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# Binding between lead ions and the high-abundance serum proteins

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## HIGHLIGHTS

- The influence of lead ion on the high abundant serum proteome is described.
- A novel formula for the heavy metal binding with protein is developed.
- Protein–protein interaction affects $Pb^{2+}$ binding with proteome.
- A micro-environmental impact factor ($F_m$) is adopted to explain the binding reaction.

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## ABSTRACT

The interaction between three of the most abundant bovine serum proteins (serum albumin, transferrin and IgG) with $Pb^{2+}$ was investigated using electrochemistry. The data was used to construct a new theoretical model of $Pb^{2+}$ binding to the high-abundance serum proteins under non-ideal conditions. The binding constants ($\beta$) of $Pb^{2+}$ to the individual proteins and a mixture of proteins were measured according to a new theoretical equation (non-ideal state) as well as the McGhee–Von Hippel equation (ideal state). Differences between the models suggested that the $\beta$ values obtained using the non-ideal state model was more realistic. Protein–protein interactions and micro-environmental influences affected binding between $Pb^{2+}$ and the high-abundance serum proteins. We included a micro-environmental influence factor for the model ($F_m$), which accurately quantified the effect of micro-environment of the proteome of $Pb^{2+}$ binding with the serum proteins. This research provides a useful reference of theoretical and experimental work regarding heavy-metal binding interactions with serum proteins.



## 1. Introduction

Metal ions are essential to mammalian life in trace quantities as they play a key role in various biological processes (Wang et al., 2010). However, heavy metals are an increasing environmental pollutant due to industrial development and there is a growing need to understand their toxicity and develop effective methods for their detection. The toxicological features of heavy metal ions are linked to their binding properties with biological molecules. The interaction of a heavy metal with proteins has increasingly attracted the interest of many research groups. One of the most common of these toxic metals is lead. Lead poisoning is a serious disease that can endanger human health; chronic poisoning may result in mental impairment and anemia when the concentration of lead in blood exceeds 100 μg $L^{-1}$ (Holz et al., 2007; Vance, 2007). Lead may combine with hemoglobin when introduced into the body, and may also interact with other proteins or small molecules in blood plasma (Marcus, 1985). These proteins include serum albumin, transferrin and immunoglobulin, which are the most abundant proteins in blood plasma (Tirumalai et al., 2003; Guo et al., 2008).

Serum is a complex body fluid. In addition to high-abundance proteins, it is composed of a large number of low-abundance proteins, some of which are key regulatory and signaling proteins (Thadikkaran et al., 2005). The understanding of the serum proteins is limited as only 15% of the approximately 10000 different proteins have been identified (Apweiler et al., 2009), which indicates that the current. There are significant differences in serum proteins between different species, and within individuals of the same species due to differences associated with gender, age, physical condition and nutritional condition. Although there are characteristic differences between bovine and human serum,

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they have similar high abundance proteins and the resultant overall functional performance is similar (Issaq et al., 2007). The bovine equivalents are often used as mimics in studies of the primary components of the high abundance serum proteome in humans: albumin; transferrin and immunoglobulin (Peters, 1985; Thadikkaran et al., 2005).

In recent years, the binding between proteins and drugs or metals has increased in prominence. Serum albumins have featured among these proteins as they are the most abundant proteins in human and animal plasma (Merrick, 2003; Thadikkaran et al., 2005). They serve as buffering agent in blood plasma, maintain osmotic pressure and serve as a carrier of small molecules, where they are involved in the transport of drugs, fatty acids, enzymes, metabolites and hormones. Compared to other proteins, serum albumins have a smaller molecular mass, are more soluble and more stable, and are easier to purify as they have a greater affinity for ligands (Lim et al., 1988; Otagiri et al., 2009; Abassi et al., 2013). Bearing these properties in mind, it is crucial to study the physico-chemical properties, biological functions, metabolic mechanism, clinical applications and genetic variations of serum albumins (Kragh-Hansen, 1981; Cahyana and Gordon, 2013; Vignesh et al., 2013). The structures of BSA, bATF, BIG are similar to human albumin, transferrin, immunoglobulin, and therefore suitable for a preliminary study of the combined lead–protein reaction mechanism.

Human serum albumin (HSA) is a single peptide chain protein which consists of 585 amino acid residues and three similar domains: domain I (residues 1–195), domain II (residues 196–383) and domain III (residues 384–585). Each tryptophan residue (Trp214) is located in a large reactive hydrophobic cavity in sub-domain II A (He and Carter, 1993). Bovine serum albumin (BSA) consists of 582 amino acid residues and is a single peptide chain protein forming three circular domains. Almost all hydrophobic amino acid residues are located within these structures, resulting in hydrophobic cavities (Huang et al., 2004). The amino acid sequence of BSA and HSA are mostly similar, although BSA contains tryptophan residues in the 134 and 212 positions and lacks the 116 amino acid sequence. The amino-terminus (N-terminus) has an aspartic acid residue and the carboxy terminus (C-terminus) has a leucine residue. The HSA has 17 disulfide bonds (mainly in the α-helixes) and also contains 18 tyrosine residues (Carter and Ho, 1994).

The basic IgG molecule consists of four peptide chains. These consist of two lower molecular weight light chains and two larger molecular weight heavy chains that are joined by disulfide bonds (Amzel and Poljak, 1979). The light chains generally consist of 210–230 amino acid residues and do not usually contain carbohydrates. Each light chain (L-chain) contains two cyclic peptides with internal disulfide bonds. The heavy chains (H-chain) generally consist of 450–550 amino acid residues and contain 4–5 cyclic peptides with internal disulfide bonds. The IgG N-terminus displays a large variation in the amino acid sequence that is referred to as the variable region (V region), while the C-terminus is relatively stable and is referred to as the constant region or C region (Burton, 1985; Liu et al., 1995). The antigenic and immunogenic properties of human IgG and bovine IgG are determined by the composition of the V regions, especially the hyper-variable regions (Jin, 2008).

The amino acid sequences of cattle and human lactoferrins display 69% homology. Human lactoferrin consists of 711 amino acid residues and the tertiary structure consists of two similar spherical lobes (N-lobe and C-lobe). The N-lobe and C-lobe consist of two α/β sub-domains denoted as N1, N2, C1 and C2. Deep fissures between sub-domains have binding sites for $Fe^{3+}$. The connected region between the N- and C-lobes plays an important role in opening and closing of iron-binding domains and in stabilizes the iron-binding structure. There are no covalent bonds between the N- and C-lobes and they rely on hydrophobic interactions to maintain structural stability. This molecular structure consisting of two spherical lobes and four domains forms the structural basis for transferrins (Levay and Viljoen, 1995; Gomme et al., 2005). Bovine lactoferrin consists of 689 amino acid residues with two "gingko leaf-type" three-dimensional structures, including a N- and C-lobe, which are connected by three-turn helices (Macedo and de Sousa, 2008; Schwarcz et al., 2008). The general characteristics and three-dimensional structure of human and bovine lactoferrin are similar, indicating that bovine lactoferrin is suitable for comparative binding studies (Sargent et al., 2005; Bai et al., 2010).

Albumins from different mammalian species display many similarities in physico-chemical properties (Carter and Ho, 1994). The interactions of lead with these serum proteins can reveal the binding and transport mechanisms that further elucidate the toxicology of the metal ion. Understanding the interaction of lead with blood plasma proteins is important to develop new targeting drugs of higher clinical value.

Chromatographic processes have been widely used to assess interactions between heavy metal cations and proteins (Ueda et al., 2003; Lee and Lee, 2004; Kung et al., 2006; Gutiérrez et al., 2007), while spectroscopic methods such as fluorescence and UV spectroscopy have recently been utilized (Zhang et al., 2011; Zhao et al., 2011; Peng et al., 2012). Although rapid and relatively easy to use, spectroscopic methods depend on light absorbance/transmittance and may be compromised by opaque or colored solutions. In these solutions an electrochemical method is advantageous, as it can function even when the analytes are present in an unclear solution. It may also provide valuable data when analyzing molecules with a weak absorption spectrum that cannot be monitored *via* UV–vis and fluorescence spectroscopy. Electrochemical methods are also suitable for investigating the interaction between proteins and metal ions because of their high sensitivity, high selectivity, speed and ease of operation (Lu et al., 2007; Zhang and Liu, 2011). Electrochemical methods provide an important method to elucidate the underlying structure and physiological action of a protein. Presently, much electrochemical work has been published regarding the interaction of small molecules with biological macromolecules (Zhu and Li, 1999; Dong et al., 2008). However, most previous studies report the interaction of drug molecules with a single biological macromolecule and there is notably less published regarding the use of electrochemical methods to study metal ions and proteome interactions. As such, we have investigated the use of an electrochemical method to study the binding mechanism of $Pb^{2+}$ with BSA, bATF or BIG and within serum proteome in the form of fetal bovine serum (FBS). The investigation of the interaction between a heavy metal and individual or multi-component proteins representing a high abundance serum proteins is a prospective topic in the field of small molecule and protein interaction research (Giroux and Schoun, 1981; Ojha et al., 2012; Roy et al., 2013). To our knowledge, binding between lead and high abundant serum proteome has not been reported.

## 2. Materials and methods

### 2.1. Apparatus and reagents

Electrochemical experiments were performed using a CHI-660 electrochemical analyzer (Shanghai CH Instrument, China) with a glassy carbon electrode (GCE) as the working electrode, a platinum wire as the counter electrode, and a saturated calomel electrode as the reference electrode (SCE).

Bovine apotransferrin was purchased from Wako Pure Chemical Industries, while bovine serum albumin, bovine immunoglobulin and fetal bovine serum were purchased from Shanghai Haoran