Detection of picomolar concentrations of lead in water using albumin-based fluorescence sensor

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Comprehensive analysis of fluorescence of albumin shows a weak fluorescence band at 430 nm, whose intensity exhibits a remarkable sensitivity to the presence of heavy ions in water. Using this fluorescence as a marker, as low as 10 pM concentration of lead can be routinely detected. Such a great sensitivity is explained in terms of electrostatic interactions in solution, which promote protein agglomeration. The latter is independently confirmed using dynamic light scattering measurements. © 2009 American Institute of Physics. [doi:10.1063/1.3246792]

Heavy metals, such as lead, mercury, cadmium, and arsenic, present a major threat to human health and society. In particular, lead is a commonly occurring environmental hazard, which is widely recognized to be toxic at concentrations as low as 1 pM.^{1,2} While environmental efforts have resulted in significant reduction in lead exhaust into the biosphere, a considerable amount of lead is still present in soil and lead-based paints, posing a danger to the society. Although the Environmental Protection Agency limits the allowable level of lead in drinking water to 15 ppb (73 nM), recent studies show that there is no threshold for the adverse effect of this universal toxic metal, which tends to substitute biological reactions mediated by calcium, iron, and other metal ions and enzymes.^{1,3} This leads to lead poisoning resulting in serious medical conditions affecting brain development, and the cardiovascular and reproductive systems.³

The deleterious effects of lead to human health and environment present an open challenge to the scientific community to develop sensors which can detect, monitor, and measure the presence of those toxic metals.^{4,5} Several methods are currently available for detecting heavy metal ions. It includes atomic absorption spectrometry, inductively coupled plasma (ICP) atomic emission spectroscopy, and ICP mass spectrometry.⁶ However, most of these techniques are either outrageously expensive or not sensitive enough in detecting the presence of toxic metals below nanomolar concentrations.

In search for an alternative strategy to detect the heavy ions in solution, we evaluated the natural transport path of those ions in a human body. The most common media for transporting heavy metal ions is blood, most probably through binding to serum albumin, which is one of the most abundant proteins in blood serum, and which is also responsible for transport function.^{7,8} The ability of serum albumins to bind lead ions is well known, and has been used to detect micromolar concentrations of lead ions in solution.^{9,10} In this report we demonstrate how the sensitivity of this detection can be increased by several orders of magnitude reaching an important level of picomolar concentrations. To achieve this goal, we take advantage of the excitation emission matrix (EEM) method for fluorescence detection, which has been proven to be a valuable method for multicomponent analysis.¹¹ The EEM method helps to point out the excitation

wavelength where there are primary changes in fluorescence spectra.¹² We found the EEM method to be equally powerful for analysis of individual molecules, since a fluorescence spectrum from a typical protein has a rather complex structure.

In the EEM method, fluorescence spectra are recorded for a number of excitation wavelengths, which are scanned across the absorption spectrum. It provides a wealth of information, which can be further utilized for identifying the most appropriate excitation conditions. At the same time, fluorescence is a very sensitive detection method capable of analysis of single molecules in solution and minute changes in environmental conditions.¹³ In a particular example of human serum albumin (HSA), we found a relatively weak fluorescence at 430 nm, excited by 350 nm light, to be the most affected by the presence of lead ions in solution.

The buffer solution, prepared in accordance with Ref. 14 by adding disodium hydrophosphate (Na_2HPO_4), sodium dihydrophosphate (NaH_2PO_4), and sodium chloride (NaCl) to de-ionized, double distilled water, was maintained at $\text{pH} = 7.0$.¹⁴ All chemicals were purchased from Sigma-Aldrich, Inc. and were used without further purification. The concentration of HSA (Sigma-Aldrich, Inc.) in buffer solution was 1 mg/ml (15 μM). The buffer solution of albumin was prepared and kept for 24 h in a dark and dry place at a temperature about 4 °C before taking all the described measurements. The lead salt solution was prepared by dissolving an appropriate amount of lead nitrate [$\text{Pb}(\text{NO}_3)_2$] (Sigma-Aldrich, Inc.) in de-ionized, double distilled water. We used a commercially available Fluorolog-3 (Jobin-Yvon, Inc.) for collecting the fluorescence spectra. The fluorescence signal from a solution, placed in a quartz cell, was collected in a back-reflection geometry, which minimizes the effect of light propagation inside the absorbing medium. An experimentally recorded EEM consists of 31 individual emission spectra (in the spectral region from 260 to 600 nm) for a range of the excitation wavelength from 250 to 400 nm with sequential increments of 5 nm. Fluorescence spectra were recorded with an integration time of 15 s using excitation and emission slit-widths of 2 nm. The semilogarithmic fluorescence intensity plot of EEM data of HSA in buffer solution is shown in Fig. 1(a). Figure 1(b) shows, for comparison, the EEM of albumin treated with 10 pM concentration of lead solution. The increase in color intensity at around 430 nm excitation wavelength region of Fig. 1(b) reveals an evolution of fluo-

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FIG. 1. (Color online) Semilogarithmic plot of excitation emission spectra of HSA in (a) a buffer solution and (b) in a buffer solution treated with lead ($\text{Pb}^{2+} = 10 \text{ pM}$).

rescence with the addition of lead. To ensure the stability of the solution, the experiment was repeated several times after the scan was completed. The spectra were not normalized to instrument response function since all the data were compared to each other and no absolute measurements were needed. We also found that the shape of the EEM was not affected by the change in albumin and/or Na^+ concentrations, which were always present in the solution. To make sure that the presence of lead salt in the solution does not contribute to fluorescence spectra, we took the whole EEM spectrum of a very high concentration lead solution. The EEM spectrum appears to be mainly dominated by Raman spectrum of water and adds no significant contribution to typical HSA fluorescence spectra, which are several orders of magnitude stronger at any excitation wavelength.

Strong fluorescence signal, excited at 275 and 295 nm wavelengths (see Fig. 1), comes from two aromatic residues of tyrosine (Tyr) and tryptophan (Trp) located in subdomain IIIA and 214th residue of subdomain IIA, respectively.^{15,16} No other fluorescence emission is observed other than a weak Raman signal from water at around 3400 cm^{-1} with respect to the excitation wavelength, which was always present and could be, if needed, digitally removed from those spectra. Next, the albumin solution was treated with lead-containing solution starting from 1 pM (10^{-12} M) concentration. No significant changes were observed in the shape of the EEM spectra for the lowest concentration (10^{-12} M); however, with as little as 10 pM concentration of lead, a fluorescence peak at around 430 nm , when excited at around 350 nm , becomes more and more noticeable. The evolution of fluorescent spectra excited by 350 nm radiation is shown in Fig. 2(a) for increasing concentration of lead ions. The amplitude of the fluorescence signal changes dramatically. In contrast, the fluorescence emission excited by 275 nm radiation shows a gradual decrease with increase in concentration of lead [Fig. 2(b)]. However, the observed changes in fluorescence emission excited by 295 nm excitation are less pronounced. At the same time, the fluorescence signal, excited by 350 nm radiation, shows a remarkable sensitivity to the presence of lead ions in solution.

The most preferred binding site for lead ion is the thiol ($-\text{SH}$) group,¹⁷ which has a high affinity constant (logarithm of equilibrium constant) of 4.9 at physiological pH level.¹⁸ The only thiol group present in albumin is associated with Cys 34, which is present in domain I of HSA.⁹ The nearest Tyr, Tyr 84, which surrounds the sulfur of Cys 34, gets affected due to the binding of lead ion with Cys.¹⁶ Fluorescence spectra for excitation at 275 nm , which corresponds to Tyr show a gradual decrease with an increase in concentration of lead ions [Fig. 2(b)]. There is no significant change in fluorescence for 295 nm excitation from which it can be inferred that lead, however, is not affecting Trp.

The increased sensitivity of visible fluorescence of HSA to the presence of heavy ions in solution is based on the electrostatic interactions in the solution. Albumin molecules have a negative surface charge, which under normal pH conditions introduces repulsive interaction between protein molecules. When a large positive heavy ion is introduced into solution, it interacts with a protein, forming a Coulomb complex with a common hydration shell (as it is illustrated in the Fig. 3). Binding of the lead ions compensates the surface charge of protein molecules, and thus the protein molecules, which were experiencing a Coulomb repulsion initially, now

FIG. 2. (Color online) Fluorescence intensity of HSA treated with lead solution. Lead concentrations are (a) 0, (b) 10^{-11} , (c) 10^{-10} , (d) 10^{-9} , (e) 10^{-8} , (f) 10^{-7} , and (g) 10^{-6} M . Excitation wavelengths are (a) 350 nm and (b) 275 nm .

FIG. 3. (Color online) Schematic cartoon diagram illustrating the interaction of HSA molecule with a lead ion forming a dipole.

starts experiencing dipole-dipole interaction. It creates favorable conditions to form macromolecular complexes (agglomerates) composed of albumin molecules. Those complexes can be the source of the observed visible fluorescence. The exact origin of fluorescence at around 430 nm is still unclear. Other reports suggest that aggregates of albumin in the solution, caused by prolonged storage of albumin in solution, can also produce a fairly strong fluorescence emission in this spectral range.¹⁹ There are also indirect indications that it might be due to the lysine residues and their interaction.²⁰

To verify the increasing presence of agglomerates in solution, we used a light scattering apparatus (Zetasizer microvolt; Malvern Instruments, Inc.), which measures the size distribution of molecular species in the solution. Indeed, starting with 10^{-9} M concentration of lead ions in solution, the protein agglomeration was observed confirming our hypothesis. At lower concentration of lead ions, the sensitivity of light scattering detection was not sufficient to determine the presence of agglomerates in the solution.

In summary, we have demonstrated the ultrahigh sensitivity of visible fluorescence signal of albumin to the presence of heavy ions in the solution. While the detection lacks

specificity, i.e., the same mechanism should be applicable to Cd^{2+} and other positive ions as well; the proposed fluorescence sensor might find its way as an early indicator of a potential threat. Given the simplicity of the detection scheme and its incredible sensitivity, which is several orders of magnitude lower than the currently known clinically relevant level of detection, the proposed sensor can be easily integrated into a microfluidic platform, providing a rapid and sensitive assessment of the water quality.

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