Review

Resistant Protein; Its Existence and Function Beneficial to Health

Norihisa KATO and Kimikazu IWAMI

Department of Applied Biochemistry, Hiroshima University, Kagamiyama, Higashi-Hiroshima 739-8528, Japan
1 Department of Biological Resource Chemistry, Kyoto Prefectural University, Shimogamo, Sakyo-ku, Kyoto 606-8522, Japan

(Received November 22, 2001)

Summary The remnants of proteins themselves or complexes with protein remaining no longer indigestible in the intestine are referred to as resistant proteins, which exert physiological functions similar to dietary fibers and are also better for health. In recent years, noticeable functions attributable to resistant proteins have become gradually apparent with regard to several proteinous items. Recent investigations have revealed that the relevant ingredients are either condensed in isolates or concentrates of vegetable proteins or causally brought about as a consequence of denaturation and/or entanglement in the process of preparation. Some protein components inherently insusceptible to mammalian digestive enzymes also belong to the group of resistant proteins in case of edibility irrespective of their sources. Among the medicinal benefits of several resistant proteins hitherto pointed out by animal experiments, there were preventive effects against hypercholesterolemia, constipation, corpulence, tumorigenesis (colon, liver, mammary gland), gallstone formation or poisoning, and wholesome improvements in enteric fermentation of short-chain fatty acids.

Key Words resistant protein, soybean HMF, buckwheat protein, silk sericin, short-chain fatty acids

Thus far, dietary proteins have been evaluated as being high or low quality by whether their composition is well satisfied with essential amino acids. For this reason, most vegetable proteins are still believed to be inferior in quality to animal proteins. Since it was announced in the mid-1970s by Carroll and Hamilton (1) that vegetable proteins compared with animal proteins were effective in improving cholesterolemia, however, much interest has recently been aroused in a third physiological function of these proteins rather than the nutritive value, flavor or taste. There are conflicting views concerning the mechanism of hypocholesterolemic action of vegetable proteins (2), in which easily digestible milk casein is unexceptionally used as a control protein. In most cases, the in vivo digestibility of vegetable proteins doesn't rank with that of casein. On reflection, it is feasible that the very remnants withstanding digestion as well as absorption in the intestine may intimately be involved in physiological manifestations. In the meantime, some dietary fibers also serve as a preventive against cholesterolemia or colonic tumor-genesis (3–5), and part of edible starch behaves like “resistant starch” insusceptible to mammalian digestive enzymes according to circumstances (6). On the analogy of this, the postdigestive remnants of protein may safely be called “resistant protein” on the whole and individually regarded as proteinous dietary fibers, despite the absence or presence of entanglement with nonprotein ingredients. To cite instances in this connection, soy protein isolate (SPI) is among the vegetable proteins effective for the suppression of cholesterolemia in cholesterol-fed experimental animals but its protease-treated derivative, i.e., a centrifugally separable high-molecular-weight fraction (HMF), exerts a far higher effect than SPI in spite of cholesterol administration (7, 8). The reason for this is because HMF has an abundance of resistant-proteinlike indigestible ingredients with a high capturing capacity for bile acids. Other leguminous or cereal protein concentrates and indigestible animal proteins should include resistant protein in a greater or lesser degree, so that the exact factors responsible for their third food functions will need to be reconsidered with a mind to the existence of resistant protein. Moreover, there is a possibility of resistant protein influencing the enteric fermentation of short-chain fatty acids because of its ammonia or amine release by the action of microflora. This review outlines up-to-date studies on resistant protein in which further advances are expected.

Correspondence to: k_iwami@love.kpu.ac.jp or nkato@hiroshima-u.ac.jp

Abbreviations: SPI, soy protein isolate; HMF, insoluble high-molecular-weight fraction from SPI digesta; DCA, deoxycholic acid; AC, aberrant crypts; ACF, aberrant crypt foci; BP, buckwheat protein; SCFA, short-chain fatty acids; PP, potato protein; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA.
1. Bile acid-capturing capacity of soybean resistant protein and its related medicinal benefits

There was a significant difference in resistance to mammalian digestive enzymes between SPI and HMF having similar amino acid composition. The feeding experiment with growing rats has revealed that about 25% of HMF is ineffective as a protein source (9), unlike SPI which is fairly comparable to casein. For convenience, the resistant protein content was roughly estimated at the difference between HMF and casein in nutritional availability. A sizable increase in fecal bile acid excretion was characteristic of the HMF intake in animal experiments. Conversely, this implies that the bile acids were largely captured by the postdigestive remnants of HMF, namely, soybean resistant protein, and were ejected from the intestine together with other excreta. In practice, washing of the feces from HMF-fed rats with a variety of buffered aqueous solutions did little to deprive them of bile acids, most of which could be extracted with 70% alcohol. The feces then deprived of bile acids could reproduce the bile acid-holding capacity to the same extent as lost. In general, deoxycholic acid (DCA) predominant in fecal bile acids of conventional rats is dangerous as a potent promoter for tumorigenesis occurring in the colon (10). Its predominance held for the feces of HMF-fed rats more obviously. What is thus necessary prior to the actual application of HMF or soybean resistant protein is to verify whether the enteric occurrence of DCA at a high level may enable the colon to be exposed to the risk of tumorigenesis.

Azoxymethane-treated and HMF-fed F-344 rats were first examined for the frequency of occurrence of aberrant crypts (AC) or crypt foci (ACF), known as neoplastic lesions, at an early stage of carcinogenesis on the colonic mucosa. These rats given HMF or casein diets in the presence and absence of supplementation with 0.2% DCA were fed over a period of 12 weeks. When AC or ACF were enumerated at intervals of 4 weeks during this period, it was observed that DCA supplementation of the casein diet caused a marked reduction in their multiplications but this result was not found with the HMF diet, irrespective of DCA supplementation (11). If this inference is valid, it follows that the DCA-supplied casein diet is safer for tumorigenesis than a diet without DCA. It is interesting that the multiplication of AC or ACF was much higher in the HMF-fed group, regardless of DCA supplementation, in comparison with the DCA-fed casein group. It was nevertheless doubtful whether the HMF intake would really become risky to tumorigenesis. To determine the truth or falsehood experimentally, the feeding was continued for another 27 weeks until a malignant tumor was generated.

The relative frequencies of AC or ACF occurrence at both 12 and 39 weeks were exactly alike among dietary groups, although the measured values themselves were individually larger at 39 weeks than at 12 weeks. The tumor incidence was six-tenths in the DCA-fed casein group and three-ninths in the DCA-fed HMF group. To be more precise, in the former there were 18 tumors with a major axis of 4.7±0.4 mm and in the latter 3 tumors with a major axis of 2.0±0.1 mm (11). A significant difference between both groups was observed in regard to the number and size of tumors. In the absence of supplementary DCA, the casein group (n=10) as a whole had only a single tumor with a major axis of 4.0 mm. No tumor was found in either the DCA-fed casein or HMF group at 12 weeks. All the observed protuberant tumors at 39 weeks turned out to be genuine adenocarcinoma as a consequence of pathological diagnosis. The isoflavonoid content in HMF doesn't go up to 0.11% on a dry weight basis. The administration of genistein at 150 mg/kg diet to azoxymethane-treated rats has been reported to cause a reduction of 34% in ACF at 4 weeks (12). In our experiment, however, the number of total ACF in the DCA-fed HMF group amounted to at least 10-fold as many as in the DCA-fed casein group at either 12 or 39 weeks. Taken altogether, neither AC nor ACF directly predict the risk of carcinogenesis in number or multiplicity. It thus seems reasonable to consider that DCA certainly carries its cancer promotion, but that the postdigestive remnants of HMF inclusive of resistant protein, have captured DCA, thereby obstructing its direct stimulatory action on epithelial cells in the colon.

The cholesterol-lowering action of HMF can also be accounted for by its excellent capacity to capture bile acids. That is, the formation or stabilization of micelles is hindered when bile acids are captured by HMF or its postdigestive remnants; thus acidic and neutral steroids are both excreted into the feces without undergoing intestinal absorption. A lowering of bile acid concentration in enterohepatic circulation raises the biosynthesis of primary bile acids within the hepatocytes, which is consequently compensated for by the plasma cholesterol. Such combinations lead to not only an improvement in cholesterolemia but also cancer prevention.

2. Healthful efficacies of buckwheat protein in various aspects

Buckwheat has traditionally been greatly enjoyed as vermicelli or mash in Japan and other Asian countries. Buckwheat protein (BP) is composed of well-balanced amino acids, but it is inadequate in digestibility (13). In animal experiments with cholesterol-fed rats, BP was found to raise fecal sterol excretion to a good extent and thereby exert a higher hypcholesterolemic effect than SPI does (14). Although repeated attempts were made to fragment BP into large or small peptides diverse in molecular size with proteases, all the attempts have failed to obtain more effective peptides than intact BP does. The tryptic digest of BP was divided into soluble “low-molecular” and insoluble “high-molecular” fractions, which were administered to the respective groups of rats as protein sources. Three weeks later, their plasma cholesterol concentrations in the former and latter groups were obtained as 3.13 and 2.80 mmol/L, respectively, in comparison with 2.52 mmol/L in the intact BP-fed group. Among the three dietary groups, the measured values in order of concentration correlated closely with their corresponding in vitro digestibilities of protein preparations (95.3%, 83.2% and 77.3%) and...
reversely with their corresponding amounts of sterols excreted into the feces (15). These relations hint strongly that the highest hypocholesterolemic effect of intact BP was principally brought about by its insufficiency in vivo digestibility and its following enhanced sterol excretion. Accordingly, the mechanism of action can for convenience be interpreted similarly to that proposed for certain dietary fibers. Taking into account the hydrophobicity as well as the cholesterol-binding capacity of BP, however, the possibility can’t be excluded that its post-digestive remnants (so-called buckwheat resistant protein) took part in the fecal excretion of dietary cholesterol much more. In this connection, the very dietary fibers semipurified from BP concentrate according to the method of Prosky et al. (16) have never therewith exerted little hypocholesterolemic effect on cholesterol-fed rats.

Disorders against which the BP intake has any effectiveness include corpulence, gallstone, constipation, cancer and poisoning. When rats were fed both BP and casein diets for 3 weeks or more, epididymal or perirenal adipose tissue and liver of the BP-fed group became significantly less in weight and triacylglycerol content, respectively, than those of the casein-fed group. In addition, the BP intake caused not only a slight reduction in intestinal fat digestion but also a suppression of the activities of glucose-6-phosphate dehydrogenase and fatty acid synthase responsible for lipogenesis (17). It was presumed from these observations that a long-term BP intake would be indissolubly linked with a lowering of body fat and in due course an improvement in health. Alternatively, the BP intake was found to suppress the formation of gallstones to some extent in cholesterol-fed hamsters (18). This finding was well explainable on the assumption that intact BP or its post-digestive remnants would have masked cholesterol in the intestine, raised its fecal excretion, accelerated its conversion to bile acids in the liver, and thus lessened its accumulation in the gallbladder.

The BP-fed rats were relieved of atropin-induced constipation as if they had been given a high dietary fiber diet (19), while BP administration to female rats elicited a significant decrease in the frequency of occurrence of 7,12-dimethylbenzanthracene-induced mammary cancer (20). The then plasma estrogen concentration was far lower in the BP group than in the casein group, a similar effect being observed for the female rats fed a high fiber diet. The plasma estrogen at a high concentration serves as a crucial risk factor of carcinogenesis in the mammary gland. Dietary BP seems to have suppressed mammary cancer in such a manner as to disturb enterohepatic circulation of estrogen. Dietary BP was also effective either for cancer prevention in 1,2-dimethylhydrazine-fed rats (21) or for growth improvement in 5% amaranth-fed rats (22). The mechanism of efficacious manifestation of dietary BP is eventually attributable to a swift dilution and smooth excretion of carcinogen or poison.

3. Novel food functions of sparingly digestible silk protein “sericin”

Sericin is a major protein enveloping the fibroin sticky layers of cocoons (23). When silk is reeled off cocoons, sericin is mostly removed and disposed without effectual utilization. The high hydrous and indigestible properties of sericin, however, are to be expected as new merits for food materials. It has been found lately that sericin indicative of antioxidant activity serves as an inhibitor of tyrosinase (24) and that sericin feeding brings about an increase in fecal nitrogen excretion as well as an improvement in evacuation in rats (25). This clearly reveals that sericin is representative of proteinous dietary fibers. Among conventional dietary fibers apart from a proteinous one, there are effective ones for cancer prevention in the colon. The above evacuation-improving effect is common with water-soluble dietary fibers, and the carcinostatic effect of certain dietary fibers is easily explainable by the rapid intestinal transit of carcinogen-containing excreta rather than by a mechanism peculiar to such dietary fibers. Incidentally, oxidative stress is said to enhance the risk of carcinogenesis (26). It therefore seems likely that sericin will function as a preventive against colon cancer, since sericin has indigestible, fibrous and antioxidative characters. In practice, the administration of a 3% sericin diet to carcinogen-treated mice suppressed not only the ACF multiplication or tumor incidence in the colon (27) but also the functional expression of c-myc and c-fos in the epithelia (28). Furthermore, dietary sericin held the intraluminal contents or space in a more antioxidative state than in the case of no dietary sericin, and it significantly depressed the epithelial occurrence of 8-hydroxy-2′-deoxyguanosine and 4-hydroxynonenal, known as oxidative stress markers, probably because intact sericin or its partial digesta could protect the epithelial cells against oxidative stress and could suppress their abnormal proliferation leading to cancer.

A characteristic of sericin in its amino acid composition is to have an abundance of serine (30%) and aspartic acid (19%), which effectively chelate several metal ions by interposition between their hydroxyl and carboxyl groups besides bringing about such a high water-holding capacity. This physicochemical feature offers advantages to solubilize several transition elements and to aid them in intestinal absorption. Actually, Zn, Fe, Mg and Ca were taken up across the intestinal wall more effectively in rats fed a 20% egg albumin diet with supplemental 3% sericin than in rats fed a 23% egg albumin diet devoid of sericin (29). A similar effect has been observed with indigestible oligosaccharides (30). As far as the water-holding capacity is concerned, severe constipation arising from atropine injection was relieved because of easy defecation of the moist feces by sericin feeding (25).

4. Effect of resistant protein on cecal short-chain fatty acid fermentation

It is usually accepted that fibrous carbohydrates in a narrow sense or resistant starches brought into the large bowel undergo microbial fermentation and pro-
duce short-chain fatty acids (SCFA) available as an energy source for epithelial cells there (31). Among SCFA, propionic and butyric acids are particularly noted in relation to their prophylactic effects. For example, a rise in propionic fermentation in the colon has been expected to slow down the de novo synthesis of cholesterol in the liver and to lower cholesterol level in the blood (32), because propionic acid proved to be inhibitory to HMG-CoA reductase in vitro. There are arguments for and against this inference. It has also been suggested that ulcerative colitis makes further progress in the insufficiency of SCFA utilization (33), but goes into remission by supplementation with butyric acid (34). Experimental results varied diversely on the efficiency in vivo. Although butyric acid was the most antineoplastic among SCFA in cultured cell lines (35), its oral administration couldn't retarded the development of colonic neoplasia in 1,2-dimethylhydrazine-treated rats (36). It is very unlikely that butyric acid exists at an ever-pharmacological concentration in the lumen, thereby serving as a preventive against carcinogenesis in vivo. Whether the prophylactic effect is true or not, SCFA are nutritionally important as metabolic fuels for colonicocytes not only in rumens but also in nonruminants (31). Above all, butyric acid is easy to arise from microbial fermentation of resistant starch relative to nonstarch polysaccharides (37). Making a comparison between both high-amylose starches from corn and potato in rats, the production of SCFA was predominant in the former compared with the latter, in which non-volatile organic acid was conspicuous (38). Besides the difference in starch source, the degree of nitrogen utilization of microflora appears to affect the molar ratio of fermentative SCFA. Incidentally, the predominant fermentative product was not SCFA, but succinic acid, in rats fed a diet containing 20% high-amylose corn starch (resistant starch) and casein (protein source); the enhanced production of butyric acid instead of succinic acid was achieved by substituting potato protein (PP) for casein in the diet (38). PP is far inferior to casein in digestibility, implying that it has an abundance of resistant protein. An exchangeable effect similar to PP has also been observed for other cereal or leguminous proteins that are not very good in digestibility (39). Part of the resistant protein may certainly be attacked by enteric bacteria to liberate degradation products, but most products will probably be different from SCFA because of the difference in the denatured indigestible protein with or without nonprotein polymers. A plausible explanation for the mechanism of action of resistant protein is that resistant protein and its fermentative products serve as prebiotics in correcting imbalanced microflora and depressing the synthesis of organic acids other than SCFA or promoting their conversion to SCFA.

Future perspective

Resistant protein belongs to the category of dietary fibers, but it differs from a physiological point of view. One distinctive feature is to be comprehensively preventive against diverse lifestyle-related diseases. Most resistant proteins, except intrinsically occurring indigestible protein, have arisen from denaturation, complication, and entanglement during processing, being practically kept concentrated in several protein preparations. For this reason it is highly probable that some functionally favorable resistant proteins would be gained not only by seeking for edible (but indigestible) proteins in nature but also by food processing without consideration for decreased protein-nutritive value. The prolonged span of human health-life is to be expected as a result of food and nutritional investigation in the 21st century. It surely contributes toward longevity and public health to elucidate the efficacious function and its mechanism of existing or novel resistant proteins. Learned investigators' participation in this immature field is eagerly awaited.

REFERENCES


