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PURIFICATION AND PROPERTIES OF A SPECIFIC, INDUCIBLE β- GLUCANASE, SUCCINOGLUCAN DEPOLYMERASE FROM *FLAVOBACTERIUM*

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SUMMARY

Succinoglucan depolymerase from the culture fluid of *Flavobacterium* sp. Strain M64 was purified by fractionation with $(NH_4)_2SO_4$, followed by chromatographies on DEAE-cellulose and Sephadex G-200. 90-fold purification was achieved and the final preparation showed about 90% purity on polyacrylamide gel disc electrophoresis. The enzyme has a molecular weight of 180 000. The optimal pH for enzyme activity is 5.8 and the enzyme is stable from pH 4.5 to 10.0. It is stable up to 35 °C, but rapidly loses activity above 35 °C. The K_m values for succinoglucan and desuccinylated succinoglucan are 1.7 mg/ml and 1.2 mg/ml, respectively.

Succinoglucan is depolymerized to yield a polymer with a degree of polymerization of 12 and the product was suggested to be a structural unit of succinoglucan. β-Glucans and β-oligosaccharides, including schizophyllan, kefiran, laminaran, lutean, luteose, carboxymethylcellulose, curdlan, β-1,6- and β-1,3-oligosaccharides, cellobiose, 6-*O*-laminaritriosylglucose and lactose are not hydrolyzed while yeast glucan and pachyman are hydrolyzed only slightly.

Succinoglucan depolymerase is only formed when succinoglucan or desuccinylated succinoglucan is present as the sole carbon source or with another carbon compound such as xylose. Compounds such as glucose and succinic acid repress depolymerase formation induced by succinoglucan. On partial acid hydrolysis or enzymolysis of succinoglucan, most of its inductive effect is lost.

INTRODUCTION

Succinoglucan, an extracellular acidic polysaccharide produced by *Alcaligenes faecalis* var. *myxogenes* 10C3, contains D-glucose, D-galactose and succinic acid and its polysaccharide moiety consists of β-1,3-, β-1,4- and β-1,6-linked glucose residues, together with a small portion of β-1,3-linked galactose residues. The succinic acid residues are attached to the polysaccharide chain by ester linkages [1–3].

Succinoglucan is resistant to several known β-glucanase preparations [4]. We found that *Flavobacterium* sp. strain M64 isolated from soil, forms an inducible β-glucanase capable of depolymerizing the polymer when grown on medium containing succinoglucan as the sole carbon source [4].

This paper describes the purification and properties of this enzyme and studies

on the hydrolysis product of succinoglucan by this enzyme and the specificity of enzyme induction.

METHODS AND MATERIALS

Cultures

Flavobacterium sp. strain M64 was used. It was isolated from soil and is capable of utilizing succinoglucan as the sole source of carbon. The defined medium used had the following composition: $(NH_4)_2HPO_4$, 0.15%; KH_2PO_4, 0.1%; $MgSO_4 \cdot 7H_2O$, 0.05%; NaCl, $MnCl_2 \cdot 4H_2O$ and $FeCl_3 \cdot 6H_2O$, each 0.0001% and a carbon source. The medium was adjusted to pH 7.2 with 1 M NaOH. Cells from the stock culture were inoculated into this medium and grown with shaking at 30 °C for 24 h. Then cells were transferred by diluting the culture 1:20 with fresh medium.

Assay of succinoglucan depolymerase activity

Reaction mixture, containing 0.5 ml of 0.5% desuccinylated succinoglucan, 0.25 ml of 0.2 M acetate buffer (pH 5.8) and 0.25 ml of enzyme solution, was incubated at 34 °C for an appropriate time and then the reaction was stopped by adding 1 ml of Somogyi alkaline copper reagent. Increase of reducing power was determined by the method of Nelson [5]. One unit of enzyme activity is defined as the amount of enzyme which liberates 1 μmole of aldehyde group as glucose per h.

Determination of protein concentration

Protein concentration was estimated by the method of Lowry et al. [6] using bovine serum albumin as a standard. The amounts of protein in effluents from columns were determined from their absorbance at 280 nm, using a Hitach 124 UV-VIS spectrophotometer.

Preparation of crude succinoglucan depolymerase

Flavobacterium sp. Strain M64 was grown aerobically at 30 °C for 3 days in the above medium containing 0.8% succinoglucan. The cells were removed by centrifugation at 20 000 $\times$ g, and the supernatant was used as the starting material for enzyme purification.

Gel electrophoresis

Polyacrylamide gel electrophoresis was carried out at pH 4.3 by the method of Davis [7] with a current of 2 mA/tube for 3.5 h. After the run, gels were stained with Amido Black 10B. To relate the position of protein bands to that of succinoglucan depolymerase, two identical gels were subjected to electrophoresis at the same time. One gel was stained immediately after electrophoresis while the other was cut into 0.2-cm sections. Each section was extracted with 0.5 ml of 0.2 M acetate buffer (pH 5.8) in a test tube and the enzyme activity of the extract was measured.

Paper chromatography

The reaction product was examined as follows. At zero time and after appropriate incubation times, samples (0.2 ml) were removed from the reaction mixture, heated in a boiling water bath for 3 min to inactivate the enzyme, cooled and

treated with Amberlite IR-120 to remove cations. The supernatant obtained was evaporated to dryness in vacuo. The residue was dissolved in 50 μl of water and subjected to paper chromatography on Toyo-Roshi No. 50 paper. The chromatogram was developed by the descending method with *n*-butanol–pyridine–water (6:4:3, by vol.) as solvent. Reducing sugars on the chromatogram were detected with alkaline silver nitrate reagent.

Substrates

Succinoglucan [2] and curdlan [8] were prepared as reported previously from the culture broths of *Alcaligenes faecalis* var. *myxogenes* 10C3, and its mutant Strain 10C3K, respectively. Desuccinylated succinoglucan was obtained by treatment of succinoglucan with dilute alkali at 70 °C. Pachyman was prepared from Bukuryo by the method of Saito et al. [9]. Yeast glucan and water soluble laminaran [10] of *Eisenia bicyclis* were kindly supplied by Dr Misaki and Drs Nisizawa and Maeda, respectively. Lutean [11] which is synthesized by *Penicillium aculeatum* var. *apiculatum* No. 2281 and luteose, which was prepared from lutean, were obtained by courtesy of Drs Tanabe and Nakamura. Kefiran [12] and schizophyllan [13] were obtained by courtesy of Drs Kooiman and Kikumoto, respectively. β-1,3- and β-1,6-oligosaccharides were isolated by partial acid hydrolyses of curdlan and luteose, respectively, followed by chromatography on a charcoal column. Other substrates were commercial products of the highest purity available.

Gas–liquid chromatography

Gas–liquid chromatography was carried out with a Hitachi K53 gas chromatograph using a column of 3% ECNSS-M on Gas Chrom Q at 190 °C. Nitrogen was used as carrier gas, at a flow rate of 60 ml/min. Sugar samples were prepared by the method of Björnoal et al. [14].

Viscosity

Measurements of viscosity were made with a Cannon–Finske viscometer at 30 °C.

RESULTS

Purification of succinoglucan depolymerase

All operations were carried out at 4 °C, unless otherwise stated. 20 l of cultures of *Flavobacterium* sp. strain M64 were centrifuged at 20 000 × *g* for 20 min. The supernatant was concentrated to about 2 l under reduced pressure at 25 °C. The solution was brought to 60% satn of $(NH_4)_2SO_4$ and the resultant precipitate was removed by centrifugation at 15 000 × *g* for 20 min. The precipitate was dissolved in 450 ml of 0.01 M phosphate buffer (pH 6.5), and the solution was dialyzed against three changes (5 l each) of the same buffer over a period of 48 h. The precipitate formed was removed by centrifugation and the supernatant obtained was applied to a DEAE-cellulose column (3.0 cm × 28 cm) equilibrated with 0.01 M phosphate buffer (pH 6.5). The column was washed with the same buffer, and then eluted with a linear gradient of NaCl in the same buffer. Depolymerase activity was eluted as a single broad peak with approx. 0.15 M NaCl (Fig. 1). Fractions 8–35 were combined. This procedure removed carbohydrates from the enzyme preparation.

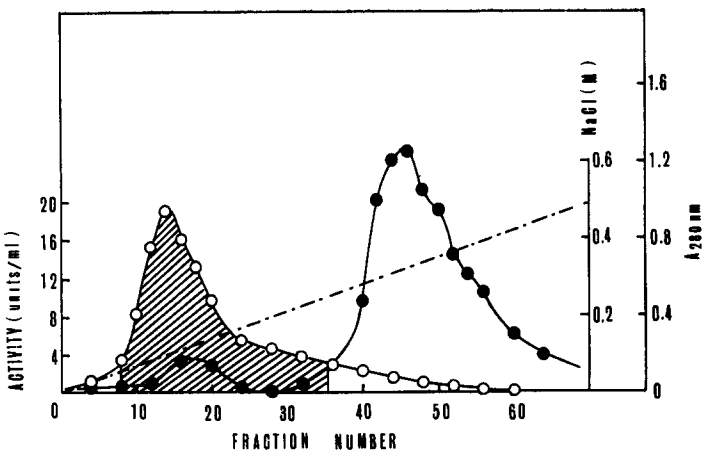

Fig. 1. Elution pattern of depolymerase from a DEAE-cellulose column. ●—●, $A_{280\ nm}$; ○—○, depolymerase activity; —·—, concentration of NaCl. The shaded area indicates fractions pooled for subsequent steps. Conditions are described in the text.

Next most of the NaCl was removed from the depolymerase solution by concentrating the solution to 10 ml by pressure dialysis against 0.01 M acetate buffer (pH 5.8). Then the depolymerase solution was applied to a column of Sephadex G-200 (3.0 cm × 40 cm) previously equilibrated with 0.01 M acetate buffer (pH 5.8). Depolymerase activity emerged as a single peak (Fig. 2). Fractions 20–30 containing depolymerase activity were pooled, concentrated to 5 ml by pressure dialysis and rechromatographed on the same Sephadex column. The active fractions were collected and evaporated to 10 ml in vacuo at 25 °C.

The purified preparation was stable on storage at 4 °C in 0.1 M acetate buffer (pH 5.8) and retained approx. 95 % of its activity after storage for 1 month. The purification procedure is summarized in Table I. The enzyme was purified about 90-fold.

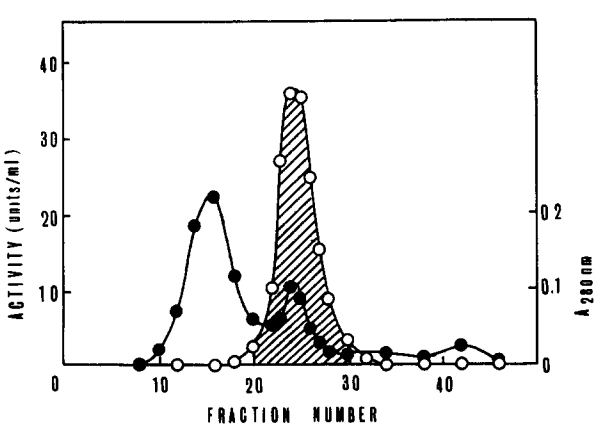

Fig. 2. Elution pattern of depolymerase from Sephadex G-200. ●—●, $A_{280\ nm}$; ○—○, depolymerase activity. The shaded area indicates fractions pooled for subsequent steps. Conditions are described in the text.

TABLE I

ENZYME PURIFICATION

Step of purification	Volume (ml)	Protein (mg)	Total activity (units)	Spec. act (units/mg)
1. Culture filtrate	17 400	1660	12 300	7.41
2. $(NH_4)_2SO_4$, 0–60% satn	540	732	8 960	12 2
3. DEAE-cellulose	10	33.7	3 880	115
4. 1st Sephadex G-200	5	1.84	980	533
5. 2nd Sephadex G-200	10	1.28	852	666

Purity of the enzyme preparation

Disc gel electrophoresis of purified depolymerase at pH 4.3 revealed a single protein component, although small bands of contaminants were detectable (Fig. 3). On extraction of slices of an unstained gel depolymerase activity was found to coincide with the main protein band, and in this way the purified depolymerase was found to be about 90% homogeneous.

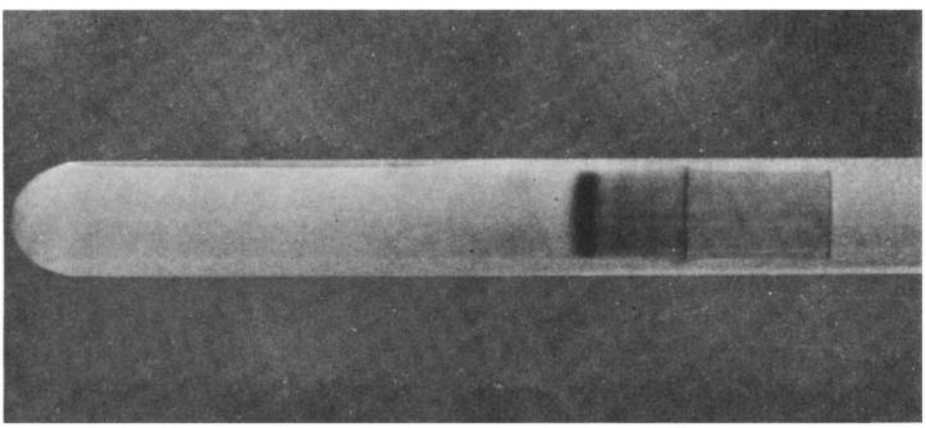

Fig 3. Polyacrylamide gel electrophoresis of purified depolymerase at pH 4.3. Approx. 0.1 mg of protein was applied to the gel. Electrophoresis was carried out for 3.5 h at 2.0 mA/gel.

Properties of succinoglucan depolymerase

The molecular weight of the enzyme was estimated by comparing its elution volume from a column of Sephadex G-200 with those of a series of proteins of known molecular weight (Mann Res. Lab.). The molecular weight of the enzyme determined by interpolation on the graph was 180 000. Maximum depolymerase activity was observed at pH 5.8 in 0.1 M acetate buffer (Fig. 4a). The activity in a 30 min reaction period increased with increase in temperature up to 45 °C (Fig. 4b). Under conditions of limited hydrolysis of the desuccinylated succinoglucan, release of reducing groups was proportional to the enzyme concentration. The depolymerase was stable on incubation for 4 h at 20 °C at about pH 4.5–10.0 (Fig. 4c). Its thermal stability at 25–

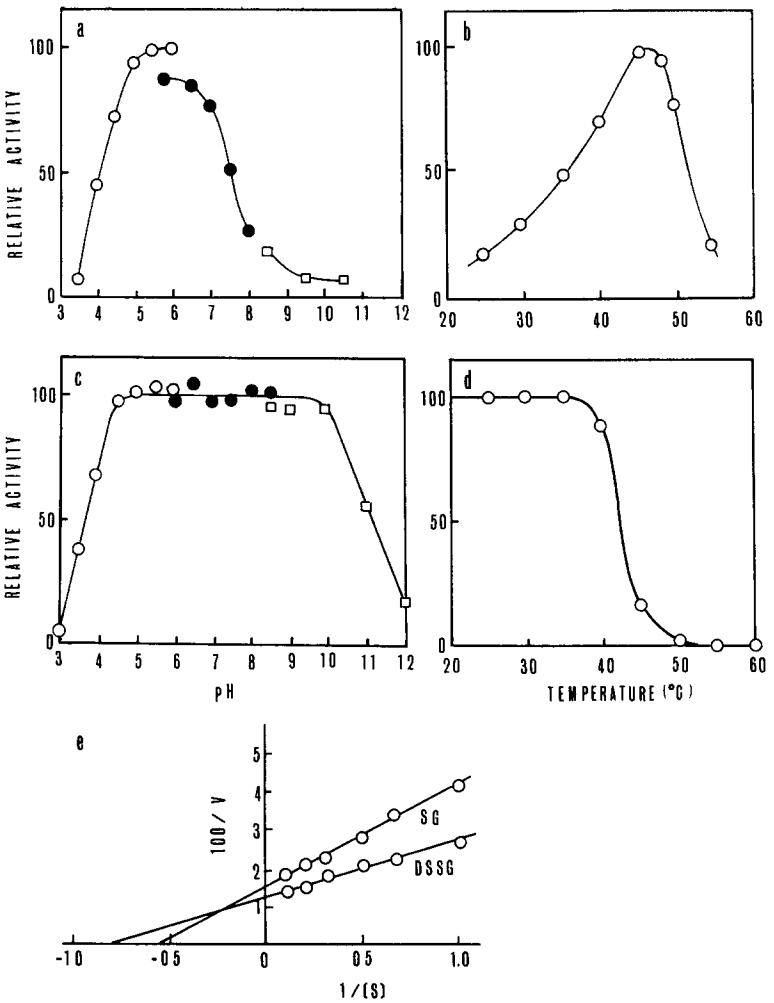

Fig. 4. Effect of pH (a,c) and temperature (b,d) on activity and stability of depolymerase, respectively, and K_m values for substrates (e) 0.1 M acetate buffer (pH 3.0–6.0) (○—○), 0 1 M ammonia buffer (pH 8.5–12.0) (□—□), 0.1 M phosphate buffer (pH 5.8–8.0) ((●—●) in (a)) and 0.1 M Tris–maleate buffer (pH 6.0–8.5) ((●) in (c)) were used. For examination of pH stability, after enzyme solutions of various pH values were incubated for 4 h at 20 °C, each enzyme solution was adjusted to pH 5.8 and residual activity was measured by the standard method. For examination of temperature stability, after enzyme dissolved in 0.1 M acetate buffer solution (pH 5.8) was incubated for 10 min at the various temperatures indicated, the activity remaining was assayed. K_m values for succinoglucan (SG) and desuccinylated succinoglucan (DSSG) were obtained from Lineweaver–Burk plot of hydrolysis of them. The reaction velocity (v) is expressed as μmoles of aldehyde groups released per h/mg of enzyme protein.

60 °C was measured in 0.1 M acetate buffer (pH 5.8). As shown in Fig. 4d, the depolymerase was remarkable heat sensitive, i.e., it was partially inactivated at 40 °C, and 80% inactivated at 45 °C, while heating for 10 min at 50 °C resulted in complete loss of activity. The reaction velocities for succinoglucan and desuccinylated succinoglucan were measured at a series of substrate concentrations, as shown in

Fig. 4e. From the graph the K_m values for succinoglucan and desuccinylated succinoglucan were estimated to be 1.7 and 1.2 mg/ml, respectively. The actions of depolymerase on various other substrates were examined (Table II). The results indicated that this enzyme has a very high specificity for succinoglucan and desuccinylated succinoglucan. It had no action on a number of oligo- and polysaccharides con-

TABLE II

SUBSTRATE SPECIFICITY

Each substrate was incubated with the depolymerase in 0.05 M acetate buffer (pH 5.8), at 34 °C at a concentration of 2 mg/ml.

Substrate	Glucosidic linkage types	Reducing sugar as glucose (μmoles/h/mg)
Succinoglucan	β-1,3-, β-1,4-, β-1,6-	574
Desuccinylated succinoglucan	β-1,3-, β-1,4-, β-1,6-	623
Yeast glucan	β-1,3-, β-1,6-	0.19
Pachyman	β-1,3-, β-1,6-	0.10
Schizophyllan	β-1,3-, β-1,6-	0
Laminaran	β-1,3-, β-1,6-	0
Kefiran*	β-1,6-	0
Lutean	β-1,6-	0
Luteose	β-1,6-	0
Carboxymethyl cellulose	β-1,4-	0
Curdlan	β-1,3-	0

* Kefiran has β-1,4- and α-1,3-galactosidic linkages in addition to a β-1,6-glucosodic linkage.

taining β-1,3-, β-1,4- or β-1,6-glucosidic linkages although traces of activities were observed with yeast glucan and pachyman. It was inactive with 6-*O*-laminaritriosylglucose, laminaripentaose, laminaritetraose, laminaritriose, laminaribiose, gentiotetraose, gentiotriose, gentiobiose, cellobiose or lactose in addition to the compounds listed in Table II.

Hydrolysis of succinoglucan by the depolymerase

As shown in Fig. 5, the viscosity of a solution of succinoglucan very rapidly decreased on incubation with the depolymerase, while the concentration of reducing sugars slowly increased. The final degree of hydrolysis was about 8.6%, calculated from the ratio of aldehyde groups formed by the depolymerase as glucose to glucose formed by complete acidic hydrolysis. Aliquots of the mixture were applied to Toyo-Roshi No. 50 paper and developed with *n*-butanol–pyridine–water solvent to examine the products of hydrolysis. The material(s) near the origin was found to be the only detectable reducing product(s) during the whole reaction period.

Product of hydrolysis with the depolymerase

Purified succinoglucan (calcium-form) was extensively hydrolyzed with the depolymerase in mixture containing the following components: succinoglucan, 500

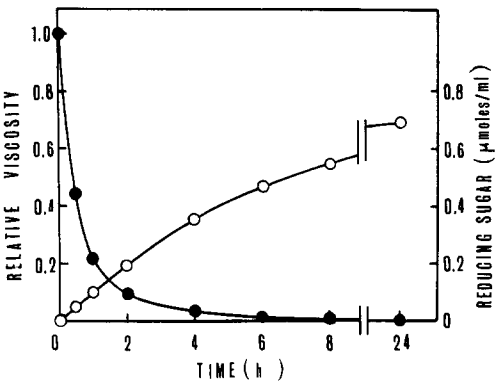

Fig. 5. Decrease of viscosity and release of reducing sugar in succinoglucan solution on treatment with the depolymerase. Succinoglucan (125 mg) was incubated with depolymerase at 30 °C in 50 ml of 0.1 M acetate buffer (pH 5.8). Aliquots were removed at the indicated times and analyzed for reducing sugars by the method of Somogyi–Nelson. Changes in viscosity were monitored using 4-ml aliquots. ●—●, viscosity relative to that of zero-time sample; ○—○, reducing sugars as glucose.

mg; acetate buffer (pH 5.8), 2.5 nmoles; toluene, 0.5 ml; and depolymerase, 780 units in a final volume of 50 ml. After 6 h and 24 h 120 units more depolymerase were added. After incubation for 48 h at 30 °C, the hydrolyzate was dialyzed against 2 l of distilled water for 6 days at 4 °C. This long dialysis time was used because the enzymatic products seemed to have too large a molecular weight to filter rapidly throughout the dialysis membrane. The dialyzable fraction was concentrated to about 10 ml and salt was removed by chromatography on a column of Sephadex G-15. Fractions in the void volume containing the product of succinoglucan formed by the depolymerase were concentrated to a small volume and mixed with 6 vol. of ethanol. The resultant precipitate was collected by centrifugation, washed with ethanol and dried in vacuo to yield 430 mg of dried material. The product gave a single symmetrical peak on gel filtration on Sephadex G-75. The fractions in the peak were applied to a DEAE-cellulose column and eluted with a linear gradient of KCl. As shown in Fig. 6, several small peaks were observed in addition to the major peak eluted with about 0.15 mM KCl. Fractions 50–70 were collected, concentrated to a small volume, and subjected to Sephadex G-15 chromatography to remove salt. Fractions in the void volume were concentrated and mixed with 6 vol. of ethanol. The resultant precipitate was washed with ethanol and dried in vacuo, yielding 240 mg of dried matter (SG-D). SG-D was composed of glucose, galactose and succinic acid. The molar ratio of glucose to galactose was determined to be 8:1 by gas–liquid chromatography. After removal of cations with resin, titration of the H-form of SG-D with NaOH revealed that the product contained 8.5% succinic acid. The degree of polymerization (*DP*) of SG-D was determined by the method of Manners et al. [15] measuring the sorbitol content of an acid hydrolyzate of SG-D reduced with boro-hydride, using sorbitol dehydrogenase. The sample gave a *DP* of 12.0. These results suggested that SG-D corresponds to a structural unit of succinoglucan (see Discussion).

Induction of depolymerase

The substrates listed in Table III were tested at concentrations of 10 mg/ml

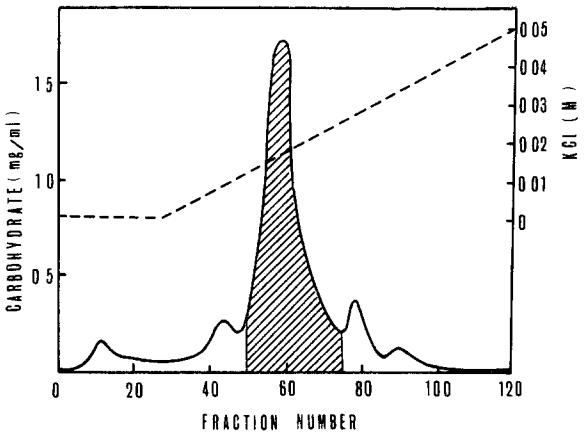

Fig. 6. DEAE-cellulose chromatography of the final product of succinoglucan formed by the depolymerase. Succinoglucan was hydrolyzed with depolymerase as described in the text. After fractionations on Sephadex G-15 and G-75, the hydrolyzate was applied to a DEAE-cellulose column (2.5 cm × 36 cm) equilibrated with 1 mM KCl. The column was eluted with 2000 ml of a linear gradient of 1–50 mM KCl (– – –). Fractions of 10 ml were collected and analyzed for sugar content (———) by the phenol–sulfuric acid method. The shaded area indicates fractions pooled for subsequent steps.

TABLE III

EFFECTS OF CARBON COMPOUNDS ON ENZYME FORMATION

The organism was incubated in chemically defined medium (5 ml) containing 1% of the carbon compounds indicated for 3 days at 30 °C. The cultures were centrifuged and the supernatants were brought to 60% satn of $(NH_4)_2SO_4$. The precipitate was collected by centrifugation and dissolved in 5 ml of 0.01 M acetate buffer (pH 5.8). The solution was dialyzed overnight against the same buffer at 4 °C. The activity of this solution was measured.

Carbon source in culture medium	Activity (units/ml)	Growth (absorbance)
Succinoglucan	0.96	4 2
Desuccinylated succinoglucan	0.83	3.8
Yeast glucan	0.01	2.5
Lutean	0.02	3.2
Luteose	0.01	4 0
Laminaran	0 01	6.5
Curdlan	0	0
Pachyman	0	0
Carboxymethyl cellulose	0	0
Soluble starch	0	2.4
Cellobiose	0	3 5
Laminaribiose	0	0
Gentiobiose	0	2 7
Lactose	0	2.8
Glucose	0	2.9
Succinate	0	1.7

TABLE IV

EFFECT OF HYDROLYSIS OF SUCCINOGLUCAN ON ENZYME FORMATION

Volumes of 5 ml of culture medium containing 1% of the carbon compounds indicated were used. Other conditions were as given in Table III.

Carbon source in culture medium	Average chain length (hexose units)	Enzyme activity (units/ml)	Growth (absorbance)
Succinoglucan	1500*	1.0	4.0
Desuccinylated succinoglucan	1500*	1.1	4.7
AH15	30**	0.23	4.2
AH60	10**	0	5.1
SG-D	12***	0.03	5.6

* Taking the molecular weight as $3 \cdot 10^5$ [2].

** Estimated from the ratio of the reducing activity on complete hydrolysis to the reducing activity of the sample.

*** Determined as described in this paper.

for their abilities to induce succinoglucan depolymerase formation. Only crude extracts of media containing succinoglucan and desuccinylated succinoglucan exhibited high activity. Weak activities were obtained in media with lutean, luteose, laminaran and yeast glucan containing β-1,6-glucosidic linkages, but gentiobiose and the other substrates tested caused no induction. The organism could not grow on carboxymethyl cellulose, curdlan, pachyman or laminaribiose.

The effect of a hydrolyzate of succinoglucan on induction of the depolymerase was studied. Succinoglucan was hydrolyzed with 0.5 M H_2SO_4 at 100 °C, and aliquots of the reaction mixture were removed after 15 and 60 min, respectively, and immediately neutralized with $BaCO_3$. The resultant precipitate of $BaSO_4$ was removed by centrifugation. The supernatant was deionized with Amberlite IR-120 and IR-45, and concentrated to dryness. These preparations and the final product (SG-D) formed by the depolymerase from succinoglucan were tested for their abilities to induce the depolymerase with above carbon compounds (Table IV). With increase in

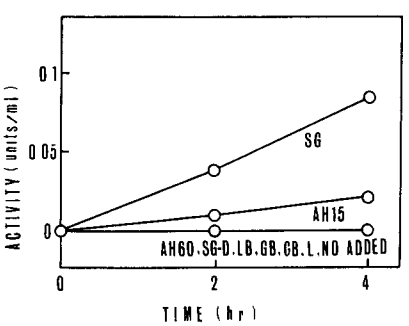

Fig. 7. Induction of depolymerase by succinoglucan and related compounds. Substrates were added at zero time to culture during exponential growth on defined medium containing 0.6% xylose as a carbon source. SG, succinoglucan; AH15 and AH60, hydrolyzates of succinoglucan formed by acid treatment for 15 min and 60 min, respectively; SG-D, final product of succinoglucan formed by depolymerase; LB, laminaribiose; GB, gentiobiose; CB, cellobiose; L, lactose.

the extent of hydrolysis induction ability decreased rapidly, although the rate of growth was not affected appreciably. The ability of this organism to grow on SG-D is due to the presence of some β-glucanase capable of acting on the substrate, present in the cells.

When succinoglucan (0.1 mg/ml) was added to cells in the defined medium containing 0.6% xylose, during the logarithmic growth phase, rapid enzyme formation occurred (Fig. 7). AH15 caused slight enzyme induction while AH60, SG-D, laminaribiose, gentiobiose, cellobiose and lactose did not. When xylose was replaced by glucose or succinic acid, no activity was induced even at the presence of succinoglucan. These carbon compounds repress formation of the depolymerase like that of other inducible enzyme.

DISCUSSION

Previously [4] we showed that *Flavobacterium* sp. Strain M64 produces different, substrate specific β-glucanases, depending on whether succinoglucan or luteose is present in the growth medium. Our succinoglucan depolymerase is the first enzyme which has been found to degrade succinoglucan well although many enzymes are known to split luteose. Succinoglucan is resistant to various pure and crude β-glucanases, including β-1,3-glucanase and β-1,4-glucanase (cellulase). A preparation of cellulase from *Basidiomycetes* can split luteose well but its activity on succinoglucan is only three hundredths of that on luteose. The structure of the polysaccharide moiety of succinoglucan is shown in Fig. 8 [3]. No other polysaccharide besides succinoglucan seems to be known which is composed of β-1,3-, β-1,4- and β-1,6-glucosidic linkages. Kefiran in kefir grain which is composed of β-1,4- and α-1,3-

→3Glc 1→4Glc 1→3Glc 1→3Glc 1→6Glc 1→4Glc 1→3Glc 1→3Glc 1→4Glc 1→3Gal 1→4Glc 1→

Fig. 8. Possible structure of the polysaccharide chain of succinoglucan [3].

galactosidic linkages and α-1,6-glucosidic linkage was cleaved at the β-1,6-glucosidic linkage [16] on long incubation (180 days) with a preparation of cellulase from *Trichoderma*. It is not clear whether the linkage of kefiran was split by cellulase itself or by another enzyme(s) contaminating the cellulase preparation. Kefiran, like succinoglucan, is quite resistant to usual glucanases. The activity of the cellulase preparation of *Trichoderma* on succinoglucan was also very weak. The succinoglucan depolymerase shows very high activity on succinoglucan.

Succinoglucan depolymerase seems to hydrolyze a specific glucosidic linkage in succinoglucan or desuccinylated succinoglucan. The presence of a succinic acid radical is unnecessary for the action of the enzyme. It is unknown which linkage is hydrolyzed. However, the fact that the *DP* of the final product of enzymatic degradation was 12 and suggests that only one linkage in each structural unit is hydrolyzed. The result also seems to provide evidence that succinoglucan consists of repeating units of about 12 degrees of polymerization.

Only succinoglucan and desuccinylated succinoglucan had the ability to induce the depolymerase and their inductive abilities were remarkably decreased by acid or enzymatic hydrolysis. These results suggest that succinoglucan itself acts as an inducer

of the depolymerase. It has been found that cellulase formation in *Pseudomonas* [17] is induced by cellulose but not by cellodextrin or cellobiose under usual culture conditions while high enzyme activities of both cellulases were attained if cellobiose was added consecutively. Formation of succinoglucan depolymerase, was not induced when 0.01 % of a partial acid hydrolyzate of succinoglucan or the final product of the action of the depolymerase was added to medium containing xylose. It seems possible, therefore, that even highly polymerized succinoglucan may reach the site for depolymerase induction without prior hydrolysis.

Torriani and Pappenheimer [18] demonstrated that a highly specific enzyme that degraded capsular polysaccharide of Type 111 pneumococcus is induced by the polysaccharide or its partial hydrolysis products in *B. palustris*. Generally, the linkages required for induction of hydrolytic enzymes are the same as those which are attacked by these enzymes. However, it is very interesting that, as in the case of our enzyme, a highly specific enzyme capable of hydrolyzing a polymer, was also induced significantly only by the same polymer.

The depolymerase is not directly useful for study of the structure of succinoglucan since the final product of its action has too large a molecular weight, with about 12 degrees of polymerization. Other enzymes acting on the final product are present in the cells of this, and other microorganisms, and studies on their actions are now in progress.

REFERENCES

1 Harada, T. (1965) Arch. Biochem. Biophys. 112, 65–69
2 Misaki, A., Saito, H., Ito, T. and Harada, T. (1969) Biochemistry 8, 4645–4650
3 Saito, H., Misaki, A. and Harada, T. (1970) Agric. Biol. Chem. 34, 1683–1689
4 Harada, T., Moori, K. and Amemura, A. (1972) Agric. Biol. Chem. 36, 2611–2613
5 Nelson, J. (1944) J. Biol. Chem. 153, 375–380
6 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
7 Davis, B. J. (1964) Ann. N.Y. Acad. Sci. 121, 404–427
8 Harada, T., Misaki, A. and Saito, H. (1968) Arch. Biochem. Biophys. 124, 292–298
9 Saito, H., Misaki, A. and Harada, T. (1968) Agric. Biol. Chem. 32, 1261–1269
10 Maeda, M. and Nisizawa, K. (1968) J. Biochem. Tokyo 63, 199–206
11 Nakamura, N., Ooyama, J. and Tanabe, O. (1961) Nippon Nogeikagaku Kaishi 35, 949–953
12 La Riviere, J. W. and Kooiman, P. (1967) Arch. Mikrobiol. 59, 269–278
13 Kikumoto, S., Miyashima, T., Kimura, K., Ookubo, S. and Komatsu, N. (1971) Nippon Nogeikagaku Kaishi 45, 162–168
14 Björnoal, H., Lindberg, B. and Svensson, S. (1967) Acta Chem. Scand. 21, 1801–1804
15 Manners, D. J., Masson, A. J. and Sturgeon, R. J. (1971) Carbohydr. Res. 17, 109–114
16 Kooiman, P. (1968) Carbohydr. Res. 7, 200–211
17 Yamane, K., Suzuki, H., Hirotani, M., Ozawa, H. and Nisizawa, K. (1970) J. Biochem. Tokyo 67, 9–18
18 Torriani, A. and Pappenheimer, Jr, A. M. (1962) J. Biol. Chem. 237, 3–13