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Molecular interaction between phosphomolybdate acid and bovine hemoglobin

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ABSTRACT

The interaction between bovine hemoglobin (BHb) and phosphomolybdate acid (PMo_{12}) was investigated by UV/vis absorption, IR, circular dichroism (CD), fluorescence, resonance light scattering spectra, synchronous fluorescence, and three-dimensional fluorescence spectra techniques under physiological pH 7.40. PMo_{12} effectively quenched the intrinsic fluorescence of BHb via static quenching. The process of binding PMo_{12} on BHb was a spontaneous molecular interaction procedure. The thermodynamic parameters, ΔH° and ΔS° were estimated to be 28.69 K J mol^{-1}, 158.20 J mol^{-1} K^{-1} according to the van' Hoff equation. This indicates that hydrophobic interaction played a major role in stabilizing the PMo_{12}–BHb complex. The effect of PMo_{12} on the conformation of BHb was analyzed using synchronous fluorescence spectroscopy, IR and CD spectra.

1. Introduction

Phosphate is an essential nutrient to all living systems. The transition element molybdenum is of essential importance for all biological systems as it is required by enzymes catalyzing diverse key reactions in the global carbon, sulfur and nitrogen metabolism. Toxicity symptoms of inorganic phosphate and molybdenum are observed [1,2]. Polyoxometalates (POMs) are receiving increasing attention in a variety of domains, including catalysis, material science, medicine, and bio-inorganic chemistry because of their chemical, structural, and electronic versatility [3,4]. Their promising antibacterial, antiviral, antitumor, anticancer, and anti-HIV activities arouse more and more research interest, which increases the interest in the association between POM species and proteins [3,5,6]. Polyanionic Keggin compound, phosphomolybdate acid (PMo_{12}) contains 12 MoO_3 and one PO_4 moiety in the molecular structure. This Keggin compound strongly inhibited the protein-tyrosine phosphatases (PTPs) [7]. Proteins are biological molecules and their incorporation into large polyatomic structures may illuminate key interactions of great importance in biochemistry. However, the binding mechanisms of POM and proteins at a molecular level remain unclear. As a consequence, preliminary studies of interactions, in a broad sense, between POM and several proteins of interest, are one of the necessary steps toward an understanding of POM's biological activity [3,8].

Hemoglobin (Hb) is well known for its function in the vascular system of animals, being a carrier of oxygen. It also aids, both directly and indirectly, the transport of carbon dioxide and regulates the pH of blood [9]. It removes hydrogen ions in the capillaries and carries them to the lungs. In addition, it is involved in many clinical diseases such as leukemia, anemia, heart disease, excessive loss of blood, etc. [10]. Concentration of Hb in plasma is about 140 g L^{-1}, which is higher than serum albumin (~40 g L^{-1}), an important carrier of biomoleculars, so Hb can accumulate some biomoleculars [11]. Hb has a molecular weight of 64.500 kDa and contains four globin chins, of which two are α-chains and two are β-chains [12]. Thus, the subunit structure of Hb is $\alpha_2\beta_2$. The α-chains contain 141 amino acids; the β-chains contain 146 amino acids. Each α-chain is in contact with β-chain [13]. There are four oxygen-binding sites on the Hb molecule. Hemoglobin can reversibly bind with many kinds of endogenous and exogenous agents such as many drugs (e.g., platinum drugs, artemisinins, flavonoids) [14–16], herbicides (e.g., Paraquat) [17], insecticides (e.g., Chlorpyrifos and Cypermethrin) [18], and nanoparticles (e.g., TiO_2, CdS) [19,20], consequently, study on binding of POM to Hb is helpful to understand transportation, distribution and bioavailability of POM in vivo. So far, most of studies are based on the interactions between POM and serum albumin [3,5,6], the reports on the binding of POM to Hb have been rather limited [13].

In this paper, the interaction between bovine hemoglobin (BHb) and phosphomolybdate acid (PMo_{12}) was investigated by UV/vis absorption, IR, fluorescence, resonance light scattering spectra, synchronous fluorescence, and three-dimensional fluorescence spectra techniques under physiological pH 7.40. Based on PMo_{12}

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quenching fluorescence and Stern–Volmer equation, the dynamic quenching constants were calculated. According to thermodynamic equations, the binding constants, the numbers of binding sites and the acting forces between PMo_{12} and BHb were discussed.

2. Materials and methods

2.1. Materials

BHb was purchased from Sigma (St. Louis, MO, USA) and used without further purification. Phosphomolybdate acid was obtained from Fluka (Switzerland). The buffer Tris was purchased from Acros (Geel, Belgium), and NaCl, HCl, etc. were all of analytical purity. BHb solution (3.0 μM) was prepared in pH 7.40 Tris–HCl buffer solution (0.05 M Tris, 0.1 M NaCl). For the CD experiments, a 0.02 M phosphate buffer of pH 7.4 was exclusively prepared in deionized water. The PMo_{12} solution (1.5 mM) was prepared by dissolving PMo_{12} in Tris–HCl buffer solution. Water was purified with a Milli-Q purification system (Barnatead, Dubuque, IA, USA) to a specific resistance >16.4 MΩ cm^{-1}. All solutions were stored in refrigerator at 4 °C in dark.

2.2. Equipments and spectral measurements

The UV/vis spectrum was recorded at room temperature on a SPECORD S 50 (Germany) equipped with 1.0 cm quartz cells. All fluorescence spectra were recorded on LS-50B Spectrofluorimeter (Perkin-Elmer, USA) equipped with 1.0 cm quartz cells and a thermostat bath. The widths of both the excitation slit and the emission slit were set to 10.0 nm/5.0 nm for BHb, respectively. The IR spectra were obtained with a Perkin-Elmer Spectroscopy 100 at room temperature. The far-UV CD spectra were measured by a Jasco J-810 spectropolarimeter (Japan Spectroscopic, Japan) using a 0.1 cm quartz cell at 0.2 nm intervals, the bandwidth was 2.0 nm and the scan speed was 100 nm min^{-1}.

2.3. Procedures

A 2.5 mL solution, containing appropriate concentration of BHb, was titrated by successive additions of a 1.5 mM stock solution of PMo_{12} (to give a final concentration of 48 μM). Titrations were done manually by using trace syringes. The fluorescence spectra were then measured (excitation at 280 nm and emission wavelengths of 290–500 nm) at two temperatures (293, 310 K). The three-dimensional fluorescence spectrum was performed under the following conditions: the emission wavelengths at 290–500 nm, the excitation at 220 nm, scanning number 11 and increment 10 nm with other parameters just the same as that of the fluorescence quenching spectra. Resonance light scattering spectra (RLS) were obtained by synchronous scanning with the wavelength range of 200–700 nm on the spectrofluorophotometer at room temperature. The CD spectra of BHb solutions containing PMo_{12} were recorded from 200 to 250 nm with two scans averaged for each CD spectrum.

3. Results and discussion

3.1. UV/vis spectra studies

UV/vis absorption measurement is a very simple method and applicable to explore the structural change and to know the complex formation. Hence, absorption spectra of BHb in presence and absence of PMo_{12} were recorded (Fig. 1).

Fig. 1 showed that the absorption wavelengths of BHb, PMo_{12} and PMo_{12}–BHb were different. The UV/vis absorption spectrum

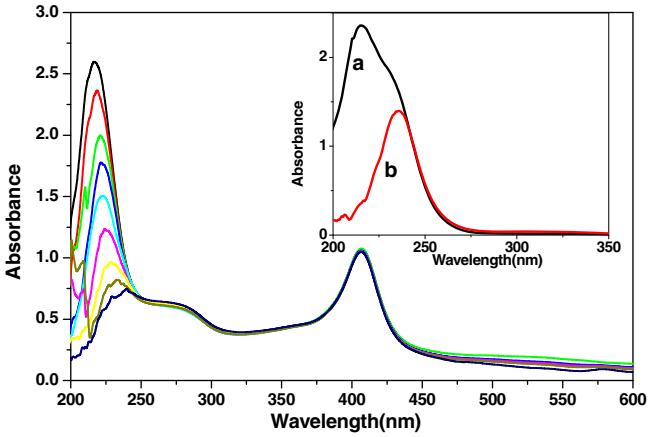

Fig. 1. Absorption spectra of BHb, PMo_{12} and PMo_{12}–BHb system. The concentration of BHb was at 3.0 μM; PMo_{12} concentrations for PMo_{12}–BHb system (from up to down) were 0, 6, 12, 18, 24, 30, 36, 42, and 48 μM. The insert shows the UV/vis spectra of PMo_{12} in presence of BHb. (a) The absorption spectrum of PMo_{12}; (b) the difference absorption spectrum between BHb–PMo_{12} and BHb. The concentration of PMo_{12} was at 30.0 μM; the concentration of BHb was at 3.0 μM.

of BHb shows a strong band in the far-UV region with a maximum at 216 nm and a weak band in the near-UV region with a maximum at 275 nm. The peak at 407 nm corresponds to the Soret-band of BHb. When the concentration of PMo_{12} in the solution is 30 μM, the peak at 216 nm is shifted to 224 nm and the absorbance is decreased 50% compared to a solution of BHb in absence of PMo_{12}. These two results indicate that there are interactions between PMo_{12} with BHb. These interactions are mainly attributed to the exposure of aromatic residues to water [21]. However the position and absorbance of the peak at 410 nm does not change significantly, which indicates that the interaction does not affect the structure of the heme group.

3.2. Fluorescence quenching of BHb by PMo_{12}

BHb contains three Trp residues in each αβ dimer, for a total of six in the tetramer: two α-14 Trp, two β-15 Trp, and two β-37 Trp. Of the three Trp residues, only the β-37 Trp is located at the dimer–dimer interface, wherein the structural differences between quaternary states are largest [22]. The intrinsic fluorescence of BHb primarily originates from β-37 Trp that plays a key role in the quaternary state change upon ligand binding [23]. Thanks to these Trp residues, BHb can emit intrinsic fluorescence. A valuable feature of intrinsic fluorescence of Hb is very sensitive to its microenvironment, especially around Trp residues. Changes in emission spectra of tryptophan are common in response to protein conformational transitions, substrate binding, or denaturizing [24]. Thus, the intrinsic fluorescence of proteins can provide considerable information about their structure and dynamics, and it is often considered on the study of protein folding and association reactions. The effect of PMo_{12} on BHb fluorescence intensity is shown in Fig. 2. As the data show, the fluorescence intensity of BHb decreased regularly with the increasing PMo_{12} concentration, the shift of the emission maximum from 340 to 343 nm is observed. PMo_{12} could acts as quenchers to decrease the fluorescence intensity of BHb. These results indicated that interaction between PMo_{12} and BHb occurs.

3.3. Fluorescence quenching mechanism

Generally speaking, the fluorescence quenching is the decrease of the quantum yield of fluorescence from a fluorophore induced