Carbohydrate Research, 128 (1984) 297–310
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

ACTION PATTERNS OF VARIOUS EXO-AMYLASES AND THE ANOMERIC CONFIGURATIONS OF THEIR PRODUCTS*

TERUO NAKAKUKI, KEIKO AZUMA, AND KEIJI KAINUMA†
National Food Research Institute, 2-1-2 Kannondai, Yatabe, Tsukuba, Ibaraki 305 (Japan)
(Received February 24th, 1983; accepted for publication in revised form, June 18th, 1983)

ABSTRACT

The product specificity, action pattern, and anomeric configuration of the hydrolysis products by four kinds of amylase have been studied comparatively. Amylases of *Streptomyces griseus*, *Pseudomonas stutzeri*, and *Aerobacter aerogenes* produced mainly the malto-oligosaccharides G_3, G_4, and G_6 at the initial stage of the reaction. The amylase of *Bacillus licheniformis* had a dual product-specificity for the formation of G_5 and G_3. Amylases of *Pseudomonas stutzeri* and *Bacillus licheniformis* catalyzed the degradation of water-insoluble, cross-linked blue starch to give respectively G_4 and $G_5 + G_3$ as the main products. All four amylases also hydrolyzed partially-oxidized potato amylose and the degree of hydrolysis increased gradually. The action patterns of the four amylases were investigated by two-dimensional paper chromatography by using ^{14}C-reducing-end-labeled malto-oligosaccharides. Three of the amylases (of *S. griseus*, *P. stutzeri*, and *A. aerogenes*) were thus characterized as exo-amylases, and that of *B. licheniformis* was an endo-amylase. The anomeric configurations of products from action of these amylases were studied polarimetrically. Three such exo-amylases, namely maltotriohydrolase, maltotetraohydrolase, and maltohexaohydrolase formed products having the α configuration, which does not fit the criterion of Reese who proposed inversion of configuration by exo-glucanases. We propose to classify this new group of amylases as "exo-alpha amylases".

The following symbols and abbreviations are used: G_1, G_2, G_3 . . . etc. denote D-glucose, maltose, maltotriose and so on; G_1, G_2*, G_3* . . . etc. denote ^{14}C-reducing-end-labeled malto-oligosaccharides; $\overline{d.p.}$ refers to average degree of polymerization; one International Unit (IU) is defined according to the International Commission on Enzymes as the amount of enzyme that hydrolyzes one μmol of glycosidic bonds per min under optimal conditions; amylases of *Streptomyces griseus*, *Pseudomonas stutzeri*, *Bacillus licheniformis* and *Aerobacter aerogenes* are abbreviated as G_3-, G_4-, G_5-, and G_6- Enz, respectively, in the Formulas, 0 refers to a D-glucose residue; — to a (1→4)-α-D-glucosidic linkage; and $\emptyset$ to a reducing-end D-glucose group. The length of the vertical arrows (↑) between D-glucose residues indicates qualitatively the frequency of bond cleavage.

†To whom requests for reprints should be addressed.

0008-6215/84/$ 03.00

INTRODUCTION

The product specificity and action pattern of an amylase from *Bacillus subtilis* [EC 3.2.1.1] were studied by Robyt *et al.*[1], who showed that the amylase had a dual product-specificity for the formation of G_3 and G_6. Okada *et al.* reported that various alpha amylases showed marked differences in their mode of action depending on their source[2]. Reese *et al.*[3] studied the anomeric configuration of products formed by the action of α-D-glucosidase [EC 3.2.1.20], exo-α-D-glucanase [EC 3.2.1.2], β-D-glucosidase [EC 3.2.1.21], and exo-(1→3)-β-D-glucanase. They concluded that exo-glucanases act on the substrate with inversion of configuration, and glucosidases with retention. They proposed classification of exo-glucanases and glucosidases according to the anomeric configuration of their products.

Recently, various oligosaccharide-forming amylases such as *Streptomyces griseus* amylase [EC 3.2.1.-][4], *Pseudomonas stutzeri* amylase [EC 3.2.1.60][5], *Bacillus licheniformis* amylase[6], and extracellular *Aerobacter aerogenes* amylase [EC 3.2.1.98][7,8] have been discovered and their characteristics studied.

In this paper, we summarize the results of studies on the product specificities for the formation of malto-oligosaccharides, action patterns of these enzymes, and the anomeric configurations of the products. We show here that such exo-amylases as exo-maltotriohydrolase, exo-maltotetraohydrolase, and exo-maltohexaohydrolase produce malto-oligosaccharides having the α configuration, which is in disagreement with the criterion specified by Reese *et al.*[3] for classification of exo-glucanases.

MATERIALS AND METHODS

Substrates. — Short-chain amylose (d.p. 23) was donated by Hayashibara Biochemical Research Laboratory, Okayama, Japan. Maltohexaose, maltoheptaose, and maltooctaose were prepared by gel filtration of a digest of short-chain amylose by bacterial liquefying amylase (BLA), as reported previously[9]. Potato amylose (Wako Pure Chemical Industries Co., Japan) was purchased commercially. Water-insoluble, cross-linked blue starch (Cibachron Blue F3GA combined starch, Neo-Amylase Test, Daiichi) was purchased from Daiichi Kagaku Yakuhin Co., Japan.

Reduced, short-chain amylose was prepared as described by Kainuma *et al.*[10]. Oxidized potato amylose (5% oxidation) was prepared according to Marshall and Whelan[11].

Enzymes. — *G_3-Forming amylase. Streptomyces griseus* amylase was kindly supplied by K. Wako of Nikken Kagagu Co., Japan. This purified enzyme-preparation showed a single band on polyacrylamide disc-gel electrophoresis[4].

G_4-Forming amylase. Pseudomonas stutzeri amylase was purified by the method of Schmidt *et al.*[12], using *Pseudomonas stutzeri* NRRL B 3389 as the microorganism.

G_5-Forming amylase. Crude *Bacillus licheniformis* amylase (Termamyl L-60) was purchased from Novo Industry Co., Japan and purified by the method of Saito[6].

G_6-Forming amylase. Extracellular *Aerobacter aerogenes* (*Klebsiella pneumoniae*) amylase was purified as described previously[13]. Crystalline bacterial liquefying alpha amylase (BLA) and *Rhizopus niveus* glucoamylase (pure grade) were purchased from Seikagaku Kogyo Co., Japan. Soybean beta amylase was a gift from Nagase Co., Japan.

Analytical methods. — The malto-oligosaccharide composition of the short-chain amylose digests formed by the action of various amylases was analyzed by high-performance liquid chromatography (l.c.). L.c. was performed as previously reported[14]. Reducing values were determined by the Somogyi–Nelson method[15] with maltose as the standard. The total carbohydrate content was determined by the phenol–sulfuric acid method[16].

Amylase was assayed by using a substrate solution consisting of 0.5 mL of 0.4% reduced, short-chain amylose and 0.4 mL of 0.1M phosphate buffer (pH 7.0). After preincubation of the substrate solution for 3 min at 40°, 0.1 mL of suitably diluted enzyme solution was added and the amount of reducing sugars released by the enzyme action for 30 min was determined by the Somogyi–Nelson method.

Ascending paper chromatography was performed with Toyo Filter Paper No. 50 (Toyo Roshi Co., Japan) with the solvent system 6:4:4 (v/v) 1-butanol–pyridine–water at 55°. Two-dimensional paper chromatography was employed[17] to survey the actions of various amylases on malto-oligosaccharides, ^{14}C-Reducing-end-labeled malto-oligosaccharides were prepared by incubating a mixture of D-[U-^{14}C]glucose (50 μCi, 35.2 μg, The Radiochemical Centre, Amersham, England) and cyclohexaamylose (20 mg) with *Bacillus macerans* amylase (10 THU) supplied by Dr. Kobayashi as described by French *et al.*[18]. These malto-oligosaccharides were chromatographed in the first direction by five ascents on a sheet of paper (30 × 25 cm, Toyo Filter Paper No. 50). The area lined by the oligosaccharides on the chromatogram was sprayed uniformly with 5 mL of amylase solution of 0.1 IU per mL. The paper was then incubated in a moist-chamber for 1 h and the saccharides newly formed by enzyme action were subsequently separated by five ascents in the second direction. The paper was air-dried and the autoradiogram prepared by placing the paper in contact with Fuji X-ray film (Fuji film Co., Japan). The oligosaccharides on the paper chromatogram were also detected by the silver nitrate dip-method[19], using unlabeled malto-oligosaccharides as the substrates.

Enzyme digests. — All reactions were performed at 40° and pH 7.0 with the enzymes buffered with 0.05–0.1M phosphate buffer. For determination of the product specificity, solutions containing 0.5% of substrate and 0.02–0.30 IU of amylases per mg substrate were used in the digest. Samples (1 mL) taken at various times of reaction were inactivated by boiling for 5 min and the saccharide composition was analyzed by l.c.

The degradation of blue starch by various amylases were studied as follows. Blue starch (10 mg) was dissolved in 10 mL of phosphate buffer (pH 7.0, 0.1M), 1 IU of each enzyme was added, and the mixture was incubated at 40°. At various intervals, 2 mL of the mixture was withdrawn and inactivated by the addition of 0.5 mL of 0.5M aqueous sodium hydroxide. The inactivated mixture was diluted fourfold with deionized water and centrifuged at 2000 r.p.m. for 10 min. The absorbance of the supernatant solution was measured at 620 nm.

The action of various amylases on oxidized amylose was examined out as follows. The substrate solution (1 mL), in which was dissolved 10 mg of oxidized amylose by boiling for 10 min, was incubated with 0.8 mL of phosphate buffer and 0.2 mL of enzyme solution (1.5 IU) at 40°. The degree of hydrolysis was determined by the Somogyi–Nelson and phenol–sulfuric acid methods.

With 1% substrate solution and 0.4 IU of amylase per mg substrate, the actions of *Bacillus licheniformis* alpha amylase on G_6, G_7, and G_8 were also studied.

Determination of anomeric configuration. — Anomeric configurations of the oligosaccharides formed by action of the various amylases were determined by the method of Robyt *et al.*[20] with a digital polarimeter DIP-181 (Nihon Bunko Co., Japan). Short-chain amylose (d.p. 23, 25 mg) was dissolved in 5 mL of 0.01M Tris · HCl buffer (pH 7.0) by boiling for 10 min, and 1–3 IU of the enzyme was added. Measurements of optical rotation were made at various intervals by using a 5-cm tube with a heated jacket (40°). When the rotation became constant, 5 mg of anhydrous sodium carbonate was added per 5 mL of digest. This brought the pH to 10.5 and accelated the mutarotation.

For control experiments with BLA, glucoamylase, and beta amylase, 50 mg of short-chain amylose was dissolved in 10 mL of 0.01M acetate buffer (pH 6.0) by boiling for 10 min and 22–50 IU of enzyme was added. Optical rotations were measured in a 10-cm tube and the temperature of reaction was 28°. When the rotation became constant, 10 mg of anhydrous sodium carbonate was added per 10 mL of digest.

RESULTS

Product specificities of various amylases. — As may be seen in Fig. 1, the major initial product of action by *Streptomyces griseus* amylase on short-chain amylose was G_3. Prolonged action led to gradual hydrolysis of G_3 and production of G_2 and G_1. G_3 was produced to the extent of ~70% (w/w) after 60 min incubation.

Fig. 2 shows the action of *Pseudomonas stutzeri* amylase on short-chain amylose. G_4 was formed predominantly at an earlier stage of reaction. Such lower saccharides as G_3, G_2, and G_1 may have arisen from the original substrates.

The action of *Bacillus licheniformis* amylase on the substrate is shown in Fig. 3. The principal initial products were G_5, G_3, and G_6. At the achroic point (30 min), G_6 had disappeared. This amylase seemed to have a dual product-specificity

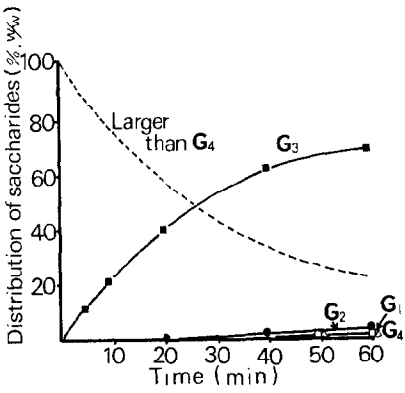


Fig. 1. The action of *Streptomyces griseus* amylase on short-chain amylose. A substrate concentration of 0.5% and 0.04 IU/mg of substrate were used in the digest.

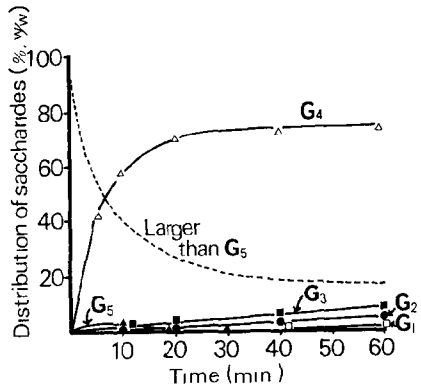


Fig. 2. The action of *Pseudomonas stutzeri* amylase on short-chain amylose. A substrate concentration of 0.5% and 0.29 IU/mg of substrate were used in the digest.

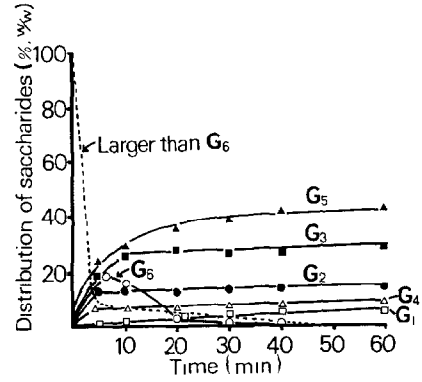


Fig. 3. The action of *Bacillus licheniformis* amylase on short-chain amylose. A substrate concentration of 0.5% and 0.16 IU/mg of substrate were used in the digest.

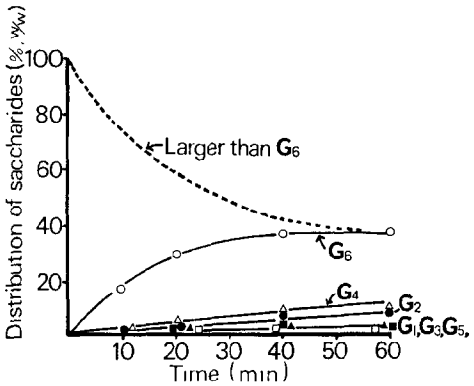


Fig. 4. The action of extracellular *Aerobacter aerogenes* amylase on short-chain amylose. A substrate concentration of 0.5% and 0.02 IU/mg of substrate were used in the digest.

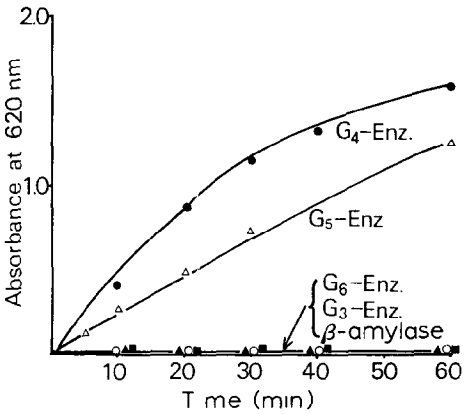


Fig. 5. The action of various amylases on water-insoluble cross-linked blue starch. Experimental details are described in the text.

for the formation of G_5 and G_3. The action pattern of the amylase resembles that of the alpha amylase from *Bacillus subtilis,* which mainly formed G_3 and G_6 as reported previously by Robyt *et al.*[1].

Fig. 4 shows the action of *Aerobacter aerogenes* amylase on the substrate. G_6 was mainly produced (~40%), and further reaction led to the gradual hydrolysis of G_6, which was degraded mainly to $G_4 + G_2$.

Furthermore, the amylases of *S. griseus, P. stutzeri,* and *A. aerogenes* caused a slower decrease in the iodine color, and the color did not disappear during incubation. In contrast, *B. licheniformis* amylase caused a fast decrease in the color, which disappeared after 30 min of incubation.

As previously reported[4,5,8], amylases of *Streptomyces griseus, Pseudomonas stutzeri,* and *Aerobacter aerogenes* degraded the borohydride-reduced, short-chain amylose by exo-attack to give G_3, G_4, and G_6, respectively, as those initial prod-

ucts. In contrast, *Bacillus licheniformis* amylase degraded the substrate by an endo-mechanism to give mainly G_5 and G_3.

Degradation of blue starch. — The actions of various amylases on blue starch are shown in Fig. 5. Among the four exo-amylases examined, only *Pseudomonas stutzeri* amylase catalyzed the degradation of blue starch. *Bacillus licheniformis* amylase also hydrolyzed the substrate. On the paper chromatogram of a *Pseudomonas stutzeri* amylase digest of blue starch, G_4 was the main product. The saccharide composition of a digest of the substrate by *Bacillus licheniformis* amylase was almost the same as that of a soluble-starch digest by the same amylase.

From these results, it is suggested that *Pseudomonas stutzeri* amylase is able to hydrolyze the blue starch by an endo-mechanism, as reported previously by Schmidt *et al.*[21]. By this characteristic action on blue starch, *Pseudomonas stutzeri* amylase differs from other exo-amylases.

Action on oxidized amylose. — A useful procedure for detection of alpha amylase contamination in exo-enzyme preparations, developed by Marshall and Whelan[11], involves the use of modified substrates containing barriers for exo-enzyme action, conveniently introduced by limited oxidation.

The results of hydrolysis of oxidized amylose by various amylases are shown in Fig. 6. An initial rapid release of maltose by the beta amylase was observed, which diminished to a negligibly low rate after ~25% hydrolysis of the oxidized amylose. Further reaction of beta amylase was blocked by the oxidized glucose residues. Although the initial rate of hydrolysis by the *Aerobacter aerogenes* amylase was the same as that of beta amylase, the rate did not decrease appreciably after 25% hydrolysis. This result suggests that the enzyme is able to bypass the oxidized glucose residue and hydrolyze the internal chain of the amylose molecule. This reaction is quite similar to the action of the enzyme in bypassing the (1→6)-α-D-glucosidic bond of beta amylase limit dextrin of amylopectin to form branched oligosaccharides, as reported previously[22]. The other three amylases and the *Aerobacter aerogenes* amylase also hydrolyzed the substrate, and the degree of hydrolysis increased gradually.

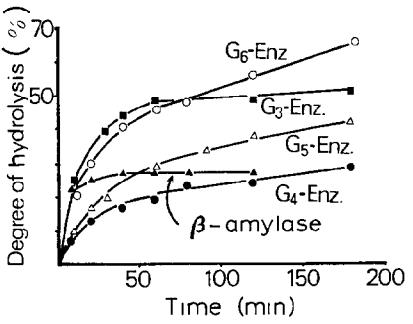


Fig. 6. The action of various amylases on partially-oxidized potato amylose. Experimental details are described in the text.

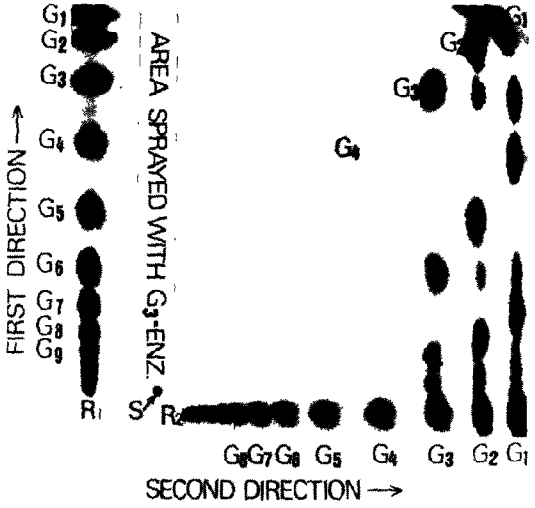


Fig. 7. Two-dimensional radioautogram showing the action of *Streptomyces griseus* amylase on ^{14}C-reducing-end-labeled malto-oligosaccharides. R_1 and R_2 are reference series for the first and second direction of the chromatogram. S is the point of application of the sample. After irrigation in the first direction, the left side of the chromatogram containing R_1 was cut off for reference. The remaining S channel was sprayed with the enzyme solution. After allowing enzyme action on the paper, the chromatogram was air-dried, reference R_2 was applied and the chromatogram was developed in the second direction.

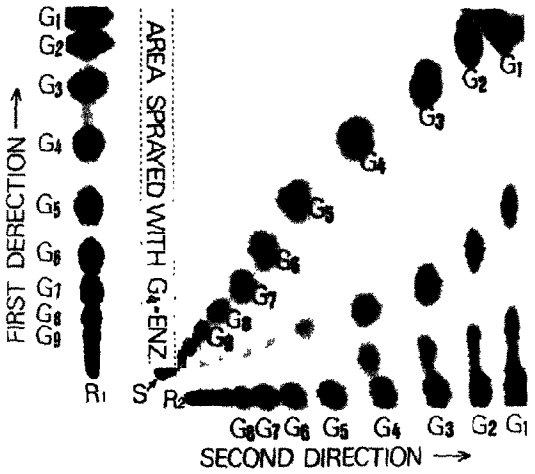
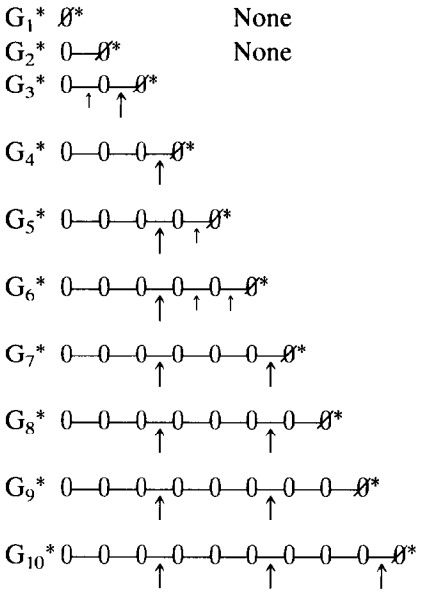


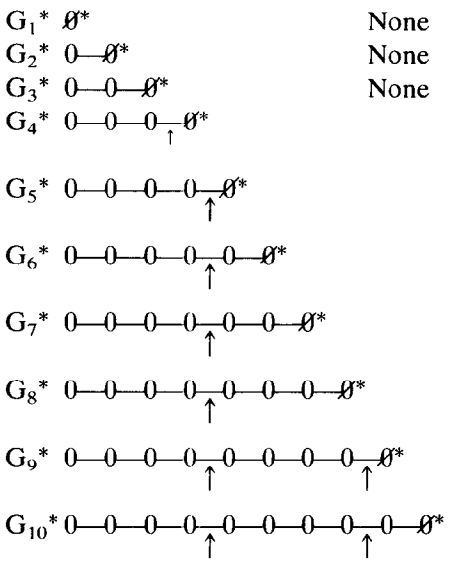
Fig. 8. Two-dimensional radioautogram showing action of *Pseudomonas stutzeri* amylase on ^{14}C-reducing-end-labeled malto-oligosaccharides. Symbols are as in Fig. 7.

Action patterns of various amylases on ^{14}C-reducing-end-labeled malto-oligosaccharides. — Streptomyces griseus *amylase.* Fig. 7 shows the result of action of *Streptomyces griseus* amylase on ^{14}C-reducing-end-labeled malto-oligosaccharides. On the autoradiogram, G_1^* + G_2^* and G_1^* were formed from G_3^* and G_4^*, respectively. G_5^* and G_6^* released G_2^* + G_1^* and G_3^* + G_2^* + G_1^*. G_1^*, G_2^*, and G_3^* were formed from G_7^*, G_8^*, and G_9^*. The results of autoradiography may be summarized schematically as follows (the arrows indicate qualitatively the frequency of bond cleavage):



These results coincide with those for unlabeled malto-oligosaccharides. Wako *et al.* presumed previously[4] from the action pattern of the enzyme on unlabeled malto-oligosaccharides that the amylase might be an exo-amylase. From our results, it is concluded that the enzyme is able to hydrolyze malto-oligosaccharides larger than G_3 to form G_3 by an exo-mechanism from the nonreducing end.

Pseudomonas stutzeri *amylase.* Fig. 8 illustrates the radioactive products formed by hydrolysis with *Pseudomonas stutzeri* amylase. G_4^* released a trace amount of G_1^*. G_5^* was hydrolyzed to form G_1^*. Other malto-oligosaccharides larger than G_5^* released G_4 by an exo-mechanism from the non-reducing end. No reaction was observed with G_2^* and G_3^*. The results of the autoradiogram may be summarized as follows (the arrows are as in Fig. 7):



From these results, it is considered that *Pseudomonas stutzeri* amylase has a high product-specificity for the formation of G_4, as described by Robyt *et al.*[5].

Bacillus licheniformis *amylase. Bacillus licheniformis* amylase is known to be a thermostable, endo-acting alpha amylase, and the enzyme characteristics were studied by Saito and Chang *et al.*[6,23]. The autoradiogram (Fig. 9) indicates that this enzyme hydrolyzes G_6* to form G_5 and G_1*. G_7* and G_8* were also hydrolyzed to form $G_5 + G_2$* and $G_5 + G_3$* by an exo-mechanism. G_3* was produced mainly from saccharides larger than G_8*. The action of the amylase on malto-oligosaccharides is summarized as follows (the arrows are as in Fig. 7):

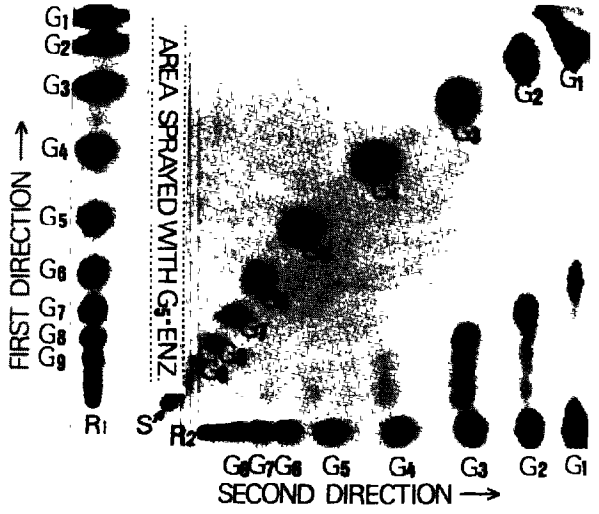
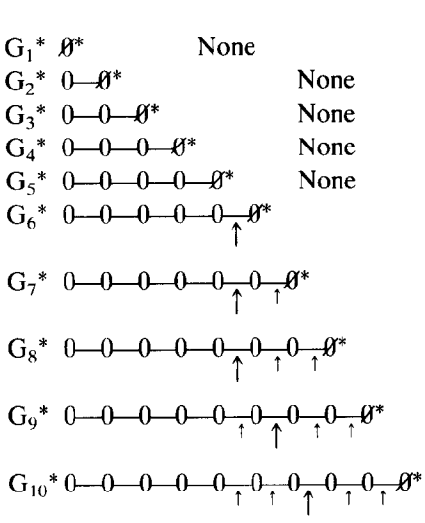


Fig. 9. Two-dimensional radioautogram showing action of *Bacillus licheniformis* amylase on ^{14}C-reducing-end-labeled malto-oligosaccharides. Symbols are as in Fig. 7.



Saito reported previously that G_6 released more $G_2 + G_4$ and $2G_3$ than $G_5 + G_1$, and that G_8 and G_9 were readily hydrolyzed to give various smaller oligosaccharides[6]. However, in our experiments, we observed that G_6 released $G_5 + G_1$ more than 2 G_3, and G_7 and G_8 were hydrolyzed to give mainly $G_5 + G_2$ and $G_5 + G_3$. The relative reaction-rate of the amylase on the substrates G_6, G_7, and G_8 was high (in the order $G_7 = G_8 > G_6$), and the initial velocity on G_7 and G_8 was five times as high as that on G_6.

Aerobacter aerogenes *amylase.* Fig. 10 shows the radioactive hydrolytic products formed by the action of extracellular amylase of *Aerobacter aerogenes.* The action of the amylase on malto-oligosaccharides may be summarized as follows (the arrows are as in Fig. 7):

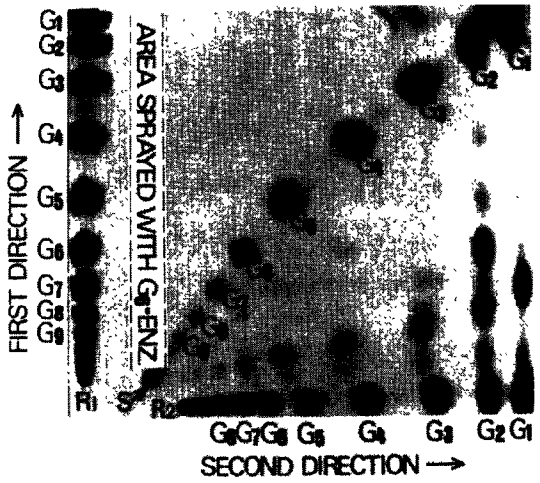
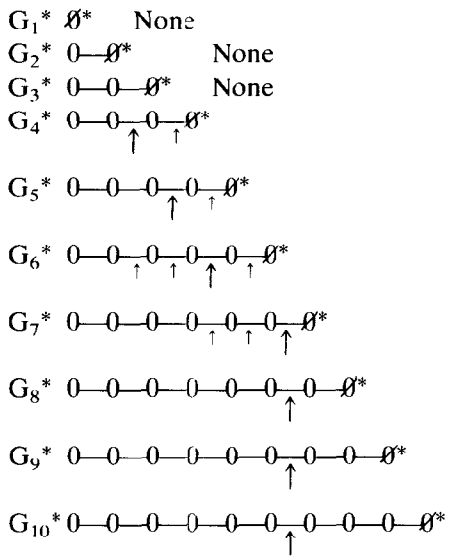


Fig. 10. Two-dimensional radioautogram showing action of *Aerobacter aerogenes* amylase on ^{14}C-reducing-end-labeled malto-oligosaccharides. Symbols are as in Fig. 7.



These results are the same as those reported on the cell-bound amylase previously described by Kainuma *et al.*[8].

Anomeric configuration. The anomeric configurations of the products obtained by action of various amylases were studied polarimetrically. Three amylases from *Bacillus subtilis*, soybean, and *Rhizopus niveus* were also studied for comparison (Fig. 11a). *Bacillus subtilis* alpha amylase, soybean beta amylase, and *Rhizopus niveus* glucoamylase gave products having the α, β, and β configurations, respectively, as reported by several authors[20,24,25]. Fig. 11b shows the results of the changes in optical rotation with time for three exo-amylases and an alpha amylase. All four amylases examined unexpectedly showed products having α anomeric configurations. The results are summarized in Table I. The anomeric form of the product of action of *Pseudomonas stutzeri* amylase is the same as that reported by Sakano *et al.*[26].

TABLE I

ANOMERIC CONFIGURATION OF PRODUCTS OF ACTION OF VARIOUS EXO-AMYLASES

Enzyme	*Anomeric form*
Glucoamylase [EC 3.2.1.3]	β
Beta amylase [EC 3 2 1.2]	β
Exo-maltotriohydrolase [EC 3.2.1.-]	α
Exo-maltotetraohydrolase [EC 3.2.1.60]	α
Exo-maltohexaohydrolase [EC 3.2.1 98]	α

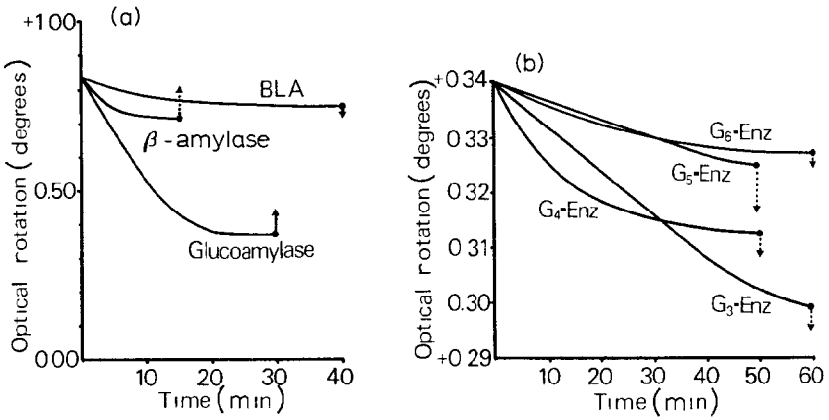


Fig. 11. Optical rotation studies of the actions of various amylases with amylose. The triangular symbol indicates the optical rotation after the addition of alkali to the digests. BLA represents alpha amylase from *Bacillus subtilis*. (a) Control: the amount of each enzyme per mg of substrate was as follows: BLA, 0.70 IU; betaamylase, 1.00 IU; glucoamylase, 0.43 IU; determinations in a 10-cm cell. (b) Various oligosaccharide-forming amylases. The amount of each enzyme per mg of substrate was as follows. G_3-Enz., 0.04 IU; G_4-Enz., 0.06 IU; G_5-Enz., 0.09 IU; G_6-Enz., 0.13 IU; determinations in a 5-cm cell.

DISCUSSION

Reese proposed a criterion to differentiate exo-glucanases and glucosidases by the anomeric configuration of their reaction products[3]. They reported that exo-glucanases act with inversion of configuration, whereas glucosidases act with retention. They made a comparative study on two sets of carbohydrases: α-D-glucosidase [EC 3.2.1.20] and exo-α-D-glucanase [glucoamylase, EC 3.2.1.13], β-D-glucosidase [EC 3.2.1.21], and exo-(1→3)-β-D-glucanase. Recently, we found that Reese's criterion seemed to fit only for glucoamylase and beta amylase as (1→4)-α-D-glucanases. In the present study, we examined three kinds of novel exo-glucanases:exo-maltotriohydrolase, exo-maltotetraohydrolase, and exo-maltohexaohydrolase. Reese's criterion is not applicable for these three exo-glucanases, which form such products as maltotriose, maltotetraose, and maltohexaose, which are of larger molecular-weight than the products of action of glucoamylase and beta amylase.

All of the exo-glucanases examined in this study unexpectedly formed malto-oligosaccharides having the α configuration. The action of these exo-amylases on partially oxidized amylose[11] revealed that the chemically modified glucose residues did not block the action of these enzymes. Normally, modified glucose residues block the action of glucoamylase and beta amylase[11], but the three exo-acting amylases, having larger binding-sites than glucoamylase and beta amylase, are capable of bypassing the modified glucose residues. The exo-maltotetraohydrolase of *Pseudomonas stutzeri* hydrolyzes a water-insoluble, cross-linked blue starch that has bulky substituents on the chains and is normally considered to be hydrolyzed

only by alpha amylase. For the comparative study, we used *Bacillus licheniformis* alpha amylase, which accumulates G_5 by endo action on starch. This amylase hydrolyzes partially oxidized amylose and blue starch, a behavior characteristic of alpha amylase. The amylase also hydrolyzed G_6, G_7, and G_8 to form maltopentaose by exo attack.

Although these results are somewhat confusing for the classification of amylases, we emphasize that Reese's criterion is applicable only for glucoamylase and beta amylase among the amylases, and there are several exo-amylases that do not fit into this classification. From these results, we propose to classify these novel exo-amylases, such as exo-maltotriohydrolase, exo-maltotetraohydrolase, and exo-maltohexaohydrolase as "exo-alpha amylase" as proposed by Sakano *et al.*[26] for exo-maltotetraohydrolase.

We also found another example of an exception to Reese's postulation in the action of dextranases. Walker mentioned that endo-dextranases caused inversion of configuration and exo-dextranases acted with retention[27].

REFERENCES

1 J. F. ROBYT AND D. FRENCH, *Arch. Biochem. Biophys.*, 100 (1963) 451–467.
2 S. OKADA, S. SUZUKI, AND M. HIGASHIHARA, *Agric. Biol. Chem.*, 33 (1969) 900–906.
3 E. T. REESE, A. H. MAGUIRE, AND F. W. PARRISH, *Can. J. Biochem.*, 46 (1968) 25–34.
4 K. WAKO, S. HASHIMOTO, S. KUBOMURA, K. YOKOTA, K. AIKAWA, AND J. KANAEDA, *J. Jpn. Soc. Starch Sci.*, 26 (1979) 175–181.
5 J. F. ROBYT AND R. J. ACKERMAN, *Arch. Biochem. Biophys.*, 145 (1971) 105–114.
6 N. SAITO, *Arch. Biochem. Biophys.*, 155 (1973) 290–298.
7 K. KAINUMA AND S. SUZUKI, *Proc. Int. Symp. Conversion Manuf. Foodstuffs Microorganisms, Kyoto*, Japan, (1971) 95–98.
8 K. KAINUMA, S. KOBAYASHI, T. ITO, AND S. SUZUKI, *FEBS Lett.*, 26 (1972) 281–285.
9 T. NAKAKUKI AND K. KAINUMA, *J. Jpn. Soc. Starch Sci.*, 29 (1982) 27–33.
10 K. KAINUMA, K. WAKO, A. NOGAMI, AND S. SUZUKI, *J. Jpn. Soc. Starch Sci.*, 20 (1973) 112–119.
11 J. J. MARSHALL AND W. J. WHELAN, *Anal. Biochem.*, 43 (1971) 316–321.
12 H. DELLWEG, M. JOHN AND J. SCHMIDT, *Eur. J. Appl. Microbiol.*, 1 (1975) 191–198.
13 T. NAKAKUKI, K. AZUMA, M. MONMA, AND K. KAINUMA, *J. Jpn. Soc. Starch Sci.*, 29 (1982) 188–197.
14 K. KAINUMA, T. NAKAKUKI, AND T. OGAWA, *J. Chromatogr.*, 212 (1981) 126–131.
15 N. NELSON, *J. Biol. Chem.*, 153 (1944) 375–380.
16 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
17 D. FRENCH, A. O. PULLEY, M. ABDULLAH, AND J. C. LINDEN, *J. Chromatogr.*, 24 (1966) 271–276.
18 D. FRENCH, M. L. LEVINE, E. NORBERG, P. NORDIN, J. H. PAZUR, AND G. M. WILD, *J. Am. Chem. Soc.*, 76 (1954) 2387–2390.
19 K. KAINUMA AND D. FRENCH, *FEBS Lett.*, 6 (1970) 182–186.
20 J. ROBYT AND D. FRENCH, *Arch. Biochem. Biophys.*, 104 (1964) 338–345.
21 J. SCHMIDT AND M. JOHN, *Biochim. Biophys. Acta*, 566 (1979) 88–99.
22 K. KAINUMA, K. WAKO, S. KOBAYASHI, A. NOGAMI, AND S. SUZUKI, *Biochim. Biophys. Acta*, 410 (1975) 333–346.
23 J. P. CHIANG, J. E. ALTER, AND M. STERNBERG, *Staerke*, 31 (1979) 86–92.
24 E. FUWA AND J. NIKUNI, *J. Agric. Chem. Soc. Jpn.*, 26 (1952) 154–159.
25 S. ONO, K. HIROMI, AND Z. HAMAUZU, *J. Biochem. (Tokyo)*, 57 (1965) 34–38.
26 Y. SAKANO, Y. KASHIWAGI, AND T. KOBAYASHI, *Agric. Biol. Chem.*, 46 (1982) 639–646.
27 G. J. WALKER, *Int. Rev. Biochem.*, 16 (1978) 89–97.