Linking molecular targets of Cd in the bloodstream to organ-based adverse health effects

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Abstract

The chronic exposure of human populations to toxic metals remains a global public health concern. Although chronic Cd exposure is linked to kidney damage, osteoporosis and cancer, the underlying biomolecular mechanisms remain incompletely understood. Since other diseases could also be causally linked to chronic Cd exposure, a systems toxicology-based approach is needed to gain new insight into the underlying exposure-disease relationship. This approach requires one to integrate the cascade of dynamic bioinorganic chemistry events that unfold in the bloodstream after Cd enters with toxicological events that unfold in target organs over time. To this end, we have conducted a systematic literature search to identify all molecular targets of Cd in plasma and in red blood cells (RBCs). Based on this information it is impossible to describe the metabolism of Cd and the toxicological relevance of it binding to molecular targets in/on RBCs is elusive. Perhaps most importantly, the role that peptides, amino acids and inorganic ions, including HCO_3^-, Cl^- and $HSeO_3^-$ play in terms of mediating the translocation of Cd to target organs and its detoxification is poorly understood. Causally linking human exposure to this metal with diseases requires a much better integration of the bioinorganic chemistry of Cd that unfolds in the bloodstream with target organs. This from a public health point of view important goal will require collaborations between scientists from different disciplines to untangle the complex mechanisms which causally link Cd exposure to disease.

1. Introduction

Cadmium (Cd), which is present in the earth's crust in form of Cd-bearing rocks and sulfidic minerals (e.g. greenockite) ranks 65th in elemental abundance.[1] While this metal is obtained mostly as a by-product of Zn-mining, the useful properties of its compounds, notably the desirable yellow pigment cadmium yellow (CdS), resulted in the increased production of Cd since the 1920s to ~25,000 t/yr in 2020.[2] While occupational exposure has been linked to adverse human health effects since 1858,[3] the danger that is associated with its inadvertent introduction of Cd^{2+} into the food supply was brought to worldwide attention in the 1960's in an outbreak of 'itai-itai' disease in Japan.[4] Subsequent systematic studies to better understand the health effects that are associated with chronic Cd exposure revealed that people aged 20-80 years who lived near industrial smelters in Belgium and had never been occupationally exposed to Cd exhibited changes in proximal tubular function of the kidneys.[5] More recent studies have provided evidence that the chronic exposure of human populations to Cd is associated with additional adverse health effects including the cardiovascular system.[6, 7] The recognition that the oral ingestion of only three times as much Cd/day was responsible for itai-itai disease compared to the Japanese diet today [8] underscores the need to minimize the dietary intake of Cd, of which 5-10% is absorbed from the GI tract into the blood circulation.[9]

While toxicological research has greatly contributed to better define maximum permissible Cd concentrations in food and drinking water[4] and metallothioneins were identified as important biomolecular targets in mammalian organs,[10] many aspects about how chronic exposure to this toxic metal exerts adverse health effects in humans remain unknown.[11-14] In principle, there are three reasons why we need to advance our understanding of the biomolecular basis underlying the chronic toxicity of Cd in humans. Firstly, the lack of a biochemical mechanism to excrete Cd

translates to a steady bioaccumulation of this metal in various internal organs over a lifetime,[15] with health consequences that are not fully understood.[13] Secondly, several recent studies indicate that chronic Cd exposure is associated with more adverse health effects in humans in addition to its established adverse effects on the kidneys, bones (osteoporosis) and its well established carcinogenicity.[4, 6, 16, 17] It has been hypothesized that chronic Cd exposure may be linked to diabetes, the prevalence of which is skyrocketing in many parts of the world.[7, 18] Last but not least, the important role that the bioinorganic chemistry of Cd in the bloodstream plays in the context of defining organ-based disease has only been recently fully recognized[19] and opens the door to develop potential palliative dietary additives to deliberately funnel Cd out of the body before organ damage occurs.[20]

While the adverse human health effects that are associated with the chronic exposure to Cd have been reviewed by others [7, 9, 13, 21-23], the underlying bioinorganic processes that ultimately determine its chronic toxicity are incompletely understood.[24] To gain deeper insight requires a systems toxicology approach[25] that integrates the bioinorganic chemistry of Cd in the bloodstream with the toxicological effects that eventually unfold in target organs.[12, 19] This approach necessitates one to establish the complete sequence of interactions of Cd with endogenous ligands to biomolecular disease process. Thus, this perspective is not intended to be a comprehensive review on Cd toxicity in humans. We first present an overview of a comprehensive literature search to identify all molecular targets of Cd in blood plasma/serum and in red blood cells (RBCs) that have been reported to date. We then provide a brief synopsis of the biomolecular mechanisms which facilitate the uptake of Cd into RBCs/target organs and summarize the mechanisms by which Cd induces organ damage. Last but not least, we identify critical knowledge

gaps that need to be addressed to establish the biomolecular mechanisms which causally link chronic Cd exposure to known adverse health effects.

1.1 Systematic literature search

PubMed and the Web of Science were used to search for articles that identify plasma and RBC proteins that are targeted by Cd. With regard to plasma proteins, a PubMed search was conducted using the keywords "cadmium", "plasma protein", "blood OR bloodstream" and "human OR humans OR Homo sapiens OR H. sapiens" and "human", which yielded 596 results (May 04, 2020). Similarly, a Web of Science search using the same keywords (except for "plasma protein") yielded 94 results (May 04, 2020). With regard to RBC proteins, PubMed and Web of Sciences searches using the keywords "cadmium", "red blood cell OR erythrocyte", "binding OR interaction OR bind" and "human OR humans OR Homo sapiens OR H. sapiens", along with "human" yielded 66 and 42 results, respectively (June 08, 2020). The title and abstract of each article were evaluated and subject to exclusion criteria, including (i) its unavailability in english (ii) no explicit mention of Cd^{2+} (iii) epidemiological studies that did not involve specific biochemical interactions (iv) the use of Cd primarily in the context of nanotechnology (e.g. quantum dots) and not free Cd^{2+} (v) the main findings were invalidated by subsequent studies (vi) a focus on Cd-binding proteins or interactions that do not occur in the blood plasma or RBCs (e.g. liver, kidney, etc.) and (vii) exclusive reliance on samples from animals, but not humans (e.g. rabbits, rats, etc.). Any additional references that were found during the literature search were used if they met these criteria.

1.2 Biomolecular Cd targets in blood plasma/serum

Eleven plasma proteins have been shown to exhibit an affinity for Cd (**Table 1**). While these protein targets are present at plasma concentrations between 1.3 and 750 μM, we have classified them into primary and secondary molecular targets based on whether there is considerable or scant evidence for Cd binding (**Figure 1**). Since key thermodynamic binding parameters of proteins (K_d values) orchestrate the metabolism of metal ions in complex biological fluids [26] and considering that values for Cd for only three plasma proteins have been reported, its dynamic exchange between these plasma protein targets is little understood. While the K_d of Cd for human serum albumin (HSA) implies a major role in its metabolism, not much is known about the K_d's of the remaining plasma proteins (**Table 1**). Indeed, a metallomics study that was conducted with rabbit plasma demonstrated that 87% of Cd that was added to plasma apparently bound to rabbit serum albumin (RSA, ~66 kDa), while 13% eluted bound to other plasma proteins (158-600 kDa).[27] Owing to the limited chromatographic resolution of the employed size-exclusion chromatography (SEC) column it is therefore possible that Cd eluted bound not only to RSA (66 kDa), but possibly also to hemopexin (57 kDa), histidine-rich glycoprotein (HRG, 75 kDa) and/or transferrin (Tf) (80 kDa). Furthermore, the binding of Cd to 158-600 kDa plasma proteins range suggests that apolipoprotein B-100 (497 kDa), immunoglobulin A (160 kDa) and G (150 kDa) must be considered as possible binding partners. The interaction of Cd with apolipoprotein A-1 and B-100 establishes a potential link between Cd exposure and the metabolism of cholesterol and fats,[28] while its binding to the immunoglobulins may affect the body's defense against bloodborne infections, which has been observed in mice [29] and could be implicated in its immunotoxicity.[30] It is entirely unclear which of the 11 plasma protein targets effectively represent chaperones which may direct its toxic cargo to specific target organs for subsequent

uptake (e.g. by receptor mediated endocytosis and/or macropinocytosis) to gain deeper insight into the exposure-disease relationship.

Table 1. Overview of studies which have identified plasma proteins that Cd^{2+} can interact with/bind to. Top-down (TD) refers to studies in which Cd^{2+} was added to plasma (P)/serum (S) and bottom-up (BU) refers to studies in which Cd^{2+} was added to pure proteins and binding was observed. All information in rows 3-4 was obtained from the first reference in that row unless otherwise indicated. Values for stability constants and serum/plasma concentrations have been converted into K_d and µM, respectively.

Protein/Metalloprotein **• Size (kDa)** **○ Plasma/Serum Concentration**	**TD (S/P)/ BU**	**Technique(s) used**	**• Protein identification** **○ Binding information**	**• Other relevant details** **○ Protein structure**
α_2-Macroglobulin [31] (α_2M) • 725 kDa ○ 1.52 – 4.28 µM$^{(M)}$[32] ○ 1.93 – 5.06 µM$^{(W)}$[32]	BU	Cd^{2+}-incubated samples of 74% pure α_2M in 0.1 M Tris* buffer (pH 7.4) were applied to a SEC* column and collected fractions were analyzed for Cd^{2+} using AAS*.	○ 4$^{\#}$ binding sites and K_d (K_d = 9.6 x 10^{-7} M)[33]	• Number of Cd^{2+} binding sites was confirmed.[33] • 2D-IEP and ^{109}Cd radiolabeling was used to demonstrate Cd^{2+} binding to α_2M in serum.[34] ○ PDB: 4ACQ
Apolipoprotein A-I[35] • 30.8 kDa ○ 36.6 – 44.8 µM[36]	TD (P)	Plasma samples (n = 29) containing 0.08 ± 0.07 ng/mL Cd^{2+} from natural exposure of the blood donors was used. Following the removal of serum albumin and immunoglobulin G from the plasma, Cd^{2+}-binding proteins were separated using a Mini Prep Cell column GE apparatus (Bio-Rad). The resulting eluent was split, with half being analyzed by ICP-MS* for Cd^{2+} and the other Cd-containing fractions were concentrated for subsequent protein identification. A single cadmium-binding protein was detected in the samples.	• After 1D page analysis and staining, the Cd-binding protein was manually excised and qualitatively identified by MS/MS after trypsin-digestion.	○ PDB: 1AV1
Apolipoprotein B-100[37] • 497 kDa ○ 2.07 ± 0.48 µM$^{(M)}$[38] ○ 1.93 ± 0.52 µM$^{(W)}$[38]	TD (S)	After loading serum onto a Cd-loaded IMAC* column, the latter was washed with 20 mM BTS buffer* and bound proteins were eluted with 0.1 M EDTA*.	• The eluted proteins underwent trypsin digestion and were identified by LC-MS/MS*.	-
Ceruloplasmin[37] • 132 kDa ○ 1.52 – 3.03 µM[39]	TD (S)	see above.	• see above.	○ PDB: 1KCW

Hemopexin[37] 57.0 kDa ○ 8.77 – 20.0 µM[32]	TD (S)	see above.	• see above.	-
Histidine-rich glycoprotein[40] (HRG) • 75.0 kDa ○ 1.33 – 2.00 µM[41]	BU	A HRG-heme complex (10 heme:1 HRG) exhibits a characteristic UV absorption spectrum. Incubation of HRG dissolved in 0.1 M phosphate buffer (pH 7.1) with $CdCl_2$ (1 HRG: 20 $CdCl_2$) followed by the addition of heme (1 HRG:10 heme) resulted in a decreased absorption band at 414 nm compared to the HRG-heme control, indicating that Cd^{2+} obstructed the binding of heme to HRG.	○ Evidence that a Gly-His-His-Pro-His motif, which is repeated 12 times in the amino acid sequence of HRG, is responsible for heme and divalent metal ion binding (including Cd^{2+}).[42]	• Confirmation of the HRG repeat motif.[42]
Immunoglobulin A[34] • 160 kDa ○ 6.25 – 18.8 µM[43]	TD (S)	Serum proteins were separated by 2D-IEP*. Incubation of the gel with radioactive metal (^{109}Cd) species followed by the exposure of gel to X-ray film revealed Cd-binding proteins.	• Proteins were identified using serum which contained monospecific antibodies.	○ PDB: 2QTJ
Immunoglobulin G[34] • 150 kDa ○ 40 – 100 µM[44]	TD (S)	see above.	• see above.	○ PDB: 1HZH
Prealbumin (Transthyretin)[34] • 55.0 kDa ○ 5.45 – 6.00 µM(M)[45] ○ 4.55 – 4.91 µM(W)[45]	TD (S)	see above.	• see above.	○ PDB: 1BMZ
Human serum albumin (HSA)[46] • 66.5 kDa ○ 544 – 808 µM[32]	BU + TD (S)	Pure HSA (2 mM) in 0.1 M phosphate buffer (pH 7.0) in D_2O was titrated with 20 – 100 mM solutions of $CdCl_2$ using ^{113}Cd and ^{1}H NMR* spectroscopy. Finally, whole serum was titrated with Cd^{2+} and analyzed by ^{113}Cd-NMR spectroscopy.	○ Results indicate that Cd^{2+} binds to sites A and B with similar affinity.[46] ○ 2 strong binding sites A+B[46], (K_d = 5 x 10^{-6} M)[47] ○ 1 weak binding site[47] ○ Evidence provided that Cd^{2+}-binding site A is comprised of His67, His247, Asn99, and Asp249.[48]	• A BU experiment which used SEC in conjunction with PBS-buffer demonstrated that 0.5 mM D,L-homocysteine mobilizes Cd^{2+} from HSA, which is relevant to hyperhomocysteinemia.[49] • A BU equilibrium dialysis study investigated the binding of Cd^{2+} to HRG as a Cd-binding protein in competition with HSA. HRG was able to bind Cd^{2+} with higher affinity than HSA, but the binding is less relevant because of the comparatively lower HRG concentration.[50]

				• A BU study using circular dichroism and electron spin resonance spectroscopy in 0.15 M NaCl (pH 7.4) demonstrated the displacement of Cu^{2+} by Cd^{2+} from it's second strongest binding site in HSA.[51] ○ PDB: 1AO6
Transferrin[52] • 80.0 kDa ○ 22.3 – 44.3 µM(M)[32] ○ 22.5 – 46.5 µM(W)[32]	BU	Differences in the UV spectra (240 – 320 nm) of apo-transferrin were recorded after titration with $CdCl_2$ solution. Protein was dissolved in 10 mM Hepes*, 5 mM sodium bicarbonate at pH 7.4.	○ Cd^{2+} has a greater affinity for the C- than the N-terminal site.[52] ○ C-terminal: $K_d = 1.1 \times 10^{-6}$ M[52] ○ N-terminal: $K_d = 1.4 \times 10^{-5}$ M[52]	• ^{13}C and ^{113}Cd NMR spectroscopy were used to confirm the presence of one His binding residue in Cd-transferrin complexes.[53] • ^{113}Cd NMR spectroscopy revealed differences in the binding of Cd^{2+} to the N- and C-lobe.[54] ○ PDB: 2HAV

***Technique and Reagent Acronyms**: Size-exclusion chromatography (SEC); Atomic absorption spectroscopy (AAS); Gel Electrophoresis (GE); Gel Electrophoresis Inductively-coupled plasma mass spectrometry (GE-ICP-MS); Immobilized metal affinity chromatography (IMAC); Liquid chromatography (LC); 2-D Immunoelectrophoresis (2D-IEP); Nuclear magnetic resonance (NMR); 2-Amino-2-(hydroxymethyl)propane-1,3-diol (Tris); bis-Tris Saline (BTS); Ethylenediaminetetraacetic acid (EDTA); 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes).

[#]Evidence suggests that a single Cd^{2+} ion will bind each of the 4 monomer subunits.

[(M)]The concentration range provided refers to levels in men.

[(W)]The concentration range provided refers to levels in women.

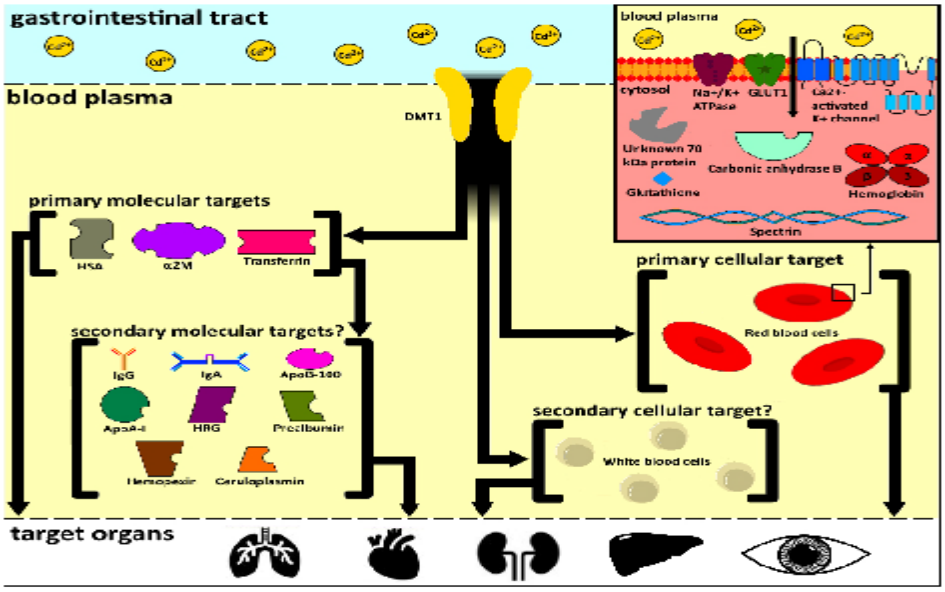

Figure 1 Cd^{2+} targets in the bloodstream. This includes plasma proteins, proteins on/in red blood cells, and white blood cells. Primary molecular targets include proteins where there is considerable evidence of Cd^{2+}-binding and a documented Cd^{2+}-binding site or a physiologically relevant K_d value has been reported. Secondary molecular targets include proteins where scant evidence is available for Cd^{2+}-binding and/or the K_d values have not been reported. Primary and secondary cellular targets been labelled based on their relative abundance in whole blood. Platelets were not considered since there is little evidence of Cd^{2+}-binding to platelet proteins in human systems. Acronyms: Immunoglobulin G (IgG); Immunoglobulin A (IgA); Apolipoprotein B-100 (ApoB-100); Apolipoprotein A-I (ApoA-I); Human serum albumin (HSA); α_2-Macroglobulin (α2M); Histidine-rich glycoprotein (HRG).

1.3 Biomolecular Cd targets on/in RBCs

Our literature search revealed eight biomolecular targets, which can be classified into three transmembrane proteins (Table 2, top half) and five cytosolic proteins/peptides (**Table 2**, lower half). Since lipid bilayer membranes also represents a possible biomolecular target for Cd, we refer the reader to comprehensive reviews that cover the interaction of Cd with various lipid model systems.[55, 56] Since Cd can target Ca^{2+} activated K^+ channel proteins, Na^+/K^+ ATPases and specific glucose uptake proteins (e.g. GLUT1), chronic Cd exposure may adversely affect the homeostasis of Na^+, K^+, Ca^{2+} [57] and glucose.[18]

In terms of cytosolic targets, hemoglobin, carbonic anhydrase, spectrin, an unknown ~70 kDa cytosolic protein [58] and the tripeptide glutathione [59] have been identified, but it is entirely unclear if any of the interaction of Cd with these may eventually compromise the integrity of the RBC membrane over its lifetime of 120 days.[60-62] Evidence in support of an adverse effect of Cd on cellular stability comes from experiments in which the exposure to 1.0 mM Cd^{2+} resulted in the transformation of disc/donut shaped RBCs to so-called 'echinocytes', which refers to RBCs that contain many small, evenly spaced thorny projections.[55] Since the actin-spectrin network gives RBCs their characteristic shape, it is not unreasonable to rationalize echinocyte formation with the binding of Cd to spectrin (Table 2). In this context it is noteworthy to mention that the lysis of RBCs will release hemoglobin, which in its free form is highly toxic to the kidneys.[63] To preclude this, the plasma protein haptoglobin sequesters free hemoglobin to form a haptoglobin-hemoglobin complex that prevents nephrotoxicity.[64] Thus, cumulative human exposure to Cd that eventually affects the integrity of the RBC membrane is an eminently important toxicological mechanism of action [65], particularly since studies with mice have revealed that chronic Cd exposure (50 ppm in drinking water) affected their half-life in the blood

circulation, possibly due to a higher accumulation of reactive oxygen species (ROS) therein.[61] Although different metallothionein (MT) isoforms (I-IV) are well established biomolecular targets of Cd in mammalian organs (e.g. the kidneys)[66], there is no compelling literature evidence in support of the presence of MT in human RBC cytosol. We do note, however, that less abundant mononuclear cells in blood have been found to express MT and thus form Cd-MT complexes therein.[67]

Table 2: Overview of RBC proteins/peptides that are located on the cell membrane and in the cytosol that have been shown to interact with Cd^{2+}. Top-down (TD) refers to studies in which intact RBCs (C) or RBC lysate (L) was used, whereas bottom-up (BU) refers to studies which utilized purer proteins. Information in row 3-4 was obtained from the first reference in that row unless otherwise indicated. Values for stability constants and serum /plasma concentrations may have different units than presented in the original source, but have been converted into K_d and μM, respectively.

Protein/Metalloprotein/ Peptide **• Size** **○ Erythrocyte Concentration**	**TD (L/C) / BU**	**Technique(s) used**	**• Protein identification** **○ Binding information**	**• Other relevant details** **○ Protein structure**
Integral membrane protein(s)				
Ca^{2+}-activated K^+ channel (IK)[68] - -	BU	Membranes were used to conduct single-channel inside-out patch clamp experiments in which the cytosolic channel segments were exposed to the solution. The current was measured when the membranes were immersed in a Hepes* buffer (10 mM, pH 7.2) to assess the activity. Cd^{2+}(1 – 100 μM) was added to the solution and compared to the current obtained for the control experiment (115 μM Ca^{2+} in Hepes* buffer).	○ Similar channel open times of Ca^{2+} to Cd^{2+} imply that these metal ions share a common binding site.	• Cd^{2+} was stimulatory at 1 - 10 μM and inhibitory at 100 μM. These results were confirmed in a follow-up study utilizing the same technique.[69]
GLUT1[70] • 54.1 kDa -	BU	Resealed RBC ghosts were treated with octyl glucoside (45 mM) to solubilize GLUT1 and the latter was then applied to a DEAE*-cellulose column followed by SEC*. After reconstituting GLUT1 into liposomes, the latter were incubated with a solution containing the unlabelled GLUT1 toxin cytochalasin B (10^{-7} M), a (^{3}H)-radiolabeled variant, and $CdSO_4$ (0 – 3 mM) for 30 minutes. After centrifugation of the	○ The percentage of cytochalasin B bound to GLUT1 decreased as Cd^{2+} concentration increased.	• 60% of cytochalasin B was bound in the absence of Cd^{2+}. This was decreased to 45% at 100 μM Cd^{2+}.

		liposomes, the resulting supernatant and pellets were analyzed (scintillation detector).		○ PDB: 4PYP
Na^+, K^+ ATPase[71] • ~152 kDa[#] -	BU	RBC ghosts were resealed in electrolyte solutions (pH 7.4) containing ATP[*] (5 mM) and either KCl (10 mM) or ouabain[†] (0.1 mM) for 30 min. These cells were exposed to $CdCl_2$ (≥ 1 nM), which allowed them to observe its effect on the activity of the pump. Cells were lysed with trichloroacetic acid, and the inorganic phosphate released from ATP hydrolysis was measured spectrophotometrically.	○ A Cd^{2+} concentration of 6.25 μM reduced the pump activity by 50% and was irreversible, even after treatment with EDTA[*].	• The inhibition was found to be non-competitive, as a reduced V_{max} was observed without change to the K_m values of Na^+, K^+, or ATP[*]. The channel saw a sharp decrease in activity between 1 – 10 μM.
Cytosolic protein(s)				
Apo-carbonic anhydrase B(I)[72] • 29.0 kDa -	BU	Carbonic anhydrase B was purified from human RBCs and Zn^{2+} was removed. The stability of protein-metal complexes was determined by equilibrium dialysis using 0.1 M acetate buffer at pH 5.5. The initial apoenzyme and metal concentrations were 4 x 10^{-5} and 3 x 10^{-5} M, while the Cd^{2+} concentration in the dialysate was determined spectrophotometrically using dithizone.	○ K_d = 6.30 x 10^{-10} M	○ PDB: 2CAB
Hemoglobin[73] • 64 kDa ○ 5.00 – 5.63 mM[74]	TD (L)	^{1}H-NMR[*] of lysate using a specific τ_2 allowed to observe the resonances for the C-4 and C-2 hydrogens on hemoglobin imidazole side chains (6.98 and 7.87 ppm, respectively).	○ $CdCl_2$ decreased the intensity of C-2 and C-4 resonances.	○ PDB: 1GZX
Spectrin[75] • 480 kDa /heterodimer -	BU	Spectrin was isolated from RBC ghosts using a buffer that contained phosphate (0.3 mM, pH 7.5), EDTA[*] (0.1 mM) and PMSF[*] (0.1 mM). After centrifugation and dialysis, the supernatant was applied onto a SEC[*] column. The obtained spectrin solution (6.3 μM) was added to a solution containing NaCl (0.1 M) and imidazole (0.05 M)(pH 6.8) and Cd^{2+} (0 – 1.5 mM). After mixing (4h), the solution was centrifuged in a Ultrafree-MC[*] (Millipore) microfiltration unit. The free Cd^{2+} present in the filtrate was analyzed by GFAAS[*] and compared to the total Cd^{2+}.	○ Binding affinities were calculated using a Scatchard plot: ○ 6 high affinity/dimer, K_d = 3.03 x 10^{-6} M ○ 8 low affinity/dimer K_d = 1.10 x 10^{-5} M	• Spectrin can form a heterodimer composed of one α and one β subunit. Dimers can then bind to each other to form a 200 nm long tetramer.[76] ○ PDB: 3LBX

Unknown Cd-binding protein[12] • ≥ 70 kDa -	TD (L)	To lysate (RBC:DI H_2O = 1:1) Cd^{2+} was added to a concentration of 178 μM, and the mixture was incubated for 2 hours at 37°C and then applied to a SEC* column using a mobile phase (100 mM Tris*, pH 7.4) which contained 2.5 mM GSH (to simulate cytosolic conditions), followed by ICP-AES* for Cd-specific detection (226.502 nm).	○ A Cd-binding protein was detected which was also found to bind Hg^{2+}.	• A previous study using mice which utilized gel filtration chromatography and ^{109}Cd detection also detected 70, 60, and 10 kDa Cd-binding proteins in RBC lysate.[77]
Cytosolic peptide(s)				
Glutathione (GSH)[73] • 307 Da ○ 0.4 – 3.0 mM[78]	TD (L)	^1H-NMR* spectra were collected for hemolyzed RBC lysate which contained 290 and 850 μM $CdCl_2$.	○ GSH resonances decreased with an increase in $CdCl_2$. ○ 2 binding sites, amino and sulfhydryl groups.[79]	• Decreasing GSH resonances were also observed when intact RBCs were incubated with 2 mM $CdCl_2$ and formation of Cd-GSH-hemoglobin complexes observed.[73]

***Technique and Reagent Acronyms**: Intermediate Conductance (IK); Graphite Furnace Atomic Absorption Spectroscopy (GFAAS); 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes); Adenosine triphosphate (ATP); Ethylenediaminetetraacetic acid (EDTA); Ethylene glycol tetraacetic acid (EGTA); Nuclear magnetic resonance (NMR); Phenylmethylsulfonyl fluoride (PMSF); Microcentrifuge (MC); Graphite Furnace Atomic Absorption Spectrometry (GFAAS); Size-exclusion chromatography (SEC); Inductively-coupled plasma atomic emission spectroscopy(ICP-AES); Dietlylaminoethyl (DEAE); 2-Amino-2-(hydroxymethyl)propane-1,3-diol (Tris).

#This value was approximated by combining known masses of the α, β, and γ subunits of human Na^+/K^+ ATPases.

‡Evidence suggests that two binding sites are present based on the degree of inhibition by $CdSO_4$ at different pH values.

†Ouabain is an inhibitor specific to Na^+/K^+ ATPases. Erythrocyte membranes contain both Mg^{2+}-ATPases and Na^+/K^+-ATPases. Ouabain-present conditions selectively measured Mg^{2+}-ATPase activity, while ouabain-absent conditions measured total ATPase activity. The difference was the Na^+/K^+-ATPase activity.

1.4 Uptake mechanisms of Cd into target organs

Comparatively few studies have focused on probing the biomolecular mechanisms that govern the uptake of Cd from plasma into endothelial [18] and target organ cells.[80] We will synopsize what is known about the uptake of Cd from plasma into RBCs and known target organs, such as the liver and/or the kidneys. Cd, however, is also uptaken by other target organs, including the eyes [81], the testes,[82] the endothelium,[18, 83, 84] and the heart,[85, 86] but little is known about the corresponding biomolecular uptake mechanisms, let alone the molecular Cd species that is uptaken.

Early studies with rat RBCs suggested that the uptake of Cd^{2+} was not an active process, but one that could be stimulated by co-exposure to Zn^{2+}.[87] Subsequent studies provided evidence in support of Cd being uptaken by cation-transport proteins in human RBCs,[88] whereas other studies demonstrated its uptake to be facilitated by L-histidine [89] and HCO_3^-/Cl^-[90] which implicated uptake by an anion exchange transporter. In view of the fact that Cl^- ions do play a role in the Cd uptake into enterocytes, namely Caco cells [91] and considering that the Cl^- concentration in plasma is about 105 mM, it is clear that the role that Cl^-, HCO_3^- and possibly other small molecular weight (SMW) metabolites play in the uptake of Cd into RBCs deserves much more attention in future research. In this context it is important to point out that Zn-deficiency is known to increase the osmotic fragility of rat RBCs,[92] which provides a potential explanation for the observed fragility of RBCs in humans following chronic exposure to Cd.[62, 93]

With regard to the uptake of Cd into the liver, cell culture studies with hepatocytes have revealed that comparatively more Cd^{2+} was accumulated than Hg^{2+}.[94] The inhibition of Cd uptake by co-exposure to Hg^{2+} indicated that a sulfhydryl-containing carrier mediates Cd^{2+} uptake and that Hg^{2+} is uptaken by an entirely different uptake mechanism.[94] Subsequent studies revealed that about

1/3 of Cd^{2+} appears to be uptaken by hepatocytes via Ca^{2+} channels [95] and that a sulfhydryl-group containing carrier mediated about 45% of the uptake, while the remaining 55% of Cd were uptaken by facilitated diffusion.[96] The fact that Cd^{2+} uptake by hepatocytes was inhibited by Zn^{2+} [97] suggests that the corresponding transmembrane protein lacks selectivity, which is not unexpected given the similarity of the physico-chemical characteristics of these metal ions.[13] Overall, comparatively few *in vitro* studies have provided evidence in support of the involvement of SMW metabolites in the uptake mechanism of Cd into RBC's, while their potential involvement in the uptake of Cd into hepatocytes,endothelial cells, cardiomyocytes and other target organ cells has not been systematically investigated.

1.5 Biomolecular mechanisms of organ damage by Cd

While it is well established that the kidneys are an important target organ for Cd,[5, 66, 98] the biomolecular mechanisms by which Cd damages this as well as other target organs are multifactorial [99] and involve the disruption of physiological signaling cascades,[100] oxidative stress,[101] the activation of cell death pathways,[102] the reprogramming of developmental signaling pathways,[23] metallohormone effects,[23] carcinogenesis,[14, 23, 103] endocrine disruption,[104] as well as epigenetic effects.[105, 106] Mitochondria and particularly complex III of the electron transfer chain appear to be a critical intracellular target of Cd toxicity.[107] In fact, recent studies have revealed that the underlying mechanism of action in mitochondria involves the decreased production of SIRT3, an SOD2 deacetylase.[102] The associated loss of the SOD2 enzyme activity then results in the build-up of reactive oxygen species (ROS) which damage mitochondria.

1.6 Knowledge gaps regarding the Cd exposure-disease relationship

Chronic human Cd exposure has long been known to be linked to lung cancer, osteoporosis, renal dysfunction [21] and to exert adverse effects on the testes [108]. Other studies, however, have suggested that Cd exposure of humans can also adversely affect the cardiovascular system [85], the endothelium [84] and is implicated in the etiology of stroke [109], atherosclerosis [110-112], age related macular degeneration [113, 114], cataract formation in the eyes [115], neurodevelopment in children [86], anemia [116], amyotrophic lateral sclerosis (ALS)[117], immunotoxicity [30], spontaneous abortion [118], renal cancer [119, 120], low birth weight [121] and diabetes [7, 122]. A comprehensive understanding of the mechanisms by which Cd adversely effects target organs would require one to know i) the structure of all molecular Cd forms (e.g. Cd-plasma protein and Cd-anion complexes) that interact with target organs,[49, 123] ii) all transmembrane proteins and mechanisms that mediate the uptake of Cd-species [124, 125] and iii) any other bioinorganic mechanisms that unfold in the blood stream [126] and may adversely affect its organ uptake as well as that of essential trace elements, such as Se [19, 127, 128]. In addition, >400 SMW metabolites have been recently identified in human plasma [129] and it has been recently demonstrated that the plasma proteome contains genetic variants.[130] Therefore, future studies should be directed at better understanding which SMW metabolites and how the genetic variability of plasma proteins modulate the metabolism of Cd to determine the degree to which certain organs are targeted (**Figure 2**).[131, 132] Establishing the biomolecular links which may causally link Cd exposure to the etiology atherosclerosis are of particular importance.[111, 133, 134]

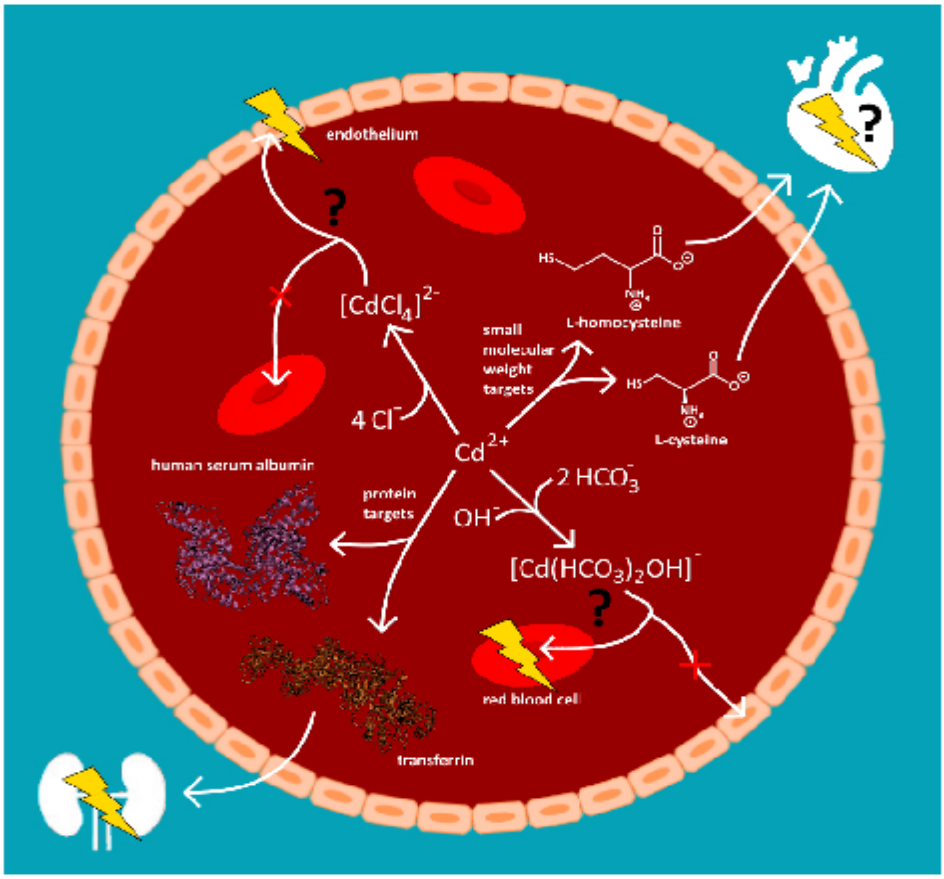

Figure 2 Conceptual depiction of the importance of Cd^{2+} interactions with ligands in the bloodstream in terms of causally linking the chronic exposure of humans to this toxic metal with its eventual damage in target organs. All protein structures were generated using the online program VMD (version 1.9.3).

1.7 Conclusion

Pollution is responsible for 9 million premature deaths per year.[135] Considering that many organ-based human diseases have not been shown to have a genetic cause [136] research that attempts to establish causal links between exposure to toxicants and the etiology of diseases is viewed by many as an important goal in the postgenomic era [136-138], but the underlying bioinorganic mechanisms of chronic Cd toxicity are incompletely understood. Motivated by the desire to establish causal links between human Cd exposure and disease, a literature review of biomolecular targets in the bloodstream revealed that in addition to HSA other plasma proteins as well as RBC target molecules play an important role in its metabolism and disposition to target organs. Arguably the biggest knowledge gap is our lack of understanding the role that plasma proteins other than HSA and SMW metabolites in plasma, such as homocysteine, HCO_3^-, Cl^- and others play in terms of directing Cd to its toxicological target cells/organs (i.e. RBCs, the endothelium, cardiomyocetes, kidneys, liver, testes, eyes), which is a prerequisite to determine the relative flux of Cd to each of the target organs and ultimately determines the toxicological effect over time. Accordingly, establishing the dynamic bioinorganic chemistry processes of Cd that unfold in the bloodstream represents an important research focus to better define the exposure-disease link.[110, 139, 140] To this end, the concerted application of advanced analytical techniques [27, 49, 141, 142] should be directed to gain insight into these fundamental toxicological processes since the increasing exposure of certain human populations to Cd via environmental sources, occupational activities [136] and/or new consumer products [143, 144] will inevitably increase its influx into the bloodstream of humans, including children. Thus, unravelling the bioinorganic processes which govern the exposure-disease link is a critical prerequisite before more stringent regulations on environmental sources of Cd can be imposed in

order to not only better protect humans from avoidable diseases,[145-149] but also to ultimately reduce health care costs.[150]

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