ELSEVIER

Spectrochimica Acta Part A 54 (1998) 2019–2024

SPECTROCHIMICA ACTA PART A

Short communication

# Study on a fluorescence-enhanced system of 1,2,4-trihydroxyanthroquinone–$Be^{2+}$–DNA

Jinghe Yang [a,*], Cunguo Lin [b], Guiling Zhang [c], Zuquan Gao [d], Xia Wu [a], Xirong Huang [a]

[a] *Department of Chemistry, Shandong University, Jinan 250100, People's Republic of China*
[b] *Branch of LUOYANG Ship Material Research Institute, Qingdao 266071, People's Republic of China*
[c] *College of Chemistry and Chemical Engineering, Qingdao Ocean University, Qingdao 266003, People's Republic of China*
[d] *Department of Biology, Shandong University, Jinan 250100, People's Republic of China*

Received 21 March 1998; accepted 24 June 1998

**Abstract**

Fluorescence-enhancement of 1,2,4-trihydroxyanthroquinone (THAQ)–$Be^{2+}$ complex enhanced by nucleic acid was studied. Experimental results revealed that double-stranded DNA can enhance remarkably the fluorescence intensity of THAQ–$Be^{2+}$, while RNA cannot. Based on these results, a fluorescence method for the selective determination of DNA in the presence of RNA was developed. Maximum fluorescence intensity was found in the pH range 3.6–4.5 with maximum excitation and emission wavelengths at 510 and 565 nm, respectively. Under optimum conditions, the calibration graph was linear over the range 0.08–18 μg $ml^{-1}$ for double-stranded fish sperm DNA (fsDNA) with the detection limit being $2.75 \times 10^{-8}$ g $ml^{-1}$. The method was applied for the determination of DNA in synthetic samples. The relative S.D. for five replicates was within 4%. In addition, the interaction mechanism of THAQ–$Be^{2+}$ with DNA was also discussed. 

*Keywords:* Fluorescence; Anthraquinone; Beryllium(II)

## 1. Introduction

Detection/determination of minute quantities of nucleic acids in many fields, such as molecular biology, biotechnology, medical diagnostics and forensic analysis, is very important. The natural fluorescence intensities of nucleic acids are so weak that direct use of fluorescence emission to study their biological properties is limited [1,2]. Usually, fluorescent probes, including fluorescent dyes, metal ions and metal complexes, are employed whilst investigating nucleic acids [3–13]. In 1966, ethdium bromide was first shown to be a useful probe for quantitating double-stranded DNA [3], which is still widely used as a general-

* Corresponding author..

1386-1425/98/$ - see front matter 
PII: S1386-1425(98)00223-6

purpose staining agent for nucleic acids after separation by gel electrophoresis. Many other dyes, such as mithramycin, the Hoechst dye 33258, 4′,6-diamidino-2-phenylindole, etc., are now used for fluorescence-based assays of nucleic acids [4–6]. In recent years, studies on nucleic acids using photochemical fluorescent probes [14], as well as monomeric and dimeric intercalating dyes [15], have also been reported. The use of small molecules, especially coordination complexes of metal ions, as probes to study DNA has attracted much attention [16–25]. Tetracycline [16], phenanthroline [17], adriamycin [23] and pyridine [24,25] are often chosen as ligands. The chosen metal ions are generally rare earth ions and transition metal ions, such as Eu, Tb, Fe, Ru, etc.

Anthraquinone is an important drug in the fight against cancer. In order to effectively design clinical anticancer drugs, it is important to understand its' medical activity mechanism at a molecular level. But, at present, choosing anthraquinone as a ligand to study nucleic acids and the medical activity mechanism of the drug is rare. In this paper, we first used THAQ as the ligand and THAQ–$Be^{2+}$ as the probe to study DNA. Experimental results showed that double-stranded DNA can enhance remarkably the fluorescence intensity of complex while RNA cannot. Based on these results, a fluorescent method was developed for the selective determination of DNA, and a satisfactory result was obtained. Also in this paper the interaction mechanism of THAQ–$Be^{2+}$ with DNA was discussed.

## 2. Experimental

### 2.1. Chemicals

Stock solutions of nucleic acids (100 μg $ml^{-1}$) were prepared by dissolving commercial fish sperm DNA (fsDNA) (Sigma) and yeast RNA (Beijing Baitai, China) in 0.05 mol $l^{-1}$ sodium chloride solution. A 0.0106 mol $l^{-1}$ stock solution of beryllium(II) was prepared by dissolving 0.1877 g of $BeSO_4 \cdot 4H_2O$ in deionised water, and then diluting to 100 ml with deionised water. A 0.001 mol $l^{-1}$ stock solution of THAQ was prepared by dissolving 0.2652 g of THAQ in 95% ethanol and then diluting to 100 ml with 95% ethanol. A 0.05 mol $l^{-1}$ Tris–HCl buffer solution was prepared by dissolving 3.03 g of Tris in deionised water, and then adjusting the pH to 4.28 by using HCl. All the chemicals used were of analytical grade and doubly deionised water was used throughout.

### 2.2. Apparatus

All fluorescence measurements were made with a Hitachi 850 spectrofluorimeter. All absorbance spectra were carried out using a UV-240 spectrophotometer (Shimadzu, Japan). All pH measurements were made with a pHS-2 acidity meter (Leici, Shanghai).

### 2.3. Procedure

To a 25-ml test-tube, solutions were added in the following order: THAQ solution, $Be^{2+}$ solution, DNA solution, and 2.0 ml of Tris–HCl buffer solution. The mixture was diluted to 10 ml with water and allowed to stand for 10 min. The fluorescence intensity was measured in a 1-cm quartz cell and the excitation and emission slits were both 10 nm.

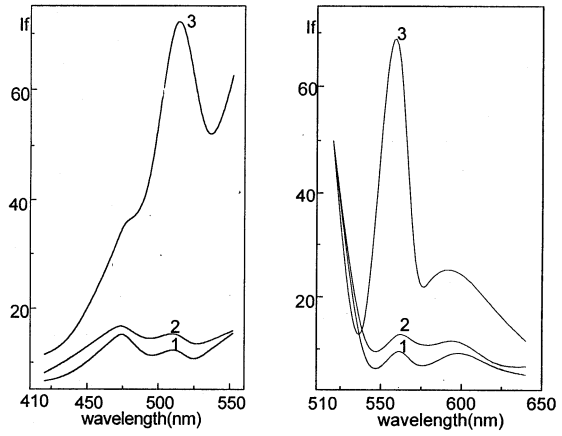


Fig. 1. Fluorescence spectra. Excitation (left) and emission (right) spectra: (1) THAQ $1.00 \times 10^{-5}$ mol $l^{-1}$; (2) THAQ $1.00 \times 10^{-5}$ and $Be^{2+}$ $1.06 \times 10^{-5}$ mol $l^{-1}$; (3) THAQ $1.00 \times 10^{-5}$ mol $l^{-1}$, $Be^{2+}$ $1.06 \times 10^{-5}$ mol $l^{-1}$, DNA $1.04 \times 10^{-5}$ g $ml^{-1}$.