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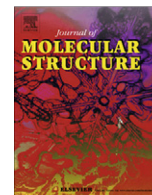

# Synthesis of dihydromyricetin–manganese (II) complex and interaction with DNA

Qingquan Guo *, Juan Yuan, Jinhua Zeng, Xiangzhu He, Daguang Li

*School of Chemical Engineering and Light Industry, Guangdong University of Technology, Guangzhou 510006, China*

## HIGHLIGHTS

- ▶ We firstly study the structure of dihydromyricetin–manganese (II) complex.
- ▶ We firstly study the interaction of complexes with DNA.
- ▶ The DMY-Mn(II) complex can interact with DNA by intercalation.
- ▶ The intercalation of complex with DNA is strong with Ksq = 1.16, Kb = 5.64 × 104 M.

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## ABSTRACT

Dihydromyricetin has many physiological functions and its metal complex could have better effects. DNA is very important in biological body, but little attention has been devoted to the relationship between dihydromyricetin–metal complex and the DNA. In this paper, dihydromyricetin–Mn (II) complex has been prepared and characterized using UV–vis absorption spectrophotometry, IR spectroscopy, elemental analysis, and thermal gravimetric analysis (TG-DTA Analysis). The interaction of dihydromyricetin–Mn (II) complex with DNA was investigated using UV–vis spectra, fluorescence measurements and viscosity measurements. The results indicate that the dihydromyricetin–manganese (II) complex can intercalate into the stacked base pairs of DNA with binding constant $K_b = 5.64 \times 10^4$ M and compete with the strong intercalator ethidium bromide for the intercalative binding sites with Stern–Volmer quenching constant, $K_{sq} = 1.16$.

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## 1. Introduction

DNA is generally the primary intracellular target of anticancer drugs, so the interaction between small molecules and DNA can cause DNA damage in cancer cells, inhibiting the proliferation of cancer cells by causing DNA damage and blocking their division [1–4]. In recent years, many researchers have been focused on the interaction of small molecules with DNA [5–9].

Small molecules can interact with DNA through the following three non-covalent modes: intercalation, groove binding and external static electronic effects. Among these interactions, intercalation is one of the most important DNA binding modes, which is related to the antitumor activity of the compound. Recently, there is a great interest on the binding of transition metal complexes with DNA, owing to their possible applications as new cancer therapeutic agents and their photochemical properties that make them potential probes of DNA structure and conformation [6,7,9]. Moreover, transition metal chelation is an excellent ways to increase the lipophilic character of the organic moiety, on coordination, ligands might improve their bioactivity profiles, while some inactive ligands may acquire pharmacological properties, consequently transition metal complexes have become an important class of structure-selective binding agents for nucleic acids. So the development of synthetic, sequence-selective DNA binding and cleavage agents for DNA itself is essential for further expected applications in molecular biology, medicine, and related fields [10–12].

Dihydromyricetin (3,5,7,3′,4′,5′-six hydroxy-2,3-dihydro flavonol, DMY), is an important flavone, which has multiple biological effects such as hypoglycemic, antithrombotic, antioxidant, immunostimulating, anti-inflammatory, and antibacterial activities [13–15]. Dihydromyricetin can chelate metal ions to form metal complexes that have better antioxidation and antitumor activity than dihydromyricetin alone [16,17]. Manganese ions, an indispensable trace elements in animal health growth, mostly exist in the various metal protein and metal enzymes in divalent forms, closely related with the biochemical metabolism in animals and nutritional status of animals [18–20]. However, unfortunately, very little research on the preparation of the dihydromyricetin–manganese (II) complex and its interaction with DNA are reported up to now.

* Corresponding author. Address: School of Chemical Engineering and Light Industry, Guangdong University of Technology, Guangzhou Higher Education Mega Center, Guangzhou 510006, China. Tel.: +86 20 3932232; fax: +86 20 3932231.

*E-mail address:* guoqingquan@gdut.edu.cn (Q. Guo).

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In the present work, we investigated the preparation and the mode of DNA binding of dihydromyricetin–Mn (II) complex. We demonstrate that the dihydromyricetin–Mn (II) complex could be bound to DNA via an intercalation mode.

## 2. Experimental

### 2.1. Materials

All chemicals and reagents were purchased from commercial sources and were used without further purification. All solutions were prepared using double-distilled water. Calf thymus DNA(CT DNA), ethidium bromide (EB) were obtained from Sigma (Sigma Chemical Co., St. Louis, MO, USA). The stock solution of DNA was prepared by dissolving DNA in Tris buffer (0.01 M Tris–HCl/50 mM NaCl, pH 7.2) and used within 5 days. The stock solution was stored at 4 °C. A solution of CT-DNA gave a ratio of UV absorbance at 260 and 280 nm of more than 1.8, indicating that the DNA was sufficiently free from protein. The concentration of CT DNA was determined using an extinction coefficient of 6600 $M^{-1}$ $cm^{-1}$ at 260 nm. Manganese acetate tetrahydrate was from Guangfu Fine Chemicals, Tianjin (China). The Tris–HCl buffer solution(pH 7.2) was prepared with triple distilled water. Dihydromyricetin (Purity > 98.0%) was gotten from our previous study.

### 2.2. Preparation of dihydromyricetin–manganese (II) complex

The solid DMY (10 mmol) was dissolved into 60 mL of ethanol(95%, *w/w*). Then the pH of the solution was adjusted to 7–8 with sodium carbonate. After 5 min, $(CH_3COO)_2$ $Mn{\cdot}4H_2O$ (10 mmol) was added to the above mixture. After being stirred and heated to reflux for 8 h at 70 °C, the reaction mixture was cooled to room temperature. The brown–black mixture was filtered and repeatedly washed with EtOH and water. The solid product was dried under vacuum for 48 h at room temperature. Yield: 68.0%.

### 2.3. DNA-binding measurements

The aim of this study was to investigate the interaction of native calf thymus DNA (CT-DNA) with DMY–Mn (II) complex in Tris buffer (0.01 M Tris–HCl/50 mM NaCl, pH 7.2) and in vitro conditions by using spectroscopic (UV–vis, fluorescence) and viscosimetric measurements. Concentrated stock solutions of DMY–Mn (II) complexes were prepared by dissolving them in a small amount of DMSO and diluted with Tris buffer solution to get the required concentrations.

UV–vis spectra were measured on a UV2450 UV–visible spectrophotometer (Shimadzu, Japan) in 0.01 M Tris buffer. Absorption titration experiments were performed by maintaining the concentration of the DMY–Mn (II) complex in sample cuvettes as constant ($4.0 \times 10^{-5}$ M) but varing CT-DNA concentrations, which the solutions ($r_i = [C_{DNA}/C_{DMY\text{–}Mn\ (II)\ complex}]$ = 0.0, 0.2, 0.4, 0.6, 0.8, 1.0) were mixed by repeated inversion. After the solutions had been mixed for 15 min, the absorption spectras were recorded. While measuring the absorption spectra, equal amounts of DNA were added to both complex and reference solutions with micro-injector to eliminate the absorbance of DNA itself.

Fluorescence measurements were made using FluoroMax-4 VPF-100 fluorescence spectrophotometer(HORIBA Jobin Yvon) with a slit width 15 nm for the excitation and emission beams. Fluorescence titrations were carried out by adding increasing amounts of buffered CT DNA directly into the sample cuvettes containing the solution of DMY–Mn (II) complex ($C = 2.0 \times 10^{-4}$ M, 0.01 M Tris buffer, pH 7.2), the concentration range of the DNA was 0–$4.0 \times 10^{-4}$ M bp. Emission spectra were recorded at excitation wavelength of 374 nm. Fluorescence quenching study was conducted by adding increasing amounts of DMY–Mn (II) complex directly into the EB-DNA system ($C_{EB} = 5.0 \times 10^{-5}$ M, $C_{DNA} = 2.0 \times 10^{-4}$ M bp, $r_i = [C_{DMY\text{–}Mn\ (II)\ complex}/C_{DNA}]$ = 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.01 M Tris–HCl buffer, pH 7.2). Emission spectra were recorded at excitation wavelength of 338 nm. All measurements were performed at 30 °C.

For viscosity measurements, a viscosimeter (NDJ-5S(SNB-2)) was used. The increasing amounts of DMY–Mn (II) complex were added directly into the buffered DNA system($C_{DNA} = 2.0 \times 10^{-4}$ M bp, $r_i = [C_{DMY\text{–}Mn\ (II)\ complex}/C_{DNA}$ = 0.0, 0.1, 0.2, 0.3, 0.4, 0.5], 0.01 M Tris–HCl buffer, pH 7.2), then kept for 24 h at 30.0 ± 0.1 °C by a constant temperature bath. Data are presented as $(\eta/\eta_0)^{1/3}$ versus binding ratio, where $\eta$ and $\eta_0$ are respectively the viscosity of DNA in the presence and absence of the complex.

## 3. Results and discussion

### 3.1. Identification of the structure of dihydromyricetin–manganese (II) complex

#### 3.1.1. UV–vis spectra studies

DMY exhibits two major absorption bands in the UV–vis region. Absorptions in the 280–310 nm range correspond to the B-ring portion (cinnamoyl system, band I), and absorptions in the 200–220 nm range correspond to the A-ring portion(benzoyl system, band II) (Fig. 1). The spectra are related to the $\pi$–$\pi^*$ transitions within the aromatic ring of the ligand molecules [20]. In the presence of metal ions, a bathochromic shift is typically observed in the absorption spectra of flavonoids. Fig. 2 showed when a complex between DMY and Mn (II) is formatted, bands I and II gradually shifted to longer wavelengths, accompanied with obvious decrease in absorption. The results indicated the formation of a complex between DMY and Mn (II).

#### 3.1.2. Infrared spectroscopy studies

Dihydromyricetin–manganese (II) complexes were analyzed by IR spectroscopy which were recorded as KBr pellets on a Vertex 70 infrared spectrometer (Bruker Optics) in the frequency range 400–4000 $cm^{-1}$. Table 1 showed that the dihydromyricetin–manganese (II) complexes have still the absorption peaks in the 1450–1600 $cm^{-1}$ with almost no change, indicating the benzene

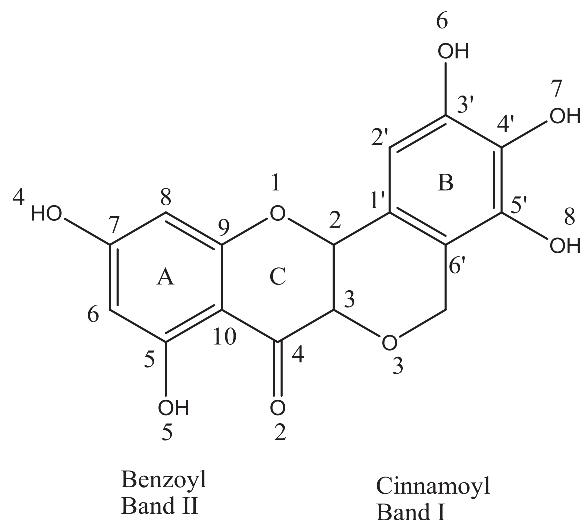


**Fig. 1.** Dihydromyricetin structure and the division of bands I and II related to UV–vis absorption bands.

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