Intestinal Probiotics: Interactions with Bile Salts and Reduction of Cholesterol

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Abstract

Probiotics mainly reside in host intestinal tract, where they interact with bile salts and ingested cholesterol. This paper reviews the recent progresses of probiotics on the following issues, i.e. bile salts effect on membrane composition; probiotic tolerance and modification of bile salts; the mechanism of probiotic removal of host cholesterol; and the roles that bile salt hydrolase plays.

1. Introduction

Probiotics are symbiotic microorganisms which reside in host intestine and play a role in promoting host health. Whether they are aboriginal or by ingestion, should be enough of living cells survived in the bile salts environment of intestine so that the microbes could play its beneficial roles. Probiotics are generally believed to help host regulate the immune response, inhibit pathogenic bacteria, enhance intestinal barrier, prevent cells from canceration, lower blood lipid concentration, etc. Among these functions, the hypocholesterolemic effect is of great significance to the maintenance of human cardiovascular health.

2. Interactions of bile salts and probiotics

Cholesterol is used by liver as a precursor to synthesize the primary bile acid (mainly chenodeoxycholic acid (CDCA) and cholic acid), which are then modified by intestinal bacteria to form...
secondary bile acid (lithocholic acid and deoxycholic acid). Before excretion, bile acids are peptide-bound with glycine or taurine and self-assembled into micelles (Fig. 1). Because of the detergent property, bile salts can alter the conformation of bacterial membrane proteins, which may lead to protein misfolding or denaturation; bile salts can plug into the membrane lipids, thus may change the integrity and permeability of the cell membrane \[^{[6]}\]. Bile salts are also demonstrated to be responsible for generating oxygen free radicals, altering RNA secondary structure, inducing DNA damage and activating DNA repair related enzymes \[^{[3]}\].

2.1. Effect of bile salts on bacterial morphology

Colony shape and cell morphology are important indicators that characterize the probiotic tolerance of bile salts. Suskovic et al found that the colonies of *Lactobacillus acidophilus* M92 performed two types of shape, i.e. smooth and rough; the rough ones were more sensitive to bile salt \[^{[10]}\]. They suggested that this difference is not caused by genetic mutation but the phenotypic response to the environment; the smooth colony cells have more compact short-chain structure, whereas the rough colony cells are longer and more vulnerable to the surfactant effect of bile salts.

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**Fig. 1** Bile acids in human digestive system

\[^{[3]}\] Bile salts are also demonstrated to be responsible for generating oxygen free radicals, altering RNA secondary structure, inducing DNA damage and activating DNA repair related enzymes. Bile acids are peptide-bound with glycine or taurine and self-assembled into micelles (Fig. 1). Because of the detergent property, bile salts can alter the conformation of bacterial membrane proteins, which may lead to protein misfolding or denaturation. Bile salts can plug into the membrane lipids, thus may change the integrity and permeability of the cell membrane. Bile salts are also demonstrated to be responsible for generating oxygen free radicals, altering RNA secondary structure, inducing DNA damage and activating DNA repair related enzymes.
2.2. Effect of bile salts on membrane lipid composition

It was found that *Lactobacillus reuteri* cells which growing stably in bile salts added culture, compared with control, have a decline in phospholipid content and a lower ratio of saturated to unsaturated fatty acids \[^8\]. Another remarkable feature of bile salts cultured bacteria is that the unusual C18:0, 10-OH and C18:0, 10-oxo fatty acids are found in the plasma membrane. Oxo fatty acids are considered to play an important role in bacterial membrane permeability and tolerance of harsh environments \[^29\]. These modifications and alterations of lipids are likely to maintain the optimal fluidity of cell membrane and an effective barrier to noxious stimulus, which help keeping the basic functions of cells \[^30\].

The changes in membrane lipid composition could lead to a consequent binding of cholesterol. In a probiotics-mediated cholesterol - bile salts coprecipitation study, about 20 percent amount of medium cholesterol bound solid to the cell wall or membrane and could not be eluted at pH 7 \[^8\]. This may due to a threesome intermolecular network in which the amino group of bile salt and the sugar hydroxyl group of membrane lipid are both hydrogen bonded with cholesterol.

2.3. Probing and modification of bile salts by probiotics

Bile salts can be perceived by probiotics directly through the two-component signaling system, i.e. a membrane associated histidine kinase sensory protein and a response regulator protein (HPK-RR) \[^3\]. When a stimulus from bile salts is detected by HPK, a signal is conducted to RR which guides the cellular response to the stimulus. Bile salts can yet be indirectly probed by monitoring membrane disintegrity or cytoplasm overflow. In addition, the conformation changes of macromolecules under bile salts condition may also transduce signals \[^2,3\]. Modifications to bile salts by probiotics include epimerization (the stereocemical inversion of hydroxyl group on C-3, 7, 12), deconjugation (removal of amino acid residual), hydroxylation and dehydroxylation \[^22\].

2.4. Bile salts tolerance and bile salt hydrolase of probiotics

Probiotics, mostly Gram-positive bacteria, is more vulnerable to bile salts than the Gram-negative bacteria. Bile tolerance is a strain-specific nature, and one cannot tell which species of bacteria is more resistant to bile salts than the others. Different pH, temperature and other environmental factors may either cause the bacteria more vulnerable to bile salts or enhance their survival rate. Intestinal bile content would increase significantly after high-fat food were ingested; but it usually maintains at a certain low level. Pre-exposure of probiotics to a low level of bile salts can enhance their ability to tolerate high concentrations of bile \[^3\].

The molecular mechanism of how probiotics tolerate bile salts is still not clear \[^23\]. Taken into account that the harmful effects of bile salts are complex, it is suggested that the molecular basis of bile tolerance should be related with proteins groups which, respectively, sense, modify and efflux bile salts, regulate
cell envelope construction, and maintain homeostasis [3, 26]. Bacterial bile salt hydrolase (BSH), which controls the deconjugation reaction of bile salts, is believed to take an active part in abating the bile salts toxicity. The amino acid released by deconjugation can be further utilized as carbon and nitrogen sources for the benefit of bacterial sustenance and survival [7]. BSH promotes the integration of cholesterol into bacterial membrane, which results in the alteration of membrane properties (e.g. potential, fluidity, tensile strength) [13], thus affecting the bile salt tolerance and the sensitivity to host defensin, and improving the intestinal viability of probiotics [3].

BSH is widely present in mammalian intestinal bacteria as Bifidobacterium spp., L. acidophilus, Lactobacillus gasseri, Lactobacillus johnsonni, L. plantarum and so on. Yet BSH is not expressed in strains including Lactobacillus delbruecki, Lactobacillus lactis, Lactobacillus helveticus, Streptococcus thermophiles, which live in non-bile environment [1]. BSH is an intracellular, non-allosteric enzyme which is non-sensitive to oxygen; the optimum pH is 5~6, and the activity of BSH is associated with biomass density [19]. It was reported that the BSH activity of stationary L. johnsonni cells increased by 3~5 times within 20 min after adding conjugated bile salts to the culture [24]. This result indicated that BSH expressions are inducible. BSH + probiotics usually have different tendencies to utilize bile salts; they prefer either taurocholic acids or glycocholic acids [3].

3. Cholesterol removing mechanisms of probiotics

Several theories of how probiotics remove host cholesterol have been put forward. They are mainly: 1) Assimilation. When cultivated at the presence of bile salts, bacteria intake cholesterol from the culture [16]; 2) Coprecipitation. Under acidic conditions, the deconjugated bile salts co-sediment with cholesterol [12]; 3) Adsorption and incorporation. In this case, cholesterol is adsorbed by or incorporated to the growing cells surface [25]; and 4) Reduction of the cholesterol absorption by host [19, 39]. Despite of inconclusive, yet, a large number of studies implied that the varieties of mechanisms may work together to lower the level of host cholesterol.

3.1. Coprecipitation and assimilation

Deconjugated bile salts will coprecipitate with cholesterol at acidic conditions (pH<5.5), thus the intestinal cholesterol can be removed [12]. Though a typical intestinal physiological pH is about neutral to slightly alkaline which is unsuitable for co-precipitation reaction, however, fermentation of dietary fiber by intestinal microbes produces short-chain fatty acids, which could transfer the microenvironment into acidic pH, ensuring the occurrence of coprecipitation [19].

Coprecipitation occurs at low pH, while assimilation does not have this limit. In a case that the coprecipitation effect was excluded, it was found that the amount of cholesterol which was removed by growing bacteria was far more than that by heat-killed bacteria [14]. Absorbed cholesterol was not degraded but stored as inclusions which may be used as a metabolic precursor [11]. The ability to assimilate cholesterol is highly dependent on the growth of probiotics: the more active cells are, the
stronger assimilation they can do; and no significant absorption is observed at the latent or resting stage [3]. It is also noticed that the presence of bile salts appears to be a prerequisite for the assimilation of cholesterol [16]. However, the uptake rate of cholesterol is irrelevant either to bile salts concentration or the nature of the amino residuals of conjugated bile salts [3].

A large number of in vitro experiments showed that both coprecipitation and assimilation make contribution to cholesterol removal. The investigation on bifidobacteria showed that 8~24 % of removed cholesterol could be eluted; 6~50 % of removed cholesterol was uptaken by bacteria cells [16]. Zhang et al periodically assayed the concentration of conjugated bile salts in the growing culture and calculated the amount of cholesterol removed by coprecipitation, and thus determine the ratio of coprecipitation and assimilation [9]. Li et al developed a mathematical model: by knowing the bacterial BSH concentration at any time, the amount of cholesterol removed respectively by coprecipitation and assimilation can be determined [28].

3.2. Adsorption and incorporation of cholesterol to membrane

Lin and Chen studied the in vivo cholesterol removing potential of six strains of L. acidophilus. They suggested that in addition to precipitation and absorption effects, cholesterol is most likely to be adsorbed on the cell surface [15]. Kimoto observed that dead Lactococci cells still could remove some cholesterol from culture, and he attributed it to the interaction of cell wall and cholesterol [14]. Another study on Micrococcus lysodeikticus, L. acidophilus, Proteus mirabilis and Bacillus megaterium found that cells grown in the culture which contains cholesterol and bile salts are more sonic resistant than the control; it is conferred that cholesterol may incorporate into the cell membrane, bringing on a mycoplasma-like membrane property [15].

3.3. Reduce the host absorption of cholesterol

Huang and Zheng [5] reported that L. acidophilus in vivo releases a soluble effector which lowers the expression of NPC1 L1 gene of human intestinal epithelial cells, and so prevents Caco-2 cells from uptaking cholesterol micelles dispersed in the intestine content. This effector also influences the liver LXR receptor; thereby can fine-tune the body’s cholesterol metabolism.

3.4. Break of bile salts homeostasis by deconjugation

Most deconjugated bile salts escape from the enterohepatic circulation; they are excreted in the feces. The loss of bile salts lowers the emulsification and absorption level of exogenous cholesterol; in order to make up the loss, the host will use the body cholesterol to synthesize bile salts, thereby reducing the cholesterol storage [17, 18]. However, the possible side effect cannot be ignored: the 7α-modified deconjugated bile salts could disturb intestinal normal flora, cause diarrhea and mucosal inflammation, and even induce cancer [20, 27].
A good deal more attention is paid to the effects of intestinal probiotics on host health. The cholesterol removing capacity of probiotics as a quasi-endogenous lipid lowering mechanism will be continuously concerned about. Though experiments in vitro and corresponding theories have become more complete and mature, animal tests and human studies are currently weak. Several barriers that limit the in vivo studies are small sample size, short duration, over-taking, great individual differences, diet control difficulty, etc. [4,25] In recent years some progress has been made by use of molecular biology method to study the mechanism of probiotics gastrointestinal tolerance and regulation of host cholesterol metabolism; however, the confirmed mechanism remains limited [4]. Thus, future work on probiotics may aim at the following points: the molecular mechanism of in vivo cholesterol removal, probiotic activity in the intestinal tract, interaction between probiotics and the host, gastrointestinal-tolerant preparations, and animal experiment with more confidence and higher throughput.

References


