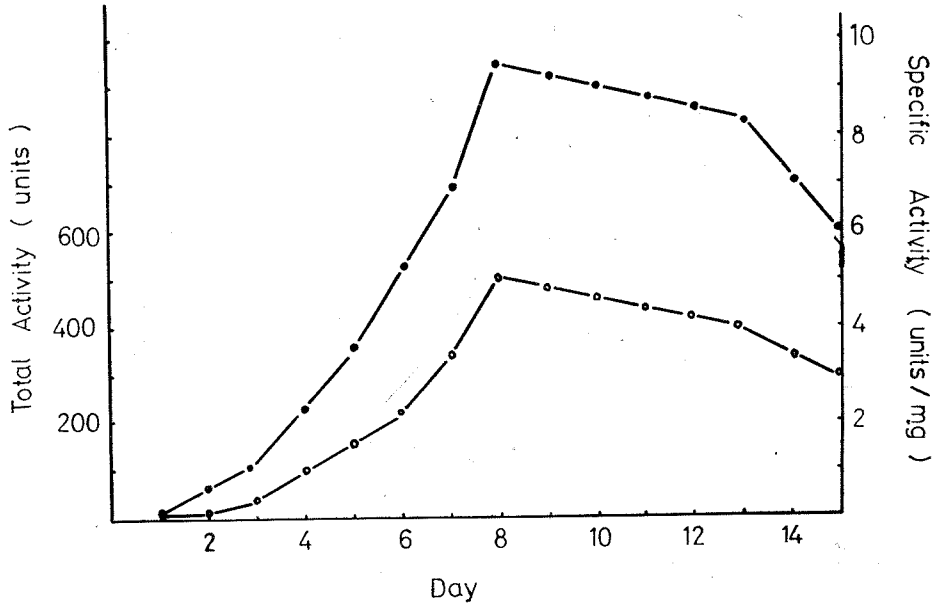

Fig. 1. The activity of amylase during germination.
20 gm of rice seeds were soaked in 0.3% H_2O_2 for 30 minutes and then in distilled water for 3 days. Swollen seeds were transfered to a box and germinated in dark at 30°C for various days.
(○-○, Total activity; ●-●, Specific activity.)

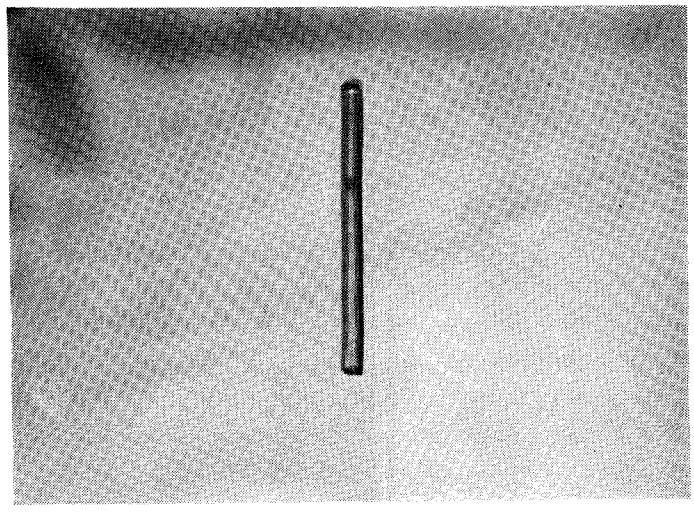

Fig. 2. Disc gel electrophoresis pattern of purified amylase.
The purity of fraction V was evaluated by 10% polyacrylamide gel electrophoresis at pH 8.9 according to the method of Gabriel (1971). The amount of enzyme is 0.1 mg.

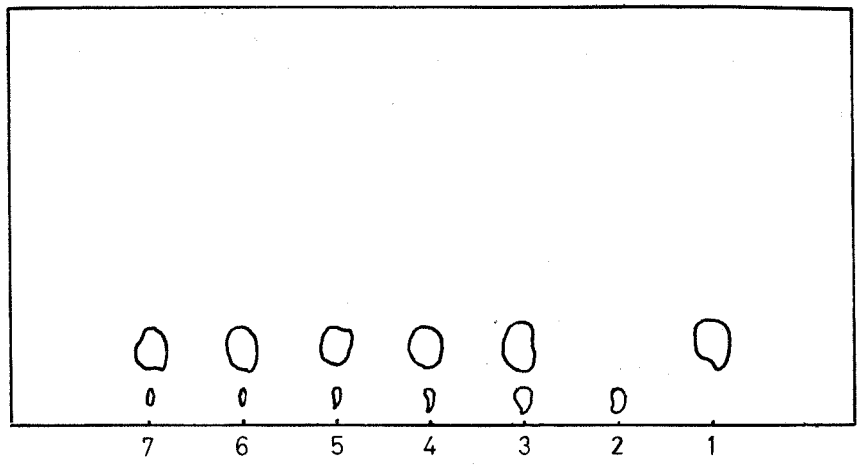

Fig. 3. Paper chromatographic pattern of the amylase catalyzed reaction products.
No. 1, 2 represent standard glucose and maltose, respectively.
No. 3 to 7 represent the products in the 30 minute reaction of starch and the amylase of fraction I to V respectively. See text for experimental details.

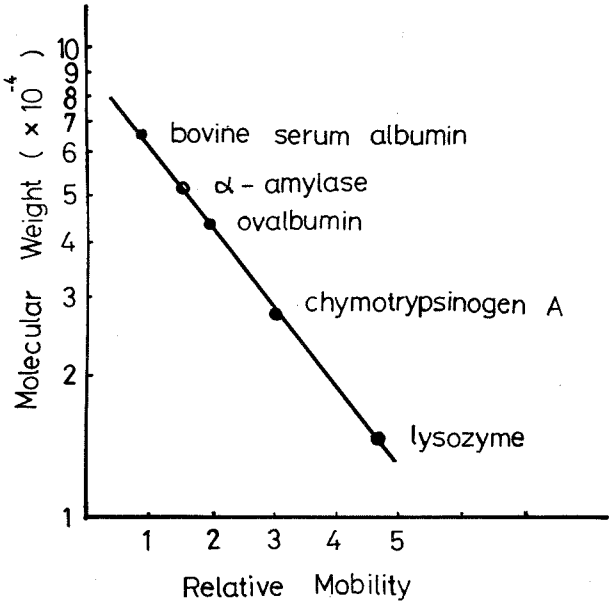

Fig. 4. The molecular weight of α-amylase was determined by the method of Webber & Osborn using 10.0% SDS polyacrylamide gel electrophoresis.

Table 2. *Calcium contents of rice α-amylase in various purification steps*

Step	μg of calcium per mg of freeze-dried material
1. Fraction I: Crude extract	0.327
2. Fraction II: Ammonium sulfate fractionation	0.699
3. Fraction III: Selective heat treatment	0.402
4. Fraction IV: Acetone fractionation	1.052
*5. Fraction V: Glycogen complex formation	1.046

* In this experiment, Fraction V enzyme is in phosphate buffer without calcium acetate.
The calcium contents were determined by Instrumentation laboratory IL-151 spectrometer. High purity water (less than 10 mho/cm), which was prepared by Millipore Milli RQ apparatus, was used in this experiment.

per mg of freeze-dried protein, which is equivalent to 1.32 gram atom of calcium per mole of protein (assuming the molecular weight of the enzyme 50,500).

The effect of calcium ion on enzyme activity

The α-amylase from germinated rice seeds has absolute requirement of calcium for enzyme activity. As shown in Fig. 5, the addition of 2 mM EDTA in the enzyme assay to remove the enzyme-bound calcium completely abolished the enzyme activity and the addition of calcium chloride gradually restored the activity.

As shown in Fig. 6, low concentration of calcium salt apparently can further increase the enzyme activity. Since calcium chloride and calcium acetate exhibited similar behavior, it was concluded that calcium ion was the activating factor.

Properties of α-amylase from germinated rice seeds

Optimum pH: The enzyme was assayed in 0.02 M buffer solution at 25°C for its pH-activity profile. As shown in Fig. 7, the enzyme was quite active between pH 4 and 10 with an optimum at pH 4.5.

Optimum temperature: The enzyme was assayed in 0.02 M phosphate buffer (pH 7.6) for its temperature-activity profile. Fig. 8, indicated that the enzyme showed the highest activity at 60°C under the assay condition.

Stability of the purified α-amylase: The purified enzyme in the presence and absence of calcium acetate was stored separately at 4°C and assayed at vavious storage time. The results shown in Fig. 9 indicated that after ten week storage the enzyme in the presence of 0.02 M calcium acetate still retained 65% of its original activity while the enzyme in the absence of calcium acetate completely lost the activity. Since calcium chloride exhibited

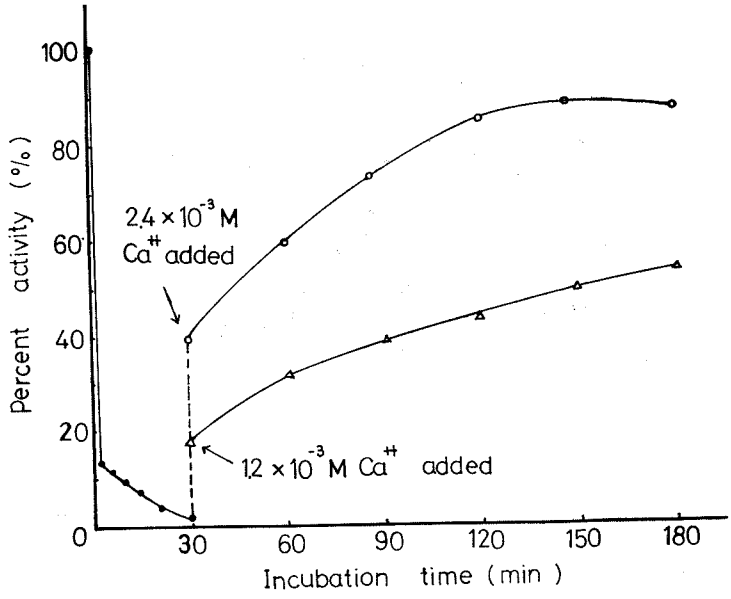

Fig. 5. Reversible inactivation of α-amylase by EDTA.
α-Amylase (4.8×10^{-4} M) was incubated with EDTA (2×10^{-3} M) at 35°C for 30 minutes and then calcium chloride (△-△, 1.2×10^{-3} M; ○-○, 2.4×10^{-3} M) was added and incubated for various time intervals. At points indicated, the incubation solution was assayed for enzyme activity.

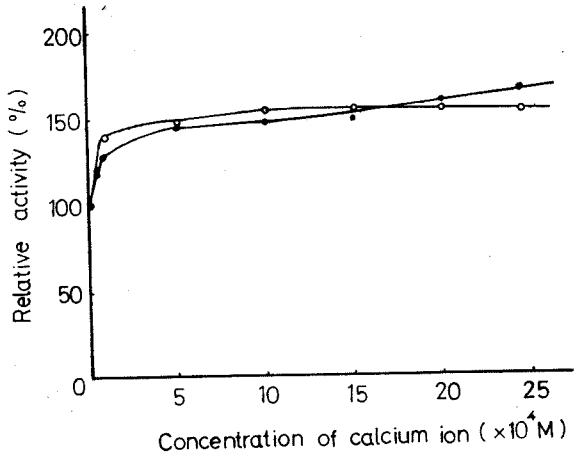

Fig. 6. The activation effect of added calcium ion on α-amylase activity.
Various concentration of calcium chloride (●) or calcium acetate (○) was added in dinitrosalicylate assay. The α-amylase concentration was 3.36×10^{-4} M.

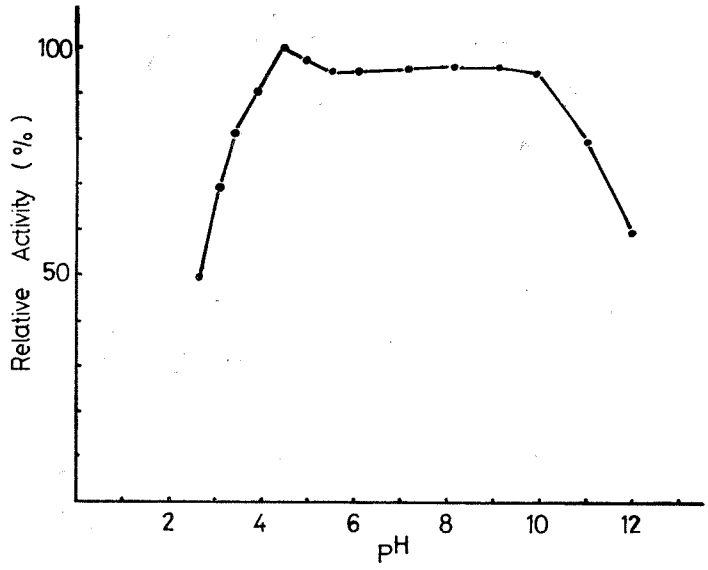

Fig. 7. Effect of pH on the amylase activity of germinated rice seeds.
The enzyme reaction was carried out at 30°C for 5 minutes at various pH value. pH 3.0-6.0, 0.02 M citrate buffer; pH 7.1-8.9, 0.02 M Tris-HCl buffer; pH 9.0-11.0, 0.02 M Glycine-NaOH buffer; pH 11.0-11.9, 0.02 M phosphate-NaOH buffer.

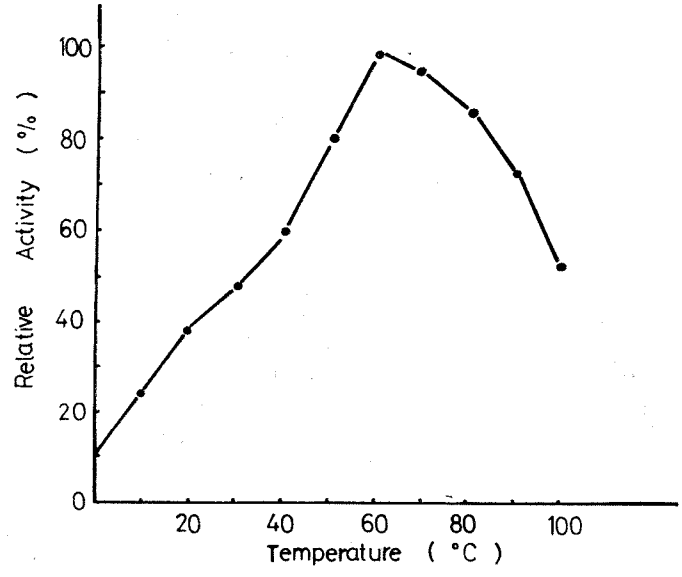

Fig. 8. Temperature-activity profile of α-amylase.
The enzyme reaction was carried out in 0.02 M phosphate buffer (pH 7.6).

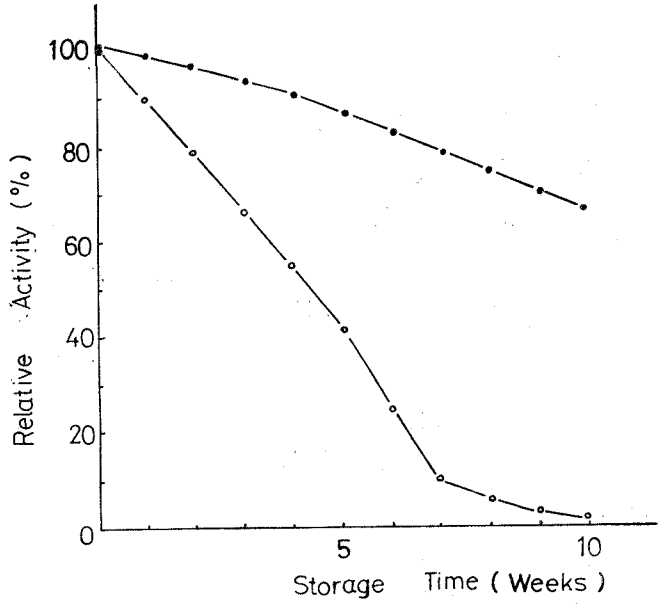

Fig. 9. Stability of the purified α-amylase of germinated rice seeds.
The purified enzyme in 0.01 M phosphate buffer (pH 7.6) with (●) and without (○) the presence of 0.02 M $CaCl_2$ were stored separately at 4°C.

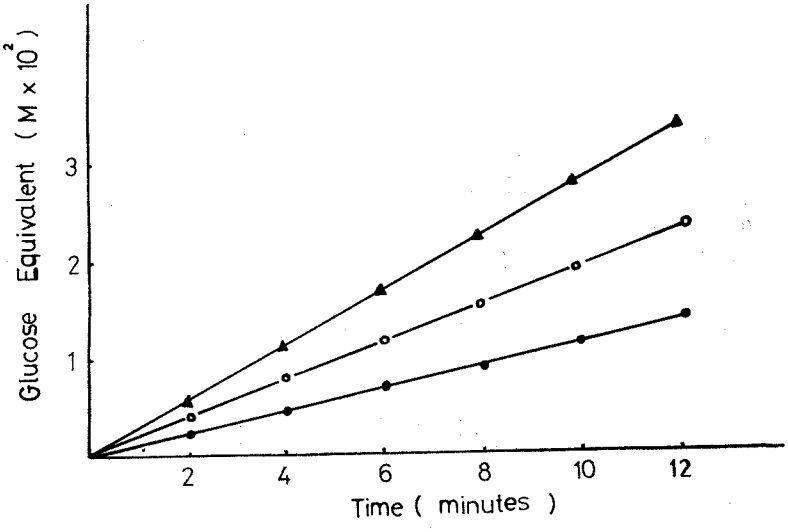

Fig. 10. Time course of α-amylase activity of germinated rice seeds.
The substrate concentrations were as follows (from the top to the bottom): 6.40×10^{-2} M, 4.94×10^{-2} M, 3.08×10^{-2} M. (where M=G/M. W.; and M=moles of glucosidic bonds per liter of solution, G=grams of starch per liter and M. W.= the molecular weight of glucose anhydride=162.) The amount of enzyme was 0.15 mg.

similar protective effect, it was concluded that calcium ion has stabilizing effect on the enzyme.

Determination of kinetic and thermodynamic parameters

Figure 10 shows that under the assay condition the time course of the α-amylase reaction is linear up to twelve minutes in the starch concentration range tested (from 3.08×10^{-2} to 6.4×10^{-2} moles of glucosidic bonds per liter).

A double reciprocal plot was used to analyze the kinetic data of purified α-amylase reaction. As shown in Fig. 11, the Michaelis constant (Km) is 2.4×10^{-2} M (moles of glucosidic bonds per liter) and the maximal velocity (V_{max}) is 1.55 mg glucose equivalent per min per mg protein. From the Arrhenius plot shown in Fig. 12, the energy of activation for this enzyme catalyzed reaction was calculated to be 14.12 kcal/mol.

Thermal inactivation studies

The enzyme used for inactivation studies were fraction V and fraction V in absence of calcium acetate. The enzyme was heated in a water bath and after various times, removed, cooled by immersion in an ice bath. Residual enzyme activity remaining in this solution was assayed in triplicate and compared to a similarly prepared but unheated control sample. The results were presented in Fig. 13. Thermal inactivation as a function of time follows first-order kinetics. Calcium acetate obviously protects the enzyme from heat denaturation. According to the equation $\ln E_t/E_0=Kd\cdot t$, where E_0, E_t represent the original activity and enzyme activity after heat treatment for time t respectively, the thermal inactivation rate constant Kd can be calculated. From these data, we can make Arrhenius plots for the thermal inactivation reaction of α-amylase (Fig. 14). The activation energies for the thermal inactivation reaction of α-amylase in the presence and absence of calcium acetate are 46.3 Kcal/mol and 20.96 Kcal/mol respectively.

Discussion

The increase of amylase activity during the germination of rice seeds is an interesting phenomenon (Fig. 1) which could conceivably be interpreted as the hydrolysis of rice starch is to supply the energy needed for germination. Preliminary results showed that the specific activities of purified α-amylases were quite different in different stage of germination (unpubished results), the increase of total amylase activity is probably not due to the biosynthesis of one unique form of α-amylase. Whether it is due to the existence of isozymes or endogenous modification of preexisted proenzyme should await further studies.

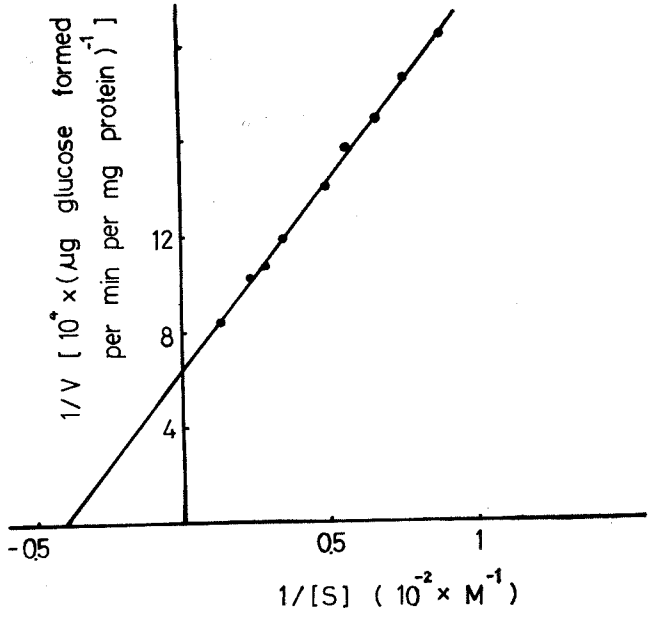

Fig. 11. Lineweaver-Burk plot of α-amylase reaction.
M=moles of glucosidic bonds per liter of solution.
Other conditions are the same as described under Fig. 10.

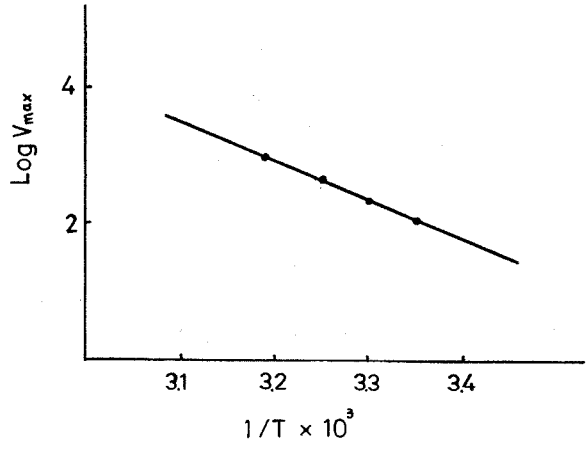

Fig. 12. Arrhenius plot of α-amylase reaction.
Amylase was assayed at various temperatures with starch as the variable substrate. Vmax was obtained from double reciprocal plots. The enzyme concentration was 1.4×10^{-3} mM.

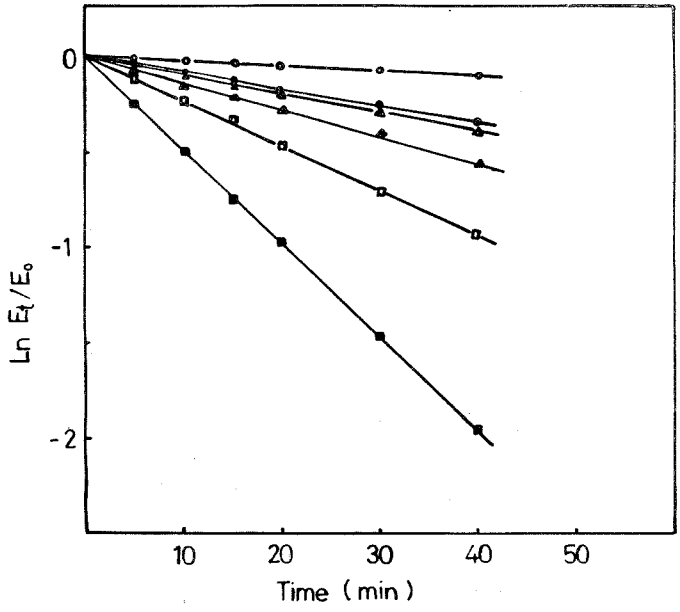

Fig. 13. Thermal inactivation of purified α-amylase.
The purified enzyme (in 0.01 M phosphate buffer pH 7.6) in the presence and absence of 0.02 M calcium acetate respectively was heated at various temperature for a period of time and then assayed for its residual activity after cooling immediately on ice. Samples with 0.02 M calcium acetate were heated at 70°C (○), 80°C (△), 85°C (□), respectively. Samples without calcium acetate were heated at 60°C (●), 70°C (▲), 80°C (■) respectively. E_0, E_t represent the original enzyme activity and the enzyme activity after heat treatment for time *t*.

We have also found that some α-amylase inhibitors also inhibit germination of rice seeds. This suggests that α-amylase could be very important in the regulation of germination.

The heterogeneity of starch molecule and its multiple potential points of cleavages by the α-amylase action made it difficult to express the enzyme activity by international unit. In the present report, we express one α-amylase activity unit as the amount of enzyme which produced one milligram equivalent of glucose per minute. In our dinitrosalicylate assay method one glucose equivalent of reducing power is equal to 1.5 maltose equivalents of reducing power.

The products of starch hydrolysis catalyzed by the purified amylase (No. 7 in Fig. 3) are largely glucose and small amount of maltose, the oligosaccharides are presumably present although they are difficult to be revealved by such experiment (paper chromatography and aniline-hydrogen phthalate spray.)

These are consistent with the α-amylase action and ruled out the possibility of β-amylase activity, whose major hydrolysis product is maltose. Since the crude extract fraction (No. 3 in Fig. 3) seems to have more maltose in its hydrodysis products, it is possible that trace amount of β-amylase existed. This conclusion is consistent with the general observation that cereal amylases are majorly α-amylase and a small fraction of β-amylase (Thoma *et al.*, 1971).

The purified α-amylase was determined by polyacrylamide gel electrophoresis to be a monomer of estimated molecular weight 50,500 (Fig. 4), with approximately 1.3 gram atom of Ca^{++} per molecule (Table 2). These are consistent with the general observations that the molecular weights of most of the α-amylases from various sources are in the range of 50,500, each molecule containing 1 gram atom of Ca^{++} (Thoma *et al.*, 1971; Kulp, 1975; Fischer and Stein, 1960).

The enzyme has absolute requirement of Ca^{++} for its activity. If we removed the Ca^{++} by EDTA, the enzyme completely lost its activity. When Ca^{++} was added back, the activity of the enzyme restored (Fig. 5). The activity of the enzyme was further increased by the addition of low calcium

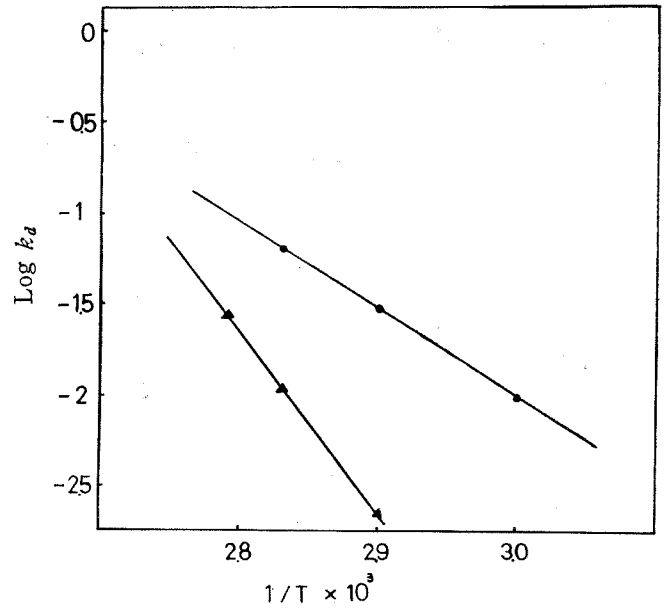

Fig. 14. Arrhenius plots for thermal inactivation of Amylase.
(▲-▲): with 0.02 M calcium acetate; (●-●): no calcium acetate. According to equation $\ln k_d = \ln A(E_a/R)(1/T)$, where k_d is the thermal inactivation rate constant calculated from Fig. 13, E_a is the energy of activation, R is the gas constant, T is the absolute temperature, A is a constant, E_a can be calculated from the slope of the plot.

salt concentrations (below 2.5 mM). Since the assay method we used was affected by Ca^{++} at concentrations higher than 2.5 mM, the effect of higher calcium concentrations could not be judged by such experiment. The addition of calcium salt apparently has stabilizing effect on the enzyme against denaturation.

The enzyme in the presence of 0.02 M calcium acetate still retained 65% of original activity while the enzyme in the absence of calcium acetate completely lost the activity after ten week storage (Fig. 9). In thermal denaturation experiments, the α-amylase with added calium salt showed much better stability against heat (Fig. 13). The activation energies for the thermal inactivation reaction of α-amylase in the presence of 0.02 M calcium acetate is much higher than that of α-amylase without calcium acetate. (46.3 Kcal/mol vs. 21.0 Kcal/mol) (Fig. 14). The effect of calcium ion is presumably due to maintaining the enzyme molecule in the optimum conformation for maxium activity and stability.

This α-amylase is quite stable between pH 4 to 10 (Fig. 7) and has an optimum temperature at 60°C (Fig. 8). It is quite stable toward heat (Fig. 13), the amylase still retained 75% activity in the absence of calcium acetate and 99% activity in the presence of 0.02 M calcium acetate after being heated 60°C (gelatinization temperature of starch) for 40 minutes.

The enzyme is also available in large amount, 400 g of rice seeds (contained approximately 11.3% water) can produce 12,590 units of crude amylase or 2610 units of purified α-amylase after germination (Table 1). From these considerations, the α-amylases from rice seeds are potentially useful for industrial applications.

Literature Cited

Blandamer, A. H. and R. B. Beechey. 1973. Dextran-gel filtration in the purification of a crustacean α-amylase. Nature **197**: 591-602.

Fischer, E. H. and E. A. Stein. 1961. α-amylase from human saliva. Biochem. Prep. **8**: 27-35.

Fischer, E. H. and E. A. Stein. 1960. α-Amylases pp 313-343 in P. D. Boyer, H. A. Lardy and K. Myrbaeck, (eds). The Enzymes 2nd ed. Vol. 4, Academic Press, New York.

Gabriel, O. 1971. Analytical disc gel electrophoresis. pp 565-568 in W. B. Jakoby (ed). Methods Vol. **22**, Academic Press, New York.

Greenwood, C. T., A. W. MacGregor and E. A. Milne. 1965. Amylolysis of starch carbohydrate Res. **1**: 229-236.

Greenwood, C. T., A. W. MacGregor and E. A. Milne. 1965. The α-amylase from broad beans. Arch. Biochem. Biophys. **112**: 459-465.

Heatley, N. G. 1958. Crystn. of amylase from pancrecatic juice. Nature **181**: 1069-1074.

Kulp, K. 1975. Carbonhydrases. pp 53-122 in G. Reed (ed.) in Enzymes in food processing 2nd ed. Academic Press New York.

Lowry, O. H., M. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein Measurement with the Folin phenol reagent. J. Biol. Chem. **193**: 265-269.

Manning, C. B. and L. L. Campbell. 1961. Thermostable α-amylase of *Bacillus stearothermophilus*. J. Biol. Chem. **236**: 2952-2959.

Minoda, Y. and K. Yamada. 1963. Acid-stable α-amylase of black aspergilli (I) detection and purification of acid-stable dextrinizing amylase. Agr. Biol. Chem. (Tokyo) **27**: 806-815.

Mutzbauer, H. and C. V. Schulz. 1965. Determination of the mole constants of α-amylases from human saliva Biochem. Biophys. Acta **102**: 526-533.

Robyt, J. and D. French. 1964. Purification and action pattern of an amylase from Bacillus Polymyxa. Arch. Biochem. Biophys. **104**: 338-344.

Takagi, T., H. Toda, and T. Isemura. 1971. Bacterial and mold amylases. pp 87-88 in P. D. Boyer (ed.). The Enzymes 3rd ed. Vol. V, Academic Press, New York.

Thoma, J. A., J. E. Spradlin and S. Dygert. 1971. Plants and animal amylases pp 115-189 in P. D. Boyer (ed.) The Enzymes, 3rd Ed. Vol. V, Academic Press, New York.

Weber, K. and M. Osborn. 1969. The Reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. **244**: 4406-4415.

Welker, N. E. and L. L. Campbell. 1967. Comparison of the amylase of *Bacillus subtilis* and *B. amylolizquefaciens*. Biochemistry. **6**: 3681-3690.

發芽稻米 α-澱粉酶之研究

蕭介夫

中央研究院植物研究所

莊麗月

國立高雄師範學院化學系

我們已自發芽稻米中，純化出一種耐熱性相當高之 α- 澱粉酶，其純化過程包括硫胺分劃，選擇性加熱，丙酮分劃及肝醣複合物之形成。此 α- 澱粉酶在 pH 4 至 10 之間活性均很高，其最適 pH 值爲4.5，其最適溫度爲 60°C，此純化 α- 澱粉酶經聚丙烯胺電泳分析測定其爲單體，分子量約爲 50,500，每一分子蛋白質緊密結合着約 1.3 克原子之 Ca^{++}，其吸光係數在 280 nm 下測定爲 $E^{1\%}_{1cm}=13.7$。此酶對有絕對 Ca^{++} 需求，因若添加 EDTA 則其活性喪失而再加鈣鹽則其活性恢復。加入 2.5 mM 鈣鹽可增進此酶之活性。

鈣離子可保護此澱粉酶抗拒變性。在 0.02 M 醋酸鈣存在下，此酶可在十週後保有 65% 之活性，而若無鈣離子存在下則活性完全喪失。在熱變性實驗中，添加鈣鹽之 α- 澱粉酶比未添加者抗拒熱變性之穩定性大爲提高。在 α- 澱粉酶之熱變性反應爲一級反應。經 Arrehnius 作圖法可估計在有及無 0.02 M 醋酸鈣存在下之活化能各爲 46.3 Kcal/mol 及 21.0 Kcal/mol。

此酶之 K_m 爲 2.4×10^{-2} moles of glucosidic bonds/liter，其 V_{max} 爲 1.55 mg glucose equivalent formed per minute per mg enzyme，其酵素反應之活化能爲 14.1 Kcal/mol。