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Research paper

Rice protein suppresses ROS generation and stimulates antioxidant gene expression via Nrf2 activation in adult rats

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ABSTRACT

To elucidate the effects of rice protein on the detoxification and antioxidant defense via the Nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, adult rats were fed casein and rice protein under cholesterol-free and -enriched dietary conditions. Nrf2 proteins and gene expressions were stimulated by rice proteins with respect to caseins accompanied by up-regulating the expression of gene encoding antioxidant and phase II detoxification in the rice protein groups. In the liver, compared with caseins, rice proteins significantly increased hepatic contents of reduced glutathione (GSH) and mRNA levels of glutamate cysteine ligase catalytic subunit (GCLC), glutamate cysteine ligase modulatory subunit (GCLM), glutathione *S*-transferase (GST), heme oxygenase 1 (HO-1) and NAD(P)H:quinone oxidoreductase 1 (NQO1), whereas hepatic accumulations of reactive oxygen species (ROS) and oxidized glutathione (GSSG) were markedly depressed in adult rats fed rice proteins. The present study demonstrates the endogenous antioxidant potential of rice proteins via mechanism through which Nrf2 activation plays an essential role in inducing Phase II antioxidants/detoxification enzymes and preventing ROS generation. This novel antioxidative function of rice protein is independent of dietary cholesterol during in adult rats.

1. Introduction

Reactive oxygen species (ROS) are very transient species that can lead to extensive damage to proteins, lipids and DNA. The high rate of ROS generation can result in oxidative stress, which is an important mechanism promoting the development of a variety of diseases, e.g. hyperlipidemia, cancer, etc (Hopps et al., 2010).

ROS can be scavenged by endogenous antioxidants/detoxification enzymes, which play a critical role in decreasing a risk of oxidative damage (Crawford et al., 2012). Accordingly, it is very important to stimulate antioxidant/detoxification enzymes for defending against ROS toxicity (Malik and Storey, 2009; Matés, 2000; Schülke et al., 2012). In this regard, much of what we know about the mechanism of protection against oxidative stress is the activation of antioxidant response element (ARE), which controls the basal and inducible expression of antioxidant/detoxification genes. ARE-mediated transcriptional activation is primarily dependent on the transcription factor, Nuclear factor erythroid 2 (NF-E2)-related factor 2 (Nrf2). Nrf2 induces and up-regulates the expression of genes encoding antioxidant and phase II detoxification, including glutamate cysteine ligase (GCL), glutathione *S*-transferase (GST), heme oxygenase 1 (HO-1), NAD(P)H:quinone oxidoreductase 1 (NQO1), etc (Li and Kong, 2009; Kim et al., 2010; Niture et al., 2014). Thus, Nrf2 pathway is regarded as playing a key role in protecting against oxidative stress through activation and up-regulation of ARE-driven detoxification and antioxidant genes (Nguyen et al., 2009).

Diet appears to exert a ROS-scavenging capacity through enhancing the endogenous antioxidative defense (Fang et al., 2002; Seifried et al., 2007). As a major dietary source for human, rice and its by-products possess lots of bioactive components to prevent the life-style diseases, exerting a variety of benefits for human health (Kubota et al., 2013; Yang et al., 2011, 2013a, 2013b; Wang et al., 2016). In particular, it has been demonstrated that rice protein can improve oxidative stress through regulating glutathione (GSH) metabolism and attenuating oxidative damage to lipids and proteins, resulting in an antioxidative response in rats (Burris et al., 2010; Yang et al., 2012a). These results suggest that the stimulation of GSH synthesis and the inhibition of GSH depletion are major contributors to the antioxidative mechanism

Abbreviations: ALT, alanine transaminase; ARE, antioxidant response element; AST, aspartate transaminase; CAS, casein; CAS-C, cholesterol-supplemented casein; GCL, glutamate cysteine ligase; GCLC, glutamate cysteine ligase catalytic subunit; GCLM, glutamate cysteine ligase modulatory subunit; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione *S*-transferase; HO-1, heme oxygenase 1; MDA, malondialdehyde; NQO1, NAD(P)H:quinone oxidoreductase 1; Nrf2, Nuclear factor erythroid 2 (NF-E2)-related factor 2; PCO, protein carbonyl; ROS, reactive oxygen species; RP, rice protein; RP-C, cholesterol-supplemented rice protein; T-GSH, total glutathione.

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exerted by rice protein. Moreover, recent studies indicate that the antioxidant response induced by rice protein is a major regulator of the hypocholesterolemic action in rats fed with rice protein, suggesting that the consumption of rice protein might be beneficial in improving oxidative stress to prevent the occurrence of hyperlipidemia (Cai et al., 2014). However, the exact molecular mechanism and the signaling pathway by which rice protein modulates Phase II enzymes gene expression involved in detoxification and antioxidant defense are still unclear. Particularly, up to now, the evidence on the ability of rice protein to regulate the ROS level via Nrf2 pathway is lacking.

Aging and dietary cholesterol are suggested as major risk factors for developing oxidative stress (Cadenas and Davies, 2000; Mahfouz and Kummerow, 2000; Salmon et al., 2010). However, antioxidative responses induced by rice proteins have been demonstrated both in growing and adult rats (Cai et al., 2014; Yang et al., 2012a). Moreover, recent findings suggest that the antioxidative potential of rice protein, through regulating glutathione metabolism in adult rats, cannot be attenuated by the addition of cholesterol in diets (Cai et al., 2014). Thus, the question arises of why rice protein can exert an antioxidant capability independently of dietary cholesterol in adult rats.

The present study, therefore, is conducted to elicit the molecular mechanism by which rice protein produces an antioxidant response to oxidative stress in adult rats fed with cholesterol-free or -enriched diets. The major aim of this study is to investigate whether and how rice protein can lead to the activation of defensive gene expression via the Nrf2 pathway, which results in the prevention of ROS generation and the enhancement of endogenous antioxidant defense against oxidative damage. Particularly, the influences of dietary cholesterol on the activation of Nrf2 and the ROS production are also investigated in the present work.

2. Materials and methods

2.1. Protein sources

Rice protein (RP) from *Oryza sativa* L. cv. *Longjing* 26 (Rice Research Institute of Heilongjiang Academy of Agricultural Sciences, Jiamusi, China) and casein (CAS) (Gansu Hualing Industrial Group, Gansu, China) were used as the dietary protein sources in the present study, in which RP was prepared by the alkaline extraction method (Yang et al., 2012b, 2012c). The protein concentrations were 873.7 g/kg and 907.6 g/kg in CAS and RP, respectively.

2.2. Animals and diets

The present experiments were approved and carried out according to the "Rules for experiments' animals" published by Chinese Government (Beijing, China).

Adult male Wistar rats (body weight 390–410 g) were purchased from the Vital River Laboratories (Beijing, China) and individually housed in metabolic cages in a room maintained at 22 ± 2 °C under a 12 h light-dark cycle (07:00–19:00 for light). Rats were allowed free access to commercial pellets (Vital River Laboratories, Beijing, China) for 3 days. After acclimatization, rats were randomly divided into four groups of similar body weight. Each group consisted of six animals.

All animals were fed ad libitum with experimental diets according to the formula recommended by American Institute of Nutrition (Reeves et al., 1993). For 2 weeks, adult rats were fed 14% (as crude protein) dietary proteins without cholesterol (CAS and RP) and with the addition of 1% cholesterol and 0.25% sodium cholate (CAS-C, cholesterol-supplemented casein; RP-C, cholesterol-supplemented rice protein) in diets. Diets were completed to 100% with starch.

2.3. Samples collection

During the feeding period, food consumption and body weight were recorded daily in the morning before replenishing the diet.

At the end of the feeding period, the rats were deprived for 18 h and then sacrificed. Blood was withdrawn from abdominal vein into a heparinized syringe under anesthesia with sodium pentobarbital (50 mg/kg body weight), immediately cooled on ice and separated by centrifugation at 12,000 ×*g* for 5 min. The plasma obtained was frozen at −20 °C until analysis. After blood collection, the liver was excised immediately, rinsed in saline and weighed after blotted on a filter paper. The whole liver was cut into several portions and quickly freeze-clamped in liquid nitrogen and stored at −80 °C until analysis.

2.4. Measurement of reduced glutathione and oxidized glutathione

Total glutathione (T-GSH) and oxidized glutathione (GSSG) in the liver were assayed with the commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Reduced glutathione (GSH) was calculated as: GSH = T-GSH − 2 × GSSG.

2.5. Determination of ROS content

The production of ROS in the liver was determined by fluorescence of 2′, 7′-dichlorofluorescin diacetate (DCF-DA) as described by the manufacturer's protocol of commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The fluorescence intensity was measured at 485 nm of excitation wavelength and 530 nm of emission wavelength. Hepatic protein levels were determined using BCA assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Data are expressed as arbitrary unit of fluorescent intensity per μg protein.

2.6. Analyses of enzyme activity

Hepatic GST activity and the activities of plasma alanine transaminase (ALT) and aspartate transaminase (AST) were determined using the methods described in the kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.7. Quantitative real-time PCR

Total RNA was extracted from rat livers using the TRIzol reagent kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. cDNA was reverse transcribed from 1 μg of total RNA using a PrimeScript™ 1st strand cDNA Synthesis Kit (Takara Bio. Inc., Otsu, Shiga, Japan). For quantitative real time PCR, cDNAs were analyzed with the ABI 7500 sequence detection system (Applied Biosystems, Foster City, CA, USA) using SYBR Green (Takara Bio. Inc., Otsu, Shiga, Japan). The primers sequences used were as following: 5′-ACAGCAACAGGGTG GTGGAC-3′ (forward) and 5′-TTTGAGGGTGCAGCGAA-CTT-3′ (reverse) for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH); 5′-CCTC CTCCTCCAAACTCAGATA-3′ (forward) and 5′-CCACAAATACCACATA GGC-AGA-3′ (reverse) for rat glutamate cysteine ligase catalytic subunit (GCLC); 5′-GGGCACAGGTAAAACCCAATA-3′ (forward) and 5′-TTCAAT GTCAGGGATGCTT-TC-3′ (reverse) for rat glutamate cysteine ligase modulatory subunit (GCLM); 5′-AACCTTTTGAGACCCTGCTGT-3′ (forward) and 5′-CTGTTTACCATTGCCGTTGA-T-3′ (reverse) for rat GST; 5′-GAATAAAGTTGCCGCTCAGAA-3′ (forward) and 5′-AAGGTTTCCCATCC TCATCAC-3′ (reverse) for rat Nrf2; 5′-GCCCTGGAAGAGGAG-ATAGAG-3′ (forward) and 5′-TAGTGCTGTGTGGCTGGTGT-3′ (reverse) for rat HO-1; 5′-TCACCACTCTACTTTGCTCCAA-3′ (forward) and 5′-TTTTCTGC TCCTCTTGAACC-TC-3′ (reverse) for rat NQO1. The results were normalized to the level of GAPDH mRNA.