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Flavonoids: The Good, the Bad, and the Ugly

by

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Abbreviations

AAPH	2,2′-azo*bis*(2-amido-propane)dihydrochloride
β-PE	β-phycoerythrin
HPLC	high performance liquid chromatography
$^{\bullet}$OH	hydroxyl radical
RO$^{\bullet}$	alkoxyl radical
ROO$^{\bullet}$	alkylperoxyl radical
ROH	alcohol
ROOH	hydroperoxide
UV	ultraviolet

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Abstract

Flavonoids are a ubiquitous component of the animal diet, being present in all foods of plant origin. They have been shown to have a number of biologically important activities, notably as antioxidants. Flavonoids have also been reported to have anticancer properties. However, there are conflicting reports of both prooxidant and carcinogenic behavior by flavonoids. This review sets forth the evidence for both modes of action, and depicts the underlying mechanisms.

Introduction

Flavonoids are polyphenolic compounds found in rich abundance in all land plants [1]. Owing to their polyphenolic nature, flavonoids often exhibit strong antioxidant properties, akin to α-tocopherol, which they structurally resemble and can replace in some model systems [1, 2]. Although animals do not directly synthesize flavonoids, their diet is supplied with them in large amounts. One estimate has the average daily consumption of flavonoids by humans as 1 gram, an amount much greater than that of other dietary antioxidants such as ascorbate or α-tocopherol [3]. Given the prevalence of these substances in our diet, and their demonstrable antioxidant activity, it is only reasonable to suppose that animals have evolved the capacity to take advantage of the beneficial properties of flavonoids.

In addition to antioxidant properties, flavonoids also exhibit an inhibitory effect on a number of enzymes. Many of these enzymes are redox-active, such as cyclooxygenase, lipoxygenase, and NADPH oxidase [4]. Flavonoids also exhibit potent anti-cancer effects, although the exact target of such inhibition has not been definitely established [5, 6]. Given the recent evidence for a prominent role for ROS in carcinogenesis, it is tempting to speculate that flavonoid-dependent inhibition of carcinogenesis is due to ROS scavenging. However, there have been an increasing number of reports that directly contradict the putative role of flavonoids as antioxidant/anticancer agents [7]. Again, due to the prevalence and apparent potency of these compounds in our diet, it behooves us to define the conditions that determine how flavonoids will behave. An understanding of the current state of knowledge is necessary for the advancement of our understanding of flavonoid biochemistry, which is the goal of this review.

Structure

Flavonoids and related polyphenols are ubiquitous in land plants, and have the general structure as shown in Figure 1 [3, 8]. Flavonoids generally consist of two benzene rings (rings A and B, Figure 1) linked by an oxygen-containing heterocycle (ring C, Figure 1). It should be noted that the chalcones are considered by many authorities to be members of the flavonoid family, despite lacking the heterocyclic ring C. The fused A and C rings are often collectively termed the flavonoid nucleus.

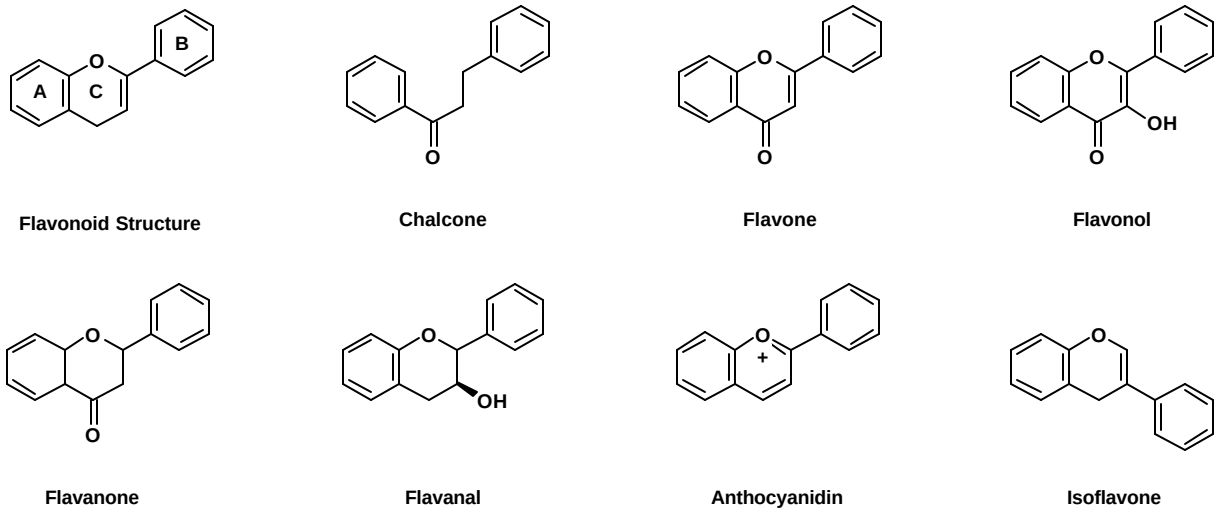

Figure 1. Structures of flavonoids.

Classification of flavonoids is based primarily upon modifications of the C ring, particularly that of oxidation state. The numbering of ring atoms follows the usual conventions. Thus the heteroatom in ring C is designated 1, and numbering continues clockwise around the heterocycle through 10. Ring B is numbered 1′, 2′, 3′, etc., beginning with the bridging carbon and proceeding clockwise through 6′. Some variation may be seen in these rules, depending on the source cited, but for consistency this review will use these conventions.

Analytical Methods

Analysis of flavonoids can be broadly separated into two categories, functional and quantitative. In this review the functional analysis of flavonoids will be restricted to their antioxidant properties, and thus will be discussed in a later section on the redox chemistry of flavonoids. Chromatographic methods are the most typically cited techniques for quantifying flavonoids, although there have been reports of spectrophotometric assays based on metal chelation by flavonoids [9]. HPLC methods utilizing UV detection systems are commonly employed, as the conjugated π-systems of flavonoids afford good UV absorption.

Redox Chemistry

Flavonoids behave as antioxidants through the mechanism of hydrogen donation, as depicted for quercetin in Figure 2.

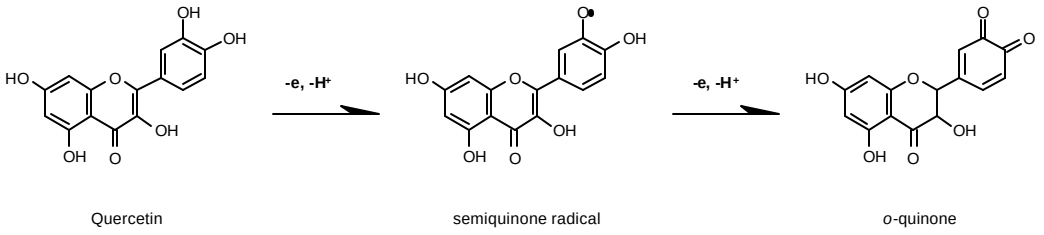

Figure 2. Oxidation of a representative flavonoid, quercetin.

Interestingly, the hydroxy groups on the chromane-like flavonoid nucleus of quercetin do not appear to participate directly in redox chemistry [10]. Instead, it is the hydroxy groups of the catechol moiety, the B ring, that donate or accept hydrogens. However, while the flavonoid nucleus does not undergo direct redox modification, it does affect the redox behavior of substituents on the B ring. Bors *et al.* has suggested the following requirements for flavonoid antioxidant activity: (1) a 3′, 4′-dihydroxy (catechol) substitution of the B ring, leading to the formation of stable phenoxyl radicals; (2) a 2,3-double bond in conjunction with a 4-carbonyl group on the C ring, allowing delocalization of the phenoxyl radical electron to the flavonoid nucleus; and (3) the combined presence of a 3-hydroxy group with a 2,3-double bond, which

increases the resonance stabilization for electron delocalization [10]. As can be seen from its structure, quercetin satisfies all these requirements, which is in keeping with its observed efficacy as an antioxidant. Table 1 summarizes the standard reduction potentials of a number of biologically important redox couples, compared to quercetin. As can be seen by its reduction potential with respect to several important radicals, quercetin is well positioned to function as an antioxidant.

Table 1. Reduction Potentials of Biologically Important Redox Couples.

Couple	**E°′/mV**
$HO^{\bullet}$, H^{+}/H_2O	2310[a]
$RO^{\bullet}$, H^{+}/ROH	1600[a]
$ROO^{\bullet}$, $H^{+}/ROOH$	1000[a]
α-tocopheroxyl$^{\bullet}$, H^{+}/α-tocopherol	500[a]
quercetin$^{\bullet}$, $H^{\bullet}$/quercetin	330[b]
ascorbate$^{\bullet-}$, H^{+}/ascorbate^{-}	282[a]

[a] Adapted from [11].
[b] Adapted from [12].

In sharp contrast to the commonly accepted role of flavonoids as antioxidants are observations that, under certain circumstances, flavonoids can act as prooxidants. One striking example reported in the literature is that of quercetin acting as a prooxidant under conditions of iron excess [7]. In that study, it was proposed that the formation of the quercetin radical *via* interaction with the mitochondrial electron transport chain or by autooxidation led to the generation of $O_2^{\bullet-}$ (see Figure 3). Also proposed in the same study was that quercetin could directly reduce Fe(III) to Fe(II), thus providing all the elements necessary to generate the highly oxidizing radical, $^{\bullet}OH$ (see Figure 3). EPR was used to demonstrate the production and identity of the indicated radicals.

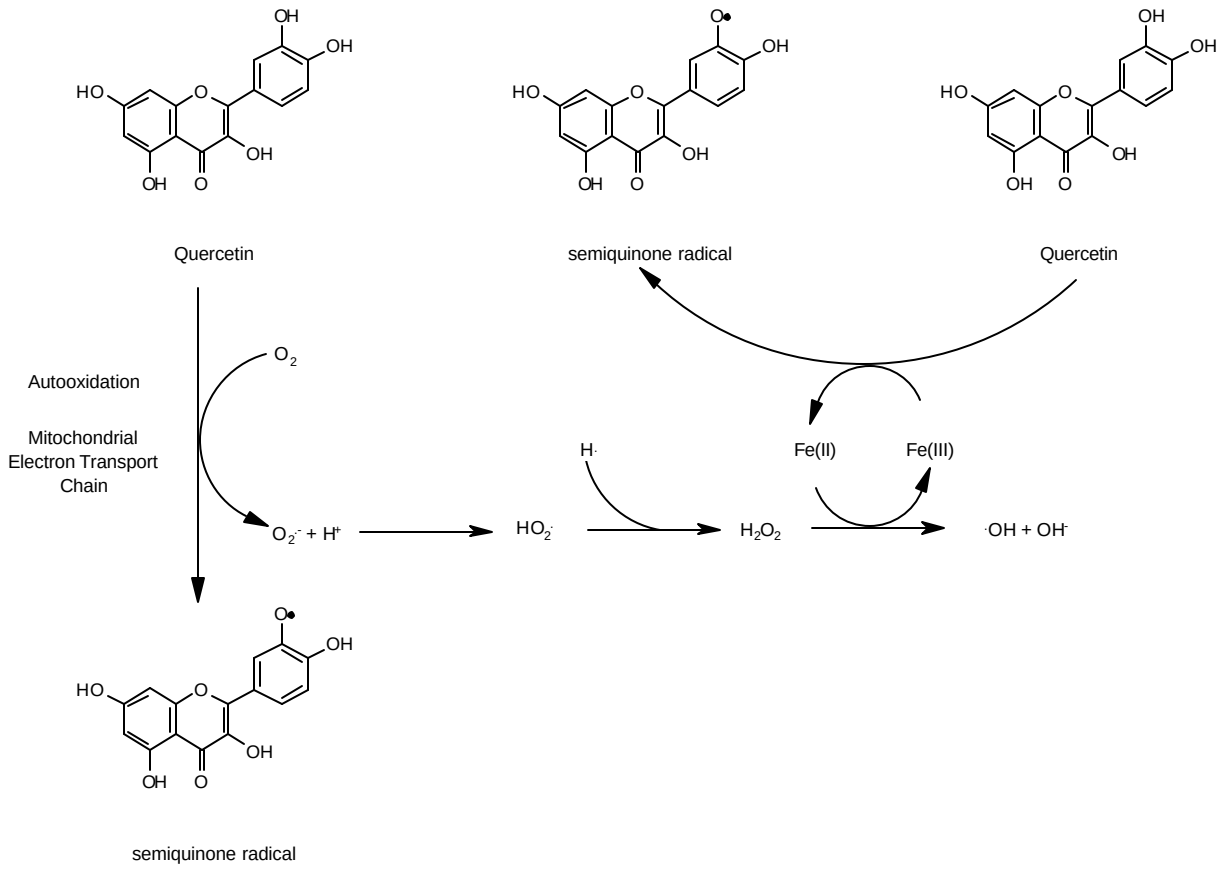

Figure 3. Iron-dependent redox cycling of quercetin.

The previously cited work of Hodnick *et al*. is supported by that of Cao *et al*., wherein the authors demonstrated that Cu^{2+} could interact with quercetin to produce a prooxidative activity [8]. In the latter study, an *in vitro* assay based on the oxidation of β-Phycoerythrin (β-PE) was used to assess the ability of quercetin and other flavonoids to suppress the oxidation of β-PE induced by a variety of prooxidants. β-PE is a fluorescent compound that loses fluorescence upon oxidation, and thus forms the basis of a general assay for antioxidant activity. The prooxidant systems used were 2,2′-azo*bis*(2-amido-propane)dihydrochloride (AAPH), a generator of peroxyl radical ($ROO^{\bullet}$); H_2O_2/Cu^{2+}, a source of hydroxyl radical ($^{\bullet}OH$) *via* Fenton chemistry; and Cu^{2+} as a source of pure transition metal oxidant. The results obtained by Cao's group showed that quercetin was able to inhibit the oxidation of β-PE in response to $ROO^{\bullet}$ and H_2O_2/Cu^{2+}, while Cu^{2+} plus quercetin actually enhanced the oxidation of β-PE, as compared to

Cu^{2+} without quercetin. Given other work demonstrating the ability of quercetin to directly reduce transition metals, this would seem to suggest that β-PE might be oxidized by the quercetin semiquinone radical, in a scheme as depicted in Figure 4.

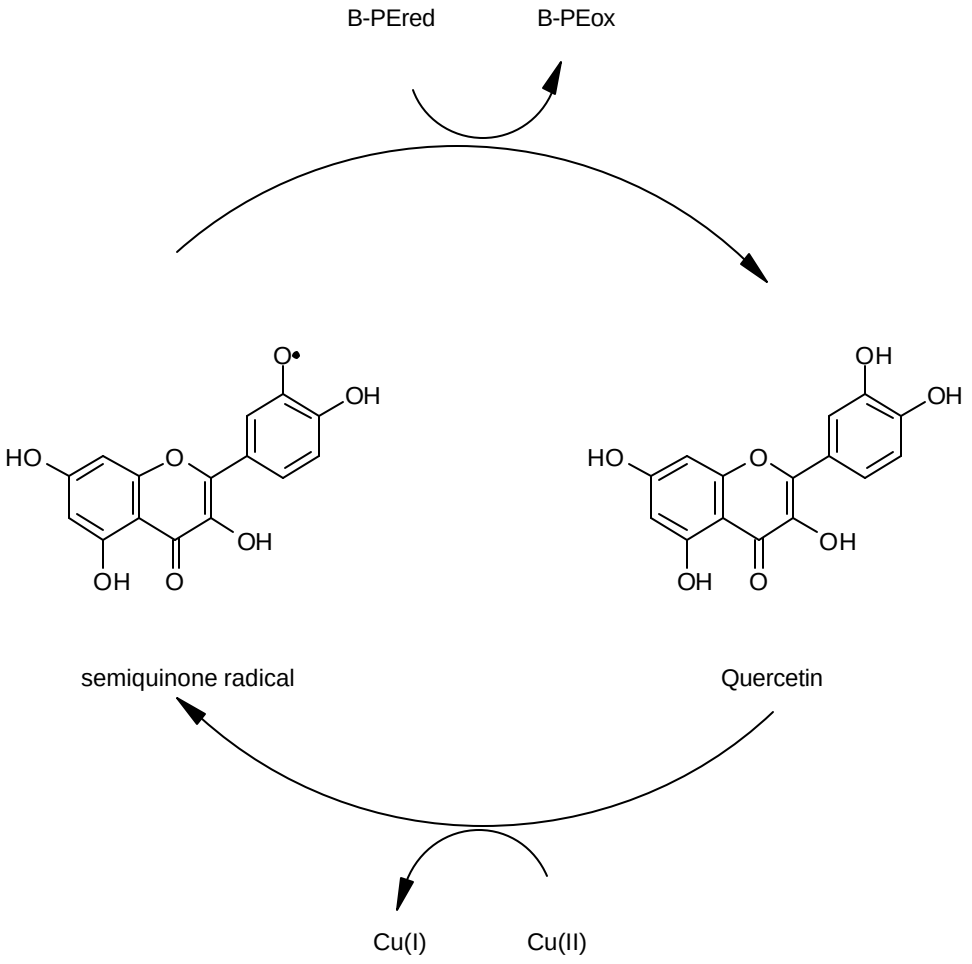

Figure 4. Quercetin-dependent oxidation of β-PE *via* reduction of Cu(II).

Biochemistry

Flavonoids are perhaps best known as antioxidants, and there is ample evidence, both *in vitro* and *in vivo*, that they can have this activity. However, antioxidant activity is not the only reported function of flavonoids. Other reported activities include the inhibition of various enzymes, particularly those involved in mediating inflammatory responses, such as the prostaglandin synthetic enzyme cyclooxygenase and the phagocytic respiratory burst generator NADPH oxidase [4, 13]. It is thought by many investigators that the inhibition of many of the

enzymes targeted by flavonoids occurs through a combination of active site recognition and redox activity [4, 13]. The latter mechanism is an especially attractive hypothesis given the fact that many of these enzymes have active site redox-labile residues, such as cysteines or metals.

Although flavonoids have demonstrated anti-cancer activities, conflicting reports showing that flavonoids can in fact be carcinogenic and/or mutagenic are accumulating. Numerous studies have shown that flavonoids (notably quercetin) in combination with copper can cause DNA strand scission, and this is phenomenon is consistent with scoring positive as a mutagen in the Ames mutagenicity assay [14]. However, it is debatable as to whether these findings are actually relevant, as many of the required elements of the experimental model are not present in the context of normal physiology. For instance, the concentration of free copper in a eukaryotic cell has been estimated to be less than one Cu^{n+} ion per cell, which would prevent the copper-dependent prooxidant processes from occurring [15]. Still, the potent redox activity and ubiquitous nature of dietary flavonoids necessitates a closer investigation into the actual *in vivo* role of these compounds.

Summary

Flavonoids are naturally occurring plant compounds with potent antioxidant and anticancer activities. Flavonoids are increasingly appreciated as being an important component of the human diet. Additionally, a role for natural and synthetic flavonoids in medical applications is being developed. While the antioxidant activities of flavonoids are well appreciated *in vitro*, our understanding of flavonoid biochemistry *in vivo* is less complete. Particularly confusing are the apparent paradoxical behavior of flavonoids in some settings, wherein they may behave as prooxidants or carcinogens. With a more sophisticated approach to modeling physiologically relevant systems, we may begin to understand the apparently dual nature of flavonoid biochemistry.

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