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Mobilization of Cd from human serum albumin by small molecular weight thiols

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ABSTRACT

Although the toxic metal Cd is an established human nephrotoxin, little is known about the role that interactions with plasma constitutents play in determining its mammalian target organs. To gain insight, a Cd–human serum albumin (HSA) complex was analyzed on a system consisting of size exclusion chromatography (SEC) coupled on-line to a flame atomic absorption spectrometer (FAAS). Using phosphate buffered saline (pH 7.4) as the mobile phase, we investigated the effect of 1–10 mM oxidized glutathione (GSSG), L-cysteine (Cys), L-glutathione (GSH), or *N*-acetyl-L-cysteine (NAC) on the elution of Cd. As expected, GSSG did not mobilize Cd from the Cd–HSA complex up to a concentration of 4 mM. With 1.0 mM NAC, ~30% of the injected Cd–HSA complex eluted as such, while the mobilized Cd was lost on the column. With 1.0 mM of Cys or GSH, no parent Cd–HSA complex was detected and 88% and 82% of the protein bound Cd eluted close to the elution volume, likely in form of $Cd(Cys)_2$ and a Cd–GSH 1:1 complex. Interestingly, with GSH and NAC concentrations >4.0 mM, a Cd double peak was detected, which was rationalized in terms of the elution of a polynuclear Cd complex baseline-separated from a mononuclear Cd complex. In contrast, mobile phases which contained Cys concentrations $\geq$2 mM resulted in the detection of only a single Cd peak, probably $Cd(Cys)_4$. Our results establish SEC–FAAS as a viable tool to probe the mobilization of Cd from binding sites on plasma proteins at near physiological conditions. The detected complexes between Cd and Cys or GSH may be involved in the translocation of Cd to mammalian target organs.

1. Introduction

The toxic metal cadmium ranks 65th in elemental abundance in the Earth's crust (0.11 mg Cd/kg) [1]. Despite this comparatively low ranking, Cd is being increasingly recognized as a 'priority pollutant' [2] since evidence is accumulating that this metal is biomagnified in the aquatic food chain [3] and may therefore adversely affect entire ecosystems [2]. In addition, the analysis of human urine and blood from the average human population has revealed that up to ~5% of the U.S. population is potentially at risk from low level exposure to Cd [4–9], predominantly via the ingestion of contaminated food [10,11] and possibly also due to the exposure to consumer products [12,13]. Furthermore, occupational exposure of Cd represents an ongoing problem [14]. Even though extensive research efforts have been directed toward a better understanding of the mammalian toxicology of Cd, considerable knowledge gaps remain [15]. The role that the bloodstream plays in the context of its toxicity and its distribution to target organs [16] represents a case in point. In principle, Cd may exert systemic toxicity by adversely affecting the distribution of essential ultra-trace elements (e.g. selenium) to organs [16,17]. Alternatively, this toxic metal is also known to exert selective toxicity (e.g. to the kidneys [18] and testes [19]), but details regarding the blood-based mechanisms which deliver Cd to these target organs remain elusive [15].

Although Cd is known to bind to human serum albumin (HSA) [20,21], transferrin [22–25], α_2-macroglobulin [26–28], and ferritin [29,30], it is currently unknown which of these or possibly other plasma proteins play a role in shuttling this toxic metal to its toxicological target organs. Studies with rats have revealed that intravenously administered Cd (0.4 mg/kg body weight) – despite its initial binding to rat serum albumin – is rapidly cleared from the bloodstream (within ~30 min) [31] by an as yet unknown mechanism. Similar experiments (also with rats; 1.0 mg Cd/kg) resulted in the initial decrease of the blood Cd concentration over a 3 h period, followed by an increase 60 h after administration [32]. The rapid disappearance of Cd from the bloodstream could involve

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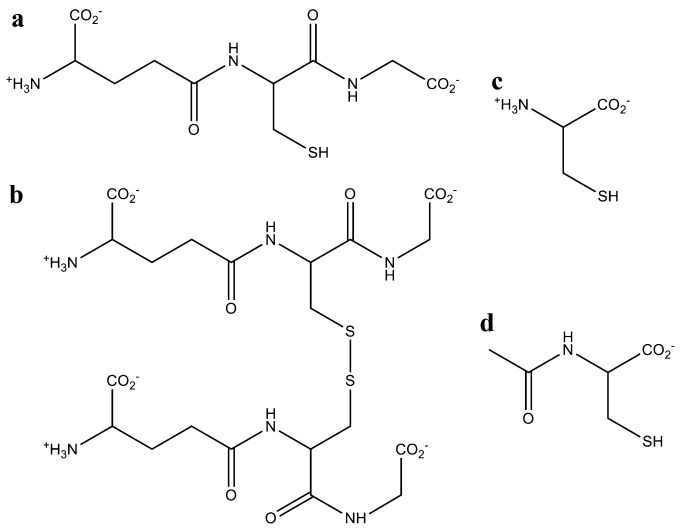

Fig. 1. Structures of L-glutathione (a), oxidized glutathione (b), L-cyteine (c) and *N*-acetyl-L-cysteine (d).

the binding of Cd to one or more plasma protein(s), followed by receptor-mediated endocytosis of the formed complex(es) [15,33] as has been reported for the uptake of Cd-metallothionein into proximal tubules in the kidney [34,35]. Alternatively, an initially formed Cd-plasma protein complex (primary interaction) may interact with endogenous or exogenous small molecular weight (SMW) ligands (secondary interaction) which may abstract Cd. The formed SMW ligand–Cd complex could then be translocated to the target organ(s) by specific membrane transport systems [36–39]. The latter mechanism does, in fact, appear to play an important role in the mammalian metabolism of Cu [40]. Considering that the co-administration of rats with Cd and L-cysteine (Cys) or L-glutathione (GSH) in one solution greatly increased the translocation of Cd to the kidneys (1 h after their co-administration [36]), it is possible that Cd–SMW thiol complexes are translocated from the blood-stream to the kidneys. Alternatively, the interaction between Cd, plasma proteins with distinct Cd binding sites and SMW thiols may play a role in the mechanism which directs Cd to its target organs [41]. The latter scenario appears feasible given that SMW plasma thiols are not in equilibrium, but in a steady state maintained in part by their transport between tissues during their metabolism [42].

To this end, we have analyzed a Cd–HSA complex on a LC system consisting of size exclusion chromatography (SEC) coupled on-line to a flame atomic absorption spectrometer (FAAS; Cd-specific detector). Using phosphate buffered saline (PBS) as the mobile phase, the effect of increasing concentrations of SMW ligands (1.0–10 mM) in the mobile phase on the elution of Cd was investigated. SMW ligands (Fig. 1) were selected based on either their presence in the mammalian bloodstream {oxidized glutathione (GSSG), Cys, GSH [42] and histidine (His) [43]} or the ability of exogenous ligands to decrease the toxicity of Cd in rats {*N*-acetyl-L-cysteine (NAC) [44]}. The results were intended to provide insight into the toxicological mechanism which is possibly involved in delivering Cd to its mammalian target organs.

2. Material and methods

2.1. Chemicals

An HSA stock solution (Alburex®—Human Albumin USP) containing 12.5 g of HSA in 50 mL of buffered diluents was obtained from CSL Behring AG (Wankdorfstrasse 10, 3000 Berne 22, Switzerland; LOT 4309900005). Phosphate buffered saline (PBS) tablets were acquired from Sigma-Aldrich (St. Louis, MO, United States). $CdCl_2$ (>99%), L-cysteine (>99%), L-glutathione (>99%), *N*-acetyl-L-cysteine (>99%), L-histidine (>99%) and oxidized L-glutathione (>98%) were obtained from Sigma-Aldrich (St. Louis, MO, United States). All solutions were prepared with distilled water from a Simplicity Water Purification System (Millipore, Billerica, MA, USA).

2.2. Solutions

An aqueous stock solution containing a Cd–HSA complex was prepared by slowly adding 300 μL of a 5000 ppm Cd stock solution (prepared by dissolving 0.083 g of $CdCl_2$ in 10 mL) to 150 mL of a 45 g HSA/L solution prepared by adding 27 mL of Alburex® 25 to the 150 mL mark with PBS buffer while stirring (HSA concentration range in healthy adults: 35–54 g/L[45]). The final pH of the solution containing the Cd–HSA complex was 7.39 (Symphony SB20 pH Meter, Thermo Electron Corporation, Beverly, MA, USA). Although the Cd:HSA molar ratio was 1:7, this solution corresponds to a de facto 1:1 Cd:HSA complex. Although commercially available HSA is likely to contain some oxidized HSA, this is irrelevant in the context of this study as Cd is known not to bind to the Cys-34, but to two binding sites with equal affinity [20,46]. One of the two binding sites consists of two His (67 in domain I and 247 in domain II), one Asp (249 in domain II), and one Asn (99 in domain I) [21,47], while the other binding site is still unknown. Aliquots of this solution (1.0 mL) which contained 10 μg Cd were transferred to Eppendorf tubes, frozen at −30 °C overnight and then transferred to a liquid nitrogen dewar until use. Mobile phase buffer was prepared by dissolving PBS buffer tablets in the appropriate volume of distilled water followed by filtration through a Whatman 0.45 μm Nylon filter membrane (GE Healthcare, Buckinghamshire, UK). All mobile phases that contained SMW ligands (1–10 mM) were prepared by dissolving one PBS buffer tablet in 180 mL distilled water followed by the addition of the appropriate amount of ligand. After adjustment of the pH to 7.4, the solution was filled up to the 200 mL mark with PBS buffer. Because of the rapid oxidation of reduced thiols at pH 7.4, all experiments were conducted immediately after the preparation of the mobile phase. Following the equilibration of the column for 1 h at a flow rate of 1.0 mL/min, the Cd–HSA complex was injected in triplicate. Owing to the poor solubility of L-cystine in water, this ligand was not investigated.

2.3. Instrumentation

The employed HPLC system consisted of a 426 HPLC Pump (Alltech Associates, Inc., Deerfield, IL, USA), a Yarra 3 μm SEC-3000 (300 × 7.8 mm) column (fractionation range between 700 and 5 kDa) in conjunction with a Rheodyne six-port injection valve (500 μL PEEK sample-loop). The flow rate was 1.0 mL min^{-1} and Cd detection was accomplished by connecting the exit of the HPLC-column to the pneumatic nebulizer of a Buck Model 200A flame atomic absorption spectrometer (FAAS)(Buck Scientific, East Norwalk, CT, USA) at 228.8 nm (Cd). The FAAS was operated with an air/acetylene flame (oxidant pressure: 241 kPa, fuel pressure: 75 kPa).

2.4. Analysis of a Cd–HSA 1:1 complex

The HPLC system was equilibrated with the mobile phase of interest using a flow rate of 1.0 mL/min (60 min). After thawing each Cd–HSA 1:1 complex aliquot at room temperature (45 min) and incubation at 37 °C (30 min), aliquots (0.5 mL) were injected onto the SEC–FAAS system and data collection was initiated. For every investigated mobile phase the Cd–HSA 1:1 complex was analyzed in triplicate and representative chromatograms are depicted. Raw