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Static quenching of tryptophan fluorescence in proteins by a dioxomolybdenum(VI) thiolate complex

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ABSTRACT

The binding of *cis*-dioxobis(dithiocarbamato) molybdenum(VI) with the proteins bovine serum albumin, human serum albumin, lysozyme, and free tryptophan was studied using fluorescence spectroscopy and Stern–Volmer kinetics. The quenching of tryptophan fluorescence was determined to be static with binding constants on the order of 10^4–10^5 M^{-1}, and with a binding site number of one. The interaction was studied over a range of temperatures, and the binding was found to be exothermic with a negative change in entropy. Quantum chemical calculations were also conducted to identify optimal spatial contacts and the resulting energetic contributions between the complex and free tryptophan.

1. Introduction

Transition metal ions are essential for a great variety of biochemical functions. These ions can be incorporated into or associate closely with proteins, giving rise to functions ranging from cell signaling, gene expression, antioxidant activity to electron transport and catalysis, and in the case of molybdenum, amino acid metabolism. Earlier studies focused on the interaction of transition metal ions with biologically important proteins, particularly with bovine serum albumin (BSA) [1,2]. More recent studies have shifted focus to interaction of proteins with transition metal complexes, including nickel, chromium and molybdenum complexes [3–7]. Based on whether the ligand is hydrophobic, hydrophilic, or possess certain chemical features, the study of a wide number of complexes can give insight into how proteins behave in different chemical environments.

Serum albumins are widely studied and well-characterized proteins [3,4,8–16]. The essential difference between human serum albumin (HSA) and BSA is the number of tryptophan residues (Trp). BSA contains two tryptophans, one on the surface of the molecule (Trp134) and one closer to the interior of the protein in a hydrophobic fold (Trp212). HSA contains only one tryptophan (Trp214), in a similar chemical environment as Trp212 in BSA [17]. The binding capabilities of serum albumins are largely due to the presence of these tryptophan binding sites. Lysozyme is a single polypeptide chain that consists of 129 amino acid residues with six tryptophans which are located in a hydrophobic fold. The tryptophan is responsible for a large portion of the reactivity in the active site of the lysozyme enzyme [18–20]. The interaction of lysozyme with various quenchers has been reported [3,21,22].

The underlying motivation for this work is our interest in identifying and synthesizing water-soluble transition metal complexes that can be used as probes for protein activity. Fluorescence quenching is a useful tool for measuring the extent and accessibility of protein binding sites to small molecules. Using Stern–Volmer kinetics and Forster's theory, the number of binding sites, the binding constant K_a, and the distance between donor and acceptor can be determined [23–25]. The fluorescence signal of a protein is derived from its aromatic residues, primarily tryptophan [3]. Tryptophan fluorescence is sensitive to its environment, and is a convenient spectroscopic probe for the structure and rotational dynamics surrounding the residue [24]. Fluorescence quenching studies are not limited to just proteins: Fluorescence quenching of non-biological systems by nanoparticles, C_{60} fullerenes and carbon tetrachloride have been reported in recent years [26–28].

In this study we have explored the binding interaction of a water soluble dioxo-molybdenum(VI) complex containing a non-aromatic diethyldithiocarbamate ligand $[MoO_2(S_2CNEt)_2]$, with BSA, HSA, lysozyme and free tryptophan (free-Trp). The structure of this dioxo-Mo(VI) complex is shown in Fig. 1. The results

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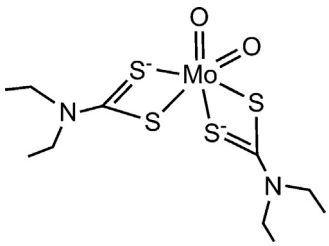

Fig. 1. Structure of *cis*-dioxobis(N,N-diethyldithiocarbamato) molybdenum(VI) [$MoO_2(S_2CNEt)_2$].

presented here complement previously published work by our group on the interaction of transition metal complexes with various proteins [3,4]. Wu and co-workers [6,29] have previously described the interaction between a highly aromatic phenylfluorone molybdenum(VI) complex with BSA and HSA. Although the ligand systems are very different, some comparison between the Mo(VI) complexes as they interact with globular proteins can be made.

2. Materials and methods

2.1. Chemicals

The proteins BSA, HSA and lysozyme, and free-Trp were purchased from Sigma–Aldrich and used without further purification. Protein solutions were prepared in 50 mM Tris–HCl buffer (pH 7.4). The dioxo-Mo(VI) complex, *cis*-dioxobis(N,N-diethyldithiocarbamato) molybdenum(VI) [$MoO_2(S_2CNEt)_2$], was synthesized using the procedures described by Moore and Moloy [30,31]. The complex was recrystallized from dichloromethane, and thoroughly dried under vacuum before use. All solutions were prepared using deionized water (reverse osmosis, 18.2 MΩ).

2.2. Instrumentation and quenching protocol

Absorption spectra were measured using a Cary 4000 UV–Vis Spectrophotometer. Fluorescence emission spectra were measured using a Perkin-Elmer LS50B Luminescence Spectrometer. For the fluorescence quenching experiments, the protein concentration was fixed at 1.0×10^{-4} M and the concentration of the dioxo-Mo(VI) complex was increased through the preparation of individual samples. The maximum dioxo-Mo(VI) complex concentration ranged from 2.1×10^{-4} M to 2.7×10^{-4} M, depending on the extent of quenching. Excitation wavelengths ranged from 279 nm to 281 nm and slit widths were varied as needed. Fluorescence spectra were obtained over a 25–55 °C range. Emission maxima were at 345 nm for the protein samples and 363 nm for tryptophan.

2.3. Data analysis

The dynamic quenching constant (K_{SV}) was determined using the Stern–Volmer equation [25,32]:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \quad (1)$$

F_0 and F correspond to the fluorescence intensities of the protein without quencher and in the presence of quencher, respectively, and [Q] is the concentration of the quencher. K_{SV} is related to the lifetime of the system according to the following equation [25]:

$$K_{SV} = k_q \tau_0 \quad (2)$$

k_q is the bimolecular quenching constant and τ_0 is the lifetime of the protein (order of 10^{-8} s).

The static quenching constant or binding constant, K_a, and number of binding sites (n) between the protein and metal complex were calculated using the following equation [26,27]:

$$\log\left[\frac{F_0 - F}{F}\right] = \log K_a + n \log[Q] \quad (3)$$

The changes in enthalpy, ΔH, and entropy, ΔS, were determined using the van't Hoff equation:

$$\ln\ K_a = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (4)$$

2.4. Computational chemistry

Quantum chemical calculations were carried out using the Gaussian09 program (Gaussian, Inc., Wallingford, CT, USA). The structures of tryptophan, the dioxo-Mo(VI) complex and various orientations of the complex were first optimized using Hartree–Fock theory and the LanL2DZ basis set. Excited states were modeled using the time-dependent self-consistent-field (TD-SCF) method following computational methodologies described in the literature [33–35]. Structures were first optimized in the gas-phase and then in water, where the Polarized Continuum Model (PCM) was used. In this model, the solvent cavity is defined as a series of interlocking spheres.

3. Results and discussion

3.1. Fluorescence quenching

The quenching of the intrinsic fluorescence of tryptophan was monitored in order to study the interaction between the dioxo-Mo(VI) complex and proteins, and free-Trp. Fluorescence quenching plots are shown in Fig. 2. The onset and extent of fluorescence quenching varied. The fluorescence of BSA was quenched 83% at a complex concentration of 2.1×10^{-4} M, with negligible change when the concentration was raised to 2.5×10^{-4} M. At the same two concentrations, the fluorescence of HSA was quenched 55%. The fluorescence of lysozyme was quenched 69% at a complex concentration of 2.7×10^{-4} M, while the fluorescence of free-Trp was quenched 59% at a complex concentration of 2.1×10^{-4} M. At lower dioxo-Mo(VI) complex concentration (1.2×10^{-5} M) the fluorescence of BSA was quenched 14%, whereas the fluorescence of HSA and free-Trp was quenched by 3% and 2%, respectively. The fluorescence of lysozyme did not measurably quench until the complex concentration reached 3.8×10^{-5} M.

The fluorescence quenching is due to the interaction of the dioxo-Mo(VI) complex with the Trp residue(s) of the protein and amino acid residues in the immediate vicinity in the protein. There are three factors that influence the quenching: (i) The hydrophobicity of the ligand, (ii) the charge of the complex, and (iii) the immediate environment of the Trp in the protein [3,4]. In all three proteins, the tryptophans are surrounded by both hydrophobic and partially hydrophobic residues such as valine (BSA, HSA and lysozyme), partially hydrophobic tyrosine (BSA and HSA), leucine and phenylalanine (BSA) and isoleucine (lysozyme) [3]. The Trp residues in BSA and lysozyme are also surrounded by negatively charged glutamine and polar uncharged amino acids such as serine (BSA and lysozyme) as well as glutamine and threonine (lysozyme). Given the presence of positively charged amines in the dioxo-Mo(VI) complex, the polar Mo=O bonds and non-polar N-diethyl groups, a strong interaction between the Trp residues and its immediate environment is expected.

It is worth noting that Wu and co-workers [6,29] reported a dramatic blue shift in the emission maxima in BSA and HSA as the concentration of their phenylfluorone-Mo(VI) complex was