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Amylase Action in Maltodextrin-Sodium Dodecylsulfate Solution

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Enzyme catalyzed hydrolysis of maltodextrin was studied with enzyme-maltodextrin and enzyme-maltodextrin-sodium dodecylsulfate systems. Sodium dodecylsulfate (SDS) is a complex forming agent for maltodextrin. When endo-enzyme such as Taka-amylase A was used as the enzyme, ratio of the initial velocity of the hydrolysis of the system containing SDS to that without SDS decreases with the increase of degree of polymerization (D.P.) of the maltodextrin and becomes zero with the maltodextrin of D. P. above about 60. When the liquefying α-amylase of Bacillus subtilis was used as an endo-enzyme, a similar result was also obtained. When exo-enzyme such as Glucoamylase of Rh. Delemar was used, the ratio decreases gradually with the increase of D.P. of maltodextrin, and remains in a relatively high level even in the case of maltodextrin of D.P. above 60. In the case of Taka-amylase A, the enzyme action is suppressed by the presence of the stiff helical segments of SDS complex and the initial velocity of the system with SDS decreases compared with the one without SDS. In the case of Glucoamylase, the presence of the stiff helical segment does not distinctively induce such a depression of the enzyme action as that in the case of Taka-amylase A. The depression is partly dependent on the inhibitory interaction of SDS with the enzyme.

According to recent studies on the conformation of amylose (or maltodextrin[*2]) molecules in

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[*2] Maltodextrin is a degraded amylose, *i. e.* a relatively low molecular weight linear polysaccharide linked by α-1,4 glucosidic linkages.

water,[1-4] amylose behaves like a random coil, while a relatively large percentage of molecule has a helical structure, the individual helical segments being connected by random segments. The amylose-iodine complex in solution is assumed to have a similar structure as stated above in which the polyiodine chain is formed in the axis of the helical segment. The amylose-sodium dodecylsulfate complex[3,5,6] is assumed to have a similar structure as the iodine complex. The character of the helical segment of amylose in the absence of the complexing agent is different from that of the complex. In the absence of the complexing agent, the thermal fluctuation in the conformation of the molecular chain occurs between the helical and random segments. Thus the helical segment has a relatively flexible character and the conformation is designated as deformed helix.[1,2,7] The helical segment in the complex is assumed to be relatively stiff owing to the holding of the iodine or sodium dodecylsulfate molecules inside the helical segment.

Studies have been carried out to elucidate the difference in the characteristics of the enzyme actions of two types of amylases (*i.e.* endo type and exo type) in enzyme-maltodextrin-sodium dodecylsulfate system.

Experimental

Enzyme. Endo-type: Taka-amylase A (TAA) (molecular weight, 53000) was prepared from "Taka-diastase Sankyô" according to the method of Akabori *et al.*[8] and kept as 4.25% aqueous solution containing 0.02M calcium acetate.

Liquefying α-amylase of Bacillus subtilis (BLA) (molecular weight, 45000) was supplied by Ueda Chemical Industry.

Exo-type: Glucoamylase of Rh. Delemar (GA) (molecular weight, 47000) was supplied by Professor J. Fukumoto of Osaka City University and Dr. Y. Tsujisaka of Osaka Municipal Technical Research Institute.

Concentration of these enzymes was determined by absorbance at 280 mμ, assuming $E^{1\%}_{1cm}$=22.0, 25.0 and 14.0 for TAA, BLA and GA, respectively.

Substrate. Maltodextrins: fractionated maltodextrins were prepared according to Hiromi *et al.*[9] A commercial product of potato amylose from Pierce Chemical Co., Illinois was partially hydrolyzed by using TAA at pH=4.5 and at 35°C for 45 min. The partially degraded amylose was precipitated by the addition of ethanol and washed with distilled water and dried. Using this preparation, the fractionation was carried out according to Everett and Foster[10] by employing dimethyl sulfoxide as solvent and ethanol as precipitant. Five subfractions were obtained and designated as maltodextrin I, II, III, IV and V. Maltodextrin VI was prepared according to the method of Hizukuri *et al.*[11] as a crystalline product from a highly concentrated aqueous solution (about 70%) of an acid hydrolyzed dextrin mixture. Determination of the carbonyl groups as the reducing end of each maltodextrin was made by a modified Somogyi-Nelson method.[12] The values of number-average molecular weight and degree of polymerization (D.P.) are listed in Table 1. These maltodextrins were the same as those used for the polarographic studies of starches by Takagi *et al.*[13]

Table 1. Number-average molecular weight ($\bar{M}_n$) and degree of polymerization (D.P.) of substrates

Substrate	$\bar{M}_n$	D.P.
Maltodextrin I	19000	116.6
II	12000	71.5
III	8200	50.5
IV	6200	38.4
V	4600	28.4
VI	2040	12.6
Maltoheptaose	1150	7.0
Maltose	342	2.0

Maltoheptaose was prepared from β-Shardinger's dextrin according to the method of French *et al.*[14]

Maltose was a commercial product and was used after recrystallization four times.

Helical Complex Forming Agent. Sodium dodecylsulfate (SDS), used as the complex forming agent with the substrate molecules, was a commerical product of Ishizu Pure Chemicals and was extracted by ethyl ether using Soxhlet extractor for 15 hr in order to remove unreacted higher alcohol.

Procedure. For the preparation of the reaction mixture suitable to the present experiment, the following three factors were considered.

(1) There should be a linear relationship between the increase of the number of reducing ends produced

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and the reaction time.

(2) The amount of SDS in reaction mixture must be high enough for the formation of a sufficient quantity of helical complex in order to detect the influence of the presence of the complex on the reaction velocity.

(3) The concentration of SDS must be lower than the one where inactivation of enzyme owing to the interaction between SDS and enzyme, and/or the formation of aggregates or precipitates of the SDS-maltodextrin complex occurs.

The procedure adopted for following the reaction of the TAA-substrate-SDS system was as follows. Twenty m*l* of 0.05% substrate solution with or without 0.015% SDS was taken into a reaction vessel immersed in a constant-temperature water bath controlled at 25°C. After about 30 min the reaction was commenced by introducing 10 μl of 2.5×10^{-2}% TAA solution to the substrate solution, and at 2 min intervals the reaction mixture was pipetted into 3 m*l* of Somogyi's reagent, and the reaction was stopped. The number of reducing ends produced was determined by means of Somogyi-Nelson method and was expressed by the value of absorbance observed at the wavelength of 530 mμ. A similar procedure as stated above was adopted for the other enzyme reaction systems. The final recipes for the reaction mixtures were as follows: TAA; 2.0×10^{-9}M, substrate; 0.05%, SDS; 0.5×10^{-3}M, and pH=5.3 for TAA system; BLA; 2.8×10^{-9}M, substrate; 0.1%, SDS; 1.0×10^{-3}M, and pH=5.85 for BLA system; GA; 1.0×10^{-8}M, substrate; 0.05%, SDS; 0.5×10^{-3}M, and pH=4.5 for GA system.

The amount of SDS in the above reaction mixture satisfies the condition of one SDS molecule per one helical turn, provided that all anhydroglucose units of maltodextrin (or amylose) take part in helix formation and 6 anhydroglucose units form one helical turn. Under this condition the complex, SDS-maltodextrin I, II or III showed a slight tendency to form an aggregate in solution, and the reaction was made under continuous stirring to prevent the formation of a large visible aggregate.

Results and Discussion

Typical examples of the change in absorbance with reaction time are shown in Figs. 1, 2 and 3. Each plot was found to show a good linear relationship within the interval of 18 min. The initial velocity was obtained from the slope of the plot shown in these figures. The ratio of the initial velocity of the system containing SDS to that without SDS was expressed in % and designated as "reactivity factor" or *R*. The value of *R* may be primarily determined by the ratio of the number of hydrolyzable α-1,4 glucosidic linkage present in the reaction mixture at the initial stage of reaction

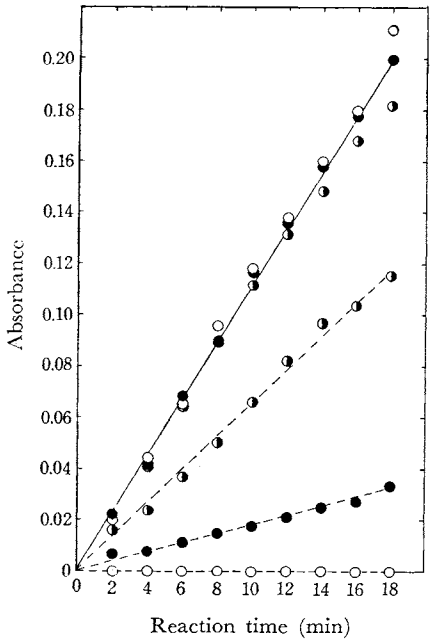

Fig. 1. The plot of absorbance *vs.* reaction time obtained with the system of TAA: 2×10^{-9}M, Substrate: 0.05%, with or without 0.5×10^{-3}M SDS, pH: 5.3, and at 25°C.
—○—: TAA-Maltodextrin I, ···○···: TAA-Maltodextrin I-SDS, —●—: TAA-Maltodextrin IV, ···●···: TAA-Maltodextrin IV-SDS, —◑—: TAA-Maltodextrin VI, ···◑···: TAA-Maltodextrin VI-SDS

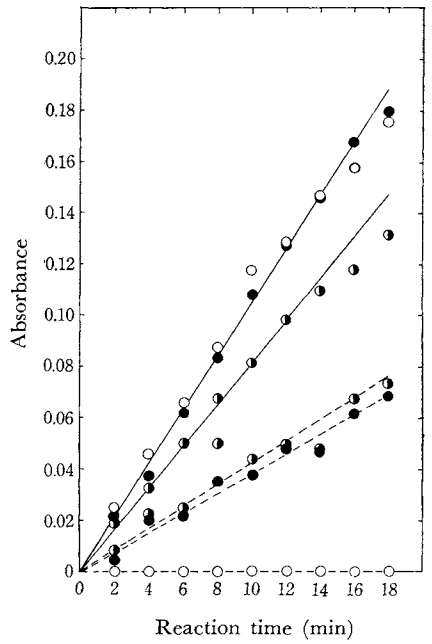

Fig. 2. The plot of absorbance *vs.* reaction time obtained with the system of BLA: 2.8×10^{-9}M, Substrate: 0.10%, with or without 1.0×10^{-3}M SDS, pH: 5.85, and at 25°C.
—○—: BLA-Maltodextrin I, ···○···: BLA-Maltodextrin I-SDS, —●—: BLA-Maltodextrin IV, ···●···: BLA-Maltodextrin IV-SDS, —◑—: BLA-Maltodextrin VI, ···◑···: BLA-Maltodextrin VI-SDS

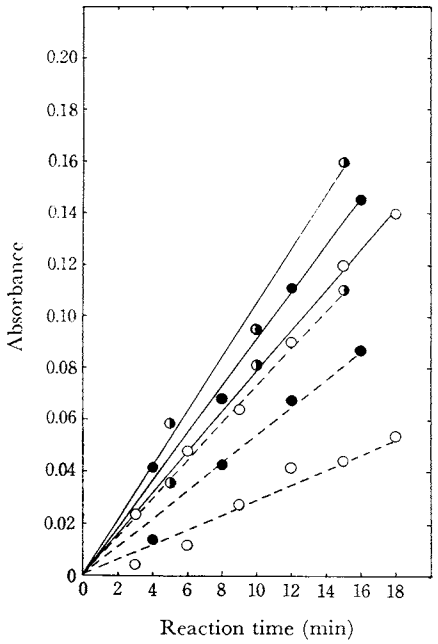

Fig. 3. The plot of absorbance *vs.* reaction time obtained with the system of GA: 1.0×10^{-8}M, Substrate: 0.05%, with or without 0.5×10^{-3}M SDS, pH: 4.5, and at 25°C.
—○—: GA-Maltodextrin I, ···○···: GA-Maltodextrin I-SDS, —●—: GA-Maltodextrin IV, ···●···: GA-Maltodextrin IV-SDS, —◑—: GA-Maltodextrin VI, ···◑···: GA-Maltodextrin VI-SDS

in the system containing SDS to that without SDS.

Figures 4,5 and 6 and Table 2 show the change of R with D.P. of substrate. In TAA (endo-enzyme) system where TAA can hydrolyze α-1,4 glucosidic linkage at random along the linear chain of substrate, the initial velocity of TAA-low D.P. substrate (maltoheptaose)-SDS system was as high as that without SDS, and the value of R

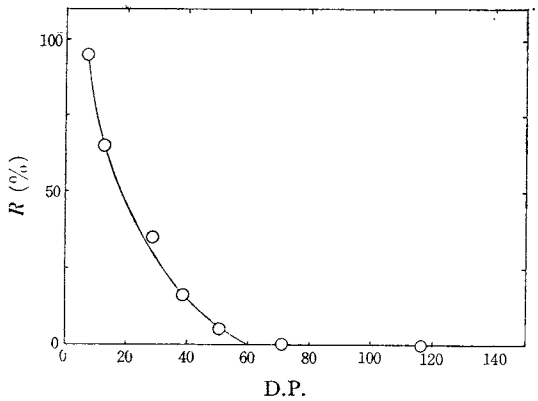

Fig. 4. The plot of R *vs.* D.P. with TAA-Maltodextrin-SDS system (TAA: 2.0×10^{-9}M, SDS: 0.5×10^{-3}M, Substrate: 0.05%, pH: 5.3, at 25°C).

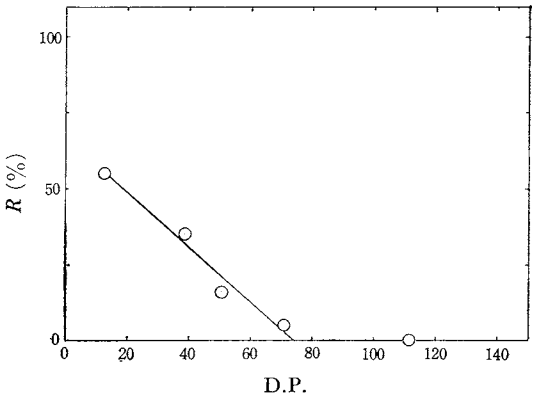

Fig. 5. The plot of R *vs.* D.P. with BLA-Maltodextrin-SDS system (BLA: 2.8×10^{-9}M, SDS: 1.0×10^{-3}M, Substrate: 0.10%, pH: 5.85, at 25°C).

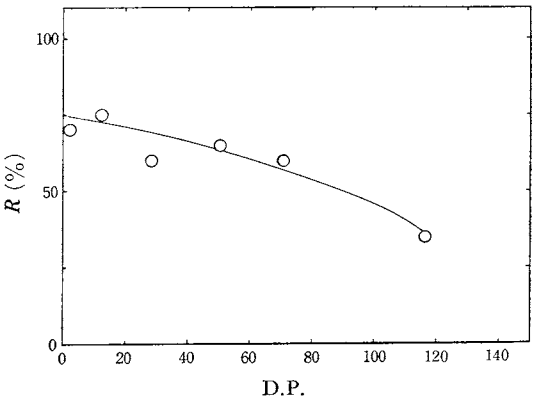

Fig. 6. The plot of R *vs.* D.P. with GA-Maltodextrin-SDS system (GA: 1.0×10^{-8}M, SDS: 0.5×10^{-3}M, Substrate: 0.05%, pH: 4.5, at 25°C).

Table 2. The relation between R and D.P. of substrate with three kinds of systems using TAA, BLA and GA as enzyme, respectively

D.P.	R (%)		
of substrate	TAA	BLA	GA
116.6	0	0	35
71.5	0	5	60
50.5	5	17	65
38.4	20	35	60
28.4	30	—	—
12.6	70	55	75
7.0	95	—	70

was as high as 95%. The value of R decreases rapidly with the increase of D.P. of the substrate and tends to 0% at the D.P. value of about 60 (molecular weight, 1×10^4). In the presence of SDS, the substrate having higher D.P. value than

60, was not hydrolyzed by TAA within the reaction time of 18 min.

As shown in Fig. 5, in BLA (endo-enzyme) system, a similar result was obtained between R and D.P. of substrate as in the case of TAA system.

In GA system (Fig. 6) where GA is an exo-enzyme which catalyzes the hydrolysis of maltodextrin producing glucose from its non-reducing end, the value of R for the substrate of low D.P. value was lower than that in the case of TAA system. However, the rate of decrease of R with the increase of D.P. was slight compared with that in the case of TAA, and a fairly high value of R of 60—65% was obtained with the substrate of D.P. 60—70 to which TAA and BLA could not react in the presence of SDS.

The difference in the characteristics of the enzyme action between the endo-enzyme and exo-enzyme as described above is expected to be brought about primarily by the difference in the contribution of the helical segments of the SDS complex on the reaction. The following experiments on the iodine complex of maltodextrin were carried out to elucidate the conformation of maltodextrin and the SDS complex in solution.

The absorption spectra of the maltodextrin-iodine complex were recorded by a Hitachi Recording Spectrophotometer Model EPS-3 at 25°C. Figure 7 shows the relation between λ_{max} (wave-

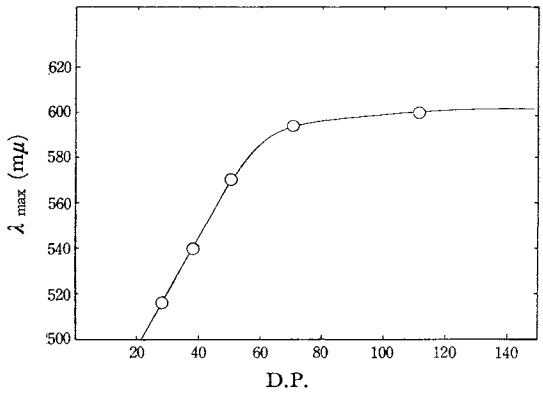

Fig. 7. The relation between the λ_{max} and D.P. of maltodextrin; maltodextrin: 3.7×10^{-3}%, KI: 8.0×10^{-3}M, I_2: 8.0×10^{-5}M, pH: 5.3, at 25°C. (This composition corresponds to the one containing minimum amount of iodine, where the value of λ_{max} takes the longest wavelength, irrespective of the values of D.P. of maltodextrins.)

length of the maximum of absorption spectra observed in the range of wavelength of 400—600 mμ) and D.P. at pH=5.3. As shown in Fig. 7, with increasing D.P. the value of λ_{max} shows a remarkable shift toward longer wavelength up to the D.P. value of about 60, and the plot of λ_{max} *versus* D.P. revealed a break point at about D.P.=60. Above D.P.=70 the value of λ_{max} changes only slightly with the increase of D.P. and the same result was also obtained at pH 4.5. In a previous publication[15] one of the present authors (S.O.) showed that the λ_{max} of the iodine complex shifts toward longer wavelength with the increase of the length of the polyiodine chain. Thus the shift of λ_{max} with the increase of D.P. up to about 60, corresponds to the increase of the length of the single polyiodine chain in the complex. We (T.W. and S.O.) also showed that the helical segments of amylose molecule (molecular weight: 1×10^6) were composed of 66 anhydroglucose units.[16] Therefore the value of about 60 corresponds to the maximum number of anhydroglucose units which take part in the formation of the helical segment.

From the results obtained with the iodine complex, the conformation of the maltodextrin of D.P. above 60—70 in the absence of complexing agent in acidic solution may be a deformed helix. The individual helical segments are connected by random segments which are composed of a certain number of the anhydroglucose units. There are the freely rotating endgroups of a certain number of anhydroglucose units which do not take part in the helix formation. The maltodextrin of D.P. below about 60 consists of a single helix with the freely rotating endgroups.

As shown in Fig. 4, in the case of TAA-substrate-SDS system, the substrates of D.P. from 7 to 60 were hydrolyzed by the enzyme. The initial velocity obtained with the system of low D.P. substrate as maltoheptaose in the presence of SDS was essentially the same as the one without SDS. It may be assumed that the SDS molecules do not inhibit the TAA action under the present experimental conditions. The decrease in the value of R with the increase of D.P. of substrate shows that the SDS complex is difficult to be hydrolyzed by TAA compared with maltodextrin uncoupled with SDS. When the length of the freely rotating endgroups is assumed to be constant independent of the D.P. of the substrate, the ratio of the number of anhydroglucose units in endgroups to that of the helical segment decreases with the increase of D.P. of substrate. Therefore the number of hydrolyzable α-1,4 glucosidic linkage decreases with the increase of D.P. and the value of R decreases with the increase of D.P. In the case of substrate of D.P. above 60 where the helical segments are stabilized by the complex formation, both random segments and endgroups which can be hydrolyzed by TAA in the absence of SDS, would not be hydrolyzed by TAA due to the steric hindrance of the stiff helical segments.

As shown in Fig. 6, in the case of GA-substrate-

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SDS system, the enzyme action can be assumed to be inhibited by free SDS in solution to some extent, since the value of R was only 70% with the system using maltose as the substrate which can not form the helical complex with SDS. The inhibition of the enzyme action of GA by SDS can be suggested by the result of the measurement of difference spectra.

Absorption spectra obtained with GA solution and GA-SDS solution are shown in Fig. 8. A

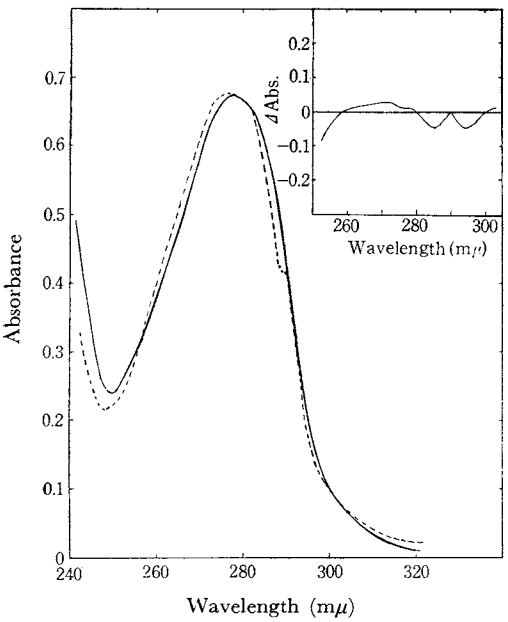

Fig. 8. Absorption spectra of 0.453% GA solution (solid line) and 0.453% GA and 0.2% SDS solution (broken line) at 25°C and difference spectrum (inset).

distinct difference in shape was observed between the two curves. The difference spectrum was recorded by a Hitachi Recording Spectrophotometer Model EPS-3, using four cells; two of them were put on one side of the light path in series, one containing enzyme solution and the other SDS solution, respectively, and on the other side were put the other two cells containing a mixture of enzyme and SDS in one cell, and the buffer solution only in the other, respectively. The spectrum is shown in the inset of Fig. 8. From these results, some interaction is expected to occur between GA and SDS. This interaction suggests the inhibition of the enzyme action of GA. The difference in absorption spectra as in the case of GA was not observed between TAA and TAA-SDS solutions.

In spite of the inhibition of GA action by SDS, the enzyme GA still hydrolyzed the SDS complex of the substrate of D.P. above 60, *i.e.* the values of R are 60% for D.P. 70 and 35% for D.P. 117 (Table 2). These results are markedly different from those obtained with TAA and BLA. When the number of anhydroglucose units of the freely rotating endgroups of maltodextrin chain is assumed to be constant independent of chain length, the ratio of the number of anhydroglucose units in the helical segment to that in whole molecule would become larger with the increase in D.P. in the case of the substrate of D.P. below 60. Thus the amount of SDS combined with the substrate would be smaller and that of SDS free in solution larger in the lower D.P. substrate system compared with the case of the higher D.P. system. In the system containing substrate of D.P. above 60, the concentration of free SDS is assumed to be constant according to the results obtained with the iodine complex as shown in Fig. 7. Thus the enzymatic activity would be more inhibited by free SDS with the system containing lower D.P. substrate, and the value of R is expected to increase with the increase of D.P. of the substrate up to about 60 and become constant with the further increase of D.P. Contrary to expectation, the value of R actually observed decreases gradually with the increase of D.P. of the substrate, though it is fairly high with the complex of high D.P. substrate which was not hydrolyzed by TAA and BLA. The fact that a relatively high value of R was obtained with the system of higher D.P. substrate may support the assumption that the endgroups of the complex are freely rotating and can be hydrolyzed by GA, irrespective of the value of D.P. of the substrate. The decrease in the value of R with the increase of D.P. of the substrate may be explained by the following reasons.

(1) The length of the endgroups of the substrate molecule becomes shorter with the increase of D.P. and consequently the affinity of the substrate for GA becomes smaller.

(2) Even though the length of the endgroups is constant, the stiff helical structure of the complex interferes with the enzymatic action.

(3) Effective concentration of the substrate decreases, which has endgroups with favorable number of anhydroglucose units to be hydrolyzable with GA.

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