In Vitro Studies on Calf Thymus DNA interaction with Quercetin-Palladium (II) Complex

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Abstract—Flavonoids are antioxidants, which are known to influence bioavailability of the metal in the body. Small organic molecules binding to nucleic acids are of great interest in modern medicine because they constitute a significant portion of the anticancer drugs. Binding studies of flavonoids with DNA are useful for the understanding of the reaction mechanism and providing guidance for the application and design of new and more efficient drugs targeted to DNA. Quercetin one of the most abundant dietary flavonoids, has been investigated in the presence of Pd (II) in 50% Ethanol. Structure of the complex was determined through UV–Vis studies. Binding interaction of this complex with calf thymus (CT) DNA has been investigated by Absorption spectroscopy. The binding constants ($K_b$) for complex with DNA were estimated to be $4.38 \times 10^3$, through spectroscopic titrations. The complex displays significant binding properties to the CT-DNA. The experimental results suggest that Quercetin–Pd(II) could bind to DNA via outside binding mode.

Keywords-CT-DNA; Quercetin-Pd(II) complex; Intrinsic binding constant; Absorption Spectroscopy.

I. INTRODUCTION

DNA plays a key role in the synthesis of proteins (gene expression) as well as its own replication making it a potential target for drugs, especially for antiviral, antibiotic and anticancer action. Thus, favorable DNA interaction patterns based on the study of small molecules that bind to nucleic acids is one of the most important parameters in the screening design for new drugs and development processes [1]. There are several types of sites in the DNA molecule where binding of metal complexes can occur: (i) between two base pairs (intercalation), (ii) in the minor and major groove, (iii) on the outside of the helix[2]. Recently, natural substances such as flavonoids, that show biological activity in many mammalian cell systems in vitro and in vivo, have been investigated as to their interaction with DNA. The flavonoid compounds occupy a prominent position among the plant polyphenols (Fig. 1).

A great number of flavonoids are able to chelate metal ions; and often the presence of a 3-hydroxyl group or a 5-hydroxyl group allows the complex formation involving the carbonyl function [3-5]. While these beneficial effects mainly come from their abilities to scavenge free radicals, complexation properties with metal ions have also been recognized to contribute to the total biological activity. Quercetin (3, 3’, 4’, 5, 7-pentahydroxyflavone) is a strong hydroxyl-antioxidant and a major dietary flavonoid most common present in nature(Fig. 2). Quercetin can chelate metal ions to form metal complexes that have better antioxidation and antitumor activity than quercetin alone [6,7]. Identification or structural information of flavonoids can be obtained by comparison of their UV–Vis spectra in 50% Ethanol and in presence of metal, the observed spectral shifts are often characteristic of their substitution [8,9]. PdCl$_2$ is widely used as a color forming reagent in spectrophotometric determinations of many drugs [10-11].

Given that some metal ions, especially transition metals, not only play vital roles in a vast number of widely differing biological processes, but also may be potentially toxic in their free state, the studies of interactions between quercetin-metal complexes with DNA are important to further the understanding of pharmacology of quercetin, we report here the preparation and DNA binding of the Pd (II) with quercetin.

II. MATERIALS AND METHODS

A. Reagents and Chemicals

The highly polymerized calf thymus-DNA (CT-DNA) and Tris-HCl were purchased from Sigma Co. Quercetin and Palladium Chloride solutions have been prepared in a mixture of water and ethanol in a 1:1 ratio, the solubility of
both components (PdCl$_2$ and quercetin) having a maximum at this composition. The palladium (II) chloride solution ($1.0 \times 10^{-2}$ M) was prepared by dissolving PdCl$_2$ in water (to which 0.4 ml of concentrated hydrochloride acid had been added) by warming the mixture on a water bath. Tris-HCl buffer solution was prepared from (tris-(hydroxymethyl)-amino-methane-hydrogen chloride) and pH was adjusted to 7.4. The stock solution of DNA was prepared by dissolving of DNA in 10 mM of the tris-HCl buffer at pH 7.4 and dialyzing exhaustively against the same buffer for 24 h and used within 5 days. A solution of CT-DNA gave a ratio of UV absorbance at 260 and 280 nm more than 1.8, indicating that DNA was sufficiently free from protein [12]. The DNA concentration (monomer units) of the stock solution ($1 \times 10^{-2}$ M per nucleotide) was determined by UV spectrophotometry in properly diluted samples using a molar absorption coefficient of 6600 M$^{-1}$ cm$^{-1}$ at 260 nm [13].

B. Preparation of the complex

The solution of quercetin was prepared by dissolving completely its powder in 50% Ethanol until the color of the solution was yellow. PdCl$_2$ solution (50% Ethanol) was added quickly the color of the solution changes to brownish stirred the solution. After stirring the reaction mixture was filtered, the filtrate was evaporating slowly at room temp. Then collect the brownish product and washed with ether to remove the uncreative part of the reagent, dried the product in vacuum desiccator, yielded the brownish product.

C. Physical measurements

The UV-Vis spectra for DNA with Quercetin- Pd (II) complex interactions were obtained using a Unico (UV 2100) spectrophotometer. Absorption titration experiments were carried out by keeping the concentration of DNA constant ($5 \times 10^{-5}$ M) while varying the complex concentration from $5 \times 10^{-5}$ M to $4 \times 10^{-4}$ M ($\text{ri} = $ [complex]/[DNA] = 0.1–0.5).

Absorbance values were recorded after each successive addition of DNA solution and equilibration (ca. 10 min). The data were then fitted to Eq. (1) to obtain intrinsic binding constant, $K_b$[14]:

$$
\frac{[\text{DNA}]}{[\text{DNA}]} = \frac{1}{\varepsilon_b - \varepsilon_f} + \frac{1}{\varepsilon_f}
$$

Where [DNA] is the concentration of DNA in base pairs, $\varepsilon_a$ corresponds to the extinction coefficient observed $\lambda_{abs}$/Pd(II), $\varepsilon_f$ corresponds to the extinction coefficient of the free compound, $\varepsilon_b$ is the extinction coefficient of the compound when fully bound to DNA, and $K_b$ is the intrinsic binding constant. In particular, $\varepsilon_f$ was determined by a calibration curve of the isolated Quercetin-Pd (II) complex in aqueous solution, following the Beer’s law $\varepsilon_f$ was determined as the ratio between the measured absorbance and the Quercetin-Pd (II) complex concentration, $\lambda_{abs}$/Pd(II)A plot of [DNA]/($\varepsilon_b - \varepsilon_f$) vs. [DNA] gave a slope of $1/(\varepsilon_b - \varepsilon_f)$ and a Y intercept equal to $1/K_b$ ($\varepsilon_b - \varepsilon_f$); $K_b$ is the ratio of the slope to the Y intercept.

III. RESULTS AND DISCUSSION

A. Interaction between Quercetin and Pd (II) (UV-Vis studies)

The UV-Vis absorbance spectra of $5 \times 10^{-6}$ M quercetin and Quercetin- Pd (II) complex (with increasing concentration) in 50% ethanol solution are shown in Fig. 3 with the main data listed in Table I. quercetin exhibits two major absorption bands in the ultraviolet/visible region. The
absorptions in the 320–385 (nm) range correspond to the B ring portion (cinnamoyl system, band I), and the absorptions in the 240–280 (nm) range correspond to the A ring portion (benzoyl system, band II) (Fig. 2). The spectra are related to the $\pi \rightarrow \pi^*$ transitions within the aromatic ring of the ligand molecules [15]. In the presence of metal ions, a bathochromic shift is typically observed in the absorption spectra of flavonoids. The UV-visible spectrum of quercetin in ethanol shows two major absorption bands at 366 nm (band I) and 259 nm (band II). It is observed that complex formation causes a bathochromic shift in the absorption bands.

### TABLE I. UV–VIS ABSORPTION SPECTRUM DATA (NM) OF QUERCETIN AND QUERCETIN–Pd(II) COMPLEX

<table>
<thead>
<tr>
<th>Compound</th>
<th>Band(II), nm</th>
<th>Band(I), nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>259</td>
<td>366</td>
</tr>
<tr>
<td>Complex</td>
<td>264</td>
<td>446</td>
</tr>
</tbody>
</table>

Figure 3. UV-vis spectra of the free Quercetin and Quercetin-Pd(II) complex in 50% Ethanol

This red shift is caused by the increased conjugative effect when the complex is formed to give a new ring. When Pd(II) was added to the solution, in comparison with quercetin absorption spectrum, that of the complex is shifted to the long-wavelength region as shown in Fig. 3. Band I red shifts by ca. 80 nm and a new stronger intense absorbance peak appear at 446 nm (band III). Band II shows a stronger intense absorbance at higher wavelength (264 nm) (Band IV). The isobastic point was observed at 328 nm, characteristic of the formation of a complex [16]. Also it was shown that, metal-flavonoid complex produces significant bathochromic shift only in one absorbance band, i.e. band I [17].

#### B. Electronic absorption and DNA binding

Electronic absorption spectroscopy is universally employed to determine the binding characteristics of metal complex with DNA. The $r_i$ values were calculated from the following equation: $r_i = [\text{complex}]/[\text{DNA}]$. The UV band of DNA at about 260 nm was monitored at the absence and presence of different amounts of Quercetin-Pd (II) complex. “Hyperchromic” and “hypochromic” effects are the spectra features of DNA concerning its double helical structure [18]. The spectral change process reflects the corresponding changes in DNA in its conformation and structure after complex binding to DNA. Hyperchromism derives from damage of the DNA double-helix structure; in contrast, hypochromism results from the contraction of DNA in the helix axis, as well as from the conformational change of DNA [19]. The absorption spectral traces of the CT DNA with increasing concentration of the Quercetin-Pd (II) complex are shown in Fig. 4. This is a typical “hypochromic” effect, which suggests that Quercetin-Pd (II) complex interact with CT-DNA through non-intercalative binding mode such as outside binding. Additionally, based on the absorbance values obtained in the spectroscopic titration, the Quercetin-Pd (II) complex-DNA binding constant, $K_b$, was calculated as described in the experimental procedure. The value obtained for $K_b$ was $4.38 \times 10^3$ M$^{-1}$, $K_b$ value is lower than those observed for typical classical intercalators, e.g. $K_b$ for ethidium bromide–DNA complex is equal to $7 \times 10^7$ M$^{-1}$ [20]. Therefore we propose that complex binds to CT-DNA through outside binding such as electrostatic interaction.

#### IV. CONCLUSIONS

This study examined the interaction of metal ion with quercetin in 50% Ethanol solution and DNA binding study of quercetin-Pd (II) complex with CT-DNA by absorption spectroscopy. The binding of complex to DNA result in hypochromism effect in UV absorption spectra beside this, the low value of $K_b$ [$4.38 \times 10^3$ M$^{-1}$], we conclude that non-intercalative modes of binding, such as outside binding, which could lead to conformational change in CT-DNA, and, therefore putative change in the gene expression/regulation activity.
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REFERENCES


