Purification and Characterization of Conjugated Bile Salt Hydrolase from *Bifidobacterium longum* BB536

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***Bifidobacterium* species deconjugate taurocholic, taurodeoxycholic, taurochenodeoxycholic, glycocholic, glycodeoxycholic, and glycochenodeoxycholic acids. The enzyme level increases in the growth phase. No increase in activity is observed for the cytoplasmic enzyme after addition of conjugated bile acids to a stationary-phase culture. Conjugated bile salt hydrolase (BSH) was purified from *Bifidobacterium longum* BB536. Its apparent molecular mass in denaturing polyacrylamide gel electrophoresis was ca. 40,000 Da. The intact enzyme had a relative molecular weight of ca. 250,000 as determined by gel filtration chromatography, suggesting that the native BSH of *B. longum* is probably a hexamer. The purified enzyme is active towards both glycine and taurine conjugates of cholate, deoxycholate, and chenodeoxycholate. The pH optimum is in the range of 5.5 to 6.5. A loss of BSH activity is observed after incubation at temperatures higher than 42°C; at 60°C, 50% of the BSH activity is lost. The importance of free sulfhydryl groups at the enzyme active center is suggested. For *B. longum* BB536, no significant difference in the initial rate of deconjugation and enzymatic efficiency appears between bile salts. The enzymatic efficiency is higher for *B. longum* BB536 than for other genera. In this paper, a new method which permits a display of BSH activity directly on polyacrylamide gels is described; this method confirms the molecular weight obtained for *B. longum* BB536 BSH.**

The indigenous microflora of the intestinal tract interact intensively with host-produced substances (33), such as mucins (27) and bile acids (8, 9, 13, 14). Bile salts are biotransformed into a number of metabolites by the intestinal microflora of humans and animals (18). The hydrolysis of conjugated bile acids is one of the most common microbial bile salt biotransformations. The conjugated bile salt hydrolase (BSH) enzyme catalyzes the hydrolysis of the amide bond that conjugates bile acids to glycine or taurine (18). It has been demonstrated that this microbial activity is related to growth depression in chickens (8) and the small bowel syndrome in humans (16, 32). BSH activity has been detected in members of the genera *Clostridium*, *Lactobacillus*, *Fusobacterium*, *Bacteroides*, *Bifidobacterium*, *Peptostreptococcus*, and *Streptococcus* (6, 28, 29). Several BSH enzymes from *Bacteroides fragilis* subsp. *fragilis* (31), *Bacteroides vulgatus* (20), *Clostridium perfringens* (15), and *Lactobacillus* spp. (24) have been purified and characterized. These purified enzymes differ in their physical, kinetic, and genetic properties, including intact enzyme and subunit molecular weights, pH optimum, $V_{\max}$, and genetic regulation (6).

Only limited information is available on bifidobacterial response to bile salts (10, 19, 22). Previous results indicated that the ability to deconjugate taurocholic and glycocholic acids was widespread among members of the genus *Bifidobacterium*. *Bifidobacterium* spp. showed growth limitation in the presence of sodium glycocholate (19). However, after propagation in TPY devoid of bile salts, the bacterial strains regained normal growth activity.

In this study, we have purified and characterized the conjugated BSH of *Bifidobacterium longum* BB536 (2). Some of its properties were determined. We compare the *Bifidobacterium* enzyme with the conjugated BSHs purified from *Bacteroides fragilis* (35), *Bacteroides vulgatus* (21), *Clostridium perfringens*

(15), and *Lactobacillus* spp. (24, 25). In addition, we describe a new method that allows the direct determination and localization of BSH protein on polyacrylamide gels.

MATERIALS AND METHODS

Materials. Sodium salts of taurocholic, taurodeoxycholic, taurochenodeoxycholic, glycocholic, glycodeoxycholic, glycochenodeoxycholic, cholic, deoxycholic, and chenodeoxycholic acids were obtained from Sigma. Fast protein liquid chromatography (FPLC; Pharmacia LKB, Uppsala, Sweden) was used for purification steps. The columns used were Mono Q HR 5/5 and Superose 12 HR 10/30 from Pharmacia and Methyl HIC (hydrophobic interaction; 5 ml) from Bio-Rad.

Bacterial strains and growth conditions. Table 1 lists the bacterial strains used in this study. *Bifidobacterium* strains were cultured anaerobically for 16 h at 37°C in TPY (34).

Conjugated BSH assays. *Bifidobacterium* strains were tested for conjugated BSH activity by thin-layer chromatography. The conjugated bile salts listed above were tested. TPY broth containing 0.75 mM bile salts was inoculated with bacteria (1%). After incubation under anaerobic conditions (20 h), the samples (10 ml) were adjusted to pH 7 with 1 N NaOH and centrifuged for 15 min at 12,000 × g to remove the cells. The supernatant fractions were adjusted to pH 1 with 1 N HCl. Extracts and controls were spotted onto silica gel plates. The chromatograms were developed as described by Gilliland and Speck (14). A new method was used to allow location of BSH protein directly after electrophoresis on polyacrylamide gels (23). After migration, the inner-layer plate was washed for 30 min in Na₂HPO₄/NaH₂PO₄ buffer (100 mM, pH 6.4) and then cut into 0.5-cm-long segments. The segments were incubated separately for 16 h at 37°C in 1.5-ml microtubes containing 0.2 ml of 0.75 mM taurocholic acid. Afterward, the free bile acids were extracted with three 1-ml portions of ethyl acetate (30). The residues were further extracted with three 1-ml portions of *n*-butanol to recover the conjugated bile acids (30).

The extracts were evaporated to dryness on a rotary evaporator under reduced pressure. The residues were dissolved in 1.5 ml of 0.01 N NaOH. Afterward, the concentrations of free and conjugated bile salts were determined colorimetrically by the method of Irvin et al. (20).

Another more sensitive method was used during the purification and characterization of BSH. An enzymatic assay was performed by measuring the release of amino acids resulting from hydrolysis of the amide bond of bile salts. The reaction mixture (80 µl) contained the protein (10 to 50 µg), 4 mM bile salts, and 100 mM Na₂HPO₄/NaH₂PO₄ buffer (pH 6.4). The reaction mixture was incubated at 37°C for 15 min. The enzymatic reaction was terminated by the addition of an equal volume of 15% trichloroacetic acid, and precipitated proteins were removed by centrifugation at 15,000 × g for 15 min. The amount of amino acids present in the supernatant was measured by the procedure of Church et al. (7). In brief, 680 µl of borate buffer, 50 mM 1% sodium dodecyl sulfate (SDS, pH

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TABLE 1. *Bifidobacterium* strains used in this study

Origin	Strain	Source ^a
Human	<i>B. longum</i> BB536	Benno et al. (2)
	<i>B. longum</i> ATCC 15707 ^T	ATCC
	<i>B. longum</i> ATCC 15708	ATCC
	<i>B. breve</i> ATCC 15700 ^T	ATCC
	<i>B. breve</i> ATCC 15698	ATCC
	<i>B. infantis</i> ATCC 25962	ATCC
	<i>B. infantis</i> ATCC 15697 ^T	ATCC
	<i>B. adolescentis</i> CIP 6458	CIP
	<i>B. adolescentis</i> CIP 6459	CIP
	<i>B. adolescentis</i> CIP 6460	CIP
	<i>B. adolescentis</i> CIP 6461	CIP
	<i>B. bifidum</i> CIP 6464	CIP
	<i>B. bifidum</i> CIP 6465	CIP
Animal	<i>B. animalis</i> ATCC 25527 ^T	ATCC
	<i>B. pseudolongum</i> ATCC 25526 ^T	ATCC
	<i>B. thermophilum</i> ATCC 25525 ^T	ATCC

^a ATCC, American Type Culture Collection, Rockville, Md.; CIP, Collection de l'Institut Pasteur, Paris.

9.5), and 80 μ l of 0.3% (wt/vol) trinitrobenzenesulfonic acid (TNBS) were added to an 80- μ l aliquot of supernatant. The mixture was incubated in darkness at room temperature for 30 min. Color development was stopped by the addition of 800 μ l of 0.6 N HCl. Amino acid concentrations were determined at 346 nm by using a standard curve prepared with free taurine or glycine.

Purification of BSH. (i) **Preparation of cell extract.** After growth, 500 ml of broth culture was centrifuged at 12,000 $\times$ g for 1 h. The pellet was washed with 50 mM Na₂HPO₄/NaH₂PO₄ (pH 6.4), and centrifuged at 12,000 $\times$ g for 30 min. The cells were disrupted by sonication three times for 2 min each at 0°C (5 μ m peak to peak) with an ultrasonic oscillator (MSE, Crawley, Sussex, England). After centrifugation at 12,000 $\times$ g for 30 min, the supernatant was used in the purification experiment. The protein concentration was determined by the Bradford assay (4).

(ii) **Mono Q anion-exchange chromatography.** The cell extract was applied to a Mono Q HR 5/5 column preequilibrated (flow rate, 1 ml/min) with 50 mM Na₂HPO₄/NaH₂PO₄ (pH 6.4)–50 mM NaCl (buffer A). The column was washed (5 times the bed volume) to remove unbound materials. The proteins were eluted with a gradient of sodium chloride in buffer A (see Fig. 3). Fractions (2 ml) were collected and immediately assayed for BSH activity. Solid ammonium sulfate (0.7 M final concentration) was added to active fractions. The resulting extract was used for hydrophobic interaction chromatography.

(iii) **Methyl HIC hydrophobic interaction chromatography.** The column was developed (flow rate, 0.5 ml/min) by a linear ammonium sulfate gradient (0.7 to 0 M) in buffer A. Because of ammonium sulfate and TNBS interaction, the fractions were dialyzed against 1 liter of buffer A for 20 h at 4°C before BSH activity determination.

BSH purity and molecular weight estimation. Protein purity was estimated by subjecting aliquots from each purification step to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 12% slab gels (23) and in non-denaturing gels of 7% acrylamide with buffers free of SDS and 2-mercaptoethanol. Both denaturing and non-denaturing gels were stained with Coomassie R250 blue. The apparent molecular weight of the subunit was estimated by comparing its migration with that of standard proteins of known molecular weight on denaturing polyacrylamide gels. In addition, the molecular weight was estimated by gel filtration with Superose HR 10/30 (flow rate, 0.5 ml/min) equilibrated with 100 mM NaCl in buffer A.

Partial characterization of BSH. The effects of Cu²⁺, Mg²⁺, iodoacetic acid, and EDTA (30 mM each), Ca²⁺, F⁻, and N₃⁻ (50 mM each), periodic acid (3 mM), and common thiol inhibitors (parachloromercuribenzoic acid [0.01 mM], *N*-ethylmaleimide [1 mM], iodoacetamide [30 mM], arsenic acid [10 mM], mercuric acetate [0.1 mM], and potassium hexacyanoferrate III [1 mM]) on BSH activity were tested after 30 min of incubation at 37°C. All compounds were dissolved in buffer A. Buffer A (pH 5.8 to 8) and Toerell and Stenhagen's citrate-phosphate buffer, 100 mM (pH 2 to 12), were used to study pH effect on BSH activity.

RESULTS

Testing strains for BSH activity. We have demonstrated by thin-layer chromatography experiments that all growing strains deconjugated the different bile salts tested and produced exclusively the corresponding free bile salt. These results show

FIG. 1. Effect of taurocholic acid on BSH activity in *B. longum* BB536. An overnight culture was divided into two aliquots; 0.75 mM taurocholic acid was added to one (□) but not to the other (△). Thereafter, cells were removed at 0, 10, 20, 30, and 60 min and assayed for BSH activity. BSH activity is expressed as millimoles of cholic acid formed per minute per liter of culture.

that all *Bifidobacterium* strains have BSH activity and deconjugate all biliary salts tested.

Effect of conjugated bile acids on BSH activity. Different *Bifidobacterium* strains (Table 1) were tested. When taurocholic acid was added to cells and samples were removed at intervals, the deconjugation product was released immediately and linearly with respect to time. This result suggested that taurocholic acid did not induce or activate the hydrolase. This hypothesis was tested with cultures grown anaerobically at 37°C to the stationary phase and divided into two aliquots. To one, 0.75 mM taurocholic acid in 5 mM sodium acetate buffer (pH 5.0) was added; to the other, an equal volume of acetate buffer was added. Cells were removed from both samples at 0, 10, 20, 30, and 60 min. Whole cells or corresponding cell extracts (obtained from cells disrupted by sonication) were tested for BSH activity. The same activity was determined for each extract (Fig. 1). The other conjugated and unconjugated bile acids listed in Materials and Methods, taurine, and glycine, were tested for the ability to stimulate BSH activity in whole cells. These different compounds have no effect on BSH activity.

Growth phase regulation of BSH activity. BSH level was examined as a function of the growth of a culture. Cells from an overnight culture of *B. longum* or *B. animalis* were inoculated into fresh TPY broth, incubated anaerobically at 37°C, and sampled every hour thereafter. After the cell density was measured in the samples, the cells were assayed for their BSH level. The enzyme level increased in exponential-phase cells (Fig. 2). A weak increase in BSH level was detected in stationary-phase cells. The same BSH level was obtained in cells grown in medium containing taurocholic acid as in cells grown in medium free of bile salts.

Purification of BSH. BSH was purified from *B. longum* BB536 by the procedure detailed under Materials and Methods (Table 2). Purification was monitored by measuring enzyme activity with taurocholic acid (4 mM) as the substrate. The intracellular extract was passed over a Mono Q anion-exchange column. Proteins with hydrolase activity were found in fractions eluted between 0.11 and 0.15 M NaCl (Fig. 3).

FIG. 2. BSH activity during growth of *B. longum*. Cells from an overnight culture were used to inoculate fresh TPY medium (1%). The cells were then removed. The cell density of the sample was determined by measuring the optical density OD at 600 nm (□), and BSH activity (△) was determined with taurocholic acid. BSH activity is expressed as millimoles of cholic acid formed per minute per liter of culture.

Active fractions of BSH were pooled and passed over a hydrophobic interaction column. Proteins with hydrolase activity were found in the fraction eluted at 0.4 M ammonium sulfate. The electrophoretogram of the active fractions at the various stages of purification is shown in Fig. 4. The purified extract contained a protein migrating as a single band in SDS-PAGE. This protein had an apparent molecular mass of 42,000 to 38,000 Da. The same single band was obtained in nondenaturing PAGE. For the purified extract, the same mass (42,000 to 38,000 Da) was determined by gel filtration.

For *B. longum* and *B. animalis* crude extracts, two fractions with BSH activity were obtained for each strain by gel filtration. The first corresponded to proteins of 270,000 to 240,000 Da and the second to proteins of 42,000 to 38,000 Da. Nevertheless, for *B. animalis* ATCC 25527, no activity was detected after passage over the Mono Q anion-exchange column.

Detection of BSH activity after electrophoresis. Crude intracellular extracts of *B. longum* and *B. animalis* were used. The same segments with BSH activity were obtained for each strain. For each extract, in nondenaturing conditions, two segments presented BSH activity. The first segment, called segment A, had a low electrophoretic mobility. A higher electrophoretic mobility was obtained for the second, called segment B. In denaturing conditions, BSH was only detected in segment B, corresponding to 42,000 to 38,000 Da. After the purification procedure for *B. longum* BB536, direct determination after

TABLE 2. Purification of BSH from *B. longum* BB536

Step	Total protein (mg)	Amino acids formed (mmol/min)		Recovery (%)	Purification (fold)
		Sp act (per mg of protein)	Total activity		
Crude extract	42.14	8.21	346	100	1
Mono Q	5.7	58.15	331.45	95.8	7.1
Methyl-HIC	0.66	270	178.2	51.5	32.9

FIG. 3. Elution profile of BSH activity of *B. longum* BB536 from Mono Q column. The column was developed with a gradient of sodium chloride in buffer A (▲). The optical density (OD) at 280 nm (□) was determined for each fraction. BSH activity (●) is expressed as millimoles of cholic acid formed per minute per fraction.

SDS-PAGE confirmed that the purified protein was the BSH enzyme.

pH and temperature optima of hydrolase. Reactions were run with the purified enzyme at 37°C for 15 min. Studies on the effect of pH on enzyme catalysis (Fig. 5) showed a broad pH optimum over the range from 5.5 to 6.5. The optimum temperature of catalysis was between 30 and 40°C (Fig. 6). The activity was determined after incubation for 30 min at different temperatures. A loss of activity was observed at temperatures higher than 42°C. At 60°C, 50% of the BSH activity was lost (Fig. 6).

Substrate specificities and kinetic analysis of BSH. Concerning the initial rates of deconjugation and V_{max}/K_m ratios which represented enzymatic efficiencies, no significant differences appeared between bile salts (Table 3).

Effects of ions and common thiol inhibitors. A complete inhibition was observed with Cu^{2+} , iodoacetic, and periodic acids (Table 4). There was no inhibition of BSH with EDTA, Ca^{2+} , Mg^{2+} , F^- , and N_3^- . The inhibition suggests that easily complexed groups (possibly sulfhydryl groups) are involved at the enzyme active site. To determine if sulfhydryl groups were

FIG. 4. Electrophoresis of cell extracts at each purification step through a 12% denaturing polyacrylamide gel. Lanes: 1, size standards; 2, crude cell extracts; 3, pooled fractions from Mono Q column; 4 and 5, pooled fractions from methyl-HIC column. The molecular weights of the standards were (top to the bottom) 94,000, 67,000, 43,000, and 30,000. The arrow indicates BSH.

FIG. 5. pH optimum of BSH activity. BSH activity is expressed as millimoles of cholic acid formed per minute per milligram of protein. Two different buffers were used: buffer A (Δ , pH 5.8 to 8) and Toerell and Stenhagen's citrate-phosphate buffer, 100 mM ($\blacksquare$, pH 2 to 12).

involved in the catalytic reaction, common thiol inhibitors were tested (Table 4). Inhibition was observed with all compounds.

DISCUSSION

Our results indicate that the ability to deconjugate bile salts is widespread among members of the genus *Bifidobacterium*. The six different bile salts are deconjugated by all strains. Thus, it may be assumed that bifidobacteria, one of the main bacterial groups in the gut, have an important role in the transformation of bile salts in the intestine. Through this activity, the bifidobacteria might perhaps contribute to the composition of the gut flora simply by virtue of the fact that through deconjugation, they accentuate the capacity of bile salts to inhibit certain microorganisms (3, 11, 12).

FIG. 6. Effect of temperature on BSH activity of *B. longum* BB536. The activity was determined at different temperatures (Δ). The enzyme was incubated for 30 min at different temperatures, and thereafter BSH activity was determined ($\square$). BSH activity is expressed as millimoles of cholic acid formed per minute per milligram of protein.

TABLE 3. Initial rate and $V_{\max}/K_m$ ratio for bile salts determined with *B. longum* BB536 purified extract^a

Bile salt	Initial rate (mM/min)	$V_{\max}/K_m$ (mM/min per mM)
Taurocholic acid	0.331 ($\pm 4.13 \times 10^{-3}$)	0.378 ($\pm 3.98 \times 10^{-3}$)
Taurodeoxycholic acid	0.250 ($\pm 3.87 \times 10^{-3}$)	0.483 ($\pm 2.77 \times 10^{-3}$)
Taurochenodeoxycholic acid	0.405 ($\pm 2.76 \times 10^{-3}$)	0.251 ($\pm 3.19 \times 10^{-3}$)
Glycocholic acid	0.378 ($\pm 3.69 \times 10^{-3}$)	0.284 ($\pm 4.22 \times 10^{-3}$)
Glycodeoxycholic acid	0.381 ($\pm 3.47 \times 10^{-3}$)	0.278 ($\pm 2.56 \times 10^{-3}$)
Glycochenodeoxycholic acid	0.372 ($\pm 3.95 \times 10^{-3}$)	0.265 ($\pm 3.11 \times 10^{-3}$)

^a Values are averages $\pm$ standard deviations for four determinations.

The BSH activity has been characterized, and the enzyme with this activity from *B. longum* BB536 has been purified. The BSH level increases during the exponential phase of growth (Fig. 2). For *Lactobacillus* sp. strain 100-100 and *B. fragilis* (35), the activity increased only when a culture of the organism entered the stationary phase (24). For *B. longum*, bacterial lysis explains the weak increase in BSH activity during the stationary phase. BSH activity in whole cells and corresponding cell extracts incubated in medium containing taurocholic acid is the same as that in whole cells and corresponding cell extracts incubated in medium free of bile salt. Therefore, bile salts apparently do not regulate the hydrolase. BSH activity in *Lactobacillus* sp. strain 100-100 is regulated by conjugated bile salts (24). For the *Bifidobacterium* genus, the same results obtained with whole cells and corresponding cell extracts incubated in medium with and without bile salts suggest that no extracellular factor regulates BSH activity. In the case of *Lactobacillus* sp. strain 100-100, an inducible extracellular factor that regulates BSH activity has been characterized (26).

We have purified the BSH from *B. longum* BB536. The molecular mass of the intact enzyme was estimated to be 270,000 to 240,000 Da, which is similar to values reported for BSH purified from *B. fragilis* (35) and *C. perfringens* (15). The native molecular weight of the BSH from *B. vulgatus* (21) is 140,000. Four isoenzymes of BSH with approximate native molecular weights of 115,000, 105,000, 95,000, and 80,000 were purified from *Lactobacillus* sp. strain 100-100 (25).

TABLE 4. Effects of different compounds on *B. longum* BB536 BSH^a

Compound or ion	Concn (mM)	% Inhibition
Cu ²⁺	30	83 $\pm$ 6
Mg ²⁺	30	0 $\pm$ 2
Iodoacetic acid	30	91 $\pm$ 4
EDTA	30	0 $\pm$ 3
Ca ²⁺	50	0 $\pm$ 2
F ⁻	50	0 $\pm$ 4
N ₃ ⁻	50	0 $\pm$ 3
Periodic acid	3	87 $\pm$ 7
para-Chloromercuribenzoic acid	0.01	80 $\pm$ 3
N-Ethylmaleimide	1	95 $\pm$ 4
Iodoacetamide	30	89 $\pm$ 6
Arsenic acid	10	85 $\pm$ 5
Mercuric acetate	0.1	96 $\pm$ 7
Potassium hexacyanoferrate	1	93 $\pm$ 6

^a Values are averages $\pm$ standard deviations for four determinations. Following incubation of the enzyme with inhibitors for 30 min at 37°C prior to the addition of the substrate, taurocholic acid, activity was measured as described in Materials and Methods.

The subunit composition of BSH enzymes from other bacteria is different. The native protein from *B. fragilis* was reported to be an octamer (subunit molecular weight, 32,500), whereas the intact enzymes from *C. perfringens* (subunit molecular weight 56,000) and *B. vulgatus* are tetramers. For *Lactobacillus* sp. strain 100-100, the native proteins are a trimer (subunit α , 42,000; subunit β , 38,000) (25). In this report, we demonstrate that the BSH of *B. longum* is probably a hexamer. The approximate molecular weight of the subunit is 42,000 to 38,000. In our conditions of purification, the structure of the complex is not stable. Hence, only the subunits are obtained after this experiment.

The BSH from *B. longum* BB536 is located in the intracellular space. The same result is obtained for *Lactobacillus* sp. strain 100-100 (24) and *C. perfringens* (15). The BSH from *B. fragilis* (35) is located both intracellularly and in the periplasmic space. The BSH from *B. longum* BB536 has similarities to the BSH from *C. perfringens*, including pH optimum and localization (15). It differs from the *C. perfringens* enzyme, however, in subunit molecular weight and enzymatic efficiency and from the hydrolase from *B. fragilis* in native and subunit molecular weight, pH optimum, and growth phase regulation. The enzymatic efficiencies, represented by $V_{\max}/K_m$ ratios, are approximately identical for the enzymes from *B. longum* BB536 and *B. fragilis*. The enzymatic efficiency of BSH from *B. longum* BB536 is higher than that determined for the *Lactobacillus* (10-fold) and *C. perfringens* (1,000-fold) enzymes. This result demonstrates an advantage of this *Bifidobacterium* strain compared with other genera of the intestinal flora (*Lactobacillus* and *Clostridium*).

Study of the substrate specificity of BSH from human fecal cultures (1, 7) has demonstrated that the portion of the amide bond and the introduction of various amino acids at or around the amide bond influence the rate of hydrolysis. Gopal-Srivastava and Hylemon (15) and Stellwag and Hylemon (35) have demonstrated that glycine and taurine conjugates of cholic and deoxycholic acids are hydrolyzed; however, taurine conjugates were hydrolyzed less readily than glycine conjugates. Concerning *B. longum* BB536, all bile salts are hydrolyzed, and no significant difference is noticed between bile salts.

Iodoacetamide, iodoacetic and periodic acids, *p*-chloromercuribenzoate, potassium hexacyanoferrate, *N*-ethylmaleimide, arsenic acid, mercuric acetate, and Cu^{2+} significantly inhibited BSH activity, suggesting the importance of free sulfhydryl groups at the enzyme active center. Such effects of a thiol group modification have been reported in earlier studies (15, 31).

Growing cells and intracellular extracts of *B. longum* and *B. animalis* deconjugate bile salts. The same segments with BSH activity were obtained after gel electrophoresis for each strain. However, BSH is not detected after passage over the Mono Q column for the *B. animalis* extract. This great difference between *B. longum* and *B. animalis* suggests a difference in the apparent stability of the protein, the presence of a factor that stimulates the intracellular BSH activity, as in *Lactobacillus* sp. strain 100-100 (25), or differences in BSH activity regulation.

In a subsequent study, purified BSH will be used for NH_2 -terminal amino acid sequence determination. The intent is to compare this sequence with those obtained for BSH from *C. perfringens*. An additional study is the cloning of the BSH gene by the direct plate assay (5). This step is important in understanding the role of these organisms in bile acid transformations in the intestinal tract.

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