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Inhibitory effects of the dietary flavonoid quercetin on the enzyme activity of zinc(II)-dependent yeast alcohol dehydrogenase: Spectroscopic and molecular docking studies

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ABSTRACT

A multispectroscopic exploration was employed to investigate the interaction between the metallo-enzyme alcohol dehydrogenase (ADH) from yeast with bioflavonoid quercetin (QTN). Here, we have characterized the complex formation between QTN and Zn^{2+} in aqueous solution and then examined the effect of such complex formation on the enzymatic activity of a zinc(II)-dependent enzyme alcohol dehydrogenase from yeast. We have observed an inhibition of enzymatic activity of ADH in presence of QTN. Enzyme inhibition kinetic experiments revealed QTN as a non-competitive inhibitor of yeast ADH. Perturbation of Circular dichroic (CD) spectrum of ADH in presence of QTN is observed due to the structural changes of ADH on complexation with the above flavonoid. Our results indicate a conformational change of ADH due to removal of Zn^{2+} present in the enzyme by QTN. This was further established by molecular modeling study which shows that the flavonoid binds to the Zn^{2+} ion which maintains the tertiary structure of the metallo-enzyme. So, QTN abstracts only half of the Zn^{2+} ions present in the enzyme *i.e.* one Zn^{2+} ion per monomer. From the present study, the structural alteration and loss of enzymatic activity of ADH are attributed to the complex formation between QTN and Zn^{2+}.

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1. Introduction

Naturally occurring small molecules have always been in the focus of study for their diverse biological activities. Among these, flavonoids have received considerable attention because of their wide range of pharmacological effects. Flavonoids are naturally occurring polyphenolic secondary metabolites that are ubiquitous in higher plants. They are low molecular weight organic compounds and categorized according to chemical structure into flavonols, flavones, isoflavones, flavanones, anthocyanidins, catechins and chalcones. The basic structure is usually characterized by two aromatic rings, ring A and ring B, which are joined by a three-carbon atoms that linked γ-pyrone ring (ring C), forming a C6–C3–C6 skeleton where polar groups, usually hydroxyl or methoxy appeared at various positions. The efficiency of flavonoids as antioxidant compounds greatly depends on their chemical structures. Three structural features being the most important as observed by Jurasekova et al. [1] and other groups [2–4]: (i) the catechol moiety in the B ring, which is a radical target site; (ii) the C2 = C3 bond in conjugation with a 4-keto function in the γ-pyrone ring, which is responsible for electron delocalization from the B ring; and (iii) the presence of both 3- and 5-hydroxyl groups for radical scavenging. Further, the catechol moiety, as well as 4-keto and additional 3- or 5-hydroxyl groups plays important role in chelating metal ions [5,6]. They possess anticancer, antiviral, antimicrobial, antiinflammatory and antiallergic potential [7–10].

Quercetin ($C_{15}H_{10}O_7$) (3,5,7,3′,4′-pentahydroxy-flavone, herein after QTN, Fig. 1), is one of the most abundant bioflavonoids and more specifically flavonol, widely distributed in edible fruits and vegetables. QTN possesses all the three structural features described earlier. It acts as an antioxidant and shows antitumor activity [11–13]. More specifically antioxidant activity of QTN is a proof of ability to act as free radical scavenger [14]. QTN can chelate with different metal ions to form metal complexes that enhance the antioxidant and antitumor activity of QTN [15,16]. Because of its strong antioxidant and metal ion chelating ability, QTN has been reported to be effective in inflammation, arteriosclerosis, bleeding, allergy and swellings [17–19]. QTN has the ability to interact with and modulate activity of a number of enzymes, namely cyclooxygenase, lipooxygenase, phosphodiesterase, alcohol dehy-

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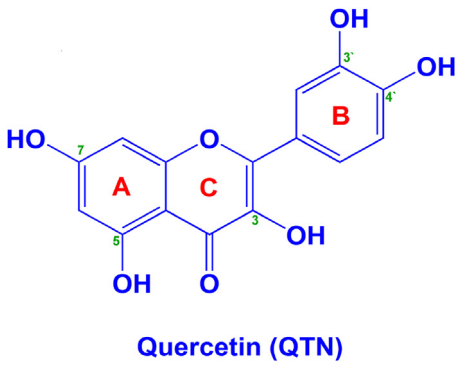

Fig. 1. Chemical structure of QTN.

drogenase, tyrosine kinase *etc* [20]. The mechanisms responsible for the cancer-preventive effects of QTN are attributed to their antioxidative activity, metal chelating ability, inhibition of enzymes that activate carcinogens and interactions with receptors and/or proteins [18]. The poor solubility nature of QTN provides restriction for the absorption by the body resulting a reduced bioavailability *in vivo*.

Metal binding property of QTN is well established [21–23]. Bivalent zinc ion [Zn^{2+}] has a vital role in the immune system affecting the cellular activity and humoral immunity [24]. Zn^{2+}, a biologically important bivalent cation, plays the central character in catalytic activity of different metallo-enzymes [25]. Complex formation between different metal ions with QTN has been studied [26]. Zinc is an essential trace element in human body. The total body zinc content has been found to be 30 mmol (2 g) [27]. Zn^{2+} has completely filled d-orbital which causes zero ligand field stabilization energy and consequently it generates flexibility in the metal coordination geometry which governs a crucial role in the ability of Zn^{2+} to catalyze a number of chemical changes.

Alcohol dehydrogenases (ADHs, EC 1.1.1.1) are Zn^{2+} containing enzymes that catalyze the oxidation of alcohols to aldehydes or ketones which is the first step in the ethanol metabolism by the liver:

$$R\text{-}CH_2\text{-}OH + NAD^+ \overset{ADH}{\rightarrow} R\text{-}CHO + NADH + H^+$$

where NAD^+/NADH are coenzymes (NAD^+, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide). Enzymes are made up from definite amino acid sequence and in case of any medium-chain length ADH ~327–376 amino acid residues are present in one chain [28]. The ADHs are generally dimeric in nature in higher eukaryotes (plants, animals *etc.*) whereas in case of prokaryotes and lower eukaryotes (yeast) they are tetrameric in nature [29]. Yeast alcohol dehydrogenase is a tetrameric enzyme with a molecular mass of ~150 kDa and it can be subdivided into four homologous subunits with 347 amino acid residues in each subunit [29]. Each subunit contains two Zn^{2+} ions among which one Zn^{2+} ion is found in the catalytic site of the enzyme and is bound to three ligands (Cys^{46}, His^{67} and Cys^{174}) [30]. The second Zn^{2+} ion is bound to four cysteines (Cys^{97}, Cys^{100}, Cys^{103} and Cys^{111}) and maintains the tertiary structure of the enzyme [30]. Thus the activity of the enzyme is directly dependent on the Zn^{2+} ion. A number of compounds reported that inhibit the enzymatic activity of yeast ADH such as dithizone, 1,10-phenanthroline, 8-hydroxyquinoline, 8-hydroxyquinoline-5-sulfonic acid, a, a'-dipyridyl, thiourea, furfural *etc* [31,32]. A dual function of ADH is reported in case of Drosophila where it catalyzes the oxidation of ethanol to acetaldehyde as well as converts this highly toxic product into acetate [33]. Considering structural features, yeast ADH is homologous with horse liver ADH [34] but when it comes to function, it shows restriction on substrate specificity in comparison to mammalian liver enzymes [35]. Moreover, increase in number of alkyl group in primary alcohol increases the chain length which in turn results a decrease in the enzymatic activity of yeast ADH [36,37]. Plapp et al. have recently reported the mechanistic implications from structures of yeast ADH complexed with coenzyme and an alcohol [38].

Considering the excellent metal chelating ability of QTN, its enhanced biological activity on metal binding and the importance of Zn^{2+} ions on activity of different enzymes, at first we have focused on the characterization of complex formation between QTN and Zn^{2+} ion in aqueous medium. In this purpose we have used different spectroscopic techniques, namely, UV–vis absorption spectrophotometry, spectrofluorimetry *etc*. We found that there was formation of complex between the said ion and QTN with high association constant [$K_a = (3.57 \pm 0.10) \times 10^4\ M^{-1}$]. Immediately after this we had the curiosity whether such association of Zn^{2+} with QTN has any consequence in the biological system. Keeping in view of this fact we extended our study on the effect of such complex formation on the activity of an enzyme containing Zn^{2+} ion. In this respect we choose a typical zinc(II) dependent enzyme yeast alcohol dehydrogenase (ADH) as the model enzyme where Zn^{2+} ion plays vital role in the structural integrity and enzyme activity.

ADH is a major enzyme for detoxification of alcohol and its inhibition may cause side effects. It is also real fact that inhibition of ADH may prove to be advantageous to study for alcohol metabolism as well as to find a way to reduce the risk of developing alcohol-related health disorders [39]. Ethanol is converted to acetaldehyde by NAD dependent ADH. Accumulation of acetaldehyde in the body is considered to be the main reason of hangovers, which are characterized by the nasty symptoms such as headache, vomiting, thirst, dizziness and other decreased sensory abilities, after the substantial consumption of alcohol [40]. The intermediate acetaldehyde is toxic, mutagenic and carcinogenic. Conversion of ethanol to acetaldehyde is thought to be an appropriate and suitable means of preventing alcoholic disorders. Keeping in mind of this negative effects of the produced aldehyde from alcohol catalyzed by ADH, our aim was to examine whether the biologically active QTN could inhibit this catalytic conversion of ethanol to aldehyde through formation of complex with the Zn^{2+} present in the enzyme. In the present study, we have demonstrated that QTN showed strong yeast ADH-inhibitory activities. From kinetic analysis we have reported the type of inhibition. Finally, we have performed molecular modeling study to locate the probable binding position of QTN into the enzyme ADH.

2. Materials and methods

2.1. Materials

QTN, acrylamide and Tris were purchased from Sigma Aldrich Corporation (St. Louis, MO, USA). Zinc nitrate and ethanol were from Merck (Germany). Alcohol dehydrogenase (lyophilized) from ex-baker's yeast and nicotinamide adenine dinucleotide (free acid) were from SRL (India) and Sigma (USA) respectively. All buffer solutions were prepared in quartz-distilled deionized water from a Milli-Q source (Millipore, USA) and were filtered through 0.45 μm millipore filters to avoid particulate matter.

2.2. Methods

2.2.1. UV–vis absorption experiments

All the UV–vis spectrophotometric studies were made on a Shimadzu model UV-1800 spectrophotometer (Shimadzu Corporation, Japan) in matched quartz cells of 1 cm path length. A thermoprogrammer was attached to it to maintain the tempera-

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