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Novel Beneficial Functions of Silk Protein, Sericin for Health

Norihisa Kato

Graduate School of Biosphere Science, Hiroshima University,
Higashi-Hiroshima 739-8528, Japan. E-mail: nkato@hiroshima-u.ac.jp

INTRODUCTION

Sericin is a silk protein that is the main constituent of silk fiber (20～30% of the total cocoon weight) (1). When cocoon is used for silk textiles, the sericin is mostly removed from the cocoon and disposed of without any use. In 1998, we provided the first evidence that sericin has a strong antioxidant action and inhibitory action of tyrosinase (2). Because of its high content of serine and aspartic acid (Table 1), this antioxidant effect appears to be mediated by its chelation of their hydroxyl and carboxyl groups to copper and other trace elements. Sericin is also known to have a skin moisturizing and anti-wrinkle effect due to its high content of serine (1). Because of these characteristic effects, sericin has been widely used in Japan as an ingredient of cosmetics. Recently we have further found that topical application of sericin markedly suppressed skin tumorigenesis (3,4). These inhibitory effects of sericin appear to be mediated through suppressing oxidative stress, cell proliferation and TNF-α (3,4).

During our study on physiological functions of buckwheat protein, we have introduced a novel concept "resistant protein" in the field of protein nutrition, being similar to dietary fibers (5-7). According to this concept, we have found several physiological functions of buckwheat protein with lower protein digestibility (7). The functions of buckwheat protein so far elucidated include hypocholesterolemic effect, anti-obese effect, anti-colon cancer effect, anti-mammary cancer effect, anti-constipation effect, etc. (5-7). Several other groups are also now investigating physiological functions of other plant proteins, including soy resistant protein, because the digest-

Table 1. Composition of amino acids of casein and sericin (mol %)

Amino acid	Casein	Sericin
Ser	5.3	30.4
Asp	6.4	19.4
Gly	3.3	12.2
Lys	6.4	10.2
Thr	4.9	6.0
Ala	4.6	4.6
Glu	19.7	4.1
Tyr	4.1	3.8
Arg	2.6	2.8
Val	7.1	2.6
Ile	5.6	1.4
His	2.6	0.9
Pro	11.6	0.8
Leu	8.7	0.6
Phe	4.2	0.4
Cys	0.2	<0.05
Met	2.6	<0.05

ibilities of plant proteins are in general lower than those of animal proteins (7). Since sericin is highly resistant to several proteases (7), we have postulated that sericin might be a resistant protein, and have some functions beneficial to health as well as dietary fibers. We have tested this hypothesis, and discovered several novel functions of sericin, implying that sericin may be beneficial to health as a component of functional food.

Anti-Constipation Effect

We have observed that sericin is highly resistant to several proteases including pepsin, pancreatin, papain, trypsin, thermolysin, etc., although sericin itself did not inhibit protease activity (8). This finding of sericin's protease resistant property together with its high water-retaining capacity prompted us to investigate whether consumption of the protein suppresses constipation. To examine this possibility, rats were injected atropine to induce typical constipation (8). Constipation by atropine is caused by reduced release of acetylcholine from the parasympathetic nervous system. One reason for the constipation in human has been thought due to a Similar mechanism. The results of this study indicated an improvement of atropine-induced constipation in rats by feeding sericin (Table 2). Our study showed higher water content in the feces of rats fed sericin, and such a rise in content might relate to the improvement in constipation. This finding implies that sericin could be a useful agent treatment of constipation as a resistant protein.

Table 2. Anti-constipation effect of dietary sericin in atropine-treated rats

	Saline		Atropine	
	Control	Sericin	Control	Sericin
Fecal wet wt (g)				
0~6 h	$0.25\pm0.05^{1)a2)}$	0.25 ± 0.03^{a}	0.12 ± 0.03^{b}	0.27 ± 0.03^{a}
0~12 h	0.42 ± 0.05^{ab}	0.46 ± 0.05^{a}	0.28 ± 0.04^{b}	0.48 ± 0.05^{a}
Fecal dry wt (g)				
0~6 h	0.17 ± 0.03^{a}	0.14 ± 0.01^{a}	0.07 ± 0.02^{b}	0.17 ± 0.02^{a}
0~12 h	0.27 ± 0.03^{a}	0.26 ± 0.02^{a}	0.18 ± 0.03^{b}	0.29 ± 0.03^{a}
Fecal water content (%)				
0~6 h	31.7 ± 3.0^{b}	38.9 ± 3.4^{ab}	32.9 ± 1.6^{ab}	39.6 ± 1.5^{a}
0~12 h	33.5 ± 2.2^{b}	40.0 ± 1.5^{a}	35.4 ± 1.5^{ab}	40.4 ± 1.5^{a}

[1)]Values are means±SE (n=11). [2)]Within a row, values followed by different letters are significantly different ($p<0.05$).

Anti-Colon Tumor Effect

Sericin is a dietary fiber-like protein with low digestibility. Consumption of dietary fibers is well known to be associated with lower incidence of colon tumors (9). Oxidative stress has been considered to be associated with tumorigenesis (10). Therefore, consumption of sericin may inhibit colon tumorigenesis by its dietary fiber-like and antioxidant properties. To test this hypothesis, mice were fed a diet containing 3% sericin for 16 weeks, and given once per week injection of 1.2-dimethylhydrazine (DMH) for the initial 10 weeks (11,12). The results showed a strong inhibitory effect of dietary sericin against the development of colon tumors in mice (Table 3) (11). Mechanistic studies have shown that consumption of sericin significantly reduced the BrdU-labeling index, a marker for colon cell proliferation and the protein expression of proliferation-related genes, c-*myc* and c-*fos*, suggesting that sericin suppresses colon tumorigenesis by reducing colon cell proliferation (Table 3). Further analysis showed that dietary sericin significantly inhibited colonic oxidative stress markers including 8-hydroxy-deoxyguanosine (8-OHdG) and 4-hydroxynonenal (4-HNE) and inducible nitric oxide synthase (iNOS) (12). From

Table 3. Anti-colon tumor effect of dietary sericin in 1,2-dimethylhydrazine-treated mice

	Control	Sericin
Mice with colon tumors (n/40)	15/40	5/40*
Tumors/mouse (n)	0.55±0.13[1)]	0.13±0.05*
Proliferative cells (%)	7.92±0.29	5.90±0.32*
c-*myc* expression cells (%)	7.63±0.24	5.46±0.24*
8-OHdG expression cells (%)	6.58±0.51	4.24±0.24*
4-HNE expression cells (%)	8.16±0.16	5.32±0.12*
iNOS expression cells (%)	9.92±0.62	8.37±0.15*

[1)]Values are means±SE (n=20). *Significantly different ($p<0.05$).

these results, sericin appears to suppress colon tumorigenesis by reducing oxidative stress, cell proliferation and nitric oxide production. Since oxidative stress is known to up-regulate the expression of c-*myc*, c-*fos* and iNOS (12), reduction in the oxidative stress by sericin intake might be associated with reduced expression of these proteins.

Because of low digestibility of sericin together with its antioxidant property, undigested sericin in the large intestine might suppress colonic oxidative stress, leading to suppression in tumor development. To examine this hypothesis, rats were fed a diet containing 3% sericin for 4 weeks, and given an injection of DMH one week before initiation of the experiment. As a result, the contents of the large intestine of rats fed sericin markedly suppressed lipid peroxidation in the homogenate of colon mucosa and Fenton-type reaction systems (Cu/H_2O_2)-induced DNA oxidation compared to those of control rats (to be published). The pattern of amino acids of higher molecular-weight fraction (proteins and peptides) in the content of large intestine of rats fed sericin was very similar to that of amino acids in sericin. We have already observed that partially digested sericin has still strong antioxidant activity, although lowering molecular weight of sericin by alkali treatment or by proteases results in lower antioxidative activity. Taken together, the antioxidant activity of undigested sericin in the large intestine appears to suppress colon tumorigenesis by reducing colonic oxidative stress.

Mineral Bioavailability-Enhancing Effect

A characteristic of sericin in its amino acid composition is to have an abundance of serine (~30%) and aspartic acid (~19%) (Table 1), which effectively chelate several metal ions by interposition between their hydroxy and carboxyl groups besides bringing about such a high water-holding capacity. This physicochemical feature offers advantages to solubilize several elements and to aid them in intestinal absorption. To test this possibility, rats were fed on the diet containing either 23% egg albumin or 20% egg albumin plus 3% sericin (13). The results indicated that consumption of sericin elevated the apparent absorption of Zn, Fe, Mg and Ca (Table 4). This findings imply that dietary sericin enhances bioavailability of these elements in rats.

Table 4. Mineral absorption-enhancing effect of dietary sericin in rats

Apparent absorption of minerals (%)	Control	Sericin
Zn	37.0±4.3[1)]	52.1±3.6*
Fe	34.4±3.9	48.6±3.0*
Mg	61.6±3.2	74.4±3.3*
Ca	61.0±3.5	71.1±2.2*

[1)]Values are means±SE (n=6). *Significantly different ($p<0.05$).

It has been reported that casein phosphopeptides (CPP) enhance the intestinal absorption of Ca, Fe and Zn in rats (14-16). These effects have been considered to be at least in part mediated by enhancing the solubilization of the elements in the intestinal tract. However, any other proteins have not been reported to have a similar effect. To our knowledge, sericin is the second protein having been found to stimulate the absorption of minerals.

There is growing evidence that consumption of several resistant sugars including fructooligosaccharides, galactooligosaccharides, lactulose and other resistant sugars enhances the bioavailability of Ca, Mg and Fe (17-20). Although the underlying mechanisms of the effects are still unclear, it has been considered that the effects might be mediated through higher absorption of them in small and large intestine (17-20). Further study is in progress in our laboratory to examine the mechanism of the effect of sericin on the absorption of the elements.

Blood Glucose-Lowering Effect

We have postulated that sericin might have some physiological functions as a dietary-fiber like protein (resistant protein). Dietary fibers are well known to have anti-obese activity. Our study has demonstrated that consumption of buckwheat protein, one of resistant proteins, caused lower adipose tissue weight in rats as well as dietary fibers (21). This effect appeared to be ascribed to reduction in hepatic fatty acid synthesis (21). Interestingly, lower digestibility of buckwheat protein was associated with lower body fat. We have considered that slow digestion of buckwheat protein diet might slow down the utilization of dietary carbohydrate and lipids, leading to lower body fat accumulation. It is of interest to examine if sericin has anti-obesity activity in rats fed high-fat diet as well as buckwheat protein. To test this possibility, rats were fed high-fat diet for 30 days. The results, however, showed no anti-obesity effect of sericin. Instead, interestingly, we have found that dietary sericin significantly improved glucose tolerance in rats after the injection of glucose. Hepatic activity of glucose-6-phosphatase was significantly reduced by sericin intake. Fasting serum glucose and parameters of lipid metabolisms, including serum lipids and hepatic lipid-metabolizing enzymes, were unaffected by sericin intake. The effect on glucose tolerance might be mediated at least in part via reduced gluconeogenesis, and not associated with lipid metabolisms. The present study provides a new possibility of potential usefulness of sericin for the prevention and treatment of type 2 diabetes. Further study is in progress in our laboratory to study whether the low digestibility and characteristic amino acids of sericin relates to this effect on blood glucose.

CONCLUSION

This review describes recent findings of functions of sericin beneficial to health, including anti-constipation effect, anti-colon tumor effect, mineral bioavailability-enhancing effect and blood glucose-lowering effect. These findings imply a potential usefulness of this protein as a material of functional food. Available data in our group indicates no adverse effect of sericin intake when animals were fed 1～4% sericin containing diets. However, more than 6% sericin containing diets appeared to some diarrhea possibly because of its water-holding capacity. Further study is necessary to evaluate the safety of sericin as a food ingredient.

REFERENCES

1. Vogel R, Meier J, Bluest R. 1993. Sericin silk protein: unique structure and properties. *Cosmetics Toiletries* 108: 101-108.
2. Kato N, Sato S, Yamanaka J, Yamada H, Few N, Nomura M. 1998. Silk protein, sericin inhibits lipid peroxidation and tyrosinase activity. *Biosci Biotechnol Biochem* 62: 145-147.
3. Zhaorigetu S, Yanaka N, Sasaki M, Watanabe H, Kato N. 2003. Silk protein, sericin, suppresses DMBA-TPA-

induced mouse skin tumorigenesis by reducing oxidative stress, inflammatory responses and endogenous promoter TNF-α. *Oncology Rep* 10: 537-543.

4. Zhaorigetu S, Yanaka N, Sasaki M, Watanabe H, Kato N. 2003. Inhibitory effects of silk protein, sericin on UVB-induced acute damage and tumor promotion by reducing oxidative stress in the skin of hairless mouse. *J Photochem Photobiol B* 71: 11-17.
5. Kayashita J, Shimaoka I, Yamazaki M, Kato N. 1997. Consumption of buckwheat protein lowers plasma cholesterol and raises fecal neutral sterols in cholesterol-fed rats because of its low digestibility. *J Nutr* 127: 1395-1400.
6. Kato N, Kayashita J, Sasaki M. 2000. Physiological functions of buckwheat protein and sericin as resistant protein. *J Jpn Soc Nutr Food Sci* 53: 71-75.
7. Kato N, Iwami K. 2002. Resistant protein; its existence and function beneficial to health. *J Nutr Sci Vitaminol* 48: 1-5.
8. Sasaki M, Yamada H, Kato N. 2000. A resistant protein, sericin improves atropine-induced constipation in rats. *Food Sci Tech Res* 6: 280-283.
9. Kritchevsky D. 1997. Dietary fiber and cancer. *Eur J Cancer Prev* 6: 435-441.
10. Guyton KZ, Kensler TW. 1993. Oxidative mechanisms in carcinogenesis. *Br Med Bull* 49: 523-544.
11. Sasaki M, Kato N, Watanabe H, Yamada H. 2000. Silk protein, sericin, suppresses colon carcinogenesis induced by 1,2-dimethylhydrazine in mice. *Oncology Rep* 7: 1049-1052.
12. Zhaorigetu S, Sasaki M, Watanabe H, Kato N. 2001. Supplemental silk protein, sericin, suppresses colon tumorigenesis in 1,2-dimethylhydrazine-treated mice by reducing oxidative stress and cell proliferation. *Biosci Biotechnol Biochem* 65: 2181-2186.
13. Sasaki M, Yamada H, Kato N. 2000. Consumption of silk protein, sericin elevates intestinal absorption of zinc, iron, magnesium and calcium in rats. *Nutr Res* 20: 1505-1511.
14. Lee YS, Noguchi T, Naito H. 1996. Phosphopeptides and soluble calcium in the small intestine of rats given a casein diet. *Br J Nutr* 43: 457-467.
15. Ait-Oukhatar N, Bouhallab S, Bureau F, Arhan P, Maubois JL, Drowdowsky MA, Bougle D. Bioavailability of caseinophosphopeptide bound to iron in the young rats. *J Nutr Biochem* 8: 190-195.
16. Hansen M, Sandstrom B, Lonnerdal B. 1996. The effect of casein phosphopeptides on zinc and calcium absorption from high phytate infant diet assed in rat pups and Caco-2 cells. *Pediatr Res* 40: 547-552.
17. Ohta A, Osakabe N, Yamada K, Saito Y, Hidaka H. 1993. Effect of fructooligosaccharides on Ca, Mg and P absorption in rats. *J Jap Soc Nutr Food Sci* 46: 123-129.
18. Brommage R, Binacua C, Antille S, Carrie AL. 1993. Intestinal calcium absorption in rats is stimulated by dietary lactulose and other resistant sugars. *J Nutr* 123: 2186-2194.
19. Chonan O, Watanuki M. 1995. Effect of galactooligosaccharides on calcium absorption in rats. *J Nutr Sci Vitaminol* 41: 95-104.
20. Schulz AG, Van Amelsvoort JM, Beynen AC. 1993. Dietary native resistant starch but not retrograded resistant starch raises magnesium and calcium absorption in rats. *J Nutr* 129: 1724-1731.
21. Kayashita J, Shimaoka I, Nakajoh M, Kato N. 1996. Feeding of buckwheat protein extract reduces hepatic triglyceride concentration, adipose tissue weight and hepatic lipogenesis in rats. *J Nutr Biochem* 7: 555-559.