Biochemical and Molecular Insights into Bile Salt Hydrolase in the Gastrointestinal Microflora - A Review -

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ABSTRACT : Bile salt deconjugation is the most biologically significant reaction among the bacterial alterations of bile acids in the gastrointestinal tract of human and animal. The responsible enzyme, bile salt hydrolase (BSH), catalyzes the hydrolysis of glycine- and/or taurine-conjugated bile salts into amino acid residues and deconjugated bile acids. Herein we review current knowledge on the distribution of BSH activity among various microorganisms with respect to their biochemical and molecular characteristics. The proposed physiological impact of BSH activity on the host animal as well as on the BSH-producing bacterial cells is discussed. BSH activity of the probiotic strains is examined on the basis of BSH hypothesis, which was proposed to explain cholesterol-lowering effects of probiotics. Finally, the potential applications of BSH research are briefly discussed. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 10 1505-1512)***

Key Words : Bile Salt Hydrolase (BSH), Gut Microbes, Probiotics, Cholesterol Lowering, Genetic Marker

INTRODUCTION

Bile acids have amphipathic characteristics due to their hydrophobic and hydrophilic moieties. The steroid ring of the bile acids has one face that is hydrophobic (that with methyl groups) and one that is hydrophilic (that with the hydroxyl groups); the amino acid conjugate is polar and hydrophilic. Their amphipathic nature enables bile acids to play an important role to emulsify the dietary lipids increasing the contact between lipases and lipid substrates. During the transit into the colon, the bile acids are exposed to approximately 2×10^{11} to 5×10^{11} bacteria per gram wet weight of human feces (Moore and Holdeman, 1974). The intestinal microflora can generate many different bile acid metabolites from cholic acid and chenodeoxycholic acid. Among biotransformation by GI microorganisms, bile salt deconjugation is the most biologically significant reaction. The classes of microbial enzymes that catalyze hydrolysis of conjugated bile salts have been collectively named bile salt hydrolase (BSH, EC 3.5.1.24).

BSH activity has been widely detected in several bacterial genera of the autochthonous gastrointestinal microbiota of animals including mice, rats, humans, chickens, and swine (Savage et al., 1977). However, it remains to be determined whether the BSH activity of the GI microbes including many commercially available probiotic strains is beneficial or detrimental to the host. While the potential positive aspects of BSH activity of the probiotics have been discussed, other possible negative concerns such as carcinogenic compounds on BSH activity have also been shown.

This review begins with a brief overview of the bile salt metabolism and follows with a distribution of BSH activity, biochemical and molecular aspect of the BSH enzymes, the proposed physiological impact of BSH activity on the host animal and its implication for BSH-producing bacterial cells are discussed. The BSH hypothesis is proposed as a means to explain cholesterol-lowering effects of probiotics. This paper describes up to date information on bile salt hydrolase and the significance of BSH research.

ENTEROHEPATIC CIRCULATION AND BILE SALT METABOLISM

Enterohepatic circulation of bile acids

Bile acids constitute approximately 50% of the organic components of bile. After synthesis from cholesterol in the liver, bile acids are conjugated with glycine and amino acid analogue taurine (Hofmann, 1977). The gallbladder stores and concentrates bile during the fasting state. Upon lipid intake, the bile salts are secreted into the duodenum, where they are intimately associated with dietary lipids and various digestive products. Conjugated bile acids are poorly absorbed by passive diffusion in the small and large intestines and mainly absorbed at the terminal ileum by the active transport mechanisms (Lack and Weiner, 1966), which is called ileum bile acid transporter (IBAT). After absorption, the mixture of bile salts is partly returned to the liver by the hepatic portal circulation in the process known as enterohepatic circulation (EHC). Roughly 600 to 800 ml of bile is produced every day. The bile acid pool is

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Table 1. Summary of purification studies for BSH from various microorganisms

Organisms	Molecular weight		Optimum pH	Location	Literatures
	Sub-unit	Native			
C. perfringens ATCC 19574			5.6-5.8	Intracellular	Nair et al. (1967)
E. faecalis			<4.8	Intracellular	Aries and Hill (1970a)
C. welchii			5.0-6.0	Intracellular	"
Bifidobacterium			5.0-6.0	Extracellular	"
B. fragilis $NCTC_{9343}$			5.0-6.0	Intracellular	"
B. fragilis ssp. fragilis ATCC 25285	32.5 kD	250 kD	4.2-4.5	Intracellular	Stellwag and Hylemon (1976)
B. fragilis 2536			4.5-5.0	Intracellular	Masuda (1981)
C. perfringens PB 6K			4.5-5.0	Intracellular	"
C. perfringens MCV 815	56.0 kD	250 kD	5.8-6.4	Intracellular	Gopal-Srivastava and Hylemon (1988)
B. vulgatus	36.0 kD	140 kD	5.6-6.4	Intracellular	Kawamoto et al. (1989)
L. acidophilus sp. 100-100	42.0 kD 38.0 kD			Intracellular	Lundeen and Savage (1990)
B. longum BB536	40.0 kD	250 kD	5.5-6.5	Intracellular	Grill et al. (1995)
L. acidophilus O16		126 kD	5.0-6.0	Intracellular	Corzo and Gilliland (1999b)
L. acidophilus L1		126 kD	3.5-4.5	Intracellular	"
L. acidophilus ATCC 43121		126 kD	3.5-4.5	Intracellular	"
B. longum SBT2928	37.3 kD	130 kD	5.0-7.0	Intracellular	Tanaka et al. (2000)
Xanthomonas maltophilia CBS 827.97	52.0 kD	100.0 kD	7.9-8.5	Intracellular	Dean et al. (2002)

approximately 1.5 to 4 g in size, whereas the daily, total circulating bile acid pool is 17 to 40 g. The entire bile acid pool recirculates 6 to 15 times per day, and approximately 0.2 to 0.5 g is lost in the feces, which is compensated by *de novo* bile acid synthesis.

Metabolism of bile acids in gastrointestinal microflora

With bile acids flowing in large amounts through the digestive tract, the complex autochthonous gastrointestinal microflora have evolved the ability to transform the bile salts to a great extent (Baron and Hylemon, 1997). The intestinal microflora can generate about 20 different bile acid metabolites from cholic acid and chenodeoxycholic acid (Drasar and Hill, 1974). Known biotransformation by GI microbes includes deconjugation of the conjugated bile salts to liberate free bile acids and amino acid moiety (Aries and Hill, 1970a), removal of hydroxyl groups principally the 7 carbon hydroxyl group of the cholic acid moiety (Gustafsson et al., 1966), oxidative and reductive reactions of the existing hydroxyl groups (Aries and Hill, 1970b), and epimerization of bile acids (Canzi et al., 1989). Among those reactions, bile salt deconjugation is the most biologically significant reaction in that it performs a 'gatekeeping' function: hydrolysis of the conjugate bile acids is a prerequisite for any sterol transformation (Batta et al., 1990).

Deconjugation of bile salts

The bile acid synthesis and conjugation is performed in the liver while in the intestine microbial activity can split the conjugates into free bile acids (Midtvedt, 1974). The classes of microbial enzymes that catalyze hydrolysis of conjugated bile salts have been collectively named conjugated bile salt hydrolases, which is also called cholylglycine hydrolases (Batta et al., 1984).

Bile salt hydrolases produced by intestinal microorganisms are involved in the first steps of bile acid transformations. The bacterial deconjugation of bile salts is considered to increase modestly in the distal ileum and markedly in the colon, since unconjugated bile acids are not present in any appreciable proportion in jejunal content (Mallory et al., 1973), but slowly increase proportionally in the ileum (Northfield and McColl, 1973). In feces most of bile acids are in the unconjugated form (Tanida et al., 1984). It was proposed that intestinal bacteria are responsible for the deconjugation from the experiment with germ-free animals in which the conjugates appear intact in the feces (Chikai et al., 1987). From the comparison of the characteristics between lactobacilli-free and lactobacilli-colonized mice, it was revealed that lactobacilli are responsible for most of the bile salt hydrolase activity in the intestinal tracts of mice (Tannock et al., 1989).

BILE SALT HYDROLASE

BSH activity has been observed mainly in bacterial species of the gastrointestinal microorganisms, including *Lactobacillus sp.* (Lundeen and Savage, 1990), *Bifidobacterium longum* (Grill et al., 1995), *Clostridium perfringens* (Gopal-Srivastava and Hylemon, 1988).

Bacteroides vulgatus (Kawamoto et al., 1989) and *Bacteroides fragilis* (Stellwag and Hylemon, 1976). In addition to autochthonous intestinal microbiota, BSH activity was also reported from an enterophathogenic strain of *Listeria monocytogenes* (Dussurget et al., 2002) and bile-adapted strain of *Xanthomonas maltophilia* (Dean et al., 2002).

Biochemical characteristics of bile salt hydrolases

Nair et al. (1967) partially purified, for the first time, the bile salt hydrolyzing enzyme from *Clostridium perfringens*, and is now commercially available (SIGMA Co.). Over the last several decades, BSH enzymes have been purified from several gastrointestinal microorganisms. The summary of purification studies on BSH from various microorganisms is shown in Table 1.

Genetics of bile salt hydrolases

In addition to the enzyme purification studies, molecular approaches have also provided new insight into the BSH of many GI microbes. For the screening of the *bsh* gene from the genomic library, a selective medium has been developed based on the principle of Dashkevicz and Feighner (1989). The first cloning experiment of the *bsh* was carried out by Christiaens et al. (1992), who cloned the gene from the genomic library of *Lactobacillus plantarum* 80. They developed a differential medium for BSH-active *E. coli* clones using LB medium with a modification. Glucose (1%) was added for the acidification of the medium during the growth of *E. coli* and calcium chloride (0.035%) was used since Ca^{2+} can enhance the precipitation of deoxycholic acid. Due to the hydrolysis of taurodeoxycholic acid and medium acidification, BSH-active colonies could be easily detected by the formation of white precipitate around colonies. For the molecular characterization of the bile salt hydrolase, the *bsh* genes have been cloned and sequenced from several bacterial species (Table 2) and BSH-negative mutants of *L. plantarum* (Leer et al., 1993) and *Lactobacillus spp.* (Tannock et al., 1997) have been constructed.

With the advent of the genomics era, many microbial genome projects are providing several *bsh* gene sequences (Table 2). The ongoing and finished genome projects on the probiotic strains have revealed more information on the *bsh* genetics. While a single locus was reported in the *B. longum* genome (Schell et al., 2002), multiple *bsh* loci were also

Table 2. Genetic information of the *bsh* genes from various microorganisms

Organisms (Accession no.)[1]	G+C (%)	ORF	Theoretical		Literatures
			pI	Mw (kD)	
L. plantarum 80 (A24002)	33.44	975 bp	5.12	37.078	Christiaens et al. (1992)
C. perfringens 13 (U20191)	27.50	990 bp	5.26	37.185	Coleman and Hudson (1995)
L. johnsonii 100-100 (AF054971)	38.38	951 bp	4.88	34.914	Elkins and Savage (1998)
L. acidophilus KS-13 (AF091248)	37.12	951 bp	5.29	35.055	Savage and Moser (1999)
B. longum SBT2928 (AF148138)	59.34	954 bp	4.71	35.155	Tanaka et al. (2000)
L. gasseri ADH (AF305888)	33.23	978 bp	4.76	36.132	Russell and Klaenhammer (2001)
L. johnsonii 100-100 (AF297873)	33.94	981 bp	5.05	36.666	Elkins et al. (2001)
L. monocytogenes EGD-e (NP_465591)	36.40	978 bp	5.02	36.848	Dussurget et al. (2002)
Organisms (derived genomic sequences)					
L. plantarum WCFS (NP_786739)	33.44	975 bp	5.12	37.042	Kleerebezem et al. (2003)
L. gasseri Lgas_3 (ZP_00046148)	33.74	978 bp	4.85	36.270	NCBI (2003a)
E. faecalis V583 (NP_814299)	30.67	975 bp	4.90	36.932	Paulsen et al. (2003)
E. faecium FAIR-E 345 (AY260046)	30.56	975bp	4.97	36.645	Wijaya et al. (2003)
C. perfringens str 13 (NP_561625)	27.47	990 bp	5.26	37.185	Shimizu et al. (2002)
B. longum NCC2705 (NP_695975)	59.54	954 bp	4.71	35.125	Schell et al. (2002)

[1] GenBank accession number.

Table 3. Summary of bile salt hydrolase (BSH) activity among various organisms

Organisms	Strains tested	Origin	BSH positive (%)	BSH negative (%)	Literatures
Lactobacillus	66	Human or animal origin	46 (70%)	20 (30%)	Dashkevicz and Feighner (1989)
Bifidobacterium	44	Feces or intestine	43 (98%)	1 (2%)	Tanaka et al. (1999)
	18	Other sources	15 (83%)	3 (17%)	"
Lactobacillus	39	Feces or intestine	23 (59%)	16 (41%)	"
	105	Other sources	28 (27%)	77 (73%)	"
Lactobacillus	30	Feces or intestine	21 (70%)	9 (30%)	Moser and Savage (2001)
	19	Other sources	7 (37%)	12 (63%)	"
Enterococcus	117	Cheese	72 (62%)	45 (38%)	Franz et al. (2001)
E. faecium	11	Cheese	9 (82%)	2 (18%)	Saavedra et al. (2003)
L. reuteri	6	Pig feces	5 (83%)	1 (17%)	Rodríguez et al. (2003)

reported in case of *L. plantarum* (Kleerebezem et al., 2003) and *L. johnsonii* (Pridmore et al., 2004) genomes, implying that they are important for their survival and persistence in the gastrointestinal tract.

Distribution of BSH activity among various microorganisms

Tanaka et al. (1999) carried out a semi-quantitative screening of more than 300 lactic strains of the genera *Bifidobacterium*, *Lactobacillus*, and the species *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Streptococcus thermophilus*. Nearly all bifidobacteria species and strains showed BSH activity, while this activity was detected only in a few species of lactobacilli, but absent in *L. lactis*, *Leu. mesenteroides*, and *S. thermophilus*. A strong correlation could be observed between the habitat of a genus or species and the presence of BSH activity. Most commonly BSH activity was found in strains isolated from the intestines or feces from mammalians from which environment rich in conjugated and unconjugated bile acids. On the other hand, strains and species isolated from other habitats like milk or vegetables, where bile salts are absent, normally did not have BSH activity. Screening of some probiotic cultures for the BSH activity is summarized in Table 3.

PHYSIOLOGICAL ROLE OF BILE SALT HYDROLASE

The proposed physiological impact of bile salt hydrolase on the host animal

Conjugated bile acids are important in the emulsification, digestion, and absorption of lipids, but unconjugated bile acids are generally considered to be less efficient detergents in the emulsification of lipids than their corresponding conjugated bile acids (Hofmann and Mysels, 1992). Furthermore, a high concentration of unconjugated bile acids derived from microbial deconjugation of bile acids in the proximal part of the digestive tract is often associated with malabsorption of lipids causing steatorrhea (Sherwood and Tabaqchali, 1969) and gallstones (Färkkilä and Miettinen, 1990) in humans and growth depression in chickens (Cole and Fuller, 1984).

High BSH activity could be influencing the lipid absorption since free bile acids have very low emulsifying capacity. As a result, the growth rate of animal species might be decreased because conjugated bile acids have an important role in lipid digestion and absorption. However, Bateup et al. (1995) did not find any significant difference in body weight between mice treated with an active BSH lactobacilli and lactobacilli-free group. In this study, growth rates of mice that consumed a nutritionally balanced diet were not affected by the presence of lactobacilli, regardless BSH activity of the strains, in the gastrointestinal tract. In some species, phospholipids play a larger role in emulsifying lipids than the conjugate bile acids. Therefore, growth depression by high levels of bile salt hydrolase might not be as important in swine and mice as in other species (Corzo, 1997).

It was proposed that enhanced bile salt hydrolase activity is not favorable because a subsequent dehydroxylation of primary bile salts by 7α-dehydroxylase active strains could generate toxic and/or mutagenic secondary bile salts (Marteau et al., 1995). It was suggested that an increase in fecal secondary bile salts upon ingestion of BSH-active strains of lactobacilli should be regarded as a potential long-term colon cancer-promoting effect (van Faassen et al., 1987). Furthermore, these toxic bile salts could disturb the normal microbiota of the gut, leading to diarrhea, mucosal inflammation or activation of harmful drugs and carcinogens in the intestinal contents (Salminen et al., 1996).

BSH activity and its implication for BSH-producing bacterial cells

Although BSH activity is a commonly observed phenomenon from many intestinal microorganisms, the importance of this enzyme to the bacterial cells is not well understood. It has been demonstrated that the released taurine from taurocholate was used by certain BSH active strain of *Clostridium* as an electron acceptor, resulting in a higher growth rate (Van Eldere et al., 1988). They suggested

that growth stimulation was due to the sulfonic acid part of taurine, which is rapidly reduced by the deconjugating strain and, after reduction, serves as an electron acceptor in strict anaerobic microorganisms.

De Smet et al. (1995) suggested that deconjugation might be a detoxification mechanism, which is of vital importance to the *Lactobacillus* cell. The protonated form of the bile salt was found to exhibit toxicity through the same mechanism as organic acids, i.e. intracellular acidification. In most of bacteria, the primary defense mechanism against intracellular acidification is pumping out of proton from the cytoplasm by H^+-ATPase at the expense of ATP (Kobayashi, 1985). The BSH enzyme converts conjugated bile salts into their deconjugated counterparts, which are weaker acids. The latter may then recapture the cotransported proton and prevent in this way the excessive expenditure of ATP to maintain pH homeostasis.

Gilliland and Speck (1977) have suggested that deconjugation of bile salts by BSH may enhance antagonist action of autochthonous microorganisms of the intestinal flora such as lactobacilli against pathogens in the intestines. Deconjugation of bile salts resulted in the formation of more toxic compound such as cholic acid and deoxycholic acid (van der Meer et al., 1991). If BSH-active strains have evolved the self-defense mechanism against these toxic compounds, deconjugated bile acids could have a higher effect on pathogenic bacteria than indigenous microorganisms in the gastrointestinal tract.

It has been proposed that BSH activity of enteric bacteria might have an important role for persistent colonization in the gastrointestinal tract (Elkins et al., 2001). Such a hypothesis was drawn partly from the facts that many of BSH active strains originated from an intestinal environment in which they are exposed to bile salts and they have to cope with the toxic nature of the reaction product (Tanaka et al., 1999). However, the advantage to the lactobacilli of producing bile salt hydrolase in relation to colonization of the intestinal tract is not readily apparent.

Bile salt hydrolase and hypocholesterolemic effect of probiotics

De Smet et al. (1994) suggested that the consumption of BSH-active strains, or cultured products containing them might bring about a lowering of serum cholesterol levels through an interaction with the host's bile salt metabolism. The proposed mechanism of hypocholesterolemic effect is comparable with that of bile salt sequestrants, which bind bile salts and prevent them from being reabsorbed.

The free bile acids produced by the enzyme BSH of some probiotics are less water-soluble and are more easily excreted via the feces. Enhanced fecal loss of bile acids may result in an increased requirement for cholesterol as a precursor for the *de novo* synthesis of bile salts to maintain bile salt homeostasis. Therefore, this drain on the bile salt pool might be regarded as a 'biological' alternative to common therapeutic interventions to treat hypercholesterolaemia.

Recently, the hypocholesterolemic effects of some probiotics, which showed high BSH activities from *in vitro* trials, have been confirmed in human (Anderson and Gilliland, 1999) as well as in animals (De Rodas et al., 1996; De Smet et al., 1998; du Toit et al., 1998). However, the hypocholesterolemic mechanism of probiotics based on the BSH hypothesis has not yet been sufficiently elucidated.

CONCLUSIONS AND POTENTIAL APPLICATIONS OF BSH RESEARCH

As discussed in the present review, it is evident that BSH activity is widely distributed in many GI microbes and many bacterial groups that have been commonly used as probiotics for human as well as animal applications. However, it remains to be determined whether the BSH activity of the probiotics is beneficial or detrimental to the host. Once it is clarified, this feature could be used as one of the selection criteria for the probiotics. Future genetic analyses should concentrate on expanding the information available on the molecular mechanisms how bacteria regulate the *bsh* gene in the GI conditions and why the GI microbes have evolved to harbor the BSH activity. This may lead to a better understanding of the interaction between animal host and microbes in their GI tracts. It is possible that information obtained from the BSH research may ultimately lead to the development of improved probiotic strains and assist in the manipulation of gut function for improving animal health.

Undoubtedly, the use of probiotics has attracted lots of attention as an alternative to antibiotics in the livestock industry. Knowledge gained through bile research will provide further insight into the survival of probiotics as well as pathogens in the GI tract. An understanding of how BSH-active probiotics have evolved the self-defense mechanism against the toxic nature of deconjugated bile acids may also explain the antagonist action of autochthonous microorganisms of the intestinal flora such as lactobacilli against pathogens in the intestines.

Potential hypocholesterolemic pharmaceuticals and food products are continuously being developed in order to control serum cholesterol levels in hypercholesterolemic patients. These pharmaceuticals are mostly based on interruption of the enterohepatic circulation of bile salts. Enhanced BSH activity of probiotics may offer potential as a biological alternative to pharmaceutical interventions to prevent and treat hypercholesterolaemia.

The *bsh* gene could be used for the development of a

new genetic marker in some bacterial groups. For example, the *bsh* gene is common in bifidobacteria, is present as single copies on the genome (Schell et al., 2002), and contains conserved nucleotide signatures that are suitable targets for PCR primers. The use of *bsh* gene may be suitable for reliable identification and phylogenetic analysis of *Bifidobacterium* species.

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