# BIOCHEMICAL STUDIES ON α-GLUCOSIDASE FROM BUCKWHEAT

## Part IV. Substrate Specificity and Action Pattern

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α-Glucosidase is the enzyme which hydrolyzes the α-glucosidic linkage of oligosaccharides or heterosides. The substrate specificity depends upon the source of the enzyme. Recently, it has been reported that some α-glucosidases hydrolyze such polysaccharides as soluble starch (5, 13, 16, 19, 9, 10) and glycogen (13, 4, 8). Regarding plant α-glucosidase, malt α-glucosidase (9) showed strong specificity for maltose and also hydrolyzed soluble starch, hetero-α-glucosides, isomaltose and sucrose; α-glucosidase from alfalfa seedling (7) showed high activity towards maltose, nigerose, isomaltose, kojibiose trehalose and sucrose; α-glucosidase from tomato (7) also cleaved nigerose. Novel amylase from mung bean (12) and glucoamylase from rice (21) showed higher activity on maltose than on soluble starch. By this reason, above two enzymes may be considered to be one of α-glucosidases. Buckwheat α-glucosidase, described in this paper, showed wide specificity. In previous paper (24), it was reported that the purified α-glucosidase hydrolyzed soluble starch as well as maltose, to glucose only. The present paper details the substrate specificity, including the action pattern on polysaccharides, oligosaccharides, gluco-disaccharides, hetero-α-glucosides and sugar alcohols. In addition, some effects of chemical reagents and glucose on buckwheat α-glucosidase are described. The biochemical significance of α-glucosidase in vivo are also discussed in relation to the transglucosylation (25) and the effect on α-amylase.

## Materials and Methods

### Materials

Buckwheat α-glucosidase was prepared by the method reported in the

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previous paper (24). α-Amylase was prepared from diastase powder by the method of AKABORI et al. (3). Soluble starch, glycogen, maltose, methyl-α-glucoside, sucrose and trehalose were obtained from commercial sources. Amylose and amylopectin were prepared from potato starch by the modified method of SCHOCH (30) and β-limit dextrin, by the method of MEYER (15). Malto-oligosaccharides were separated from the acid-hydrolysate of potato starch by carbon column chromatography and isomaltose and isomaltotriose, from the hydrolysate of dextran by the same method. Phenyl-α-glucoside and cresyl-α-glucoside were synthesized chemically (6). Nigerose and kojibiose were prepared by the transglucosylation action of present enzyme as described in the previous paper (25). Sugar alcohols were prepared according to ABDEL-AKHER et al. (1). Panose was kindly supplied by prof. S. SUGAWARA.

**Assay of the enzyme**

The reaction mixture for substrate specificity experiment consisted of 0.1 ml of enzyme solution (2.5 units), 1.9 ml of 0.1 M acetate buffer, pH 5.0 and 2 ml of 1% substrate solution. The reaction was carried out at 37°C and the degree of hydrolysis was calculated from the amount of glucose liberated as described in the previous paper (24). After 24 hrs when the hydrolysis was observed to proceed slowly, a further 0.1 ml of enzyme solution was added to the reaction mixture.

In order to examine the effect of glucose upon the hydrolysis of the substrates, following experiments were carried out. The mixture consisting of 0.2~1 ml of 2% substrate solution, 0.2~0.8 ml of 5% or 2% glucose solution, 0.7~0.9 ml of 0.1 M acetate buffer, pH 5.0 and 0.1 ml of enzyme solution was made up to 2.0 ml with distilled water. After an incubation period of 10 min in the case of maltose and of 40 min for soluble starch at 37°C, 0.5~2 ml of the reaction mixture was pipetted out to determine the increase in reducing power. The activity was expressed as the amount of glucose increased in 1 ml of reaction mixture for 10 min.

The reaction mixture for determining the effect of buckwheat α-glucosidase on α-amylase consisted of 20 ml of 1% soluble starch, 18 ml of 0.1 M acetate buffer, pH 5.0, 1 ml of α-amylase solution and 1 ml of present enzyme or 1 ml of one of these enzymes and 1 ml of distilled water. In the other case, the reaction mixture contained 5 ml of 10% soluble starch, 3 ml of 0.1 M acetate buffer, pH 5.0, 1 ml of α-amylase and 1 ml of buckwheat α-glucosidase or 1 ml of one of these enzymes and 1 ml of water. The reaction was carried out at 37°C. At a definite interval, 5 ml of the

reaction mixture in the former case or 0.5 ml in the latter case was assayed for its reducing power, respectively.

**Chromatography**

Thin-layer chromatography was conducted as in part I of this series (23) and paper chromatography, as in part II (24). The sugar alcohols were detected by means of benzidineperiodate reagent and silver nitrate (27).

## Results

**Substrate specificity**

Polysaccharides consisting of α-1, 4 and α-1, 6 linkages were hydrolyzed by present enzyme as shown in Fig. 1. The rate of hydrolysis was found to be as follows: soluble starch>amylopectin>glycogen>amylose>β-limit dextrin. Soluble starch and amylopectin were hydrolyzed more rapidly. However, the rates of hydrolysis of glycogen, amylose and β-limit dextrin were below 30% even after 48 hours. To determine the hydrolysis limit of each substrate, the enzyme solution, which had 8 times higher activity compared with that in the normal method, was used in the experiment and thereafter the same enzyme solution was added every 24 hours. After

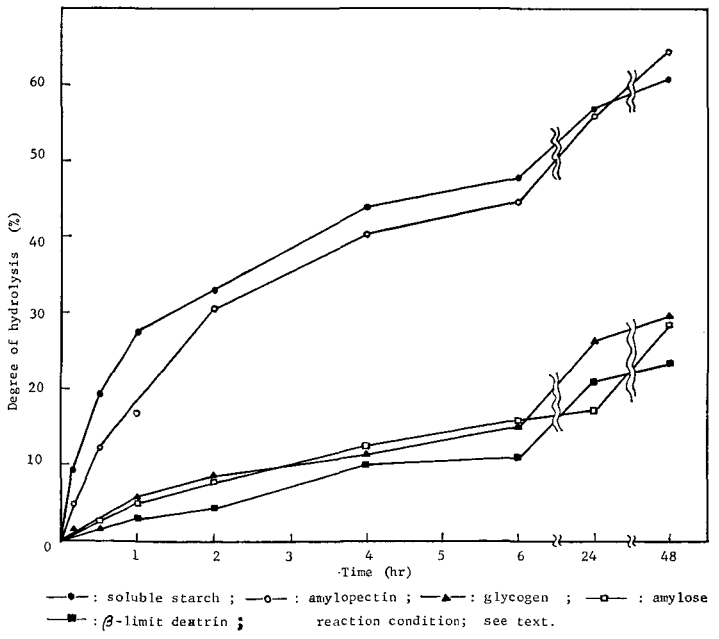



**Fig. 1.** Hydrolysis of Polysaccharides by Buckwheat α-Glucosidase.

72 hours of incubation, following values were obtained and in every case the iodine color was still observed: soluble starch (bluish violet), 72.7%; amylopectin (violet), 93.7%; glycogen, 63%; amylose (blue), 70%; $\beta$-limit dextrin (violet), 61.7%. The hydrolysis of malto-oligosacharides consisting

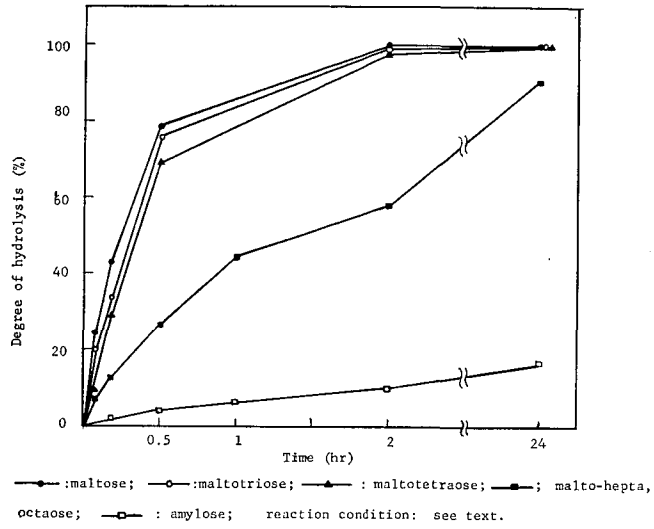



**Fig. 2.** Hydrolysis of Malto-oligosaccharides by Buckwheat $\alpha$-Glucosidase.

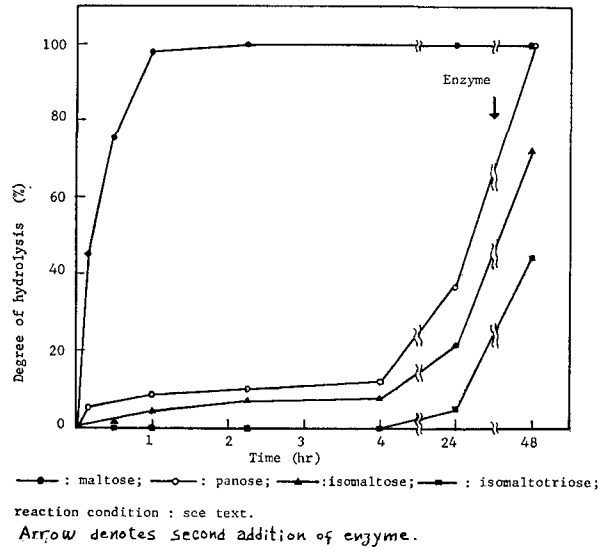



**Fig. 3.** Hydrolysis of Isomalto-oligosaccharides by Buckwheat $\alpha$-Glucosidase

of α-1, 4 linkage only is shown in Fig. 2. The rates of hydrolysis were found to be as follows: maltose>maltotriose>maltotetraose>mixture of malto-heptaose and -octaose>amylose. These results indicate that the increase of α-1, 4 linkage number is accompanied by the decrease in the rate of hydrolysis. The action of present enzyme on oligosaccharides containing α-1, 6 linkage is shown in Fig. 3. Panose, isomaltose and isomaltotriose were hydrolyzed very slowly in this order compared with maltose. The action on gluco-disaccharides which contain α-1, 1, α-1, 2, α-1, 3, α-1, 4, or α-1, 6 linkage is presented in Fig. 4. The hydrolysis degree of each disaccharide was considerably smaller than that of nigerose or maltose. Trehalose (α-1, 1) was not attacked under the conditions of present experiment. Heteroglucosides were also hydrolyzed as shown in Fig. 5. The rates of hydrolysis were as follows: p-cresyl-α-glucoside> phenyl-α-glucoside>methyl-α-glucoside. The slight hydrolysis of sucrose was also observed after longer incubation. As shown in Fig. 6, sugar alcohols were also attacked.

In Fig. 1~Fig. 6, the activity on maltose is used as the standard. The results reveal buckwheat α-glucosidase has a high specificity for maltose, malto-oligosaccharides and nigerose, and also a wide specificity for many other substrates. Concerning the substrate specificity the enzyme is similar to acid α-glucosidase of *Candida tropicallis*, which is reported to hydrolyze gluco-disaccharides containing α-1, 2, α-1, 3, α-1, 4 or α-1, 6 linkage, methyl-

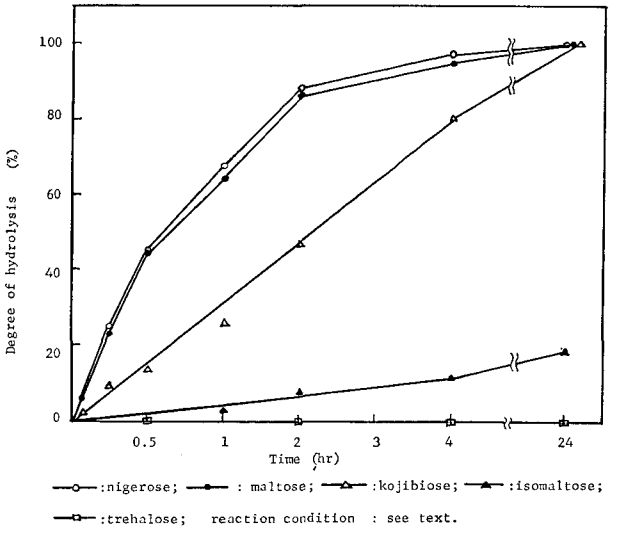



**Fig. 4.** Hydrolysis of Gluco-disaccharides by Buckweat α-Glucosidase.

α-glucoside, phenyl-α-glucoside and soluble starch, but not to hydrolyze trehalose and sucrose.

From the point of view that buckwheat α-glucosidase hydrolyzes polysaccharides, it seems to be of significance to compare the activity of the

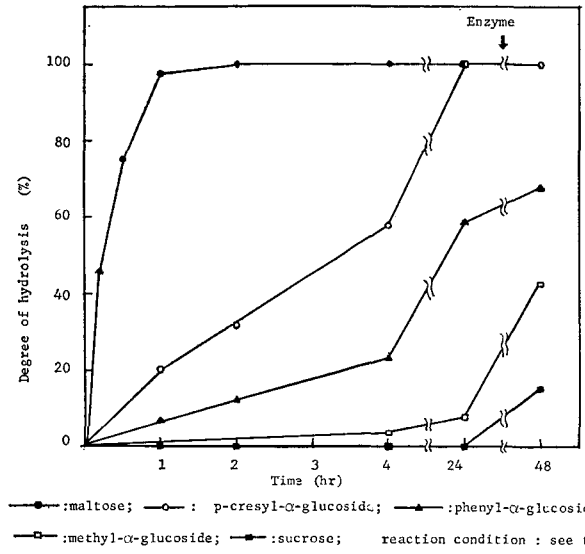



**Fig. 5.** Hydrolysis of Heteroglucosides by Buckwheat α-Glucosidase.

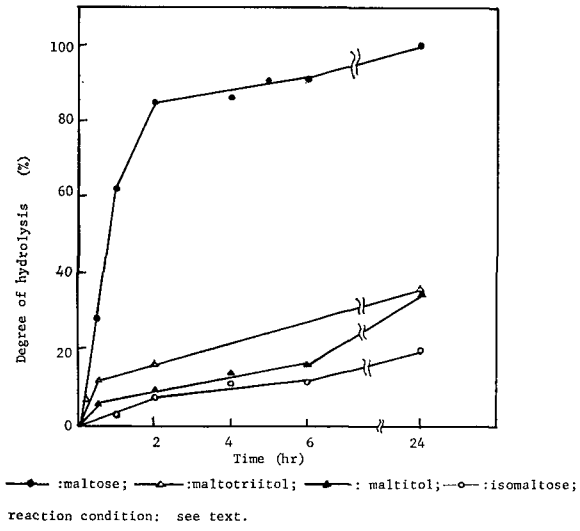



**Fig. 6.** Hydrolysis of Sugar alcohols by Buckwheat α-Glucosidase.

enzyme witn that of glucoamylase. As is wellknown, glucoamylase from *Rhizopus delemar* (28) and from *Asp. oryzae* (17, 18) hydrolyzed both soluble starch and maltose at the initial ratio of 4 : 1. Glucoamylase from *Asp. niger* is reported to hydrolyze maltose, malto-oligosaccharides (2) and each of α-1, 2, α-1, 3, α-1, 6 glucosidic linkages excluding α-1, 1 linkage (11). Saccharogenic amylase from *Asp. awamori* (29) also hydrolyzed glucodisaccharides including β, β-trehalose. These glucoamylases show a very wide substrate specificity like buckheat α-glucosidase, except their higher specificity for polysaccharides than for disaccharides.

**Initial action pattern on substrates**

It has been already reported that this enzyme hydrolyzed soluble starch to glucose unit (24). To confirm the action pattern of this enzyme, the intermediary products from malto-oligosaccharides were examined. In the initial stage of reaction, glucose and maltotriose were recognized from maltotetraose and also the thin-layer chromatograms of the intermediary products from the mixture of malto-heptaose and -octaose indicated that these were hydrolyzed to glucose unit as shown in Fig. 7. The action patterns on some sugar alcohols and panose were examined. As is known from Fig. 8, maltotriitol was converted to glucose and maltitol and isomaltotriitol, to glucose and isomaltitol. The results showed the non-reducing end of each original sugar (maltotriose or isomaltotriose) was attacked first. However, from panitol, all the expected products were recognized, that is, isomaltose, maltitol, sorbit and glucose. This result indicates that both the

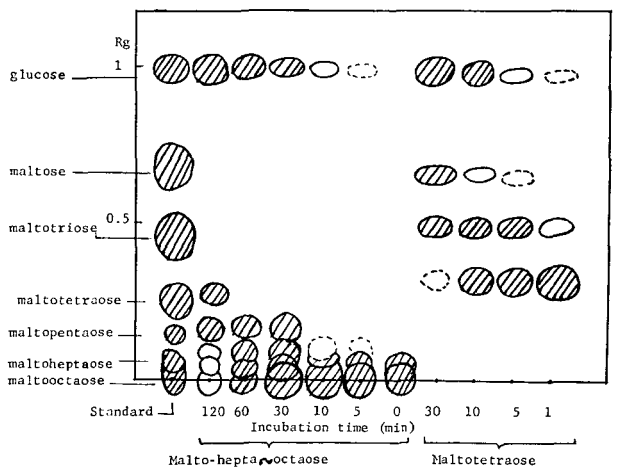
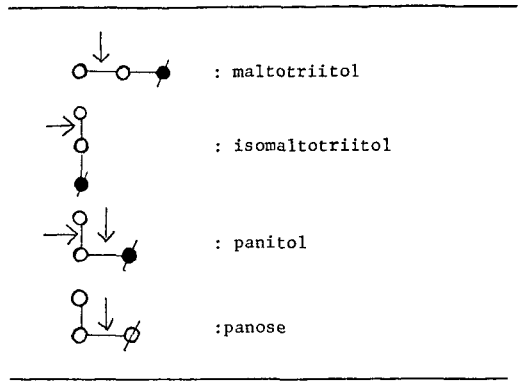



**Fig. 7.** Thin Layer Chromatogram of the Products from Oligosaccharides by the action of Buckwheat α-Glucosidase.




○ :glucose unit ; ● : sorbit unit; ○—○ : α-1,4 linkage;
: α-1,6 linkage; ∅, (●): reducing end; reaction condition, see text.

**Fig. 8.** Action Pattern on Sugar Alcohols and Panose by Buckwheat α-Glucosidase.

α-1, 4 and α-1, 6 linkage were hydrolyzed at the same time. Panose was hydrolyed to glucose and isomaltose, which shows panose was cleaved first at the α-1, 4 linkage of reducing end. These results may be interpreted as follows: the affinity of the enzyme to maltose (α-1, 4 linkage) is much higher than to isomaltose (α-1, 6 linkage) (Fig. 3) and the affinity to maltitol is nearly the same as that to isomaltose (Fig. 6). The fact that amylopectin was attacked more rapidly than amylose suggests that the present enzyme hydrolyzed the substrate to glucose by attack at the non-reducing end but the substrate may be possibly hydrolyzed from the reducing end when the non-reducing end of the substrate shows a very low affinity for the enzyme, as in the case of panose for instance.

**Lineweaver-Burk plots**

Using maltose and soluble starch in the range of 0.1% to 1.2% concentration in 0.05 M acetate buffer, pH 5.0, a Lineweaver-Burk plot was obtained as shown in Fig. 9. The plots revealed linear relationship in the range of the concentrations investigated. However, when the maltose concentration exceeded 1%, the initial velocity became lower as a result of transglucosylation action as described in previous paper (24). The value for the apparent km was 5.0 mg/ml for maltose and 7.78 mg/ml for soluble starch.

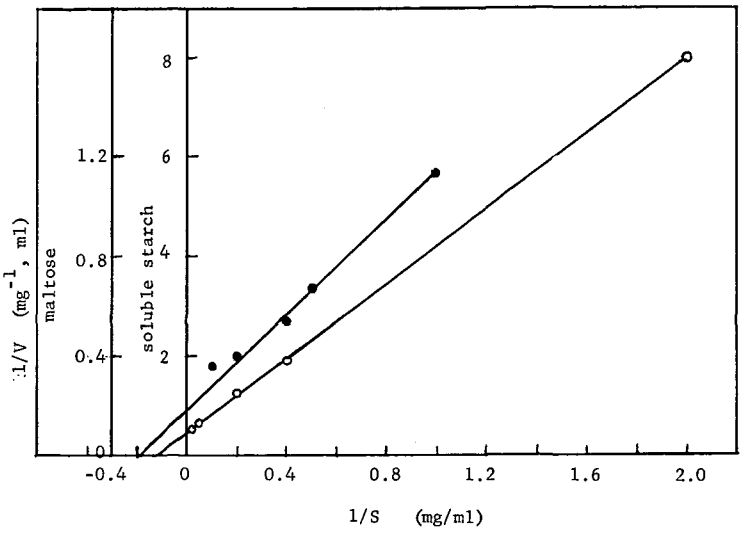



**Fig. 9.** Lineweaver-Burk plots of Buckwheat α-Glucosidase

**Effects of some chemical reagents**

The effects of metal ions and SH-reagents were examined. The protective effect of $Ca^{2+}$ on heat denaturation of the enzpme was not recognized with both substrates, maltose and soluble starch, as shown in Table 1. EDTA (0.5 M) also showed no effect on α-glucosidase activity. The effects of SH-reagents are presensed in Table 2. The enzymic activity was inhibited completely by PCMB ($10^{-4}$ M) and 86% of the activity was still recognized

TABLE 1. Effect of Calcium Ion on Buckwheat α-Glucosidase

| Temperature (°C) | Activity (%) on Maltose | | on Sol. Starch | |
|---|---|---|---|---|
| | Control | $+Ca^{2+}$ | Control | $+Ca^{2+}$ |
| 30 | 100 | 95.6 | 100 | 96.8 |
| 55 | 76.4 | 73.5 | 79.0 | 77.4 |
| 59 | 37.9 | 33.3 | 41.2 | 38.4 |

A reaction mixture containing 1 ml of Ca-acetate ($10^{-2}$ M), 1 ml of enzyme solution and 1 m of 0.1 M acetate cuffer, pH 5.0 was preincubated for 10 min. at 37°C, followed by adding 2 ml of 1% substrate solution.

in the presence of monoiodoacetic acid ($10^{-3}$ M). The addition of cysteine to the PCMB-containing mixture brought about the recovery of about 90% of original activity. The complete inhibition was observed with $Hg^{2+}$ and $Cu^{2+}$ (Table 3). From these results it seems that buckwheat $\alpha$-glucosidase is a sulfhydryl enzyme.

TABLE 2. Effect of SH-Reagents on Buckwheat $\alpha$-Glucosidase

| SH-reagent | Cysteine | Remaining Activity | |
|---|---|---|---|
| | | maltose | Sol. Starch |
| PCMB ($10^{-4}$ M) | 0 | 0 | 0 |
| MIA ($10^{-3}$ M) | 0 | 85.7 | 86.6 |
| PCMB ($10^{-4}$ M) | $10^{-3}$ M | 88.2 | 89.1 |
| MIA ($10^{-3}$ M) | $10^{-3}$ M | 90.3 | 88.2 |

A reaction mixture containing 1 ml of PCMB ($10^{-3}$ M) or monoiodoacetate (MIB, $10^{-2}$ M), 1 ml of enzyme solution and 1 ml of acetate buffer, pH 5.0 was preincubated for 5 min. at 37°C. The reaction was started by adding 2 ml of 1% substrate solution. The effect of cysteine was examined by adding 1 ml of cysteine ($10^{-2}$ M) to the reaction mixture and preincubating for 30 min. prior to the addition of the substrate.

TABLE 3. Effect of Metal Ion on Buckwheat $\alpha$-Glucosidase

| Metal ion ($10^{-3}$ M) | Remaining Activity on Maltose (%) |
|---|---|
| $MgSO_4$ | 92.7 |
| $HgCl_2$ | 0 |
| $CuSO_4$ | 0 |
| $FeSO_4$ | 91.7 |
| $ZnSO_4$ | 84.7 |

The reaction condition are the same as in Table 2, except for the addition of 1 ml of metal ion ($10^{-2}$ M) instead of the SH-reagents.

**Inhibitory effect of glucose on the hydrolysis of substrates**

The effect of the hydrolysis product (glucose) was examined on the hydrolysis of maltose and soluble starch. The results in Table 4 showed that the hydrolysis of both substrates at any concentration were strongly inhibited by adding glucose and the lower concentration of substrate and the higher concentration of glucose added brought about negative activity

TABLE 4. Inhibitory Effect of Glucose on Substrates: maltose and soluble starch.

| added glucose (mg/ml) | Activity (glucose mg/ml) | | | |
|---|---|---|---|---|
| | Substrate concentration: maltose mg/ml | | | |
| | 2 | 5 | 8 | 10 |
| 0 | 0.383 (100) | 1.050 (100) | 1.136 (100) | 0.696 (100) |
| 2 | 0.320 (83.5) | 0.426 (40.6) | 0.852 (75.0) | 0.639 (91.8) |
| 5 | 0.220 (57.4) | 0.500 (47.6) | 0.568 (50.0) | 0.184 (26.4) |
| 10 | −1.16 (−302.9) | −0.568 (−54.1) | −0.142 (−12.5) | 0.028 (4.0) |
| 20 | −2.27 (−592.8) | −1.704 (−162.2) | −1.874 (−164.9) | −1.874 (−269.3) |
| added glucose (mg/ml) | Swbstrate concentration: soluble starch mg/ml | | | |
| | 2 | 5 | 8 | 10 |
| 0 | 0.105 (100) | 0.185 (100) | 0.263 (100) | 0.284 (100) |
| 2 | 0.039 (37.1) | 0.110 (59.5) | 0.174 (66.1) | 0.213 (75.0) |
| 5 | 0.059 (56.2) | 0.080 (43.2) | 0.098 (37.3) | 0.115 (40.5) |
| 10 | 0.027 (25.7) | 0.009 (4.9) | 0.060 (22.8) | 0.062 (21.7) |
| 20 | −0.178 (−169.5) | −0.156 (−86.5) | −0.107 (−40.7) | −0.142 (−50.0) |

values. These facts may be explained in terms of the glucosyl group transfer of the substrate to added glucose, which acts as an acceptor of glucosyl moiety. This results in the production of disaccharides, trisaccharides etc., which have lower reducing activity compared with glucose added. Thus it is suggested that the synthetic reaction (transglucosylation) is preffered to the hydrolysis reaction in the case of higher concentration of glucose.

**Effect of buckwheat α-glucosidase on α-amylase action**

It has been already recognized that a rapid appearance of α-amylase activity is observed at the initial stage of buckwheat germination, being accompanied by a decrease in starch content of the seedling (23). At this stage α-amylase coexists with α-glucosidase (23). Therefore it seems to be

of significance to investigate the effect of α-glucosidase on the hydrolysis of starch by α-amylase.

Two kinds of enzyme concentration (a and b) were applied into two different concentrations (0.5% and 5%) of soluble starch. The results are shown in Table 5. In both substrate concentrations (0.5% and 5%), the hydrolysis was found to be faster and more efficient in the co-existence of both enzymes, than in the presence of either single enzyme. The substrate (0.5% concentration) was hydrolyzed by 96% in the presence of both enzymes, but the hydrolysis degree obtained in the presence of only one enzyme never rose above 50% even after 24 hours reaction. The difference in the ratio of enzyme concentration (a and b) did not bring about any remarkable change of hydrolysis degree. But the difference in the substrate concentration showed some effects on the hydrolysis: at the low concentration (0.5%) the rate of hydrolysis by the both enzymes was slightly higher

TABLE 5. Action of α-Glucosidase and α-Amylase on Soluble Starch

| Substrate conc. (%) | Enzyme | Degree of hydrolysis (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 5 | 10 | 30 min | 1 | 2 | 4 | 24 hr |
| *1 a) 0.5 | α-Amylase only | 1.7 | 4.2 | 9.1 | 15.1 | 21.6 | 27.0 | 34.0 |
| | α-Glucosidase only | — | — | — | 1.1 | 2.2 | 4.3 | 9.9 |
| | α-Glucosidase and α-Amylase | 2.2 | 3.7 | 9.6 | 16.4 | 28.1 | 36.1 | 58.8 |
| *2 b) 0.5 | α-Amylase only | 2.1 | 4.4 | 11.3 | 19.3 | 30.1 | 38.1 | 49.2 |
| | α-Glucosidase only | 2.0 | 3.5 | 9.6 | 16.8 | 25.9 | 34.1 | 48.4 |
| | α-Glucosidase and α-Amylase | 4.4 | 9.1 | 28.1 | 49.1 | 66.7 | 76.4 | 96.1 |
| *1 a) 5.0 | α-Amylase Only | 2.2 | 8.5 | 15.1 | 28.1 | 37.2 | 41.9 | 48.2 |
| | α-Glucosidrse only | — | 2.8 | 3.9 | 7.4 | 11.4 | 18.2 | 33.9 |
| | α-Glucosidase and α-Amylase | 4.6 | 10.6 | 18.7 | 32.9 | 43.6 | 53.6 | 89.5 |
| *2 b) 5.0 | α-Amylase only | 2.1 | 2.8 | 5.7 | 9.7 | 16.9 | 28.3 | 48.6 |
| | α-Glucosidase only | — | 2.2 | 4.0 | 6.8 | 11.9 | 17.9 | 41.8 |
| | α-Glucosidase and α-Amylase | 1.7 | 4.5 | 8.5 | 15.6 | 27.1 | 41.2 | 81.8 |

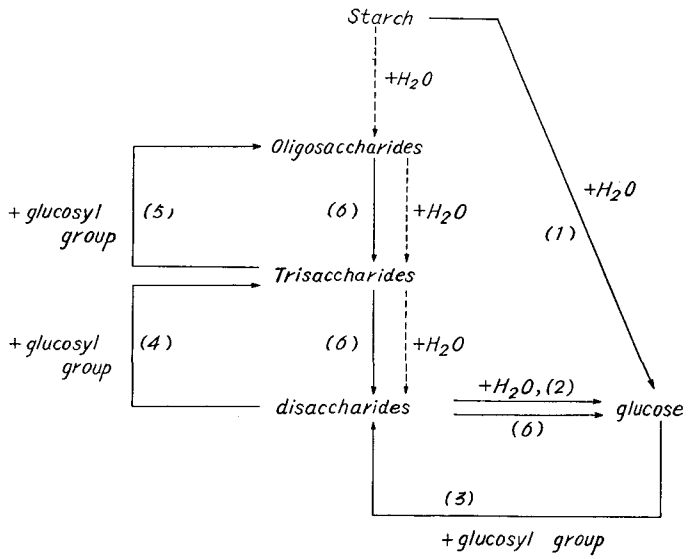
*1 a) α-amylyse activity is higher than α-glucosidase activity.

*2 b) α-Amylase activity and α-glucosidase activity are almost equal.

than the sum of the hydrolysis rate by each enzyme, while at the high concentration (5%) the former was lower than the latter. These results may be interpreted as follows: at a high substrate concentration transglucosylation by α-glucosidase will occur upon the hydrolysis products. However, the final degree of starch hydrolysis was not affected so much. With reference of these results, the metabolic role of α-glucosidase *in vivo* will be described in the discussion.

## Discussion

In the previous paper (24) it was shown that buckwheat α-glucosidase hydrolyzed not only maltose but soluble starch to glucose. In present paper the results of various chemical modifications and action patterns on substrates revealed that the hydrolysis of both substrates was caused by the single enzyme and the enzyme was not contaminated by α-amylase or β-amylase. The enzyme was found to have a wide substrate specificity upon polysaccharide, α-1, 2, α-1, 3, α-1, 4, and α-1, 6 glucosidic linkages and heteroglucosides. As was already reported (25), present enzyme transfered an activated glucosyl group from the substrate to either of C–2, –3, –4 or –6 of the glucosyl group of substrate or reaction products to yield disaccharides and oligosaccharides. On one hand, glucose showed strong inhibitory effect on hydrolysis and the synthesis of oligosaccharides by transglucosylation was presumed to occur at the higher concentration of substrate.



From these results the metabolic role of buckwheat α-glucysidase *in vivo* may be considered as described below :

(1) Starch is hydrolyzed directly to glucose by α-glucosidase.
(2) Starch is hydrolyzed to oligo-, tri-(maltotriose) and disaccharide (maltose) by α-amylase or β-amylase and these sugars are then hydrolyzed to glucose by α-glucosidase.
(3) At the high concentration of glucose produced, the glucosyl groups liberated by α-glucosidase are accepted by free glucose, not by water, at position C–2, –3, –4 or –6 to yield such disaccharides as kojibiose, nigerose, maltose or isomaltose.
(4) Glucosyl groups may be also transfered to disaccharides, forming trisaccharides.
(5) Glucosyl groups may be transfered to trisaccharides in similar fashion.
(6) Transglucosylation products are hydrolyzed again by α-glucosidase to glucose.

In the early stage of buckwheat germination, starch will be hydrolyzed slowly to glucose by process (1). At the successive stage where the rapid hydrolysis of starch is required, α-amylase appears to attack starch to glucose under the cooperation with α-glucosidase (process 2). Rapid appearance of α-amylase at this stage has been already reported (23) and it has been just indicated in this paper that starch was hydrolyzed more efficiently under the coexistence of α-amylase and α-glucosidase. On one hand, it has been reported (20) that the cooperation of pancreatic amylase with maltase from *Asp. oryzae* accelerated the hydrolysis of starch. The same effect has been observed on green gram enzyme (12). Thus it is possible to consider that when the rate of glucose metabolism is insufficient to keep pace with the rate of hydrolysis of starch to glucose, glucose will act as the acceptor for glucosyl group. Accordingly, the accumulation of glucose will be prevented, which results in the formation of disaccharides such as kojibiose, nigerose, maltose and isomaltose (process 3). The transfer of glucosyl group to the disaccharides produced will lead to the formation of trisaccharides (process 4) and oligosaccharide may be produced in a similar way (process 5). Furthermore it is presumed that the amount of glucose supply for glycolysis is regulated through transglucosylation action. These transglucosylation products are also hydrolyzed by the same α-glucosidase (process 6). In this step the rate of hydrolysis will also be dependent upon the type of linkage of saccharide. In this metabolic circumstance, the ability of α-glucosidase to hydrolyze α-1, 2 and α-1, 3 linkages will be required in addition to the ability to cleave α-1, 4 and α-1, 6 linkages. Concerning nigerase activity

Hutson et al. (7) assumed the following: although α-(1, 3)-glucosidase activity is distributed in nature, the existence of α-1, 3-glucoside is limited to nigeran, isolichenin and certain dextrans; this means nigerase has little or no metablic importance and represents one catalytic feature of a general α-glucosidase. However, it is possible to think that nigerase activity is required for the cleavage of α-1, 3 linkage which was formed by transglucosylation and therefore this activity is widespread in nature. The fact that transglucosylation was recognized in the case of starch hydrolysis (25) will show that this reaction occurrs not only *in vitro*, but also *in vivo*. At a certain stage of germination, the existence of isomaltose has been demonstrated by the authors (23) and it may be considered as an intermediary product by transglucosylation.

While the transglucosylation action by α-glucosidase is now known, examples of its biological significance are few. LUKOMSKAYA (14) considered that the oligosaccharides produced by rabbit transglucosidase would be the intermediates in glycogen synthesis. TONOMURA (26) reported that a fungus transglucosidase produced certain oligosaccharides such as isomaltose and panose, which were presumed to play an inductive role in the synthesis of α-amylase.

Further studies are necessary in order to decide the significance of transglucosidation *in vivo*. However, the present results will explain partly the role of α-glucosidase in the carbohydrate metabolism of plant.

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**References**

1. ABDEL-AKHER, M., HAMILTON, J. K. and SMITH, F. 1951. J. Am. Chem. Soc, 73: 4691.
2. ABDULLAH, M., FLEMING, I. D., TAYLOR, P. M. and WHELAN, W. J. 1963. Biochem. J. 89: 359.
3. AKABORI, S., IKENAKA, T. and HAGIHARA, B. 1954. J. Biochem. 41: 557.
4. BRUNI, C. B., AURICCHO, F. and CONELLI, I. 1969. J. Biol. Chem. 244: 4735.
5. FRENCH, D. and KNAPP, D. W. 1950. J. Biol. Chem. 187: 463.
6. HELFRICH, B. und SCHMITE-HILLOBRECHT, E. 1933. Ber. 66: 378.
7. HUTSON, D. H. and MANNERS, F. J. 1965. Biochem. J. 94: 783.
8. JEFFREY, P. L., BROWN, D. H. and BROWN, B. I. 1970. Biochem. 9: 1403.
9. JØRGENSEN, O. B. 1963. Acta Chem. Scand. 17: 2471.
10. ———————— 1964. ibid. 18: 53, 1115, 1975.

11. KAMIYAMA, H., FUKINBARA, T. and TERUI, G. 1965. Hakko Kogaku Zasshi. 43: 228, 475, 481.
12. KASUGAI, I. 1966. Private Letter.
13. LIEBERMAN, I. and ETO, W. H. 1957. J. Biol. Chem. 225: 899.
14. LUKOMSKAYA, I. S. 1962. Enzymolo. 24: 327.
15. MEYER, K. H., BERNFELD, P., BOISSONAS, R. A., GURTHER, P. and NOELTING, G. 1949. J. Phys. Colloid Chem. 53: 319.
16. MILLER, K. D. and COPELAND, W. H. 1958. J. Biol. Chem. 231: 997.
17. OKAZAKI, H. 1955. Nippon Nogeikagaku Kaishi 28: 48, 51.
18. ———— 1956. ibid 29: 181, 273, 277.
19. SAWAI, T. 1960. J. BIOCHEM. 48: 382.
20. SCHWIMMER, S. 1945. J. Biol. Chem. 161: 219.
21. SHIMOMURA, T. and AKIMOTO, F. 1968. Memoirs of Fac. of Agr., Hokkaido Univ. 6: 325.
22. STAHL, E. 1962. Dunnschicht-Chromatographie, Springen-Verlag. p. 514.
23. TAKAHASHI, M. and SHIMOMURA, T, 1968. Agr. Biol. Chem. 32: 923.
24. ———— and ———— 1968. ibid 32: 929.
25. ————, ———— and Chiba, S. 1969. ibid 33: 1399.
26. TONOMURA, K., FUTAI, F. and TANABE, O. 1961. Hakko Kogaku Zasshi 19: 1.
27. TREVELYAN, W. E., PROCTER, P. P. and HARRISON, J. N.1950. Nature 166: 444.
28. TSUJISAKA, Y., FUKUMOTO, J. and YAMAMOTO, T. 1958. Nature: 181: 770.
29. WATANABE, K. and FUKINBARA, T. 1966. Hakko Kogaku Zasshi 44: 25.
30. WILSON, J., SCHOCH, JR. T. J. and HUDSON, C. S. 1943. J. Am. Chem. Soc. 65: 1380.