Arthrobacter globiformis W31の産生する2種のエンドデキストラナーゼの精製および特性

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Purification and Characterization of Two *Endo*-dextranases from *Arthrobacter globiformis* W31

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Two *endo*-dextranases (dextranases I and II) were highly purified from cell-free culture broth of *Arthrobacter globiformis* W31 by consecutive column chromatographies. The purified enzymes were judged to be homogeneous on Native- and SDS-PAGE as well as isoelectric focusing, and their p*I*s and molecular masses to be 4.3, 107 kDa (dextranase I) and 4.5, 70 kDa (dextranase II). Both purified enzymes had an identical N-terminal amino acid sequence of NH_2-S-V-A-P-L-A-S-T-P-T-L-T-T-W-. Optimum pH values of both enzymes were pH 6.0. The anomeric configurations of the enzymatic hydrolysis products from dextran T-2000 were α-form, indicating that the α-glucosidic linkages in the substrates are retained. The enzymes split dextran T-2000 in an *endo*-lytic fashion to produce predominantly isomaltotriose, a series of isomaltooligosaccharides having a DP of more than 4 and an appreciable amount of unknown oligosaccharides. Furthermore, neither enzyme had any action on isomaltose, isomaltotriose or even isomaltotetraose.

Many dextranases have been isolated from various microorganisms,[1)] and most of these dextranases hydrolyzed dextran in an *endo*-wise. Some papers have reported about isomaltotriose-producing *endo*-dextranases.[2–4)] The hydrolysis products from dextran molecules by these dextranases were mainly isomaltotriose and a series of isomaltooligosaccharides having a DP of more than 4.

On the other hand, various kinds of dextranases such as glucodextranase,[5,6)] isomalto-dextranase[7)] and *endo*-dextranase[8)] have been reported from *Arthrobacter* genus. In the course of our dextranase studies, we isolated a new strain of *Arthrobacter* producing two *endo*-dextranases from soil. These dextranases released isomaltotriose from dextran as a primary product. A series of unknown sugars was also detected on a paper chromatogram between each isomaltooligosaccharide having a DP of more than 3.

In this paper, we describe the purification and characterization of two *endo*-dextranases from *Arthrobacter globiformis* W31.

MATERIALS AND METHODS

Chemicals. Dextran T-2000 (a product of *Leuconostoc mesenteroides* NRRL B-512F, M_r 2×10^6), carrier ampholyte (pH 3.5–5), DEAE-Sepharose, and Mono Q HR 5/5 were products of

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Abbreviations : DEAE-, diethylaminoethyl-; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodesylsulfate; NBS, *N*-bromosuccinimide; EDTA, ethylenediaminetetraacetic acid; DP, degree of polymerization.

Pharmacia Biotech AB, Uppsala, Sweden. Phenyl Toyopearl 650M was a product of Tosoh, Japan, Bio-Gel P-100 from Bio-Rad Inc., crystalline bovine serum albumin from Miles Laboratories Inc., USA, polypeptone from Daigo Eiyo Kagaku Co., Ltd., Japan, and meat extract from Kyokuto Seiyaku Kogyo Co., Ltd., Japan. Standard proteins (SDS-6H) were products of Sigma Chemical Company, St. Louis, USA. Clinical dextran (Meito 70) was kindly supplied by Meito Sangyo Co., Ltd., Japan. Isomaltooligosaccharides (DP 3–8) were prepared from the partial acid hydrolyzate of dextran T-2000 using carbon-celite and Bio-Gel P-2 column chromatographies.[9] All other reagents were of analytical grade.

Microorganism and culture conditions. The microorganism used for the production of dextranases was isolated from soil, and identified as a gram positive bacterium, *Arthrobacter globiformis* W31.

The standard culture medium for enzyme production was composed of 3 L of a liquid medium containing 1.3% clinical dextran (Meito 70), 0.2% NH_4NO_3, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.04% $CaCl_2 \cdot 2H_2O$, 0.3% polypeptone, 0.1% asparagine, and 0.3% meat extract. The medium was sterilized in an 11.5 L jar fermentor (Model M-200, Tokyo Rikakikai Co., Ltd.) at 120°C for 20 min.

The precultured broth (200 mL) of *A. globiformis* W31 grown in the same medium for 2 days, was inoculated into the standard culture medium, and then the cultivation was performed at 30 °C for 70 h at the airation rate of 7.5 L/min and the rotation speed of 100 rpm. The cell-free culture broth was obtained by refrigerated centrifuge (10,000×g), and used as a starting material for enzyme purification.

Substrates and enzyme assay. Dextran T-2000 was used as a substrate in the enzyme assay. Dextran-saccharifying activity was used as a standard assay. The reaction mixture contained 0.5 mL of 1% dextran T-2000, 1.0 mL of 40 mM acetate buffer (pH 6.0) containing 10 mM calcium acetate, and 0.5 mL of the enzyme solution. The mixture was incubated at 30°C for an appropriate period. Reducing sugars produced per mL of the mixture were measured by the method of Somogyi[10]-Nelson.[11] One unit of enzyme activity was defined as the amount of enzyme that catalyzed the liberation of reducing sugars equivalent to 1.0 μmol of D-glucose from the substrate per min under the above conditions.

Dextrans from *L. mesenteroides* NRRL B-523S, B-742S, B-1355S and B-1399 were the generous gifts from Dr. A. Jeanes of the Northern Regional Research Center, Peoria, Ⅲ., USA. Total sugar was measured by the phenol-sulfuric acid method[12] with D-glucose as a standard. The extent of hydrolysis toward the substrate by the enzyme was calculated as the percentage of reducing sugar per total sugar amount in the reaction mixture.

Protein assay. Protein concentration was measured by the method of Lowry *et al.*[13] with crystalline bovine serum albumin as a standard. The absorbance at 280 nm was used for monitoring protein in column effluents.

Purification of the enzymes. Unless otherwise stated, all purification procedures for the enzyme were done in a cold room (4°C).

Step 1. Ammonium sulfate fractionation. Solid ammonium sulfate was added to the cell free culture broth to 90% saturation. After being kept overnight, the solution was subjected to centrifugation at 10,000×g. The precipitates were dissolved in about 130 mL of 20 mM acetate buffer (pH 6.0) containing 5 mM calcium acetate.

Step 2. DEAE-Sepharose column chromatography. The precipitate solution was then put on a column of DEAE-Sepharose (2.2 ϕ×50 cm) preequilibrated with 20 mM acetate buffer (pH 6.0) containing 5 mM calcium acetate. After washing the column with the same buffer, the enzyme was eluted with the same buffer containing 0.2 M NaCl. The fractions contained most of the dextranase activity were collected and then concentrated. The concentrated solution was dialyzed overnight against 20 mM acetate buffer (pH 6.0) containing 5 mM calcium acetate.

Step 3. Bio-Gel P-100 column chromatography. The dialyzed sample was loaded on a column of Bio-Gel P-100 (1.8 ϕ×110 cm) preequilibrated with 20 mM acetate buffer (pH 6.0) containing 5 mM calcium acetate. The enzyme was eluted

with the same buffer. Two protein peaks containing dextranase activity (F-I and -II) were obtained. Each active protein peak was collected and then concentrated, separately.

Step 4. DEAE-Sepharose column chromatography for F-I. The concentrated sample of F-I was put on a column of DEAE-Sepharose (1.2 ϕ ×45 cm) preequilibrated with 20 mM acetate buffer (pH 6.0) containing 5 mM calcium acetate. After washing the column with the same buffer, the enzyme was eluted with a linear gradient of NaCl (0–0.3 M). The fractions containing most of the dextranase activity were collected and concentrated. The concentrated solution was dialyzed overnight against 20 mM acetate buffer (pH 6.0) containing 5 mM calcium acetate, and solid ammonium sulfate was added to the dialyzate to 1 M.

Step 5. Phenyl-Toyopearl 650M column chromatography for F-I. The dialyzed sample of F-I after Step 4 was loaded on a column of Phenyl Toyopearl 650M (1.2 ϕ×45 cm) preequilibrated with 20 mM acetate buffer (pH 6.0) containing 5 mM calcium acetate and 1 M ammonium sulfate. The enzyme was eluted with a liner gradient of ammonium sulfate (1–0 M). The fractions containing most of the dextranase activity were collected and concentrated. The concentrated solution was dialyzed overnight against 20 mM acetate buffer (pH 6.0) containing 5 mM calcium acetate.

Step 6. DEAE-Sepharose column chromatography for F-II. The concentrated sample of F-II was put on a column of DEAE-Sepharose (1.2 ϕ ×45 cm) preequilibrated with 20 mM acetate buffer (pH 6.0) containing 5 mM calcium acetate. After washing the column with the same buffer, the enzyme was eluted with a liner gradient of NaCl (0–0.3 M). The fractions containing most of the dextranase activity were collected and concentrated. The concentrated solution was dialyzed overnight against 20 mM imidazole–HCl buffer (pH 7.0).

Step 7. Mono Q column chromatography for F-II. The concentrated sample of F-II after Step 6 was loaded on a column of Mono Q HR5/5 (0.5 ϕ×5 cm) preequilibrated with 20 mM imidazole–HCl buffer (pH 7.0). After washing the column with the same buffer, the enzyme was eluted with a linear gradient of NaCl (0–0.5 M). The fractions containing most of the dextranase activity were collected and concentrated. The concentrated solution was dialyzed overnight against 20 mM acetate buffer (pH 6.0) containing 5 mM calcium acetate.

Electrophoresis. Native-PAGE was done in 7.5% polyacrylamide with Tris-diethyl barbituric acid buffer (pH 7.0) according to the method of Davis.[14] SDS-PAGE was done in 7.5% polyacrylamide containing 0.1% SDS by the method of Leammli.[15] In both cases, the gels were stained in 0.25% Comassie-Brilliant Blue R-250–50% methanol–10% acetic acid. The p*I*s of the purified enzymes were measured, using LKB Ampholine electrofocusing apparatus (110 mL), by the method of Vesterberg and Svensson.[16]

N-terminal amino acid sequence analysis. N-terminal amino acid sequences of the purified enzymes were determined by repetitive Edman degradation with a gas-phase protein sequencer (Model 477A-120A; Applied Biosystems Inc., Foster, Calif.).

Polarimetry. A sample of 1.0 mL of buffered enzyme solution at pH 6.0 (13.4 U of each dextranase) was added to 4.0 mL of 6% dextran T-2000 solution, and mixed thoroughly. Then a part of this mixture was quickly transferred to a 0.1-dm polarimeter cell and the changes in the rotation were measured at 30°C at intervals with a Union Giken automatic digital polarimeter (Model PM-101). After incubation for 9 min, one drop of concentrated ammonium hydroxide was added to the polarimeter cell, and then the rotation was again measured after thorough mixing.

Viscometry. The changes in viscosity during the enzymatic hydrolysis of dextran were measured at intervals with a Cannon-Fenske viscometer (c =0.0369). The enzyme activity was expressed in terms of the specific fluidity, $\phi_{sp.}$(*i.e.*, $1/\eta_{sp.}$).

Paper partition chromatography (PPC). Enzymatic hydrolysis products from dextran T-2000 were detected and identified by descending paper chromatography. Chromatography was done on Whatman No. 1 filter paper with *1*-butanol–pyridine–water (6 : 4 : 3, by volume) at room temperature for 20 h. Reaction products were detected by

the dipping procedure using alkaline silver nitrate reagent.[17]

High pressure liquid chromatography (HPLC).

HPLC analysis of sugars was done to identify and determine the enzymatic hydrolysis products from dextran using RI-detector. The column used was Aminex HPX-42A (8 $\phi \times 300$ mm, Bio-Rad Laboratories) with water (flow rate, 0.6 mL/min) at 65°C.

RESULTS

Purification of the enzymes.

The enzymes were purified by ammonium sulfate fractionation and consecutive column chromatographies. Table 1 summarizes the overall purification steps. Two purified enzymes had the high level of specific activities of 45.9 (dextranase I) and 84.2 (dextranase II) U/mg protein, and were used for the subsequent characterization.

Purity, molecular mass and N-terminal amino acid sequence of the enzymes.

Each purified enzyme gave a single protein band on both Native- and SDS-PAGE. The molecular masses of the purified enzymes were estimated to be about 107 kDa for dextranase I and 70 kDa for dextranase II by comparison of their relative mobilities on SDS-PAGE with those of standard proteins. Both purified enzymes had an identical N-terminal amino acid sequence of NH_2-S-V-A-P-L-A-S-T-P-T-L-T-T-W-.

Isoelectric point.

The purified enzymes were put on isoelectric focusing to find their p*I*s and also to check their homogeneities. For each, a sharp and symmetrical protein peak containing only dextranase activity was obtained. The enzymes were judged to be homogeneous, their p*I* values being 4.3 for dextranase I and 4.5 for dextranase II.

Effects of pH and temperature on the activity of the enzymes.

The effects of pH and temperature on the activity of the purified enzymes were studied under the standard assay conditions at 30°C for 10 min using various pH of 50 mM β, β'-dimethylglutaric acid–NaOH buffer containing 5 mM calcium chloride and at different reaction temperatures for 10 min. Dextranase I showed a single maximum at pH 6.0 and at 45°C, and dextranase II at pH 6.0 and at 40 °C.

Stabilities of the enzymes toward pH and temperature.

Each solution containing an equal amount (0.2 mL) of the purified enzyme (12.2 U for dextranase

Table 1. Recoveries and activities of the enzymes during purification.

Purification step	Total protein[a] (mg)	Enzyme activity		
		Total unit	Sp. activity (U/mg protein)	Yield (%)
$(NH_4)_2SO_4$ fraction (90% saturation)	3100.0	4768	1.5	100
DEAE-Sepharose	140.6	2489	17.7	52.2
Bio-Gel P-100				
F-I	49.5	1327	26.8	27.8
F-II	13.7	717	52.3	15.0
DEAE-Sepharose				
F-I	27.0	918	34.0	19.3
F-II	7.2	387	53.8	8.1
Phenyl-Toyopearl 650M				
Dextranase I	16.8	771	45.9	16.2
Mono Q				
Dextranase II	2.6	219	84.2	4.6

[a] Measured by the method of Lowry *et al.*[13]

I and 23.7 U for dextranase II) was individually adjusted to a pH from 3.5 to 7.5 by adding 2.8 mL of 50 mM β,β'-dimethylglutaric acid–NaOH buffer containing 5 mM calcium chloride. Both enzymes were completely stable over the range of pH 5.0–7.5 at 4°C for 24 h.

The purified enzymes (17.5 U for dextranase I and 25.6 U for dextranase II) in 0.5 mL of 20 mM acetate buffer (pH 6.0) containing 5 mM calcium acetate were heated at various temperatures for 10 min, and then cooled immediately in an ice-bath. Each treated enzyme solution was diluted with the same buffer for 130-folds (dextranase I) or 200-folds (dextranase II). The remaining enzyme activity was then measured with 0.25 mL of each enzyme solution by the standard assay at 30 °C for 10 min. Dextranase I retained the original activity on heating below 45°C, and dextranase II retained it below 40°C.

Effects of various metal ions and several enzyme inhibitors on the activity of the enzymes.

The enzymes were incubated with an equal volume of each metal ion or inhibitor solution (10 mM, pH 6.0) at 30°C for 30 min. The remaining enzyme activity per 0.5 mL of each reaction mixture containing 0.03 U of dextranases I or II was examined by the standard assay at 30°C for 10 min. The inactivation patterns of both enzymes were similar. The inactivations of both dextranases I and II were partial with 5 mM Zn^{2+} being 50 and 60% inhibition, respectively. On the other hand, Fe^{3+}, Hg^{2+}, Cu^{2+}, Ag^{+}, $KMnO_4$ and NBS inactivated both purified enzymes completely. EDTA and other metal ions tested had no inhibitory effect on the activity of the enzymes.

The effect of rose bengal (50 μM) on the activity of the enzymes was studied. Rapid inactivation curves of both enzymes were observed. Under the conditions used, the time for 90% inactivation was about 6 min in both enzymes.

Anomeric configuration of hydrolysis products.

The anomeric configurations of the reaction products formed by the enzymes were examined. The optical rotation of dextran T-2000 gradually decreased in a negative direction after addition of each enzyme. However, immediate downward mutarotations were observed in both enzymes upon the addition of ammonium hydroxide to the reaction mixtures (Fig. 1). These results indicate, at least qualitatively, that the enzymes release α-form of reaction products, and that the α-glucosidic linkages in the substrates are retained.

Mode of actions of the enzymes toward dextran T-2000.

The mode of action of each purified enzyme to-

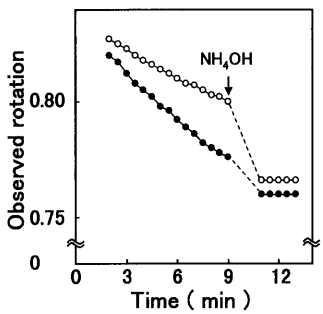

Fig. 1. Changes in optical rotation during the hydrolysis of dextran T-2000 by the purified enzymes and after base-catalyzed mutarotation.

●, dextranase I; ○, dextranase II. A drop of concentrated ammonium hydroxide was added to the digest at the point indicated by the arrow, and the optical rotation was followed by 13 min incubation. Experimental details are given in the text.

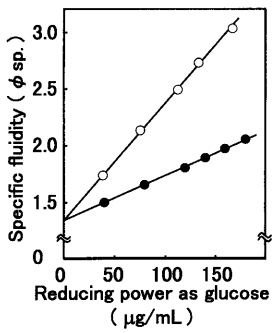

Fig. 2. Relationship between the increases in specific fluidity and reducing power during hydrolysis of dextran T-2000 by the purified enzymes.

Symbols are the same as in Fig. 1. Experimental details are given in the text.

ward dextran T-2000 was analyzed by viscometer. The reaction mixture was composed of 4 mL of 2% dextran T-2000, 2 mL of 40 mM acetate buffer (pH 6.0) containing 5 mM calcium acetate, and 2 mL of the enzyme solution (0.33 U of each dextranase). Each mixture was incubated in a Cannon-Fenske viscometer at 30°C. The decrease in viscosity of the incubation mixture and the formation of reducing sugars were measured every 5 min. Then, the increase in specific fluidity ($\phi_{sp.}$) was plotted against the increase in reducing power. As shown in Fig. 2, the specific fluidity increased with increases in reducing power in both cases. These results indicate that the mode of action of both purified enzymes is *endo*-lytic.

Substrate specificities of the enzymes.

The substrate specificities of the enzymes were studied using dextrans from different strains of *L. mesenteroides* and a series of isomaltooligosaccharides, and the hydrolysis products from these substrates were analyzed by PPC and HPLC. The changes in the reaction products from dextran T-2000 by dextranases I or II are shown in Fig. 3. As can be seen in this Figure, the action patterns on dextran T-2000 were quite similar in both enzymes, and the main reaction product was isomaltotriose. However, no glucose and faint isomaltose spots were detected on the chromatograms. On the other hand, a series of unknown spots was detected between each isomaltooligosaccharide having a DP of more than 3.

The hydrolysis products from a series of isomaltooligosaccharides due to the action of dextranases I or II are summarized in Table 2. Neither enzymes had any action on isomaltose, isomaltotriose or even isomaltotetraose. The main reaction product from isomaltopentaose to isomaltooctaose was isomaltotriose. In an early stage of incubation, almost the same amounts of isomaltotriose and isomaltopentaose were produced from isomaltooctaose with a small amount of isomaltotetraose, and a small amount of isomaltose also appeared in a late

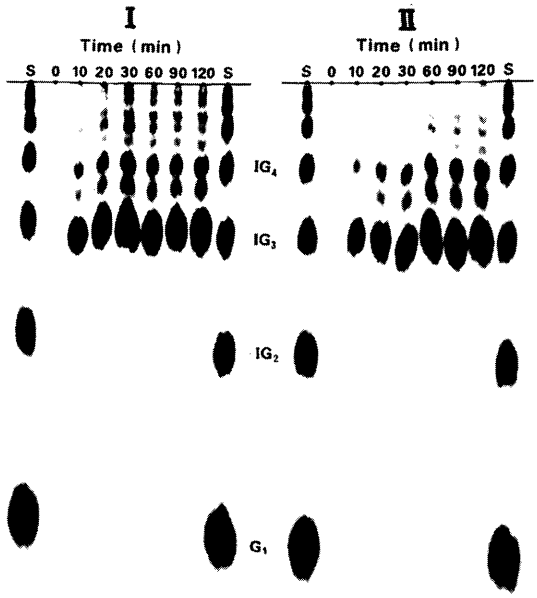

Fig. 3. A paper chromatogram of hydrolysis products from dextran T-2000 by the purified enzymes.

I, dextranase I; II, dextranase II; S, isomaltooligosaccharide standards. Experimental details are given in the text.

Table 2. Hydrolysis products from a series of isomaltooligosaccharides due to the action of dextranases I or II.

Substrate	Product	
	Early stage	Late stage
IG2	—	—
IG3	—	—
IG4	—	—
IG5	IG3=IG2	IG3=IG2
IG6	IG3	IG3
IG7	IG3=IG4	IG3=IG4
IG8	IG3=IG5≫IG4	IG3>IG5≫IG4=IG2

IG2–IG8, Isomaltose–Isomaltooctaose; —, No action.

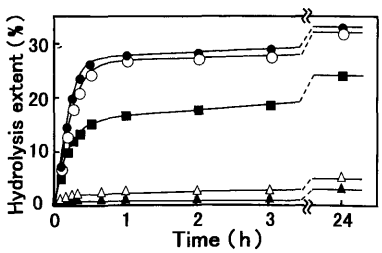

Fig. 4. Hydrolysis extents toward various dextrans by dextranases I or II.

●, Dextran T-2000; ○, B-523S dextran; ▲, B-1355S dextran; △, B-742S dextran; ■, B-1399 dextran. Experimental details are given in the text.

stage, with the decrease in the amount of isomaltopentaose.

The extent of hydrolysis toward various dextrans by dextranase I or II was measured under the same conditions. As shown in Fig. 4, the extents of hydrolysis toward dextran T-2000, dextrans from *L. mesenteroides* NRRL B-523S, B-1399, B-742S and B-1355S were about 32, 31, 24, 4.5 and 2.4%, respectively.

DISCUSSION

Two *endo*-dextranases from *Arthrobacter globiformis* W31 were highly purified by consecutive column chromatographies. The purified dextranases I and II showed the specific activities of 45.9 and 84.2 U/mg protein, respectively, and were homogeneous on Native- and SDS-PAGE as well as isoelectric focusing. The optimum pHs (pH 6.0) of the purified enzymes are close to those of a glucodextranase from *Arthrobacter globiformis* I42 (pH 6.0),[5] and an *endo*-dextranase from *Streptococcus mutans* K1-R (pH 5.5).[2]

The N-terminal amino acid sequences of dextranases I and II were identical. However, the molecular masses of these two enzymes were extremely different from each other. From these results, it may be suggested that a domain (~30.7 kDa) in the C-terminal region of dextranase II was deleted by some specific proteolysis during cultivation. The N-terminal amino acid sequences of the present dextranases (1st–14th) showed no homology with those of dextran hydrolyzing enzymes from any other *Arthrobacter* genus.[6,8,18] Oguma *et al.*[6] found that the 10th and 14th amino acids from the N-terminal of the known dextran hydrolyzing enzymes from *Arthrobacter* genus are glycine and threonine, respectively. However, the above two corresponding positions in our dextranases I and II are occupied with different amino acids, threonine and tryptophan, respectively.

The results of viscometry, polarimetry and PPC analysis indicate that the purified enzymes release a large amount of α-isomaltotriose, a series of isomaltooligosaccharides having a DP of more than 3 and also a series of unknown oligosaccharides from dextran molecules in an *endo*-lytic fashion. In contrast, many *endo*-dextranases from fungal and bacterial sources release mainly both isomaltose and isomaltotriose from dextran molecules. On the other hand, isomaltotriose-producing dextranases were reported from *Brevibacterium fuscum* var. *dextranlytytium*,[19] a *Fusarium* sp.,[4] *Streptococcus mutans* K1-R[2] and *Flavobacterium* sp. M-73.[3] Among them, only *B. fuscum* var. *dextranlytium* secreted an *exo*-type dextranase, and the other three microbes produced *endo*-type dextranases. These *endo*-type dextranases split dextran molecules to produce both isomaltose and isomaltotriose. The purified enzymes were unique in the sense of producing almost no isomaltose from dextran. Moreover they had no actions toward isomaltose, isomaltotriose or even isomaltotetraose. From these results, it is clear that the enzymes have no productive binding actions on these isomaltooligosaccharides. Isomaltooctaose was hydrolyzed to isomaltotriose and isomaltopentaose in an early stage of incubation. However, a small amount of isomaltose appeared in a late stage. This isomaltose was formed from isomaltopentaose, secondarily.

Substrate specificities of the enzymes toward dextran T-2000 resembled those of dextranase II from *Flavobacterium* sp. M-73[3] and an *endo*-dextranase from a *Fusarium* sp.,[4] the extents of hydrolysis of these three enzymes being about 32%. However, our purified enzymes produce hardly any isomaltose from dextran molecules. Therefore, our purified enzymes have a small discrepancy in substrate specificity compared to dextranase II from *Flavobacterium* sp. M-73[3] and an *endo*-dextranase from a *Fusarium* sp.[4] Kobayashi *et al.*[3] also reported that dextranase II from *Flavobacterium* sp. M-73 produces, an unknown oligosaccharide having PPC mobility between isomaltotriose and isomaltotetraose just like our present case. Our unknown oligosaccharides are presumably transglycosylation products. We are intensely interested in the formation of the structurally unknown oligosaccharides. Now we are going to isolate and identify the unknown oligosaccharides from the reaction mixtures to investigate their formation mechanisms.

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Arthrobacter globiformis W31 の産生する2種のエンドデキストラナーゼの精製および特性

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グラム陽性土壌細菌 *Arthrobacter globiformis* W31 が菌体外に産生する2種のデキストラナーゼ (dextranase I, II) を各種カラムクロマトグラフィーを組み合わせて精製し, Native-および SDS-PAGE においてそれぞれ単一の染色バンドを示す高純度標品を得た. 精製酵素の p*I* および分子質量は, それぞれ 48.3, 107 kDa (dextranase I), 4.5, 70 kDa (dextranase II) であり, 両酵素は同一の N 末端アミノ酸配列 (NH_2-S-V-A-P-L-A-S-T-P-T-L-T-T-W-) を示した. 反応至適 pH は両酵素とも pH 6.0 であった. 両酵素をデキストラン T-2000 に作用させた際の反応生成糖のアノマー型はα型を示し, 基質分子内のα-グルコシド結合が保持されることが示唆された. 両酵素はデキストラン T-2000 に対しエンド型の水解様式を示し, 主反応生成糖としてイソマルトトリオースを, また DP 4 以上の一連のイソマルトオリゴ糖や相当量の各種構造未知糖を生成した. さらに, 両酵素はイソマルトース, イソマルトトリオースおよびイソマルトテトラオースに対し, 全く水解作用を示さなかった.