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Characterization of interactions between organotin compounds and human serum albumin by capillary electrophoresis coupled with inductively coupled plasma mass spectrometry

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ABSTRACT

Thermodynamic data such as the binding constants are vital parameters describing interactions between exotic trace compounds and biomolecules in biochemical property modeling. In this study, the stability constants of organometallic compound and protein complexes were studied by using capillary electrophoresis coupled with inductively coupled plasma mass spectrometry (CE-ICP-MS), considering its low detection limits and low sample demand. Four organotin compounds (trimethyltin (TMT), tripropyltin (TPrT), tributyltin (TBT), triphenyltin (TPhT)) and human serum albumin (HSA) were used as model organometallic compounds and protein, respectively. Affinity capillary electrophoresis (ACE) and nonequilibrium capillary electrophoresis assays of equilibrium mixtures (NECEEM) were performed and compared by using ICP-MS as the detector to determine the binding constants of organotin compounds and HSA in 1:1 molar ratio assumption. Constant measurements of the two methods were both simple, however, ACE assays were more accurate and more appropriate for the constant determination of the organotin–HSA complexes, considering the errors of the NECEEM method. A good precision of the binding constants ($\log K_b$) using the ACE method was proved by different mathematical calculations, and the values were 6.13 ± 0.51 (TMT), 5.72 ± 0.38 (TPrT), 5.68 ± 0.34 (TBT), 6.05 ± 0.38 (TPhT) respectively for each of the organotin–HSA complexes, showing non-covalent interaction between organotin compounds and HSA. Meanwhile, this study also confirms the suitability of CE-ICP-MS method for further studies on organometallic complexation.

1. Introduction

Non-covalent molecular complex is an important part in regulatory biological processes, such as signal transduction, gene expression and the immune response [1–4]. Non-covalent interactions between biological molecular and exotic compounds, such as trace metals and drugs, have been aroused great interests in biology and medicine studies. Most metallic compounds could form non-covalent complexes with macromolecules in the biological fluid and organs. As one kind of biological molecules, proteins are important parts of organisms and participate in virtually every process within cells. The interaction between proteins and metallic compounds has been considered as an important aspect in the biological study, which does not only regulate the uptake and accumulation of the metallic compounds in the human body but also determine the overall distribution, excretion, differences in efficacy, and toxicity. Metallothionein (MT) is one kind of metal binding protein synthesized primarily in the liver and kidneys, which provide protection against metal toxicity and oxidative stress. Besides, metal ions and compounds could be used as the active sites in the metalloproteins, working as biocatalysts for the reactions during metabolism and so forth. The binding parameters such as dissociation constants, binding constants and stoichiometry are important to describe the non-covalent interactions. Hence, the characterization of the binding phenomenon and the determination of binding parameters are essential for the evaluation of bioaffinity of the metallic chemicals and the interactions between proteins and these compounds.

As a group of the organometallic compounds, organotin compounds were widely applied as PVC stabilizers, pesticides and additives in many industrial and chemical areas [5]. The extensive applications of organotin compounds have resulted in extensive distribution of these compounds in environmental matrixes and various biological samples such as human blood, urine, liver and hair. It is well known that the organotin compounds could cause damage to the neurological system and sexual system. In addition, tributyltin (TBT) and triphenyltin (TPhT) could interfere with

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the cytotoxic function, which have association with the cancer incidences. Although the toxicity of organotin compounds has been well established, there was still a lack of data concerning on the kinetics and the toxicological mechanism of the interaction between organotin compounds and the biomolecules. HSA is a large globular protein with a good essential amino acid profile and it is one of the most abundant proteins in human plasma. In its three-dimensional structure, it bears some drug binding sites which show high selectivity to exotic chemicals and also contributes significantly to the transport of many endogenous and exogenous ligands [6]. Thus, HSA is an important plasma protein in blood and may determine fate, disposition, metabolism, and the toxicity of small chemical compounds in human bodies [7,8]. The investigation of interactions between organotin compounds and HSA were important to illuminate the mechanism of the transportation and biological function of organotin compounds in human bodies. However, no comprehensive studies on the interactions and the kinetics of organotin compounds with HSA have been reported elsewhere.

Nowadays, several techniques have been developed to qualitatively and quantitatively study the binding of organometallic compounds to proteins such as HSA and transferrin protein [9–11]. Among these different methods available for the biomolecular interaction analysis, capillary electrophoresis (CE) has emerged as a powerful technique for quantification of binding interactions. Compared with traditional biological binding assays and NMR techniques, CE requires little sample volume and the operation and separation procedures could be achieved under physiological conditions to avoid the impact on weak interaction of biomolecules in denature conditions. Besides, CE could be used not only for the determination of the binding constants or relative affinity, but also the determination of interaction kinetics is possible. Among the various biomolecular non-covalent interaction investigation methods, ACE and NECEEM methods are the most commonly used methods. ACE is fast and low-consuming for studying molecular interactions. It is performed by dissolving one of the interactants in varying concentrations in the running buffer to observe the changes of the other substrate's migration mobility. ACE method can be performed in physiological solutions to preserve biological interactions between chemical compounds and biomolecules, and is thus more promising than the techniques operated in denaturing mode. In addition, ACE does not suffer from the complication resulting from two-phase chromatographic systems and is therefore particularly suited to study complex formation reactions [12]. NECEEM is another analytical approach, which could also be used to determine the binding constants of the non-covalent interaction between biomolecules and compounds. The method was first introduced to study of protein–probe and DNA–protein interaction in 2002 [4,13,14]. In NECEEM method, a short plug of the equilibrium mixture was introduced into the capillary and subjected to electrophoresis under non-equilibrium conditions. Kinetic parameters such as binding constant (K_b) and monomolecular rate constant (k^{-1}) could be determined in a single experiment which requires only a little amount of the protein in a few minutes [15–17].

Due to the much lower detection limits, CE-ICP-MS system is more attractive for the study of covalent and non-covalent organometallic compounds–protein complexes at trace level compared with CE-UV technique. CE-ICP-MS has already been used in various studies of the interaction of metal drugs and other metal compounds, such as mercury species, with protein or other biomolecules [10,18–20]. However, despite the extensive use, CE-ICP-MS has never been explored to investigate the non-covalent interactions of xenobiotic heavy metals with HSA by using ACE and NECEEM methods. In this study, the applications of ACE and NECEEM were tested and compared to investigate the binding constants of the interaction between the four organotin compounds (TMT, TPrT, TBT, and TPhT) and HSA, while using ICP-MS as an on-line detector.

2. Materials and methods

2.1. Chemicals and reagents

HSA (96–99%, fraction V) was purchased from Sigma–Aldrich (St. Louis, MO). A protein stock solution of 5 mg mL^{-1} was prepared by dissolving 20.0 mg HSA into 4 mL distilled deionized water (DDW) (18 MΩ cm), which was obtained from Milli-Q Advantage A10 system (Millipore, Bedford, MA). Working solutions of HSA were prepared by serial dilution of the stock solution with the phosphate buffer. The phosphate buffer (10×, pH = 7.4) used for incubation was purchased from Sigma–Aldrich (St. Louis, MO).

Trimethyltin chloride (TMT, 99% purity), tributyltin chloride (TBT, 97% purity), tripropyltin chloride (TPrT, 98% purity) and triphenyltin chloride (TPhT, 96% purity) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4{\cdot}2H_2O$) was from Beijing Chemical Co. (Beijing, China); ammonium acetate (NH_4Ac) and disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$) was from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). All the reagents were at least analytical grade. The electrolyte buffer of phosphate was filtered through a 0.22 μm membrane and degassed in ultrasonic bath before use.

2.2. Instrumentation

The schematic diagram for the instrument of CE-ICP-MS system has been published elsewhere [10,19,21]. In brief, the separations of the ACE and NECEEM methods were operated on HP^{3D} CE system (Agilent, Germany). Bare fused-silica capillaries (50-cm long × 75-μm i.d.) used in the experiment were obtained from Yongnian Optical Fiber Company (Hebei, China). The sample solution was introduced into the capillaries by using a hydrodynamic method under a gas presser of 20 mbar for 5 s. Separations were performed under a positive voltage of 20 kV. Every new capillary was conditioned by flushing with 1 mol L^{-1} NaOH for 60 min, 0.1 mol L^{-1} NaOH for 60 min, DDW for 10 min, and then, electrolyte running buffer for 60 min. Prior to each separation, the capillaries were flushed with 0.1 mol L^{-1} NaOH and electrophoresis buffer for 2 min, separately.

An Agilent 7500ce ICP-MS (Agilent, USA) was coupled with the HP^{3D} CE system for the analysis. A MicroMist nebulizer (GE, Australia) was used with a nominal flow rate of 0.1 mL min^{-1}. The plasma gas flow rate was 15 L min^{-1}; carrier gas flow rate was 0.7 L min^{-1} and the makeup gas flow rate was 0.4 L min^{-1}. The plasma RF power was 1500 W. Signals from the four isotopes (^{117}Sn, ^{118}Sn, ^{119}Sn, ^{120}Sn) of tin were monitored in Full-Quant mode (Table 1).

Interfaces of CE coupled with ICP-MS were considered as a key part in the hyphenation technique. Detailed diagram of interface used in this study could be found elsewhere [21]. Briefly, a Pt electrode, a sheath flow and the CE capillary were introduced into the interface based on a cross design. The Pt electrode was grounded. The sheath flow was used to wet the outlet of capillaries and satisfy the demands of closing the electrical circuit from CE. In addition, the sheath flow was also used to transport the outlet of CE capillaries to the MicroMist nebulizer by self-aspiration. To minimize the diffusion of sample zone, the CE capillary was inserted to the end of the nebulizer through a 1/16-in. PEEK tube. 10 mM NH_4Ac solution was used as the sheath flow. The experimental conditions of the CE-ICP-MS hyphenated technique have been listed in Table 1.