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Review Article

How do nutritional antioxidants really work: Nucleophilic tone and para-hormesis versus free radical scavenging *in vivo*

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ABSTRACT

We present arguments for an evolution in our understanding of how antioxidants in fruits and vegetables exert their health-protective effects. There is much epidemiological evidence for disease prevention by dietary antioxidants and chemical evidence that such compounds react in one-electron reactions with free radicals *in vitro*. Nonetheless, kinetic constraints indicate that *in vivo* scavenging of radicals is ineffective in antioxidant defense. Instead, enzymatic removal of nonradical electrophiles, such as hydroperoxides, in two-electron redox reactions is the major antioxidant mechanism. Furthermore, we propose that a major mechanism of action for nutritional antioxidants is the paradoxical oxidative activation of the Nrf2 (NF-E2-related factor 2) signaling pathway, which maintains protective oxidoreductases and their nucleophilic substrates. This maintenance of "nucleophilic tone," by a mechanism that can be called "para-hormesis," provides a means for regulating physiological nontoxic concentrations of the nonradical oxidant electrophiles that boost antioxidant enzymes, and damage removal and repair systems (for proteins, lipids, and DNA), at the optimal levels consistent with good health.

Contents

Abbreviations: ARE, antioxidant response element; EpRE, electrophile response element; GSH, glutathione; Nrf2, nuclear factor erythroid 2-related factor 2; SOD, superoxide dismutase; Trx, thioredoxin.

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Preface

Here we present arguments for the mechanism of action of nutritional antioxidants that are both a synthesis of evolving ideas that better explain almost all so-called "antioxidants," and a refutation of the concept that unselective supplementation can be useful. Our thesis is written from an historical perspective in order to enhance the foundations for our proposal of nucleophilic tone and para-hormesis, and in an attempt to make these concepts (which are supported by extensive chemical evidence) more accessible to the general reader. We admit to the drawbacks of diminished comprehensiveness and a bias engendered by our involvement for 40 or more years in the field. We also apologize to anyone who feels their work should have been cited here, but note that this applies to thousands of important publications that could not all be included.

Introduction

The dawn of agriculture, approximately 10,000 years ago, was a major achievement in human evolution, which resulted in easier availability of metabolic energy from carbohydrates, fats, and proteins. In the first half of the last century, studies on metabolism and bioenergetics led to the identification of inorganic and organic compounds, including vitamins, not directly required for energy, but nevertheless indispensable for life. Analysis of deficiency syndromes, by nutritionists, provided the scientific information that today still drives recommendations for prevention of specific diseases directly caused by inadequate intake of specific nutrients. Of course, it was recognized long before the scientific era that the vegetal kingdom also provides a large number of molecules that act as poisons and/or drugs in addition to being a major source of metabolic energy and essential vitamins.

In recent decades, however, a view has emerged about another important impact of nutrition on health. It became clear that many fruits and vegetables contain phytochemicals that may reduce the risk of diseases [1–3], without being related to any specifically defined pharmacological effect or deficiency syndrome. This opinion, first suggested by folk traditions about healthy diets and nonconventional medicine, has frequently been corroborated by epidemiological/statistical evidence of decreased relative risk of various diseases. Animal and *in vitro* studies of specific phytochemicals have often supported such views.

A major outcome of all this information is the popular recommendation about the importance of a regular intake of fruits and vegetables to minimize the risk of degenerative diseases and cancer [4]. The fact that just a minimal, if any, lowering of risk can be observed in subjects adopting a diet optimized [5] according to the major guidelines, does not limit the relevance of the issue. Instead, such evidence suggests that it is the nonoptimal intake that leads to an increased risk of disease. As an example, the concept of cancer prevention, and possibly reversion, by phytochemicals present in fruit and vegetables is usually discussed with regard to the alleged antioxidant effect brought by a plethora of antioxidant compounds present in vegetal foods [6].

In this review, we describe how redox prone "antioxidant" phytochemicals present in fruits and vegetables affect cellular signaling, increasing the protective effects of the Nrf2/EpRE pathway that results in a more reductive/electrophilic environment, which we refer to as "nucleophilic tone." On the basis of available chemical and biological data we propose that antioxidants present in fruit and vegetables paradoxically act together to produce an additive increase in electrophilic signaling that results in the induction of protective Phase II enzymes and increased nucleophilic substrates, such as glutathione, thioredoxin and NADPH. Furthermore, such nucleophilic substrates are all maintained in a reduced state through increased pentose shunt utilization of glucose. Our nucleophilic tone concept contrasts markedly with the kinetically unrealistic free radical scavenging proposal that has dominated antioxidant discussions for several decades.

A brief history of antioxidants

First, we will review how antioxidants became synonymous with free radical scavenging, and how kinetic constraints limit the ability of free radical scavenging to explain dietary antioxidant actions, with the notable exception of vitamin E.

The first semiempirical use of antioxidants was in the 19th century when several molecules were used to control the process of rubber production and to prevent "fatigue" of the polymers [7]. Soon, the same or similar molecules were introduced in the food industry to prevent rancidity, the most marked outcome of oxidative degradation of stored foods [7]. The chemistry underlying these effects is the quenching of peroxyl radicals and the reduction of hydroperoxides. The most typical examples of compounds acting through these two mechanisms, quenching of free radicals and reduction of electrophiles, are natural or synthetic phenolic compounds and sulfite, respectively.

In the first half of the 20th century, studies on the chemistry of oxidation of organic molecules and the involvement of free radical intermediates led to the generalization by Michaelis (best remembered for his famous description of enzymatic kinetics) that "all" biological oxidations involved free radicals [8]. While this (rather extreme) proposal was subsequently refuted, a consensus was reached that a significant number of biological oxidations (catalyzed by enzymes) do indeed involve the formation of free radical intermediates [9]. Interest in the biological significance of free radical chemistry led Albert Szent-Györgyi (Nobel laureate for the discovery of various Krebs cycle intermediates and vitamin C) to elaborate the concept that incorrect free radical formation or elimination is the ultimate cause of cancer [10]. The free radical in the cancer problem, which Szent-Györgyi characterized as "an electronic problem" led him to describe life as having negative entropy or "syntropy" [11]. The concept of syntropy is therefore an evolution of the concept of negative entropy introduced by Erwin