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Food Factors

Proceedings of the 2nd International Conference on Food Factors (ICoFF)

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FOOD FACTORS

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Volume 1

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Contents

Preface

These two volumes of "Food Factors" contain the proceedings of the 2nd International Conference on Food Factors (2nd ICoFF).

Inheriting the success of the 1995 International Conference on Food Factors (ICoFF) at Hamamatsu, Japan, the 2nd ICoFF was held from December 12 through 17, 1999 in Kyoto, Japan. The old historical city of Kyoto, with an astounding seventeen UNESCO world cultural heritage sites, attracted scientists from eighteen countries all over the globe and offered an excellent environment to present and discuss topical science in a congenial atmosphere. While in Hamamatsu discussion was centered on food factors for cancer prevention, the 2nd ICoFF covered a wide scope of research on food factors of a variety of physiological significance. The actual goal of the conference was to establish a role of food factors in disease prevention and health promotion from the scientific base.

The proceedings of the conference present recent research data and review lectures by numerous experts and will be of special interest and relevance to all who are concerned with food factors in disease prevention and health promotion. A great deal of the contributions has been selected for publication as the most informative mini-reviews or original research reports. These include recent topics in cancer prevention and antioxidants as well as vitamin E, minerals and trace elements, peptide and amino acids, flavones and flavonols, isoflavones, dietary fibers, oligo- and polysaccharides, lipids, catechins, carotenoids, polyphenols, terpenoids and sulfur-containing compounds.

We would like to take this opportunity to thank the authors for their excellent contributions and cooperation in the development of these proceedings. We would also like to thank the many others whose support made the conference a success.

Tokushima, July 2000
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Novel Aspects of Food Factors

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Mini-review

Chemoprotection against cancer by induction of Phase 2 enzymes

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Abstract. Induction of Phase 2 enzymes is an effective and sufficient strategy for achieving protection against the toxic and neoplastic effects of many carcinogens. It is proposed that the concept of Phase 2 enzymes as being responsible only for the conjugation of functionalized xenobiotics with endogenous cellular ligands such as glutathione (glutathione S-transferases) and glucuronic acid (UDP-glucuronosyltransferases) be expanded to include proteins with the following common characteristics: (a) coordinate induction by a broad range of chemical agents that all have the capacity to react with sulfhydryl groups; (b) possible regulation by common promoter elements; and (c) catalysis of reactions that lead to comprehensive protection against electrophile and reactive oxygen toxicities, by a wide variety of mechanisms. These mechanisms include: conjugation with endogenous ligands, chemical modification of reactive features of molecules that can damage DNA and other macromolecules, and generation or augementation of cellular antioxidants. In addition to the above conjugating enzymes, a provisional and partial list of Phase 2 proteins might include: NAD(P)H:quinone reductase, epoxide hydrolase, dihydrodiol dehydrogenase, γ-glutamylcysteine synthetase, heme oxygenase-1, leukotriene B_4 dehydrogenase, aflatoxin B_1 dehydrogenase, and ferritin.

Keywords: Glutathione transferase, glutathione, NAD(P)H:quinone reductase, heme oxygenase, γ-glutamylcysteine synthetase, epoxide hydrolase, ferritin, electrophile toxicity, redox cycling, antioxidants, reactive oxygen species

1. History and evolution of the Phase 1 and Phase 2 enzyme concept

More than 30 years ago R.T. Williams [42] of Saint Mary's Hospital Medical School in London formally suggested that the metabolism of xenobiotics could be viewed as resulting from sequential actions of two families of enzymes: Phase 1 enzymes that catalyze "asynthetic" reactions, which functionalize compounds largely through oxidations and reductions, and Phase 2 enzymes which promote "synthetic" conjugations of Phase 1 products with endogenous ligands such as glutathione (GSH), glucuronic and amino acids, leading usually to more water-soluble and more easily excretable products. Much subsequent work has established that the enzymes belonging to the two groups are induced by a wide variety of synthetic and natural chemical agents, and are regulated by distinct and separate mechanisms.

This somewhat limited view of the nature and functions of Phase 2 enzymes (i.e., synthesis of conjugates) is gradually giving way to a much broader concept of their scope and importance. The notion is evolving that they should perhaps be defined by the following properties: (a) coordinate induction by many types of inducers that also induce classical Phase 2 enzymes such as glutathione *S*-transferases (GSTs); (b) regulation by mechanisms that are very similar and may involve common promoter elements (e.g., the Antioxidant Responsive Element, or ARE) [13,32]; and (c) catalysis of a

wide variety of reactions that serve to protect cells against the toxicities of electrophiles and reactive oxygen species by converting them into less toxic products [14,35,37]. Indeed, there is now extensive evidence that Phase 2 enzymes not only provide a major mechanism by which cells combat the toxicities of electrophiles and reactive oxygen species, but that their induction is a highly effective and sufficient condition for protecting cells against the toxic and neoplastic challenges of many types of carcinogens. It is therefore not surprising that the discovery that many edible plants contain substantial quantities of potent Phase 2 enzyme inducers has stimulated great interest in utilizing such plants, and their inducer components, to achieve protection against cancer [9,29,43,44]. This paper reviews evidence that supports these suggestions for a broader role of Phase 2 enzymes and their important participation in protection against cancer and electrophile toxicity.

2. Roles of Phase 1 and Phase 2 enzymes in carcinogenesis and protection against cancer

A critical advance in understanding the mechanisms of carcinogenesis was the recognition by James and Elizabeth Miller [17] that most carcinogens are quite unreactive and innocuous procarcinogens, which require conversion by cellular (Phase 1) enzymes to highly reactive, electrophilic, ultimate carcinogens that react directly with nucleophilic centers of macromolecules such as DNA, to produce a series of damaging events that can evolve into cancer. These workers also demonstrated that Phase 1 enzymes were inducible, and that their activities often gave rise to nonelectrophilic detoxication products. Both electrophilic and nonelectrophilic products of Phase 1 enzyme activities are substrates for Phase 2 enzymes, which are likewise inducible, and promote detoxication reactions. Consequently the outcome of carcinogen exposure is controlled in large part by the balance between Phase 1 enzymes that can generate ultimate carcinogens and Phase 2 enzymes that detoxify these products. Although these families of enzymes are under genetic and hormonal control, they are also regulated by inducers, and the shifting of this balance by induction toward the dominance of Phase 2 enzymes has emerged as an important strategy for achieving chemoprotection against electrophile toxicity and malignancy.

An important milestone in research on chemoprotection against cancer was the discovery by Wattenberg (see reviews [38,39]) that the phenolic antioxidants BHA and BHT could substantially reduce the development of a variety of tumors evoked by numerous carcinogens in rodent models. Since these antioxidants are widely used as food preservatives, these discoveries suggested for the first time that cancer could be blocked by agents already in the food chain, and therefore presumably of relatively low toxicity. Our early biochemical and molecular studies [1–3] disclosed that administration of these antioxidants to rodents evoked marked changes in the metabolism of carcinogens and that these changes were attributable to elevation of the specific activities of Phase 2 enzymes: glutathione transferases (GSTs), epoxide hydrolase, and NAD(P)H:quinone reductase (QR) in the livers and peripheral tissues [1–3]. Furthermore, more detailed analysis, including mRNA measurements, established that these elevations resulted from enhanced rates of gene transcription and enzyme synthesis [20].

Inducers of Phase 2 enzymes are of two types: monofunctional and bifunctional [30]. Polycyclic aromatics, azo dyes, dioxin, and flavones (all large planar aromatics) are bifunctional in that they induce both Phase 2 and certain Phase 1 enzymes. These bifunctional agents bind with high affinity to the *Ah* (Aryl hydrocarbon) receptor and induce several cytochromes P450 that may activate carcinogens, e.g. CYP1A1 and 1A2. The induction of Phase 1 enzymes is mediated by the *Ah* receptor or the gene products under its control. In contrast, monofunctional inducers have no common structural features (see below), induce Phase 2 enzymes without significantly elevating Phase 1 enzymes, and are independent of *Ah* receptors or their functions [30]. One important consequence of this dichotomy of inducer types

is that it focuses on both the desirability and practicality of selecting monofunctional inducers as agents for achieving chemoprotection, thereby minimizing the hazards of activating carcinogens.

3. Determination of potencies of Phase 2 enzyme inducers

Recognition of the importance of Phase 2 enzyme induction as a mechanism of chemoprotection suggested the need to devise methods for detecting and quantifying the inducer potencies of pure chemicals and of extracts of natural products. Quinone reductase (QR) was selected as a convenient target enzyme, because of its coordinate induction with other Phase 2 enzymes, wide distribution in mammalian tissues, large inducer response, and ease of measurement by a coupled tetrazolium dye reduction assay. A robust and highly useful murine hepatoma cell line (Hepa 1c1c7) grown in 96-well microtiter plate wells provided a simple system for the highly reproducible quantitative assays of inducer potencies of single compounds, mixtures, or of plant extracts [9,28,29]. The results obtained from this system have reliably predicted the behavior of inducers in animal systems. Furthermore, the availability of mutant Hepa cells defective in cytochrome P-450 activity or aryl hydrocarbon receptor function has provided a simple method for making the important distinction between monofunctional and bifunctional inducers [30]. Potencies of inducers, conveniently expressed as concentrations required to double the QR activity, have been found to vary by 5 orders of concentration magnitudes [22,23].

4. Chemical characteristics of monofunctional Phase 2 enzyme inducers

Important insight into the chemical nature of inducers emerged from studies of analogues of the widely used food antioxidant BHA [27]. They pointed to *tert*-butylhydroquinone, a metabolite of BHA, as the active inducer, and showed that demethylation to phenols was required for induction. The orientations of the hydroxyl groups of these diphenols were critical. Only 1,4-diphenols (hydroquinones) and 1,2-diphenols (catechols), both of which are readily oxidizable to quinones, were inducers, whereas 1,3-diphenols (resorcinols) which are not oxidizable were inactive. Other substituents on the aromatic rings had little effect on inducer potency. Although oxidizability was clearly required for inducer activity, these experiments did not disclose whether the quinone products were the ultimate inducers or whether the redox process (possibly generating reactive oxygen species) produced the signals for induction. This issue was resolved by the demonstration that many Michael reaction acceptors (i.e., olefins or acetylenes conjugated to electron-withdrawing groups) were efficient inducers and that their inducer potencies correlated closely with their Michael reactivity [36]. This generalization not only explained the inducer activities of many compounds, but also permitted the correct prediction of inducer properties of novel structures. Since quinones are excellent Michael acceptors, we ascribed the inducer properties of BHA and its diphenol metabolite to their ability to undergo oxidations to quinones.

Subsequently, many different types of monofunctional inducers have been recognized. In addition to oxidizable diphenols and phenylenediamines, quinones and other Michael reaction acceptors, the range of inducers includes: isothiocyanates and their thiol addition products (e.g., dithiocarbamates); 1,2-dithiole-3-thiones; trivalent arsenicals; heavy metals such as mercury and cadmium; hydroperoxides; and vicinal dimercaptans [22,23] . More recently certain carotene metabolites have also been shown to be inducers [15]. Although these classes of inducers appear to have few common properties, they are all chemically reactive. They include many electrophiles, a single class of nucleophiles, as well as powerful oxidants and antioxidants. All inducers can modify sulfhydryl groups either by alkylation or by redox