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Catalase is inhibited by flavonoids

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ABSTRACT

Catalases, heme enzymes, which catalyze decomposition of hydrogen peroxide to water and molecular oxygen, belong to the antioxidant defense system of the cell. In this work we have shown that catalase from bovine liver is inhibited by flavonoids. The inhibition is, at least partially, due to the formation of hydrogen bonds between catalase and flavonoids. In the presence of some flavonoids the formation of unreactive catalase compound II has been detected. The most potent catalase inhibitors among the tested flavonoids have appeared myricetin, epicatechin gallate and epigallocatechin gallate. The relationship between the degree of enzyme inhibition and molecular structure of flavonoids has been analyzed.

1. Introduction

Catalase, a four subunits heme enzyme (EC 1.16.1.6) prevents cells from the so called reactive oxygen species (ROS) by decomposing hydrogen peroxide (H_2O_2) to water and molecular oxygen [1]. This reaction proceeds in two steps:

$$\text{Catalase} + H_2O_2 \rightarrow \text{compoundI} + H_2O \quad (1)$$

$$\text{CompoundI} + H_2O_2 \rightarrow \text{catalase} + H_2O + O_2 \quad (2)$$

where compound I is an oxoferryl porphyrin π-cation radical. The rate constants of both reactions are of the order of $10^7\ M^{-1}\ s^{-1}$ [2]. Catalase, under some conditions (low H_2O_2 concentration), is able to act as peroxidase, i.e. it uses H_2O_2 (or other relatively small peroxides) to oxidize secondary two-electron and one-electron donors [1]. In the latter case catalase compound I is reduced to compound II, oxoferryl derivative without radical site. The completion of catalytic cycle requires the reduction of compound II to the ferric catalase. Unfortunately, most of one-electron donor molecules do not react with catalase compound II, i.e. do not restore native enzyme. Compound II does not take part in the catalytic cycle with hydrogen peroxide and therefore its accumulation leads to inhibition of catalase activity [1].

Flavonoids are a group of polyphenolic compounds with different chemical structure and properties. Over 9000 varieties of flavonoids have been identified so far [3]. Being widely distributed in fruit, vegetables, nuts, seeds, and beverages such as wine and tea, they are an integral part of human diet [4]. Flavonoids are classified according to their chemical structure. The major flavonoids classes include flavonols, flavones, flavanones, flavanols (catechins), anthocyanidins, and isoflavones [5]. Flavonoids are known as efficient antioxidants. Due to polyphenolic structure these compounds may scavenge free radical species and other oxidants, as well as they may chelate divalent cations [6]. Acting as antioxidants, flavonoids exhibit some health benefits such as anti-inflammatory, antiallergic, antiviral, and anticancer activity [6–8]. On the other hand, their prooxidant activity has also been reported [8–11]. In some cases prooxidant activity of flavonoids can be beneficial due to upregulation phase II detoxification enzymes, and also antioxidant enzymes [11–13].

The mechanism of some of the described biological effects of flavonoids may be related to their interactions with proteins [11,14,15]. It has been reported that flavonoids are inhibitors of some enzymes, e.g. cytochromes P450, lipoxygenases, cycloxygenases, or xanthine oxidase [16]. The influence of flavonoids on catalase activity has been reported in some papers, the results are, however, contradictory. Some authors have found an increase of catalase expression and/or activity in cells in the presence of flavonoids [17–21]. This effect probably results from flavonoid-induced activation of redox-sensitive Nrf2 signaling pathway, which regulates expression of phase II detoxifying and antioxidant enzymes [21]. Others have observed no effect or even a decrease of catalase expression and activity in cells after flavonoid treatment/digestion [22,23]. Only few works concerning flavonoid–catalase interaction in model systems have been published so far [24,25]. In the work of Doronicheva et al. [24] catalase activity in the presence of flavonoids has been measured using chemiluminescence method in which hypochlorous

Abbreviations: BLC, bovine liver catalase; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate.

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acid has been used to oxidize luminol. However, we have recently show that flavonoids efficiently react with HOCl [26] and thus may disturb catalase activity determination by luminol/H_2O_2-chemiluminescence method.

In this paper we studied the influence of flavonoids and appropriate model polyphenols on catalase activity in homogenous aqueous solution determined spectrophotometrically by following H_2O_2 decomposition. Twelve flavonoids from flavonols, flavones, and flavanols (catechins) subclasses, as well as three model compounds were studied (Table 1). We found that all investigated compounds inhibited catalase in buffer solution at neutral pH. The relationship between the degree of catalase inhibition and molecular structure of flavonoids and related compounds was analyzed.

2. Materials and methods

2.1. Materials

Catalase from bovine liver (BLC), hydrogen peroxide (H_2O_2), (+)-catechin, (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin gallate (EGCG), kaempferol, astragalin, quercetin, rutin, galangin, apigenin, gallic acid, pyrogallol, and catechol were obtained from Sigma, whereas luteolin and myricetin from Alexis Biochemicals and used as received. All other chemicals were of analytical grade. A stock solution of BLC ($\sim 10^{-7}$ M) was freshly prepared in 10 mM sodium phosphate buffer (pH 7.0). Enzyme concentration was determined spectrophotometrically using ε_{405}, of $3.24 \times 10^5\ M^{-1}\ s^{-1}$ for catalase tetramer [27]. H_2O_2 solution for activity measurements was prepared daily in 10 mM sodium phosphate buffer (pH 7.0) from a 30% stock solution. Its concentration was determined spectrophotometrically, taking ε_{240} as $40\ M^{-1}\ cm^{-1}$ [28]. Stock solutions ($\sim 10^{-3}$ M) of catechins, gallic acid, pyrogallol, and catechol were prepared in nano-pure water from a MilliQ system (Millipore), whereas those of flavones and flavonols in dimethylsulfoxide (DMSO). Stock solutions of each polyphenol were prepared daily prior to use.

2.2. Catalase activity assay

Catalase activity was measured spectrophotometrically by detecting H_2O_2 decomposition at 240 nm [29]. The reaction was initiated by an injection of 20 μl of stock solution of catalase to 2 ml 10 mM H_2O_2 without or with flavonoids and other polyphenols. A blank cell contained the reaction mixture excluding H_2O_2. The kinetic traces of absorbance (A) decrease were fitted with an exponential equation $A_t = A_o \exp(-kt)$, and the first order rate constants of H_2O_2 decomposition (k), dependent on the kind and concentration of flavonoids and model polyphenols, were obtained. These k values, normalized for the same enzyme concentration, were the measure of catalase activity. In other experiments ~1 μM catalase was incubated with some flavonoids at 10 mM phosphate buffer at pH 7.0. At appropriate time intervals 40 μl aliquots were taken from the incubated mixture. 20 μl of them were injected to 2 ml 10 mM H_2O_2 and enzyme activity, defined as above, was assayed. Phosphate buffer (2 ml) with the remaining 20 μl of the incubated mixture was used as a blank. All experiments were performed at ambient temperature (23 ± 1 °C).

2.3. Absorption spectra

Absorption spectra of catalase in the presence of flavonoids at different times of incubation were also taken. An appropriate buffered solution of flavonoid was used as a blank. Hewlett Packard 8452A and Specord S600 (Carl Zeiss Jena) diode-array spectrophotometers with a quartz cell of 1 cm path length were applied.

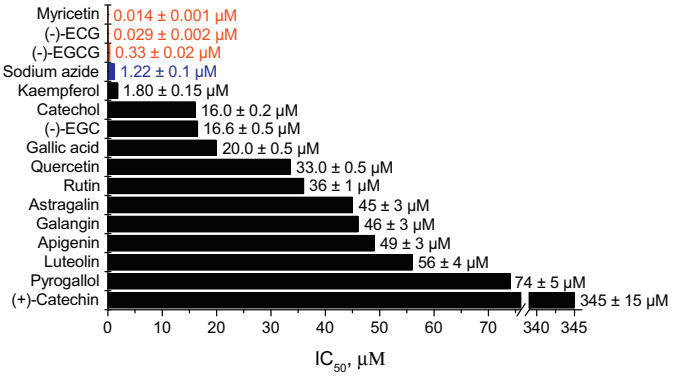

Fig. 1. The inhibitory potency of flavonoids and model polyphenolic compounds against catalase (1 nM) as characterized by IC_{50}. IC_{50} value determined for azide was also included for comparison. Catalase activity was measured immediately after injection of the enzyme into 10 mM H_2O_2 at 10 mM phosphate buffer, pH 7.0, without or with polyphenols.

2.4. Data analysis and statistics

The linear regression coefficient was calculated using OriginPro 8.6 software. Each individual assay was performed in triplicate. Results are expressed as mean values with the corresponding SD values.

3. Results

3.1. Catalase activity in the presence of flavonoids and model polyphenols

We investigated the influence of different flavonoids, as well as their structural components, catechol, pyrogallol, and gallic acid (Table 1), on the activity of catalase and found that all polyphenolic compounds used in these studies inhibited BLC. Catalase activity decreased remarkably with increasing concentration of flavonoids and related compounds. We found that flavonoids and model polyphenols, at the concentrations used in these studies, did not induce H_2O_2 decomposition. The values of IC_{50} defined here as the concentration of flavonoid or model polyphenol that caused 50% inhibition of 1 nM catalase were estimated from the inhibition curve (% activity = f[polyphenolic compounds]). The inhibition of catalase by its well known inhibitor, sodium azide, was also studied for comparison. The results are presented in Fig. 1. Among the investigated flavonoids (+)-catechin is the poorest inhibitor ($IC_{50} = 345 \pm 15$ μM) and the myricetin is the strongest one ($IC_{50} = 0.014 \pm 0.001$ μM). The value of IC_{50} determined by us for azide (1.22 ± 0.01 μM) is comparable with that reported by others [30]. We also investigated the influence of the incubation time of catalase (up to 30 min) with five selected flavonoids (EGCG, luteolin, myricetin, quercetin and rutin) on enzyme activity. In all cases 50-fold excess of flavonoid versus catalase was applied. The first activity assay was taken after 1 min incubation. The activity of catalase after 30 min incubation in the presence of luteolin, rutin, quercetin and EGCG lowered by ~10, 20, 50 and 75%, respectively (Fig. 2). Catalase exhibited ca. 30% of its initial activity after 1 min incubation with myricetin. This activity decreased down to about 15% of the initial value within next several minutes of incubation and then remained constant during the rest our observation time (Fig. 2). It should be noted that the activity of catalase after 30 min incubation at room temperature in the absence of flavonoid remained unchanged, within experimental error.