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Effects of rutin on the redox reactions of hemoglobin

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ABSTRACT

Flavonoids are widely used to attenuate oxidative damage in vitro and in vivo. In this study, we investigated the influence of rutin (quercetin-3-rhamnosylglucoside) on hemoglobin (Hb)- dependent redox reactions, i.e. oxidative stability of Hb and its cytotoxic ferryl intermediate. It was found that rutin induced generation of H_2O_2, which in turn oxidized Hb rapidly. Meanwhile, rutin exhibited anti-oxidant effect by effectively reducing ferryl intermediate back to ferric Hb at physiological pH. In comparison with quercetin, rutin had stronger capability on reducing ferryl species while lesser pro-oxidant effect on H_2O_2 generation, thus it exhibited more protective effect on H_2O_2-induced Hb oxidation. Circular dichroism spectrum showed no significant change in the secondary structure of Hb after flavonoid addition, while molecular docking revealed different binding modes of quercetin and rutin with Hb. These results might provide new insights into the potential nutritional and physiological implications of rutin and quercetin with redox active heme proteins regarding their ani- and pro-oxidant effects.

1. Introduction

Heme proteins carry out various biological functions, such as oxygen storage and transport, redox catalysis and electron transfer, etc. [1–4]. Recently, it is shown that hemoglobin (Hb) and myoglobin (Mb) play important roles in the pathologies of certain disease states, such as renal dysfunction following rhabdomyolysis and vasospasm following subarachnoid hemorrhages [3–6]. The pathophysiology is linked to the interaction of heme proteins with peroxides.

In the reaction of heme with hydrogen peroxide (H_2O_2), most noteworthy Hb species in this toxic pathway is ferryl heme (Fe^{4+}) accompanied by protein radicals. Both ferryl heme and protein radicals are highly reactive species, which could induce oxidation of protein, lipid, and nucleic acid, and even tissue damage [3–6]. The supplementation with antioxidants, reducing agents and iron chelators have been attempted to control oxidative side reactions [7–11]. Thus, the attenuation of heme proteins-dependent redox reactions by the administration of certain antioxidants is an interesting medical and biological subject.

As typical antioxidants, plant polyphenols have been widely used to attenuate oxidative damage in vitro and in vivo [12,13]. Quercetin has been reported as the most predominant flavonol and rutin as one of the most common flavonol glycosides in the human diet (Fig. 1A) [13–16]. The glycoside of quercetin is more abundant than quercetin in medicinal herbs and plant foods, and has been reported to exert numerous pharmacological activities. Particularly, rutin has attracted much attention due to its high abundance in human diet and relatively high anti-oxidant capacity. The biological, medicinal, and pharmacological application of this typical antioxidant are attributed to its anti-oxidant activities in scavenging free radicals and chelating irons [13]. Therefore, the dietary intake and therapeutic use of quercetin and rutin can provide significant health benefit [15–18]. Some flavonoids could also act as reducing agents to remove high oxidation states of heme iron, such as ferryl Hb and Mb, thus prevent the oxidation of biological molecules [10,11]. However, the efficiency of rutin in deactivation of cytotoxic ferryl species at physiological pH remains unclear.

Although the anti- and pro-oxidant properties are the well-known phenomenons for flavonoids [13,19], there is lack of data on the effects of rutin on heme proteins-dependent redox reactions. Given the nutritional and pharmacological significance of rutin, we tested this typical flavonoid for its effects on Hb-triggered redox reactions (i.e. the oxidative stability of Hb and its cytotoxic ferryl intermediate). In addition to its ability as a typical free radical

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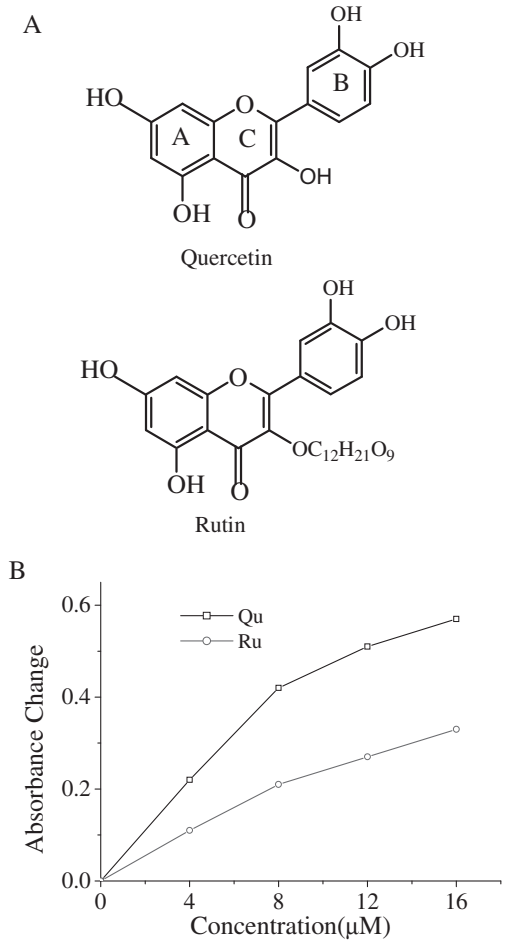

Fig. 1. (A) Schematic structures of quercetin and rutin. (B) The effects of quercetin and rutin on scavenging $ABTS^{\cdot+}$ radical.

scavenger, rutin could effectively reduce ferryl Hb back to ferric form with anti-oxidant effect. However, this polyphenol compound could exhibit pro-oxidant effect by triggering Hb oxidation through producing H_2O_2. These results in this study showed that rutin possessed anti- and pro-oxidant activities through interfering in Hb-dependent redox reactions.

2. Materials and methods

2.1. Materials

Bovine hemoglobin (Hb) and catalase were purchased from Sigma. 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) was purchased from Amresco. Quercetin (Qu) and rutin (Ru) were purchased from Shanxi Huike Botanical Development Co. Ltd., and then recrystallized in methanol. The purities of the flavonoids were above 98% according to HPLC analysis.

2.2. Anti-oxidant activity assay

Scavenging of $ABTS^{\cdot+}$ was widely used to measure the free radical scavenging activities of flavonoids [20]. Briefly, stock solution of $ABTS^{\cdot+}$ was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm. Upon addition of various concentrations of flavonoid into diluted $ABTS^{\cdot+}$-ethanol solution, the absorbance reading at 734 nm was undertaken exactly 2 min after initial mixing. The decrease in absorbance represented the anti-oxidant activity of flavonoids.

2.3. Detection of H_2O_2 in the reaction between Hb and rutin

In order to investigate the potential roles of widespread H_2O_2 in oxidation process, catalase was used to indirectly detect the formation of H_2O_2 [21]. In the presence or absence of catalase, Hb (20 μM, final concentration, the same below) was treated with rutin (20 μM) or H_2O_2 (20 μM) at 37 °C for 10 min. The obtained reaction samples were used in optical spectra determination.

2.4. Ferryl Hb reduction by rutin

Ferryl Hb was generated by addition of H_2O_2 into Hb in a 1:1 ratio at pH 7.4. After 15 min, conversion of ferric Hb to ferryl Hb was greater than 95% [7,9]. Catalase was added (10 U) to remove excess H_2O_2, and then antioxidant was added. The final antioxidant concentrations were 0, 10, 20, 50, 100 and 200 μM for the experiments. The optical changes following addition of antioxidant were monitored and the time courses for reduction of ferryl Hb were fitted to a double exponential function using Microsoft Excel [7,8].

2.5. Interaction between flavonoid and Hb by circular dichroism and molecular docking

Circular dichroism (CD) spectrometer (MOS-450/AF-CD) was used to acquire CD spectrum, and the secondary structure composition of Hb was calculated. Flavonoids were docked to the bovine Hb-ray crystal structure (PDB ID: 2QSP) using the AutoDock 4.0 software, as described previously in Ref. [22].

2.6. Statistical analysis

All data were expressed as the means ± SD of three independent experiments. Significance was assessed by using the one-way ANOVA. ($P < 0.05$ as significant).

3. Results and discussion

Heme proteins have played an important role in investigation of free radicals in biology and medicine not only because they are endowed by heme groups with the electron redox reactivity, but also because they are well characterized and ubiquitous [3,4]. Rutin is the most common and investigated member of polyphenolic flavonoids [13–16], which makes it an important contributor to the potential protection against Hb-related redox reactions in vitro and in vivo.

3.1. Pro-oxidant property of rutin in Hb oxidation

Scavenging of $ABTS^{\cdot+}$ has been widely applied to measure the free radical scavenging activities of flavonoids [20]. In the present study (Fig. 1B), rutin exhibited significant anti-oxidant effect on scavenging $ABTS^{\cdot+}$ radical. The higher the concentration of rutin, the more efficient the $ABTS^{\cdot+}$ radical-scavenging ability. Compared with rutin, quercetin had stronger activity in $ABTS^{\cdot+}$ radical-scavenging, which had been widely reported [13,18].

In this study, we used spectrophotometric method to monitor Hb redox transition in the presence of rutin. Fig. 2A showed the spectral changes of Hb upon the addition of rutin or H_2O_2. The increase in the absorbance at 420 nm and the decrease at 404 nm revealed that the addition of H_2O_2 led to the oxidation of ferric Hb to ferryl Hb [8,9,11]. The spectral changes of Hb were similar to that formed in the reaction of Hb with H_2O_2. After incubation with rutin, the spectrum of Hb shifted from its ferric state to ferryl intermediate. It implied that rutin exerted significant pro-oxidant effect on Hb oxidation, and H_2O_2 might be involved in this process. Meanwhile, quercetin, with stronger free radical scavenging capability