APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1980, p. 40-47
0099-2240/80/07-0040/08$02.00/0

Vol. 40, No. 1

Purification of Glycoside Hydrolases from *Bacteroides fragilis*

JAN-OLOF BERG, LENNART LINDQVIST, AND CARL E. NORD*

Department of Bacteriology, National Bacteriological Laboratory, and Department of Oral Microbiology, Karolinska Institute, S-105 21 Stockholm, Sweden

Six glycoside hydrolases in the culture medium of *Bacteroides fragilis*—α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, β-*N*-acetylglucosaminidase, and α-L-fucosidase—were systematically purified by ammonium sulfate precipitation, gel filtration chromatography, and density gradient isoelectric focusing. The isoelectric focusing resolved the glycosidases into distinct, well-separated fractions and revealed three differently charged forms of β-*N*-acetylglucosaminidase and of α-L-fucosidase. Furthermore, α-glucosidase and β-*N*-acetylglucosaminidase were shown to possess dual affinities for the respective galactoside substrates, and β-galactosidase also hydrolyzed β-D-fucoside. α-Glucosidase was purified to homogeneity, as indicated by a thin-layer isoelectric focusing zymogram technique. The glycosidases, with exception of β-glucosidase and the acid α-L-fucosidase, were each separated from other glycosidic activities to 99%. The molecular weights varied between 58,000 and 125,000. The pH optima ranged from 4.8 to 6.9.

The energy sources of the intestinal bacteria in humans have attracted a great deal of attention (17). The stability of the intestinal microflora has led to the hypothesis that mucins and other endogenous substances are important nutritional factors for these bacteria (11). Nutritional balance studies have shown digestion of dietary carbohydrates that cannot be degraded by mammalian enzymes. The disappearance of such carbohydrates has been explained by the hydrolytic activities of intestinal bacteria (7). On the basis of these studies, it has been postulated that the natural energy sources of colon bacteria are complex polysaccharides (17). To utilize the various sugars, the polysaccharides must be degraded enzymatically.

Bacteroides fragilis is a rich source of glycoside hydrolases which can cleave low-molecular-weight polysaccharides and glycosyl moieties of glycoproteins or glycolipids into monosaccharides. The formation of α-glucosidase, β-galactosidase, and β-*N*-acetylglucosaminidase has been reported in a previous study (1). The glycoside hydrolases appeared during the late logarithmic growth phase and in the stationary phase of *B. fragilis* cultures, mainly in association with the cells but also to a fair extent in the culture supernatant.

The present paper deals with the purification of α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, β-*N*-acetylglucosaminidase, and α-L-fucosidase from culture supernatants of a strain of *B. fragilis*.

MATERIALS AND METHODS

Bacterial strain. *B. fragilis* B 70 was chosen for the present investigation because it produced greater amounts of the glycoside hydrolases than other strains previously tested (1).

Culture conditions. *B. fragilis* B 70 was grown in 1-liter fermentors (FL 101, Biotec, Stockholm, Sweden). The pH (7.0) was kept constant by means of an automatic titrator (LP-300, Biotec), and sodium hydroxide (1 M) was used for titration. An autoclavable combined glass reference electrode (GK 4031, Radiometer, Copenhagen, Denmark) was used. Temperature control was effected by the use of a controlling thermometer placed into a pocket immersed in the culture, a transistorized relay, and a 40-W cartridge heater. The temperature was 37 ± 0.01°C. Agitation was performed by an impeller stirring at 200 rpm (LP-300, Biotec). An anaerobic atmosphere was maintained by bubbling a mixture of oxygen-free nitrogen (92%, vol/vol), carbon dioxide (5%, vol/vol), and hydrogen (3%, vol/vol) through the culture at a rate of 0.05 liter/min. The medium contained proteose peptone yeast extract, glucose, cysteine-hydrochloride, and resazurin (1).

Glycoside hydrolase assays. The glycoside hydrolase activities were assayed with *p*-nitrophenyl glycosides (5 mM) as substrates (13) (Table 1). All enzyme assays were conducted in 0.05 M sodium phosphate buffer, pH 6.2, at 37°C.

The substrate buffer mixture (0.5 ml) was incubated with 0.2 ml of enzyme solution, and the reaction was stopped by addition of 2 ml of 0.6 M potassium borate buffer, pH 10.4. Hydrolysis of the *p*-nitrophenyl glycosides was determined spectrophotometrically at 410 nm in a Beckman spectrophotometer 24. Enzyme activity was expressed in katals, i.e., the amount of

TABLE 1. *Enzyme activities of and assays for glycosidases of B. fragilis*

Enzyme	EC no.	*p*-Nitrophenyl substrate	Activity[a]
α-Glucosidase	3.2.1.20	*R*-α-Glucoside	+
β-Glucosidase	3.2.1.21	*R*-β-Glucoside	+
α-Galactosidase	3.2.1.22	*R*-α-Galactoside	+
β-Galactosidase	3.2.1.23	*R*-β-Galactoside	+
α-*N*-Acetylglucosaminidase	3.2.1.50	*R*-α-2-Acetamido-2-deoxy-D-glucoside	−
β-*N*-Acetylglucosaminidase	3.2.1.30[b]	*R*-β-2-Acetamido-2-deoxy-D-glucoside	+
α-*N*-Acetylgalactosaminidase	3.2.1.49	*R*-α-2-Acetamido-2-deoxy-D-galactoside	−
β-*N*-Acetylgalactosaminidase	3.2.1.53[b]	*R*-β-2-Acetamido-2-deoxy-D-galactoside	+
α-Mannosidase	3.2.1.24	*R*-α-Mannoside	−
β-Mannosidase	3.2.1.25	*R*-β-Mannoside	−
α-L-Fucosidase	3.2.1.51	*R*-α-L-Fucoside	+
β-D-Fucosidase	3.2.1.38	*R*-β-D-Fucoside	+

[a] Presence (+) or absence (−) of activity in the culture supernatant.

[b] For the nomenclature of the β-hexosaminidase activities, see text.

enzymatic activity transforming 1 mol of substrate per s under the standard conditions.

Protein determination. Protein was assayed after extensive dialysis by the Lowry method (10) with bovine serum albumin as a standard or by using the absorbance at 280 nm as a rough determination of the proteins in the fractions at the different purification steps.

Isoelectric focusing. A 440-ml column (8100, LKB Produkter, Stockholm-Bromma, Sweden) was used for density gradient electrofocusing. Carrier Ampholines, pH 3.5 to 10 and pH 5 to 7, in equal parts were used in a final concentration of 2% (vol/vol) to establish the pH gradient. The electrofocusing was run for 48 h at 4°C with a final potential of 400 to 500 V (2 to 4 mA). Isoelectric focusing in thin-layer polyacrylamide gels was performed on a Multiphor LKB 2117 (LKB Produkter). Ampholine polyacrylamide gel plates (pH 3.5 to 9.5, LKB Produkter) were used. The electrofocusing was run for 2 h at 4°C with a power of 30 W.

Staining of the gels. Proteins were stained by the method of Söderholm et al. (18) with Coomassie brilliant blue. Demonstration of α-glucosidase activity was performed by immersing the gel into 0.10 M phosphate buffer (pH 6.0) containing 0.03% 2-naphthyl-α-D-glucopyranoside and 0.1% fast red violet RB salt. The incubation was carried out at 20°C for 30 min.

Gel filtration. Columns of Sephadex G-200 (150 by 5 cm, 100 by 2.6 cm, or 35 by 2.6 cm) equilibrated with 0.10 M tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 7.5, containing 0.20 M KCl as standard solvent and of Sephadex G-25 (25 by 5 cm), equilibrated with 0.5% Ampholine, were used.

Purification procedures. Unless otherwise stated, all purification steps were performed at 4 to 6°C. Centrifugations were done at 13,700 × *g* for 15 min in a Sorvall RC 2-B centrifuge. After centrifugation of the culture, the supernatant was precipitated by ammonium sulfate in two steps by the method of Glasgow et al. (3). The first precipitation was performed at 70% saturation with ammonium sulfate. The precipitate was dissolved in and dialyzed against distilled water (step 2). A second precipitation of this material was done to obtain the fraction between 25 and 70% saturation with ammonium sulfate, and the precipitate was dissolved in 70 ml of 0.10 M tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 7.5 (step 3). The enzyme solution was passed through a Sephadex G-200 column (150 by 5 cm) using the standard buffer as eluant (step 4). The fractions containing glycosidase activities were collected and concentrated by ammonium sulfate precipitation (70% saturation). The precipitate was dissolved in 50 ml of a 0.5% Ampholine solution and desalted by Sephadex G-25 column chromatography. The column was equilibrated and eluted with a 0.5% Ampholine solution (pH range, 3.5 to 10; average pH, 7.2) (step 5). The desalted enzyme fraction was collected and used for density gradient isoelectric focusing (step 6). Further purification was obtained by Sephadex G-200 gel filtration chromatography of each separate enzyme (steps 7 and 8).

Molecular weight determination. The molecular weight of the glycosidases was determined by Sephadex G-200 gel filtration chromatography with blue dextran, bovine λ-globulin, transferrin, bovine albumin, ovalbumin, α-chymotrypsinogen, myoglobin, and ribonuclease as markers. The molecular weight markers were chromatographed, and a plot of the logarithms of the molecular weight of the markers versus the partition coefficients (k_{av}) was used for evaluation of the molecular weight of the glycosidases.

Chemicals. All chemicals were of analytical grade unless otherwise stated. *p*-Nitrophenyl-α- and β-D-glucopyranosides, *p*-nitrophenyl-α- and β-D-galactopyranosides, *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide, *p*-nitrophenyl-*N*-acetyl-β-D-galactosaminide, *p*-nitrophenyl-α- and β-mannosides, *p*-nitrophenyl-α-L-fucoside, *p*-nitrophenyl-β-D-fucoside, fast red violet RB salt, 2-naphthyl-α-D-glucopyranoside, bovine λ-globulin, transferrin, bovine albumin, ovalbumin, myoglobin α-chymotrypsinogen, and ribonuclease were obtained from Sigma Chemical Co., St. Louis, Mo. *p*-Nitrophenyl-*N*-acetyl-α-D-glucosaminide and *p*-nitrophenyl-*N*-acetyl-α-D-galactosaminide were obtained from Koch-Light Laboratories Ltd., Colnbrook, United Kingdom. Coomassie blue R 250 was purchased from ICI, Manchester, United Kingdom. Salts and constituents for buffers were obtained from Merck AG, Darmstadt, West Germany. Ampholine carrier ampholytes were purchased from LKB-Produkter, Stockholm, Sweden, and blue dextran and Sephadex G-25 and G-200 were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Culture media were purchased from Difco Laboratories, Detroit, Mich.

RESULTS

Separation of glycosidases. A summary of the purification is given in Table 2. The glycosidases from 1 liter of culture supernatant were concentrated and purified by ammonium sulfate

TABLE 2. *Purification of glycosidases from B. fragilis*

Enzyme and purification	Step no.	Vol (ml)	Total protein (mg)	Total activity (nkat)	Sp act (nkat/mg)	Yield (%)	Purification factor
α-Glucosidase							
Culture supernatant	1	950	18,350	3,030	0.165	100	1
2nd $(NH_4)_2SO_4$ precipitation	3	71	1,020	2,120	2.08	68	13
1st G-200 gel chromatography	4	65	224	1,060	4.73	35	29
Isoelectric focusing	6	20	48.4	727	15.0	24	91
2nd G-200 gel	7	26	4.7	506	108.0	17	654
3rd G-200 gel chromatography	8	22	0.880	395	449.0	13	2,720
β-Glucosidase							
	1	950	18,350	31.6	0.0017	100	1
	3	71	1,020	21.7	0.0212	69	13
	4	65	224	20.0	0.0892	63	52
	6	14	10.1	4.66	0.461	15	268
	8	20	0.280	0.800	2.86	3	1,660
α-Galactosidase							
	1	950	18,350	48.3	0.0026	100	1
	3	71	1,020	38.3	0.0375	79	14
	4	65	224	25.0	0.112	51	43
pI 6.2, 54%	6	22	9.90	7.50	0.758	15	291
	8	14	0.090	1.57	17.4	3	6,709
β-Galactosidase							
	1	950	18,350	840	0.0458	100	1
	3	71	1,020	523	0.513	62	11
	4	65	224	390	1.74	46	38
2nd G-200 gel chromatography	7	31	0.710	66.7	93.9	8	2,050
β-*N*-Acetylglucosaminidase							
	1	950	18,350	1,240	0.0676	100	1
	3	71	1,020	967	0.948	78	14
	4	65	224	800	3.57	65	53
pI 4.4, 14%	6	19	49.5	41.7	0.842	3	12
	7	25	1.60	16.3	10.2	1	150
pI 5.8, 55%	6	14	10.3	163	15.8	13	233
	7	25	0.950	61.7	64.9	5	960
pI 6.0, 31%	6	22	9.95	91.7	9.21	7	136
	7	25	1.07	36.7	34.3	3	506
α-L-Fucosidase							
	1	950	18,350	348	0.0190	100	1
	3	71	1,020	293	0.287	84	15
	4	65	224	265	1.18	76	62
pI 5.9, 8%	6	14	10.2	10.0	0.980	3	52
	7	24	1.07	1.67	1.53	0.5	80
pI 6.3, 40%	6	23	7.18	47.2	6.57	13	345
	7	24	0.520	14.6	28.2	4	1,484
pI 8.2, 52%	6	27	7.64	61.2	8.01	17	421
	7	30	0.540	19.5	36.1	6	1,901

precipitation in two steps with a 11- to 15-fold increase in specific activity for the different enzymes. The subsequent Sephadex G-200 chromatography step separated the main enzyme fraction from high-molecular-weight components (Fig. 1). All glycosidases showed a tendency to bind to material eluting in the void volume peak, but this binding behavior was limited by the pH and ionic strength of the buffer solution. The glycosidases were recovered within the included volume, representing molecular weights between 60,000 and 120,000. The isoe-

lectric focusing resolved the glycosidases into distinct fractions in the pH range 4.3 to 8.3 (Fig. 2). One α-glucosidase fraction possessing a minor α-galactosidase activity with pI 4.9, one β-glucosidase fraction with pI 5.6, one α-galactosidase fraction with pI 6.2, and one β-galactosidase fraction with pI 7.0 were obtained. β-*N*-acetylglucosaminidase was recovered in three fractions with pI 4.4, 5.8, and 6.0. α-L-Fucosidase was separated into two major and one small fraction with pI 6.3, 8.2, and 5.8.

Purification of α-glucosidase. Contaminating ampholytes and proteins were removed by a two-step Sephadex G-200 gel filtration chromatography (steps 7 and 8). The first gel filtration step was performed with the standard buffer. The second step made use of the retardation of the α-glucosidase by the Sephadex gel equilibrated with 0.02 M sodium phosphate buffer, pH 6.0 (Fig. 3). The pooled enzyme fraction (26 ml) from the gel filtration step 7 was applied

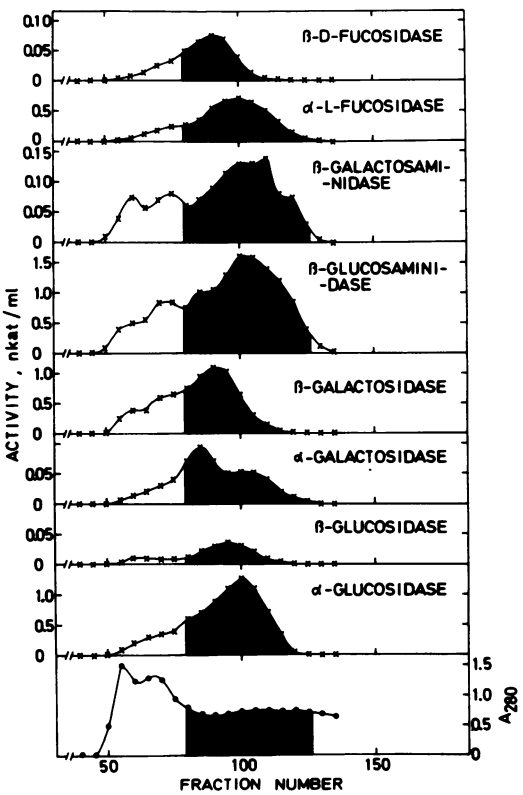

FIG. 1. *First gel filtration on Sephadex G-200 (step 4) of glycosidases from B. fragilis. The material from step 3 (71 ml) was applied on a column (150 by 5 cm) and eluted with 0.10 M tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 7.5) containing 0.20 M KCl. Flow rate: 15 ml/h. Fraction volume: 10 ml. Presence of named glycosidases in fractions indicated by shading.*

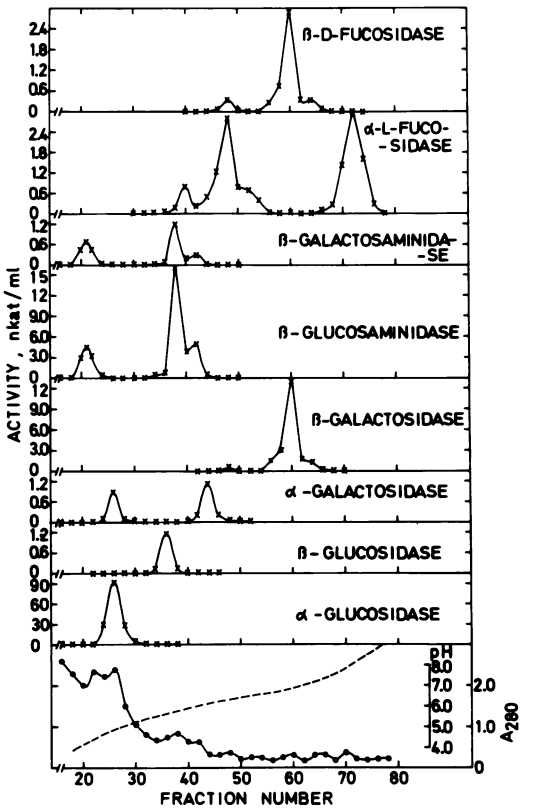

FIG. 2. *Isoelectric focusing of glycosidases from B. fragilis. The desalted material from step 5 (120 ml) was applied to the column in a 2% Ampholine solution (1%, pH 3.5 to 10, and 1%, pH 5 to 7) stabilized with a sucrose gradient. After 48 h at 400 to 500 V (2.0 to 0.8 W) and at 14°C, the column was drained into 5.0-ml fractions.*

onto the gel, and then elution was performed with 0.10 M tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 7.5) containing 0.30 M KCl. The "adsorption" of α-glucosidase by the Sephadex gel was inhibited by the rise in ionic strength and pH of the eluting buffer solution. Thus, α-glucosidase was recovered as a highly purified preparation (Fig. 4). The α-glucosidase fractions from the last three steps contained slight α-galactosidase activity, averaging 0.16% of the α-glucosidase activity.

Purification of β-glucosidase. Further purification after the isoelectric focusing step was obtained by two gel filtration chromatographies on Sephadex G-200 equilibrated and eluted with the standard buffer solution. The enzyme was separated from other glycosidases except for β-*N*-acetylglucosaminidase. The final yield was 3%, with a 1,660-fold increase in specific activity.

Purification of α-galactosidase. The isoelectric focusing resolved α-galactosidase activity

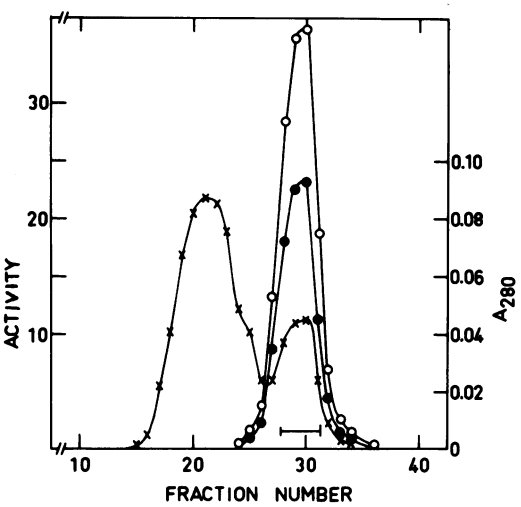

FIG. 3. *Gel filtration of α-glucosidase from B. fragilis utilizing the retardation of the enzyme on Sephadex G-200. The pooled enzyme fraction from step 7 (26 ml) was applied on a column (25 by 2.6 cm) equilibrated with 0.02 M sodium phosphate buffer, pH 6.0, and immediately eluted with 0.10 M tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 7.5, containing 0.30 M KCl. Fraction volume: 5 ml. Symbols: ●, α-glucosidase activity, nanokatals per milliliter; ○, α-galactosidase activity, picokatals per milliliter; ×, absorbance at 280 nm.*

into two separate fractions, namely one fraction with pI 4.9 together with α-glucosidase and one fraction with pI 6.2 with no other glycosidase activity. The pI 4.9 fraction was α-glucosidase with affinity for α-galactoside (purified as described above, Fig. 3). The latter fraction contained 54% of the total α-galactosidase activity recovered by the isoelectric focusing technique and was designated as α-galactosidase. The final purification of the α-galactosidase pI 6.2 was achieved by gel filtration chromatography twice on Sephadex G-200 with a 6,709-fold purification based on the initial specific activity on *p*-nitrophenyl-α-galactoside given by α-glucosidase and α-galactosidase together.

Purification of β-galactosidase. The main β-galactosidase fraction with pI 7.0 was separated by isoelectric focusing from all other glycosidases except β-D-fucosidase. The ratio between the β-D-fucosidase activity and the β-galactosidase activity was 1:15 in all fractions from the isoelectric focusing column and in the final Sephadex G-200 chromatography step. The purification was 2,050-fold, with an 8% yield.

Purification of β-*N*-acetylglucosaminidase. The relative distribution of β-*N*-acetylglucosaminidase activity between the differently charged forms separated by the isoelectric focusing was 14% in the pI 4.4 form, 55% in the pI 5.8 form, and 31% in the pI 6.0 form. The β-*N*-acetylgalactosaminidase activity in these fractions was 8% of the β-*N*-acetylglucosaminidase

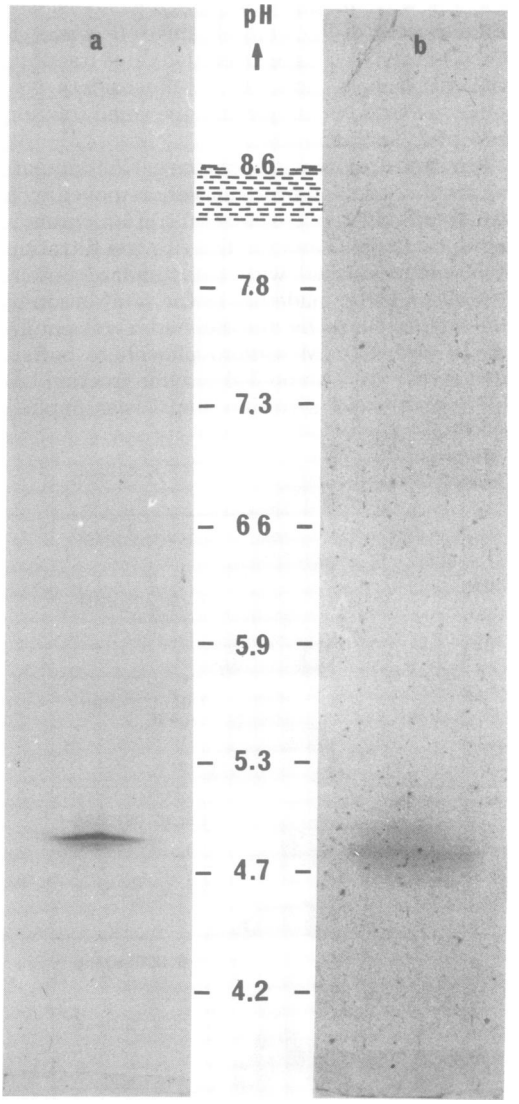

FIG. 4. *Isoelectric focusing in thin-layer polyacrylamide gel of α-glucosidase from B. fragilis. A 2.5-nkat quantity (20 μl, 5.5 μg) of the concentrated and desalted α-glucosidase fraction from step 8 was absorbed into filter paper (10 by 5 mm) and applied to the gel. The electrofocusing was run for 2 h with a power of 30 W. After termination of the focusing, the gel was cut in two slices perpendicular to the electrode strips. (a) Stained for demonstration of proteins with Coomassie brilliant blue. (b) Stained for demonstration of α-glucosidase activity with 2-naphthyl-α-D-glucopyranoside. The shaded area marks the position of sample application.*

activity in the pI 4.4 form, 4% in the pI 5.8 form, and 1% in the pI 6.0 form. Further purification was obtained by Sephadex G-200 gel filtration chromatography, and the final purification fold was 150 for the enzyme with pI 4.4, 960 for the pI 5.8 form, and 506 for the pI 6.0 form, based on the initial specific activity for all β-*N*-acetylglucosaminidase forms together.

Purification of α-L-fucosidase. α-L-Fucosidase was also separated into three differently charged fractions by the isoelectric focusing technique. The acid α-L-fucosidase was not separated from the β-*N*-acetylglucosaminidase with pI 5.8 either by isoelectric focusing or by gel filtration chromatography. The remaining α-L-fucosidases representing 40% (pI 6.3) and 52% (pI 8.2) of the total yield were separated from other glycosidases, except particularly for β-D-fucosidase in the case of the pI 6.3 form, after a final Sephadex G-200 chromatography step. The purification reached 1,484-fold (pI 6.3) and 1,901-fold (pI 8.2) based on the initial specific activity for all α-L-fucosidase forms together.

Glycosidase activities in the purified enzyme preparations. The purified glycosidases were assayed with *p*-nitrophenyl-glycosides listed in Table 1. Substrates where no enzyme activity was detected in the culture supernatant were not tested (Table 3). Activity on two substrates was regarded as dual substrate affinity of the enzyme if the results from the isoelectric focusing step and the following Sephadex G-200 gel filtration chromatographies showed a constant ratio between the two activities. Dual substrate affinities were found in the following preparations: α-glucosidase and β-*N*-acetylglucosaminidase showed slight affinities for the respective galactoside substrates, and β-galactosidase hydrolyzed β-D-fucoside. An increase in the ratio between the main enzyme activity and other glycosidic activities during the last purification steps (steps 6 to 8) indicated removal of contaminating glycosidases and in some cases the presence of small amounts of contaminating glycosidase in the final preparation, e.g., the presence of β-*N*-acetylglucosaminidase, β-galactosidase, and β-D-fucosidase in the pI 6.3 form of α-D-fucosidase (Table 3).

Physical properties of the purified glycosidases. Estimated molecular weights, pH optima, and pI's are given in Table 4. The molecular weights varied from 125,000 ± 12,000 (α-galactosidase) to 58,000 ± 6,000 (β-*N*-acetylglucosaminidase, pI 5.8). The pH optima ranged from pH 6.9 (β-galactosidase) to pH 4.8 (β-*N*-acetylglucosaminidase, pI 6.0).

DISCUSSION

This paper reports the purification of six different exoglycosidases and of three differently charged forms of β-*N*-acetylglucosaminidase and α-L-fucosidase, respectively. The initial Sephadex G-200 gel filtration chromatography was used to separate the glycosidases from high-molecular-weight components which tended to adsorb the enzymes and obstruct further purification. This binding behavior has also been reported for *Bacillus subtilis* β-*N*-acetylglucosaminidase (14). This adsorption was reduced by using potassium chloride at a 0.2 M concentration in the buffer solution. Higher ionic strength inhibited some of the glycosidases irreversibly.

The use of isoelectric focusing as a preparative step in the purification revealed differently charged forms of β-*N*-acetylglucosaminidase and α-L-fucosidase. β-*N*-Acetylglucosaminidase

TABLE 3. *Glycosidic activities in the purified glycosidases from B. fragilis*

Purified enzyme prepn	Activity (% of purified enzyme prepn activity)							
	α-Glucosidase	β-Glucosidase	α-Galactosidase	β-Galactosidase	β-*N*-Acetylglucosaminidase	β-*N*-Acetylgalactosaminidase	α-L-Fucosidase	β-D-Fucosidase
α-Glucosidase	100	<0.01	0.16	<0.01	<0.01	<0.01	<0.01	<0.01
β-Glucosidase	0.90	100	<0.01	<0.01	70.5	2.75	<0.01	<0.01
α-Galactosidase	0.13	<0.01	100	<0.01	1.6	<0.02	<0.01	<0.01
β-Galactosidase	<0.01	<0.01	<0.01	100	<0.01	<0.01	0.20	14.0
β-*N*-Acetylglucosaminidase								
pI 4.4	65	<0.01	0.10	<0.01	100	8.0	<0.01	<0.01
pI 5.8	0.90	<0.02	<0.02	<0.01	100	3.9	1.7	<0.01
pI 6.0	0.30	<0.01	0.80	0.60	100	1.1	0.8	<0.01
α-L-Fucosidase								
pI 5.9	5.9	0.16	<1.0	0.30	660	20	100	<1.0
pI 6.3	0.05	<0.01	0.10	0.60	1.43	<0.01	100	0.30
pI 8.2	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	100	<0.01

TABLE 4. *Physical properties of the purified glycosidases from B. fragilis*

Enzyme	pI	Mol wt[a]	pH optimum
α-Glucosidase	4.9	60,000 ± 5,000	ND[b]
β-Glucosidase	5.6	93,000 ± 10,000	5.7
α-Galactosidase	6.2	125,000 ± 12,000	5.5
β-Galactosidase	7.0	58,000 ± 5,000	6.9
β-*N*-Acetylglucosaminidase	4.4	ND	6.4
β-N-Acetylglucosaminidase	5.8	58,000 ± 6,000	5.5
β-N-Acetylglucosaminidase	6.0	74,000 ± 10,000	4.8
α-L-Fucosidase	5.8	68,000 ± 7,000	6.3
α-L-Fucosidase	6.3	68,000 ± 8,000	6.3
α-L-Fucosidase	8.2	85,000 ± 10,000	6.1

[a] Mean value ± standard deviation of three determinations.
[b] ND, Not determined.

has been purified from *Bacillus subtilis* (14), *Escherichia coli* (19) and *Streptococcus pneumoniae* (6), but none of these studies reported more than one enzyme with β-*N*-acetylglucosaminidase activity.

The purified α-glucosidases had weak activity on *p*-nitrophenyl-α-D-galactoside. The isoelectric focusing and the final two-step Sephadex G-200 chromatography indicated that this activity was due to one enzyme, α-glucosidase, with a low affinity on α-galactoside. Most investigations on α-glucosidase report a strong substrate specificity. α-Glucosidase from *Pseudomonas fluorescens* (4) showed a specificity for maltose but very limited action against glucose polymers and no activity on sucrose or isomaltose as substrates. Yeast α-glucosidases have been reported to be either maltases or isomaltases (9). The affinity of β-*N*-acetylglucosaminidase for β-*N*-acetylgalactosaminide is well known (12). The three charged forms of β-*N*-acetylglucosaminidases showed varying levels of β-*N*-acetylgalactosaminidase activity (1 to 8%). These activities co-purified. So far it has not been possible to classify the enzymes as EC 3.2.1.30 β-*N*-acetylglucosaminidase or EC 3.2.1.52 β-*N*-acetylhexosaminidase, since both enzymes are reported to be active on β-*N*-acetylgalactosaminide.

The β-galactosidase was active against β-D-fucoside. This double affinity has been reported earlier for *S. pneumoniae* β-galactosidase (5).

The vast spectrum of glycosidases produced by *B. fragilis* indicates its ability to hydrolyze and ferment polysaccharides from various sources. Salyers et al. (16) have reported fermentation of monosaccharide components of mucin by *B. fragilis* but not hydrolysis of complete polysaccharides. It has also been shown that bacterial β-*N*-acetylglucosaminidases are capable of releasing terminal 2-acetamido-2-deoxy-D-glucose residues from desialylated glycoproteins (6). The findings by Salyers et al. (16) indicate that the *Bacteroides* glycosidases are capable of participating in the degradation of mucin.

B. fragilis has also been reported to ferment various plant polysaccharides (16). α-Glucosidase participates in the hydrolysis of α(1-4)glucans such as maltose, amylose and amylopectin. The breakdown of β(1-4)glucans cannot be performed by human glycosidases. Degradation of such glucans has been reported in the human colon (2, 15, 17), and one of the microorganisms with cellulase activity may be *B. fragilis*.

Galactosylsucrose from soybeans is another type of plant oligosaccharide that is not digestible by human intestinal enzymes. Thus, α-galactosidase of bacterial origin has been suggested to have a nutritional role in the breakdown of soybean oligosaccharides (8).

The present investigation has shown the range of glycosidic activities of *B. fragilis*. α-Glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, β-*N*-acetylglucosaminidases, and α-L-fucosidases have been purified. Characterization of the kinetic properties and substrate specificities is in progress and will be reported in a following paper. Purified and characterized glycosidases may be valuable tools for further studies of the ecology of the microflora indigenous to humans.

LITERATURE CITED

1. **Berg, J.-O., C. E. Nord, and T. Wadström.** 1978. Formation of glycosidases in batch and continuous culture of *Bacteroides fragilis*. Appl. Environ. Microbiol. **35:** 269-273.
2. **Betian, H. G., B. A. Linehan, M. P. Bryant, and L. V. Holdeman.** 1977. Isolation of a cellulolytic *Bacteroides* sp. from human feces. Appl. Environ. Microbiol. **33:** 1009-1010.
3. **Glasgow, L. R., J. P. Paulson, and R. L. Hill.** 1977. Systematic purification of five glycosidases from *Streptococcus* (Diplococcus) *pneumoniae*. J. Biol. Chem. **252:**8615-8623.
4. **Guffanti, A. A., and W. A. Corpe.** 1976. Partial purification and characterization of alpha-glucosidase from *Pseudomonas fluorescens W.* Arch. Microbiol. **107:** 269-276.
5. **Hughes, R. C., and R. W. Jeanloz.** 1964. The extracellular glycosidases of *Diplococcus pneumoniae*. I. Purification and properties of a neuraminidase and a β-galactosidase. Action on the α_1-acid glycoprotein of human plasma. Biochemistry **3:**1535-1543.
6. **Hughes, R. C., and R. W. Jeanloz.** 1964. The extracellular glycosidases of *Diplococcus pneumoniae*. II. Purification and properties of a β-N-acetylglucosaminidase. Action on a derivate of the α_1-acid glycoprotein of human plasma. Biochemistry **3:**1543-1548.
7. **Jeanes, A.** 1975. Digestibility of food polysaccharides by man: a review, p. 336-347. *In* A. Jeanes and J. Hodge

(ed.), Physiological effects of food carbohydrates. American Chemical Society, Washington, D.C.

8. **Kawamura, S., T. Kasai, and S. Tanusi.** 1976. Purification and properties of α-galactosidase from *Escherichia coli* subsp. *communior* IAM 1272. Agr. Biol. Chem. **40**:641–648.
9. **Lai, H-Y.L., and B. Axelrod.** 1975. The specificity of the synthetic reaction of two yeast α-glycosidases. Biochim. Biophys. Acta **391**:121–128.
10. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
11. **Moore, W. E. C., and L. V. Holdeman.** 1975. Discussion of current bacteriological investigations of the relationship between intestinal flora, diet and colon cancer. Cancer Res. **35**:3418–3420.
12. **Nomenclature Committee of the International Union of Biochemistry.** 1979. Enzyme nomenclature. Academic Press, Inc., New York.
13. **Nord, C. E., L. Linder, T. Wadström, and A. A. Lindberg.** 1973. Formation of glycoside-hydrolases by oral streptococci. Arch. Oral Biol. **18**:391–402.
14. **Ortiz, J. M., J. B. Gillespie, and R. C. W. Berkeley.** 1972. An exo-β-N-acetylglucosaminidase from *Bacillus subtilis B*; extraction and purification. Biochim. Biophys. Acta **289**:174–186.
15. **Salyers, A. A., J. K. Palmer, and T. D. Wilkins.** 1977. Laminarase (β-glucanase) activity in *Bacteroides* from the human colon. Appl. Environ. Microbiol. **33**:1118–1124.
16. **Salyers, A. A., J. R. Vercellotti, S. E. H. West, and T. D. Wilkins.** 1977. Fermentation of mucin and plant polysaccharides by strains of *Bacteroides* from the human colon. Appl. Environ. Microbiol. **33**:319–322.
17. **Salyers, A. A.** 1979. Energy sources of major intestinal fermentative anaerobes. Am. J. Clin. Nutr. **32**:158–163.
18. **Söderholm, J., P. Allestam, and T. Wadström.** 1972. A rapid method for isoelectric focusing in polyacrylamide gel. FEBS Lett. **24**:89–92.
19. **Yem, D. W., and H. C. Wu.** 1976. Purification and properties of β-N-acetylglucosaminidase from *Escherichia coli*. J. Bacteriol. **125**:324–331.