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Protein binding studies of luminescent rhenium(I) diimine complexes

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ABSTRACT

Interaction of four luminescent rhenium(I) diimine complexes, $[Re(CO)_3(N–N)L]PF_6$ ((N–N = 2,2-bipyridine, L = py-3-COOH) **1a**, (N–N = 2,2-bipyridine, L = py-3-$CONH_2$) **1b**, (N–N = 1,10-phenanthroline, L = py-3-COOH) **2a**, (N–N = 1,10-phenanthroline, L = py-3-$CONH_2$) **2b** with bovine serum albumin (BSA) at physiological pH has been examined using UV–Vis absorption and luminescence spectroscopy, excited state lifetime measurement and circular dichroism (CD). In the presence of BSA, the luminescence of Re(I) complexes is quenched due to the locking-in of the probe into the protein environment. Interestingly the probe is released from the protein environment in the presence of sodium dodecyl sulfate (SDS) resulting in the restoration of the original luminescence along with a red shift in the emission maximum. These observations are explained in terms of binding constants (K_a) of probe with protein and surfactant and the nature of the binding has been investigated from Scatchard plot and Hill's coefficient (n) value. These studies point out that the interaction between Re(I) complexes and BSA is cooperative in nature.

1. Introduction

Proteins, the most versatile macromolecules in living organisms, serve crucial functions in essentially all biological processes [1]. The expression of protein functions relates not only to the primary structure, but also to the spatial structures [2]. Proteins are the main target of damage both inside and outside of the cells [1,3]. Proteins are a major target for oxidants as a result of their abundance in biological systems, and their reactivity. Davies [3] discussed oxidative pathways including reactive species, such as peroxides, which can induce further oxidation and chain reactions (within proteins and via damage transfer to other molecules) and stable products. The major fate of oxidized proteins is catabolism by proteosomal and lysosomal pathways, but some materials appear to be poorly degraded and accumulate within cells. The accumulation of such damaged materials may contribute to a range of human pathologies. In addition to the physiological factors (temperature, pH, etc.) the exogenous environmental pollutants can also trigger the alteration of protein conformation, including the denaturation of the protein, leading to disease [1,4]. Dowling et al. and Dalle-Donne et al. have shown that diabetes, cancer, cardiovascular diseases are closely related to the structural damage of proteins [4,5]. The most abundant protein in blood plasma is serum albumin [6–9]. It binds and transports many drug molecules, which are poorly soluble in water, through the blood stream. In addition, albumin is the most multifunctional transport protein and plays an important role in the transport and deposition of a variety of endogenous and exogenous substances in blood [10].

Bovine serum albumin (BSA) is highly stable and comparatively cheap, and its structure is similar to HSA (human serum albumin) in 76% [11]. BSA is composed of a single chain of 583 amino acid residues and can be divided into three homologous domains, each domain can be subdivided into two sub-domains (A and B). In general BSA has been used extensively in the past years, partly because of its structural homology with HSA [12,13].

Nowadays, the study of the targeting and non-covalent interaction of transition metal complexes with proteins and other biomolecules is an emerging field that links bioinorganic chemistry with chemical and synthetic biology [14–16]. Transition metal complexes are appealing candidates in the search for new diagnostic and therapeutic agents. They represent a unique modular system, wherein the metal center holds its ligands in a precisely defined three-dimensional structure. These ligands can be varied relatively easily, in order to change the characteristics of the complex in either subtle or dramatic fashion. Transition metal complexes also offer rich photophysical and photochemical properties, expanding their utility beyond chemical recognition. On the other hand, labeling of biological species with fluorescent markers is a very common procedure for diagnostic applications nowadays [17,18]. The commonly used organic labeling reagents have several disadvantages such as short emission lifetimes, strong pH dependence, self-quenching effects, and low photostability. In this regard, rhenium(I)-polypyridine complexes are very promising candidates

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for biological labeling in view of their remarkable luminescent properties [19–21]. The photoluminescence of rhenium(I)-diimine complexes can be exploited in the development of luminescent biological labeling reagents for the following reasons [22]. First, the use of various diimine ligands for the rhenium(I) complexes allows control on the metal-to-ligand charge-transfer (MLCT) emission energy and thereby offers a series of multicolor luminescent labeling reagents. Also, the large Stokes shifts usually observed for these complexes can minimize the self-quenching effects commonly encountered in multiple labeling of biomolecules with fluorescent organic dyes [23]. Moreover, the long emission lifetimes of rhenium(I)-diimine complexes, compared to those of organic fluorophores, could be applied in time-resolved detection techniques that can provide improved sensitivity [24]. The luminescent rhenium(I)-diimine units also provide environment-sensitive emission. This property renders them promising candidates as luminescent labeling reagents and probes for biological molecules.

In this study, we report the synthesis, characterization and the binding properties of four simple Re(I) complexes with BSA. This study has been carried out using UV–Vis, luminescence spectroscopy, circular dichroism and luminescence lifetime measurement. The Scatchard and Hill's equations were used to calculate binding constants and the nature of the binding. The aim of this work is to explore the interaction of Re(I) complexes with protein specifically aiming at the conformational changes of BSA. The obtained results show that the Re(I) complexes disturb the tertiary structure of BSA with loss of α-helix stability.

2. Results and discussion

2.1. Synthesis and photophysical studies

The structure of the Re(I) complexes synthesized for the present study are shown in Chart 1. *fac*-$[Re^{I}(NN)(CO)_3L]^+$ complexes have been prepared by an easy, well defined two step synthetic procedure. The reaction of starting material $Re(CO)_5Br$ with the bidentate ligands (NN = 2,2′-bpy and 1,10-phen) containing sp^2 nitrogen donors belonging to an extended π-system leads to the formation of *fac*-$[Re^{I}(NN)(CO)_3Br]$ species which are air and moisture stable and kinetically inert, this latter feature being typical of the low spin d^6 Re^{I} cation. The Br^- can be removed in a second separate step through reflux with $Ag(CF_3SO_3)$ and substituted with L (where, L = nicotinic acid and nicotinamide), to get *fac*-$[Re^{I}(NN)(CO)_3L]^+$ complexes which are also air and moisture stable and kinetically inert.

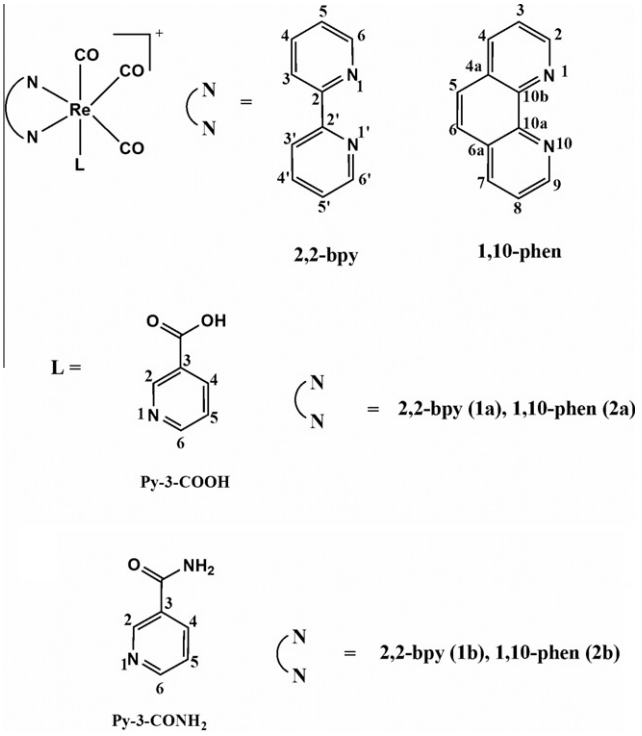

Chart 1. Structures of the rhenium(I) complexes.

The absorption and emission spectra of **1a** in acetonitrile are shown in Fig.1 and the spectral data collected in Table 1. The electronic absorption spectra of Re(I)-tricarbonyl complexes **1a**–**2b** reveal strong absorption bands at 200–315 nm and less intense absorption shoulders *ca.* 345–368 nm (Table 1). With reference to previous studies on related Re(I) complexes [25–27,17], these strong high energy absorption bands are assigned to the ligand centered (LC) π–π* transition and low energy absorption shoulders to spin allowed metal to ligand charge transfer MLCT transition from the Re $d\pi-$ orbital to the π* orbital of the ligand ($d\pi$ (Re) → π* (diimine). The ^{3}MLCT emission of these complexes are observed in the region 542–548 nm in acetonitrile at RT. Lifetimes of these complexes are close to the literature values of the related Re(I) complexes [28,29].

2.2. Absorption and emission titrations

All the four Re(I) complexes (**1a**–**2b**) show an enhancement in its MLCT absorption (≈350 nm) on the addition of BSA. For example, Fig. 2 shows the absorption titration spectrum of complex **1a** with BSA. It is important to note that BSA has no absorption at 350 nm. This shows the interaction between Re(I) complex and BSA in the ground state. The absorbance intensity of 80 μM of **1a** is 0.30 at 350 nm (corresponds to MLCT absorption of Re(I) complex) which shows an enhancement in the presence of BSA (intensity increases from 0.30 to 0.69 at 350 nm), which is attributed to the non-covalent interactions between Re(I) complex and BSA. In order to confirm the interaction between Re(I) complexes and BSA, luminescence titrations are also carried out for the same sample solutions. The emission titration results show that the emission intensity of the Re(I) complexes is quenched with slight blue shift, 5–6 nm, in the presence of BSA. As a sample, results of the emission titration of complex **1a** with BSA are illustrated in Fig. 3. Luminescence quenching refers to any process with a decrease of the

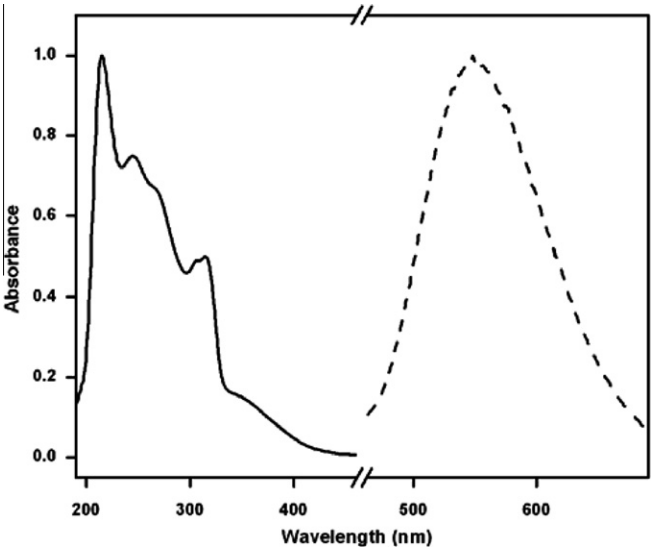

Fig. 1. Normalized absorption and emission spectra of complex **1a** in acetonitrile. Excitation maximum = 355 nm.