Mechanisms Underlying Food Functionality *via* Molecular Chaperones: Chemical Training Hypothesis

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ABSTRACT

Most physiologically functional food factors are derived from plants. Although those chemicals exhibit versatile bioactivities, their mechanisms of action are not fully understood. It is important to indicate that phytochemicals, but not plant nutrients, xenobiotics in humans and thus induce expressions of self-defense molecules, including anti-oxidative and xenobiotic function as metabolizing enzymes *via* the Keap1/Nrf2 system. In addition, recent reports have shown that several phytochemicals are capable of up-regulating the activities of molecular chaperones such as heat shock proteins (HSPs) and proteasomes. For example, sulforaphane, a sulfur-containing phytochemical present in cruciferous plants, was recently reported to induce HSP27 expression and increase proteasome activity. On the other hand, accumulating evidence shows that some toxins exert health beneficial effects under non-toxic doses, a phenomenon termed hormesis. Importantly, those beneficial effects are abrogated or disappear when given in high doses. Collectively, chronic exposure to phytochemicals, *i.e.*, 'chemical training', may increase self-defense mechanisms, thereby contributing to health promotion and disease prevention.

Key words: phytochemical, molecular chaperone, heat shock protein, stress adaptation.

INTRODUCTION

Many physiologically functional food factors are secondary plant metabolites such as flavonoids and terpenoids. For example, resveratrol, which exerts numerous bioactivities including a longevity effect, is a well-described phytoalexin, a type of plant toxin that protects against invading microorganisms and insects. However, it remains unknown why and how those phytochemicals exhibit bioactivities that are beneficial for humans. The antioxidant effects of phytochemicals have been intensively studied and proposed to be one of the major mechanisms of action to account for their health promotion and disease prevention effects. On the other hand, it is controversial whether they have adequate efficacy to actually exhibit antioxidant effects *in vivo,* while some investigators have described side-effects due to pro-oxidation, especially at high doses[1]. In addition to their antioxidant activities, analyses of the interactions of phytochemicals with biological proteins are essential to understand the molecular mechanisms underlying their bioactivities. In 2004, Tachibana and coworkers identified the receptor for (-)-epigallocatechin-3-gallate (EGCG) as a 67kDa laminin receptor[2]. That pioneering work stimulated many other researchers to search for other receptors and binding proteins of EGCg, as well as those of other phytochemicals.

Sulforaphane[3], a sulfur-containing, chemopreventive agent found in cruciferous plants, has an electrophilicity chemical characteristic that is derived from an isothiocyanate (ITC) group. Curcumin, the major yellow pigment in turmeric, exhibits multiple bioactivities and also has an electrophilic α,β-unsaturated carbonyl group[4]. In addition, zerumbone, a chemo- preventive sesquiterpene present in *Zingiber zerumbet* Smith, has a similar functional group that is indispensable for its bioactivities[5]. Those electrophilic phytochemicals induce nuclear factor-E2-related factor (Nrf)2-dependent expression of self-protecting enzymes[6].

Heat shock proteins (HSPs) are a class of stress-inducible proteins that play essential roles as molecular chaperones and protect cells against proteotoxic damage due to a variety of stimuli[7]. The key transcription factor for HSP induction is heat shock factor (HSF)1, which is dormant when associated with HSP90 in a normal state. When exposed to heat stress, HSF1 is liberated from HSP90 and translocated into the nucleus to induce transactivation of stress-adapting genes including *hsps*. A recent report discovered that electrophilic 4-hydroxy-2- nonenal (HNE), a degradation product of lipid peroxidation, activates HSF1 for inducing HSP70[8]. Collectively, electrophiles may be characterized as having notable activities for inducing self-defensive proteins including anti-oxidative, drug metabolizing and molecular chaperones. This mini-review article highlights stress responses induced by electrophilic phytochemicals and discusses their relationships to bioactivities.

NRF2-DEPENDENT PROTEINS

I. *Anti-Oxidative and Xenobiotic-Metabolizing Enzymes*

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The Kelch-like ECH-associated protein 1 (Keap1)/ Nrf2 system adaptively functions to protect cells from oxidative and electrophilic damages. In a normal state, the transcription factor Nrf2 is continuously ubiquitinated by the Cul3-Keap1 ubiquitin E3 ligase complex and thereby rapidly subjected to degradation in proteasomes (Figure 1). Electrophilic chemicals and oxidative stresses oxidize the reactive cysteine residues of Keap1 in both direct and indirect manners[9]. This critical step stabilizes Nrf2, thereby inducing robust expression of a battery of cytoprotective genes. In addition, prior to translocation of Nrf2 into the nucleus, its transcription activity is modulated by several protein kinases, which are simultaneously activated by stimuli.

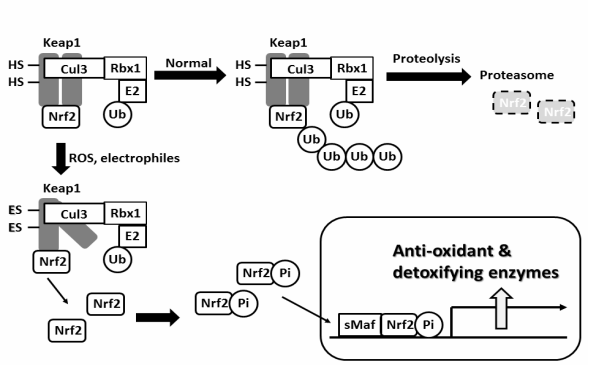

Figure 1. Action mechanism underlying Nrf2 activation. In a normal state, Nrf2 is continuously ubiquitinated and thereby rapidly subjected to degradation in proteasomes. Electrophilic chemicals and oxidative stresses oxidize the reactive cysteine residues of Keap1. This critical step stabilizes Nrf2 and induces robust expression of a battery of cytoprotective genes.

II. *Modulations by Phytochemicals*

Sulforaphane has been shown by many investigators to have a substantial ability for inducing antioxidant enzymes including superoxide dismutase[10]. This efficacy is considered to have close associations with its potent anti-carcinogenesis activities in chemically and biologically induced carcinogenesis in several organs[3]. The critical moiety of this agent for activating Nrf2 has been shown to be an ITC group, as the same class of compounds including phenethyl ITC have comparable activities[11]. Na *et al*. documented that Nrf2 mediates EGCG-induced expression of some representative antioxidant enzymes, possibly *via* Akt and ERK1/2 signaling [12]. However, the molecular mechanisms underlying EGCG activation of Nrf2 remain to be fully elucidated. One possibility is that EGCG is auto-oxidized to generate reactive oxygen species, which are capable of oxidizing reactive Keap1 cysteine and activating Nrf2, as mentioned above. The other possibility is that auto-oxidation of EGCG converts it into an *o*-quinone counterpart which may form a covalent bond with the reactive cysteine(s) of Keap1 *via* Michael addition. This notion is supported by the observation by Ishii *et al.,* who reported that conversion[13]. We previously reported that zerumbone functioned as a marked chemopreventive agent in chemical carcinogenesis models of the skin[14], colon[15], and lungs[16]. Interestingly, zerumbone is an electrophilic phytochemical that elevates the expression of antioxidant and drug metabolizing enzymes *in vivo*[14], suggesting it to be an Nrf2 activator. Thus, we generated zerumbone-bound Sepharose gels to explore its binding proteins[17]. Incubation of cell lysates with this chemical probe resulted in identification of Keap1 as *in vitro* binding protein of this compound.

PROTEIN QUALITY CONTROL

I. *HSPs*

HSPs are highly conserved families of proteins expressed by all types of cells and organisms. In addition to constitutive isoforms, physical, biological, and chemical stressors are known to up-regulate their inducible isoforms. In addition, some isoforms are actively secreted or released by cellular damage to confer stress signaling[18]. HSPs play central roles in protein quality control by binding to unfolded proteins (Figure 2). There is also a great body of evidence that maintenance of HPSs at high levels substantially contributes to longevity[19]. In a normal state, HSP90, the major constitutive isoform, is associated with heat shock factor1 (HSF1) and biologically dormant. Heat shock and some chemicals are capable of dissociation from the heterodimer complex, after which the resultant free HSF1 translocates into the nucleus for induction of HSPs. Thus, proteotoxic stressors at appropriate levels may strengthen the protein quality control system, making it more efficient than that before stress exposure.

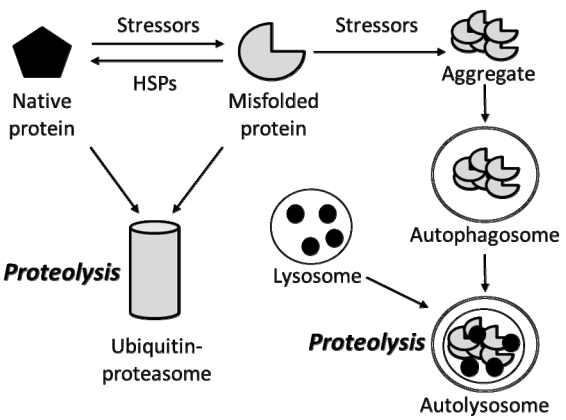

Figure 2. Proteolysis of denatured and aggregated proteins *via* ubiquitin-proteasomes and autophagosomes.

II. *Proteasomes and Autophagosomes*

Unfolded, denatured cellular proteins not refolded by molecular chaperones may aggregate by non-specific hydrophobic interactions. This critical process is involved

in the onset of many neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases[20]. The aggregated unused proteins are sequestrated by autophagosomes, which are generated by Atg family members, LC3-II, and others[21]. This leads to proteolytic processes through biological fusion of autophagosomes and lysosomes, acidic organelles that contain a cocktail of proteases (Figure 2). On the other hand, some housekeeping proteins, together with denatured proteins, are known to be degraded *via* the ubiquitin-proteasome system[22].

III. *Modulation by Phytochemicals*

Hu et al. identified Nrf2-dependent and sulforaphane-inducible genes which included HSPs and ubiquitin/proteasome subunits, using Nrf2(-/-) mice[23]. In accordance with their findings, a recent study by Gan et al. showed that sulforaphane activates HSF1 for inducing the expression of HSP27 and stimulating proteasome activity[24]. Meanwhile, HSP-16.2, a putative stress-sensitive reporter related to longevity in *Caenorhabditis elegans*, was found to be up-regulated by EGCG[25], though that finding was contradicted by Abbas *et al.*[26], possibly due to differences in experimental conditions. Additionally, EGCG has been described as an inhibitor od HSP90[27] and proteasomes[28]. Curcumin, a marked HSPs inducer[29], modulated proteasome activity in a concentration-independent manner[30]. Namely, curcumin (1 μM) increased proteasome activity by 46% as compared to untreated keratinocytes whereas significant inhibition was seen at a concentration of 10 μM. These observations suggest that beneficial stress responses of phytochemicals, including HSP expression and increased proteasome activity, are associated with their concentrations and treatment time. A recent study by Aoki et al. showed that curcumin inhibited tumor growth in xenograft model of U87-MG cells, which was accompanied with autophagy[31]. In addition, PEITC induced autophagic and apoptotic cell death in PC-3 human prostate cancer cells in Atg5-dependent manner[32].

CONCLUSION

Most plant secondary metabolites are biosynthesized for the purpose of self-defense and adaptation to environmental stresses. In general, they are structurally simple and small-molecules as compared with designed drugs. Therefore, it is not surprising that phytochemicals bind to biological proteins in highly non-specific manner, though it is now evident that they also possess several specific binding proteins that mediate their bioactivities. Off-target protein modifications by phytochemicals may induce HSP expression and activate proteasome- and autophagosome-dependent proteolysis systems, particularly in the later stages of protein denaturation. However, it is important to note that those chemical stressors may cause beneficial effects when their modification intensity is below adaptation capacity, which is related to the concepts of 'hormesis'[33] and 'xenohormesis'[34]. On the other hand, overdoses likely induce side-effects and toxicity. Humans are continuously exposed to xenobiotic phytochemicals from daily food intake, thus biological systems are considered to be chronically activated to counteract or adapt to those stresses. Such a continuous process may be termed 'chemical training', and presumably has effects on health and longevity.

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