Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 130 (2014) 553–560

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

A selective, long-lived deep-red emissive ruthenium(II) polypyridine complexes for the detection of BSA

Eththilu Babu [a,b], Paulpandian Muthu Mareeswaran [a,c], Subramanian Singaravadivel [a], Jayaraman Bhuvaneswari [a,d], Seenivasan Rajagopal [a,*]

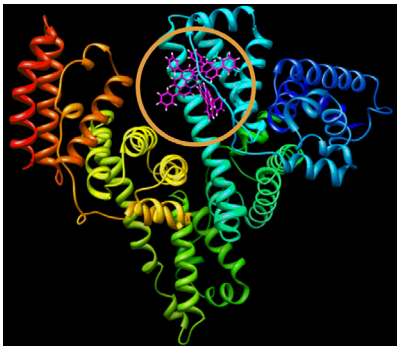
[a] Department of Physical Chemistry, School of Chemistry, Madurai Kamaraj University, Madurai, Tamil Nadu, India
[b] Department of Chemistry, VV College of Engineering, Tisaiyanvilai, Tirunelveli, India
[c] Graduate School of EEWS (WCU), Korea Advanced Institute of Science and Technology, Daejeon, South Korea
[d] Department of Chemistry, SRI G.V.G Visalakshi College for Women, Udumalapet, Tamil Nadu, India

HIGHLIGHTS

- Binding of ruthenium(II) complexes is efficient with BSA.
- Sensing of ruthenium(II) complexes with BSA and HSA is higher than other proteins.
- The α helicity of BSA has decreased with the addition of ruthenium(II) complexes.
- Mode of binding is established by docking studies.

GRAPHICAL ABSTRACT

A novel luminescence turn-on detection method for BSA have been developed using Ru(II) complexes, *via* non-covalent interactions.



ARTICLE INFO

Article history:
Received 14 February 2014
Received in revised form 2 April 2014
Accepted 10 April 2014
Available online 20 April 2014

Keywords:
Ruthenium(II) complex
Luminescence
Bovine serum albumin (BSA)
Biosensor
Docking

ABSTRACT

A selective, label free luminescence sensor for bovine serum albumin (BSA) is investigated using ruthenium(II) complexes over the other proteins. Interaction between BSA and ruthenium(II) complexes has been studied using absorption, emission, excited state lifetime and circular dichroism (CD) spectral techniques. The luminescence intensity of ruthenium(II) complexes (**I** and **II**), has enhanced at 602 and 613 nm with a large hypsochromic shift of 18 and 5 nm respectively upon addition of BSA. The mode of binding of ruthenium(II) complexes with BSA has analyzed using computational docking studies.

Introduction

Fluorescent probes are having wide range of applications in the field of biosensors and proteomics [1–3]. Protein detection is one of the important field, since proteins playing various roles like

* Corresponding author. Tel.: +91 4522458246; fax: +91 4522459139.
E-mail address: rajagopalseenivasan@yahoo.com (S. Rajagopal).

http://dx.doi.org/10.1016/j.saa.2014.04.060
1386-1425/

transporter, building blocks and pathogens in biology. Most of the molecular probes and sensors are fluorescent organic molecules. Their photophysical properties like emission intensity and excited state life time are used as sensing pathways. However, the use of such probes to reveal the local biological environment is scarcely addressed in the literature [4]. Even few reports are investigated on the mechanism of protein detection by light-up probes [4,5] and these light-up probes are mainly used for molecular recognition such as DNA, proteins and as stains in gel electrophoresis [6–9].

The organic fluorophores have limitations, such as, lower excited state lifetime, poor photostability and lower wavelength absorption [10,11]. The fluorescence of probe molecule should not overlap with background fluorescence from proteins. In this regard, the use of luminescent transition-metal complexes as sensors continues to attract considerable interest because of their long lifetimes compared to their purely organic counterparts and higher wavelength absorption [12–14]. Luminescent transition metal complexes, especially those with d^6 electronic configuration such as ruthenium(II), osmium(II) and rhenium(I), are receiving much interest in imaging and luminescent probe for biomolecules [15,16]. Because of the high photostability, low-energy absorption, and relatively long lived luminescence of ruthenium(II)-polypyridine complexes [17], we envisage ruthenium(II)-polypyridine complexes as promising candidates for luminescent biological probes.

The most abundant protein of circulatory system is serum albumins. Serum proteins play an important role in the transportation and delivery of drug molecules in the blood [18]. Among the serum albumins, bovine serum albumin (BSA) has a wide range of physiological functions involving the binding, transportation and delivery of fatty acids, porphyrins, bilirubin, steroids, etc. It is also consumed as dietary proteins. This heart shaped protein, BSA has three homologous domains I–III and each domain is made up of two subdomains, A and B, having unique binding properties. BSA is a single-chain 582 amino acid protein with 17 cystine cross-linked residues [19–22]. BSA has two tryptophans, Trp-134 and Trp-212, embedded in the first sub-domain IB and sub-domain IIA, respectively [23,24]. Several techniques such as electrochemistry [25], Rayleigh light scattering [26], two-photon excitation [27], seeded liquid beam desorption mass spectrometry [28], Raman scattering [29] and optical sensor [30] have been applied for the detection of BSA. Recently, many optical techniques have been utilized to investigate the interaction of proteins with ligands, because these methods are sensitive and relatively easy to use [31–33]. Among these, fluorescence technique is the most useful method to study the biomolecular interactions since it is sensitive to low concentrations and amicable with physiological environments [34].

Herein we report a label free biosensor using Ru(II) complexes for selective recognition of BSA in phosphate buffer solution (pH 7.4). This approach relies on the hydrophobic interaction with probe molecule using steady state emission signal change. The mode of binding is also analyzed using docking studies.

Experimental

Materials

The commercial samples of $RuCl_3{\cdot}nH_2O$, 4,7-diphenyl-1,10-phenanthroline disulfonic acid (dpsphen), 4,7-diphenyl-1,10-phenanthroline (dpphen), ammonium hexaflurophosphate and other chemicals are procured from Sigma. Bovine serum albumin (BSA), human serum albumin (HSA), lysozyme, cytochrome c, myoglobin, thrombin, platelet derived growth factor (PDGF) are purchased from Sigma Aldrich and used as such. The stock, 1×10^{-3} M, solution of all the proteins were prepared using PBS buffer. Sodium chloride, potassium chloride, disodium phosphate, sodium phosphate and all the solvents are purchased from Merck and used as such. The luminophores $[Ru(dpphen)_3]^{2+}$ (**I**) and $[Ru(dpsphen)_3]^{4-}$ (**II**) (Chart 1) are prepared using reported literature methods and characterized using ESI-MS spectroscopy (Figs. S1 and S2) [35,36].

Synthesis of tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) chloride (I)

The $[Ru(dpphen)_3]Cl_2$ is synthesized using literature method [35]. $RuCl_3{\cdot}3H_2O$ (1 mmol) and 4,7-diphen-1,10-phenanthroline (3 mmol) is taken in ethylene glycol under nitrogen atmosphere and heated to reflux for 72 h and the crude product is chromatographed using silica gel. The solution on evaporation yielded orange red crystals. Yield = 85% ESI-MS (m/z) 548.6496 ($M\text{-}2Cl^-$ doubly charged species).

Synthesis of tetrasodium tris[1,10-phenanthrolinediyl-4,7-di(benzenesulphanato) ruthenate(II) hexahydrate(II)

The $Na_4[Ru(dpsphen)_3]$ is synthesized using literature method [36]. The blue solution (0.5 mmol, 5 mL) is prepared by refluxing 1 g of $RuCl_3{\cdot}3H_2O$ in 50% ethanol for 4–5 h in a hot water bath. This solution is transferred into a degassed solution of the ligand, 4,7-diphenylphenanthroline disulphonic acid (1 g, 2 mmol), in water (20 mL) and the mixture is heated at reflux under nitrogen for 12 h. The resultant red solution is filtered hot, and the red filtrate evaporated to dryness to give an orange-brown solid. This is recrystallized from water–ethanol mixture, and then dissolved in water (5 mL) and then chromatographed using water as eluent. The red band is collected and evaporated to dryness to give a brown solid. ESI-MS (m/z): 263.6496 (6^- charged species) [36].

Apparatus and spectral measurements

All the solutions of Ru(II) complexes and proteins are prepared using phosphate buffer at pH 7.4. The UV–vis absorption spectra are recorded at room temperature on Analtik Jena Specord S100 diode-array spectrophotometer – equipped with 1.0 cm path length quartz cells. The UV–vis absorption spectral studies are carried out in the wavelength range from 300 nm to 700 nm. During the spectral measurement the concentration of the complex is maintained constant and the concentration of BSA was varied. The emission spectra are recorded using JASCO FP6300 Spectrofluorimeter with 10 mm path length cuvette. Emission spectra are recorded in the range of 500–800 nm. Titrations are done manually *via* a micro-syringe and the fluorescence intensity of complex in the absence and in the presence of the BSA is measured after incubation for 5 min. Excited state lifetime of Ru(II) complexes are determined by time dependent luminescence decay measurements using a Czerny–Turner monochromator and analyzed by Hamamatsu R-928 photomultiplier tube. Circular dichroism (CD) measurements are performed on JASCO J810 spectropolarimeter at room temperature over the wavelength 200–360 nm. Parameters are set as follows: path length, 50 mm; resolution, 0.5 nm; scan speed, 50 nm min^{-1}; band width, 1 nm; response 1 s. All the measurements are done at room temperature and repeated three times using freshly prepared samples and the results are reported as the average.

Determination of binding constant

Scatchard equation

The binding constants between the probe and the protein have been determined from the fluorescence intensity data using Scatchard plot. The Scatchard equation is shown in Eq. (1) [37,38]