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Bioinorganic Explorations of Zn(II) Sequestration by Human S100 Host-Defense Proteins

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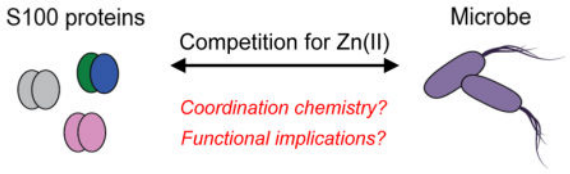
Abstract

The human innate immune system launches a metal-withholding response to starve invading microbial pathogens of essential metal nutrients. Zn(II)-sequestering proteins of the human S100 family contribute to this process and include calprotectin (CP, S100A8/S100A9 oligomer, calgranulin A/B oligomer), S100A12 (calgranulin C), and S100A7 (psoriasin). This perspective highlights recent advances on the Zn(II) coordination chemistry of these three proteins, as well as select studies that evaluate Zn(II) sequestration as an antimicrobial mechanism.

Graphical Abstract



Zn(II) is a ubiquitous d-block metal ion that performs structural, catalytic, and signaling roles in biology.[1,2] In the context of the host/microbe interaction and infectious disease, invading microbial pathogens must obtain this essential metal nutrient from the host to colonize and promote virulence.[3,4] The mammalian innate immune system, which provides a first line of defense against pathogenic invaders, employs one of two opposing strategies to modulate Zn(II) levels at infection sites and thereby inhibit the growth of pathogens: Zn(II) limitation and Zn(II) intoxication.[4,5] This perspective focuses on the former phenomenon, which is a component of the metal-withholding innate immune response. This process is often termed “nutritional immunity.”[6–8] In response to Zn(II) limitation and in an attempt to fulfill their nutritional requirements, pathogens express high-affinity Zn(II)-uptake systems to acquire Zn(II) from the host environment.[4,5,9,10] Thus, a tug-of-war for a limited supply of metal nutrient occurs at the host/pathogen interface, and the outcome of this competition impacts the progression of infection. Zn(II)-sequestering host-defense proteins are important contributors to this host/microbe interaction and, in humans, include three proteins from the S100 family: calprotectin (CP, S100A8/S100A9 oligomer, calgranulin A/B oligomer), S100A12 (calgranulin C), and S100A7 (psoriasin) (Figures 1, 2).

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Human S100 proteins include 21 relatively small (≈10 kDa) α-helical Ca(II)-binding polypeptides.[11] Apo S100 proteins typically exist as homodimers, or as a heterodimer in the case of CP, that each form a stable four-helix domain with a hydrophobic core. Each S100 polypeptide contains two EF-hand domains where the C-terminal EF-hand is described as "canonical" or "calmodulin-like" and the N-terminal EF-hand is described as "non-canonical." A "canonical" EF-hand domain affords a heptadentate coordination sphere for Ca(II), whereas a "non-canonical" EF-hand binds Ca(II) with lower coordination number.[12] A comparison of the structures of CP, S100A12, and S100A7 highlights some additional similarities (Figures 2, 3). Each hetero- or homodimer exhibits two transition-metal-binding sites that are independent from the Ca(II)-binding sites. The transition-metal-binding sites form at the homo- or heterodimer interface and are composed of two or more metal-binding residues from each subunit. Despite these commonalities, biological and biochemical studies performed over the past three decades have demonstrated that each protein exhibits distinct expression patterns, as well as Zn(II)-binding and -sequestering strategies.

Human CP is produced and released by white blood cells and epithelial cells.[13,14] Neutrophils constitutively express CP, which constitutes ≈40% of total cytoplasmic protein in these immune cells. Monocytes and macrophages also express this protein.[15,16] Apo CP exists as a heterodimer of S100A8 (α, 10.8 kDa) and S100A9 (β, 13.2 kDa). Coordination of Ca(II) ions at the EF-hand domains, or a divalent transition metal ion at the His_6 site, causes two heterodimers to self-associate and form a heterotetramer.[17–21] CP exhibits broad-spectrum antimicrobial activity, and early studies identified a link between its growth inhibitory activity and Zn(II).[22–26] More recent investigations have (i) defined how CP contributes to Zn(II) sequestration,[27,28] (ii) examined how select microbial pathogens respond to Zn(II) limitation imposed by CP,[29–33] and (iii) uncovered that CP can withhold additional divalent first-row transition metals, including Mn(II),[34–36] Fe(II),[37] Ni(II),[38] and Cu(II).[39]

Human S100A12 is also produced and released by neutrophils.[40,41] This protein is abundant, at least based on studies of porcine neutrophils, where it was found to constitute up to 8% of total cytoplasmic protein.[42] Other white blood cells that include monocytes, macrophages and eosinophils, as well as epithelial cells, also express S100A12.[43–48] It is a 21-kDa homodimer that self-associates into tetramers and hexamers in the presence of metal ions.[49–53] S100A12 is accepted to participate in nutritional immunity, and the current model suggests that it selectively sequesters Zn(II).[54,55] It exhibits antifungal activity against *Candida* spp and antibacterial activity against some pathogens including *Listeria monocytogenes*.[54,55]

Human S100A7 (psoriasin) is a 22-kDa homodimer that is expressed by epithelial cells and secreted into the upper epithelia.[56–59] In contrast to CP and S100A12, higher-order oligomers of S100A7 have not been observed. S100A7 has been identified as an antibacterial factor of the skin,[60] tongue,[61] and female genital tract.[62] Recent studies reported that S100A7 possesses antifungal activity.[63] To date, three different mechanisms of action have been proposed for the host-defense function of S100A7, one of which involves Zn(II) sequestration.[60,64]

Beyond participating in the metal-withholding response, S100 proteins have been associated with a variety of intracellular and extracellular processes, including cytoskeletal interactions, protein phosphorylation, membrane trafficking, calcium homeostasis, cell growth and migration, regulation of the cell cycle and transcription factors, tissue development and repair, cell migration and chemotaxis, and innate and adaptive immune responses.[11,65,66] Metal ions have been shown to mediate some of these processes, including the interaction of S100A12 with the receptor for advanced glycated end products (RAGE), which triggers a proinflammatory response.[67] While these topics are outside of the scope of this review, we look forward to future studies that address how transition metal ions contribute to the functions of S100 proteins in a variety of contexts.

In the following sections, we present a selection of recent work that has advanced our knowledge of the Zn(II)-binding properties of human CP, S100A12 and S100A7. Together, these vignettes describe new facets of the biological coordination chemistry of Zn(II) and inform our molecular understanding of how S100 proteins contribute to innate immunity, the host/microbe interaction, and the homeostasis of metal ions.

Calprotectin (S100A9/S100A9 Oligomer)

Among the Zn(II)-chelating S100 proteins, CP is unique because of its heterooligomeric (S100A8/S100A9) composition. This structural attribute affords four different EF-hand domains and two transition metal-binding sites with different amino acid compositions per heterodimer (Figure 2A–C). Moreover, S100A9 is the longest human S100 polypeptide and it exhibits a C-terminal extension or “tail” that we define as residues 96–114 (Figure 3). These unusual features define the coordination chemistry of CP and its remarkable metal-sequestering ability. The transition-metal-binding sites are located at the S100A8/S100A9 heterodimer interface and are commonly referred to as site 1 and site 2. Site 1 is a His_3Asp motif composed of His83 and His87 from S100A8, and His20 and Asp30 from S100A9 (Figure 2C). Site 2 is a biologically unprecedented His_6 motif defined by His17 and His27 from S100A8, and His91, His95, His103, and His105 from S100A9 (Figure 2C).[28,37,38,68–70] His103 and His105 are located in the flexible C-terminal tail of the S100A9 subunit. Both sites coordinate Zn(II) with high affinity and provide a 2:1 Zn(II):CP heterodimer stoichiometry, as described further below. Because no crystallographic or solution structure of Zn(II)-bound CP has been reported to date, current understanding of Zn(II) chelation by CP is based on results from solution studies.

A recent investigation established that the Zn(II) ion coordinated at site 2 is ligated by the six residues of the His_6 motif.[28] Prior to this work, it was unclear whether His103 and His105 of the S100A9 C-terminal tail contribute to Zn(II) binding at this site. During investigations of the protease stability of CP, a serendipitous observation provided compelling evidence for the existence of an unprecedented Zn(II)-His_6 site. Whereas proteinase K cleaved the S100A9 polypeptide of Ca(II)-bound CP after His104, addition of 2 equivalents of Zn(II) protected this cleavage site.[21] When the CP variants His103Ala and His105Ala were examined, proteinase K cleaved the S100A9 subunit of the Zn(II)-free and Zn(II)-bound protein after His104, suggesting that both His103 and His105 are involved in coordinating Zn(II) at site 2. This notion was supported by X-ray absorption spectroscopy of

the Zn(II)-bound ΔHis$_3$Asp variant of CP, which has the four residues of the His$_3$Asp site mutated to Ala residues. The X-ray absorption near-edge structure (XANES) region of the spectrum indicated that the Zn(II) ion is coordinated in a six-coordinate geometry, and the extended X-ray absorption fine structure (EXAFS) region was consistent with Zn(II) in a His$_6$ coordination environment.[28] This result is consistent with studies of Mn(II), Fe(II) and Ni(II) coordination at this site; the promiscuous His$_6$ motif allows CP to compete with microbes for multiple divalent first-row transition metal ions.[37,38,68,69] The coordination number of Zn(II) bound at the His$_3$Asp site is currently unclear and a topic for future investigation. In particular, whether the Asp residue is mono- or bidentate is unknown.

A variety of experiments have been performed to ascertain the Zn(II)-binding affinities of sites 1 and 2, including direct Zn(II)-binding titrations monitored by isothermal titration calorimetry (ITC) and Zn(II) competition titrations (Table 1). In the latter experiments, a fluorescent small-molecule Zn(II) sensor of known Zn(II)-binding affinity (apparent dissociation constant value, $K_{d,Zn(II)}$) and CP are allowed to compete for Zn(II), and the fluorescence observed from the sensor provides a quantitative measure of the relative affinities of the two competitors. Because CP also binds Ca(II) ions, titrations with Ca(II)-insensitive Zn(II) sensors are important for interrogating the effect of Ca(II) ions on Zn(II) binding. In early work, the Ca(II)-insensitive sensor Zinpyr- 4 (ZP4, $K_{d,Zn(II)}$ = 650 pM, pH 7.0)[72] revealed that Ca(II) ions modulate the Zn(II) affinities of both sites 1and 2 (Table 1).[28] In particular, ZP4 showed that Ca(II)-bound CP coordinates Zn(II) with higher affinity than the Ca(II)-free form. This discovery provided the foundation for the current model where the Ca(II) ion concentrations in the cytoplasm and extracellular space modulate the functional properties of CP (*vide infra*).[27]

One limitation of the ZP4 competition experiments was that CP outcompeted ZP4 for Zn(II) in the presence of excess Ca(II) ions; thus, only upper limits to the Zn(II) affinities could be determined from this method. Later, a new fluorescent Ca(II)-insensitive Zn(II) sensor named HNBO-DPA that binds Zn(II) with higher affinity than ZP4 was reported (HNBO-DPA $K_{d,Zn(II)}$ = 12 pM, pH 7.0).[73] Competition experiments performed with HNBO-DPA provided a new set of $K_{d,Zn(II)}$ values of Ca(II)-bound CP, and indicated that Ca(II)-insensitive Zn(II) sensors with greater Zn(II) affinity are needed because one Zn(II)-binding site out-completed HNBO-DPA for Zn(II) (Table 1).[28] Moreover, Zn(II) competition titrations with HNBO-DPA and a variant of CP that has no His residues in the S100A9 C-terminal tail (His103Ala/His104Ala/His105Ala variant) provided two additional important insights: (i) the tail His residues of CP contribute to high-affinity Zn(II) binding at the His$_6$ site and (ii) perturbation of the His$_6$ site lowered the Zn(II) affinity of the His$_3$Asp site. The latter observation provided the first hint of allostery between sites 1 and 2.[28] Further defining the Zn(II)-binding affinities, assessing the binding order, and deciphering crosstalk between the His$_3$Asp and His$_6$ sites are avenues that warrant continued investigation. In summary, the $K_{d,Zn(II)}$ values determined for CP to date are consistent with its role as a Zn(II)-sequestering host-defense protein. Indeed, recent work demonstrated that both sites 1 and 2 of CP sequester Zn(II) from microbes and thereby contribute to its growth inhibitory properties.[28]

As noted above, one major finding from the initial Zn(II) competition studies with ZP4 was that the presence of excess Ca(II) ions enhances the Zn(II) affinities of both sites 1 and 2.[27] This work, as well as subsequent studies of how CP coordinates other divalent transition metals,[36,37,74] indicated that CP morphs into its high-affinity, metal-sequestering form in the extracellular milieu where Ca(II) concentrations are orders of magnitude higher than those found in the cytoplasm of resting cells (e.g. 10–100 nM versus ≈2 mM). The molecular basis for how Ca(II) complexation enhances the Zn(II) affinities requires elucidation. Ca(II) binding causes formation of S100A8/S100A9 tetramers[17–19] and enhances the proteolytic stability of the protein,[21] which suggests that changes in conformation or dynamics may be at work. These observations also suggest that CP is predominantly a heterodimer when stored in the cytoplasm and forms heterotetramers in the extracellular space (Figure 1). In agreement with the notion that Ca(II) ions enhance metal sequestration, several studies have shown that the presence of Ca(II) ions enhances the ability of CP to deplete transition metals from microbial growth medium and its antimicrobial activity.[27,28,34,37,75]

S100A12

The human S100A12 homodimer houses two His_3Asp sites at the dimer interface that are composed of residues His15 and Asp25 of one subunit, and His85’ and His89’ of the other subunit (Figure 2D–F). Five crystal structures of S100A12 have been reported to date, including a structure of the Zn(II)-bound protein.[49–53] In this structure, each Zn(II) ion is coordinated at a His_3Asp site in a distorted tetrahedral geometry, affording a 2:1 Zn(II):S100A12 stoichiometry.[49] On the basis of crystallographic and solution studies, S100A12 exhibits complex oligomerization behavior.[49–53] For instance, Zn(II) binding causes two S100A12 dimers to self-associate into tetramers, and coordination of both Ca(II) and Zn(II) results in the formation of hexamers.[49,50] We note that a crystal structure of Cu(II)-bound S100A12 shows a 2:1 Cu(II):S100A12 stoichiometry where the Cu(II) ions bind at the His_3 Asp sites.[50,51] It was also hypothesized that the interplay between S100A12 and Cu(II) contributes to the anti-parasitic activity of S100A12;[76] however, to the best of our knowledge, there is no reported experimental evidence that supports this notion. Further investigations are required to evaluate Cu(II) binding by S100A12 in solution and decipher its biological significance.

The crystal structure of Zn(II)-bound S100A12 provided a foundation for recent solution studies addressing its Zn(II)-chelating properties. Zn(II) competition experiments with a series of small-molecule Zn(II) sensors, including the fluorescent Zn(II) sensor FZ3 ($K_{d,Zn(II)}$ = 9 nM, pH 7.4),[77] established that human S100A12 binds Zn(II) with subnanomolar affinity (Table 1).[55] These results are reminiscent of early studies of porcine S100A12, which reported that this orthologue binds Zn(II) tightly ($K_{d,Zn(II)}$ = 10 nM),[42] and refute a prior study that employed intrinsic protein emission to study human S100A12 and reported $K_{d,Zn(II)}$ values of >10 μM for each site.[49] Although further work is required to determine the $K_{d,Zn(II)}$ values of S100A12 and determine how Ca(II) ions affect Zn(II) binding, the current data are consistent with the notion that S100A12 functions as a Zn(II)-sequestering protein. Moreover, results from several other experiments indicated that Ca(II)-bound S100A12 has higher Zn(II) affinity than the apo protein. For instance, selective Zn(II) depletion from microbial growth medium by S100A12 was enhanced when the medium was

supplemented with ≈2 mM Ca(II).[55] Moreover, the antimicrobial activity of S100A12 was enhanced in the presence of excess Ca(II) ions.[55] These observations support the notion that Ca(II) ions modulate the functional properties of S100A12 such that its Zn(II)-sequestering ability is enhanced in the extracellular space.

Indeed, several recent reports provide compelling evidence that S100A12 functions as a Zn(II)-withholding protein. One investigation revealed that S100A12 is more prevalent within *Helicobacter pylori*-infected gastric tissue compared to healthy tissue, and S100A12 was shown to inhibit the growth of *H. pylori* through a Zn(II)-reversible mechanism.[54] Another report identified S100A12 as an antifungal agent that displays Ca(II)-dependent growth inhibitory activity against *Candida* spp.[55] The antifungal activity of S100A12 was lost when the protein was pre-incubated with two equivalents of Zn(II), supporting a Zn(II)-starvation mode of action.

Although both S100A12 and CP sequester Zn(II) and appear to have similar Ca(II) dependence, there are marked differences in the antimicrobial profiles of these proteins. S100A12 exhibits a relatively narrow spectrum of antimicrobial activity,[55] whereas CP provides growth inhibition against a variety of bacterial and fungal pathogens.[27,28,68] CP is functionally versatile because of its ability to sequester multiple first-row transition metals, which are essential nutrients, at the His_6 site. In contrast, S100A12 (and the His_3Asp site of CP) is more selective and sequesters Zn(II) but not Mn(II), Fe(II) and Ni(II).[55] Thus, it is possible that microbes that are more sensitive to Zn(II) deprivation are more susceptible to S100A12.

Because neutrophils produce and release S100A12 and CP, it is likely that these two proteins act together in the extracellular space. Metal substitution experiments with CP indicate that the His_6 site binds a divalent transition metal rapidly and with slow exchange, and that it will entrap the metal it encounters first.[37] Given that Zn(II) is ubiquitous and relatively abundant, we propose that Zn(II) sequestration by S100A12 may boost the ability of CP to capture other nutrient metals like Fe(II), Mn(II), and Ni(II) at the His_6 site.[55] Most studies consider S100A12 and CP independently, and investigating these two proteins together may improve our understanding of metal sequestration by the host innate immune system.

S100A7 (Psoriasin)

The human S100A7 homodimer contains two His_3Asp motifs at the dimer interface.[58,59] These Zn(II)-binding sites are composed of His87 and His91 from one monomer, and His18' and Asp25' from the other monomer (Figure 2G–I, Figure 3).[58,59] Two crystal structures of Zn(II)-bound S100A7 have been reported and both show a 2:1 Zn(II):S100A7 stoichiometry with each Zn(II) ion coordinated at a His_3Asp site in a distorted tetrahedral geometry.[59]

S100A7 has several unusual structural attributes. Its N-terminal EF-hand is truncated; thus, the loop is three residues shorter than the corresponding loops of S100A12 and CP (Figure 3). The N-terminal EF hand also contains a Ser residue at position 30. This position corresponds to an Asp/Glu residue that binds Ca(II) in S100A12, S100A8, and S100A9 (Figure 3). Another striking structural feature of S100A7 is that each polypeptide contains

two Cys residues, Cys47 and Cys96, which can form an intramolecular disulfide bond (Figures 2, 3).[58, 59] As a result, the S100A7 homodimer can exist in the reduced form ($S100A7_{red}$) with four free Cys thiolates or in the oxidized from ($S100A7_{ox}$) with two intramolecular disulfide bonds. Moreover, the Cys47–Cys96 disulfide bond is in close proximity to the Zn(II)-binding motifs; Cys96 is separated from the Zn(II)-coordinating residue His91 by a Gly-Ala-Ala-Pro loop. These structural features motivated examination of the effects of disulfide bond formation and Ca(II) ions on the Zn(II)-binding properties and antibacterial activity of S100A7.[64] Investigation of the disulfide redox behavior of S100A7 revealed that (i) the midpoint potential of S100A7 falls in the physiological range; (ii) Ca(II) binding depresses the midpoint potential by 40 mV from −255 mV for the apo protein to −298 mV for the Ca(II)-bound protein at pH 7.0; (iii) Zn(II) coordination appears to further depress the midpoint potential such that the value cannot be determined using standard redox buffer systems, (iv) apo $S100A7_{ox}$ is a substrate for the mammalian thioredoxin system (Trx/TrxR), and (v) metal-bound $S100A7_{ox}$ is not readily reduced by Trx/TrxR.[64] Taken together, these observations indicated that both $S100A7_{ox}$ and $S100A7_{red}$ can exist under physiological conditions, and that metal-bound S100A7 is more likely to be in the oxidized form. The former conclusion is in agreement with prior *ex vivo* analyses that reported the isolation of $S100A7_{ox}$ and $S100A7_{red}$ from skin samples.[60,63]

Zn(II) competition experiments demonstrated that $S100A7_{ox}$ and $S100A7_{red}$ each coordinate two equivalents of Zn(II) with sub-nanomolar affinity in the absence and presence of Ca(II) ions (Table 1), and that the cysteine thiolates in $S100A7_{red}$ do not form a third high-affinity Zn(II) site.[64] This work provided revision to a previously reported $K_{d,Zn(II)}$ value for S100A7 of 100 μM.[57] The results also refuted a prior hypothesis that a third high-affinity Zn(II)-binding site created by the Cys thiolates conferred antifungal activity to $S100A7_{red}$.[63] Moreover, Zn(II) competition titrations with ZP4[72] revealed similar $K_{d,Zn(II)}$ values for $S100A7_{ox}$ and $S100A7_{red}$ in both the absence and presence of excess Ca(II) ions (Table 1). These results indicated that (i) the redox state of the Cys residues in S100A7 has a negligible effect on the apparent $K_{d,Zn(II)}$ value obtained using ZP4, and (ii) apo- and Ca(II)-bound S100A7 have similar Zn(II) affinities. The latter observation indicates that S100A7 does not share the same Ca(II) dependence as CP and S100A12.

Although the Zn(II) competition studies described above revealed negligible difference in Zn(II) affinities of $S100A7_{red}$ and $S100A7_{ox}$, several other lines of experimental evidence indicated that $S100A7_{ox}$ is more effective at Zn(II) sequestration than $S100A7_{red}$. For instance, experiments that probed metal exchange at the His_3Asp sites demonstrated that the metal substitution rate occurs more slowly for $S100A7_{ox}$ than $S100A7_{red}$. Moreover, investigations of antibacterial activity showed that $S100A7_{ox}$ is more active than $S100A7_{red}$.[64] In total, these results suggested that S100A7 can exist in more than one form depending on the redox environment and presence of divalent cations like Ca(II) and Zn(II). In addition, it appears that S100A7 uses redox cues to tune its Zn(II)-sequestering ability and antibacterial activity, and that $S100A7_{ox}$ may be a more important contributor to Zn(II) withholding in the extracellular space than $S100A7_{red}$. We expect that further biophysical studies designed to elucidate how disulfide bond formation and reduction affects the protein scaffold and Zn(II) coordination at the His_3Asp sites will be informative.

Outlook

Over the past decade, our understanding of how the host and pathogen compete for Zn(II) and other essential metal nutrients has markedly advanced, and S100 proteins have emerged as key players in the metal-withholding innate immune response. The remarkable coordination chemistry exhibited by human CP, S100A12, and S100A7 provides new examples of how metal-binding properties can be tuned by environmental cues. Studies of Zn(II) chelation by CP revealed that Ca(II) binding enhances the Zn(II) affinity of this protein, and provided a paradigm for considering how Ca(II) ions affect its ability to sequester other metal nutrients as well as its antimicrobial activity. Subsequent investigations of Zn(II) sequestration by S100A12 and S100A7 built upon this work and afforded some similarities as well as striking differences, highlighting that metal-sequestering S100 proteins must be evaluated on a case-by-case basis. Similar to CP, current work indicates that Ca(II) ions modulate the Zn(II)-binding affinity of S100A12 and that this protein has the capacity to sequester Zn(II) in the extracellular space. S100A7, in contrast, appears to tune its Zn(II)-sequestering capacity through intramolecular disulfide-bond redox chemistry. Curiously, the disulfide bonds of S100A7 are more difficult to reduce when the protein is Ca(II) bound, which suggests an indirect modulatory effect of Ca(II) ions on the Zn(II)-binding properties of this protein. Taken together, a compelling picture emerges where CP, S100A12 and S100A7 are exquisitely designed to limit Zn(II) availability in the extracellular space.

From a physiological perspective, these three S100 proteins are abundant and involved in Zn(II) homeostasis and innate immunity. They are also implicated in other pathological processes that include the inflammatory response and tumorigenesis.[11] In order to fully appreciate how CP, S100A12 and S100A7 contribute to human physiology and pathology in broad terms and understand whether the Zn(II)-bound forms are important players, it is essential to understand the molecular underpinnings of Zn(II) coordination by each protein. In closing, we believe that the three case studies illustrated in this perspective convey the importance of deciphering the interplay between Zn(II) and S100 proteins at the host/pathogen interface from the fundamental and molecular perspectives, and provide a foundation for further refining current models that describe how S100 proteins contribute to Zn(II) biology.

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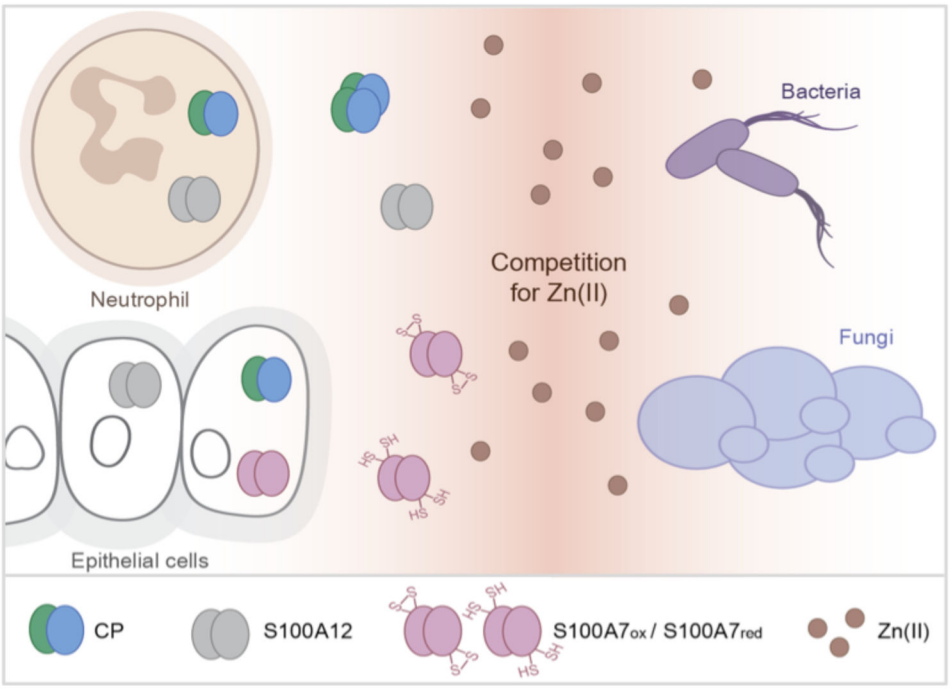

Figure 1.

Extracellular roles of CP, S100A12, and S100A7 in Zn(II) sequestration. Neutrophils, other types of white blood cells including monocytes and macrophages (not shown), and epithelial cells release these host-defense proteins into the extracellular space as described in the cartoon and main text. These proteins compete with microbes for bioavailable Zn(II) in the extracellular space.

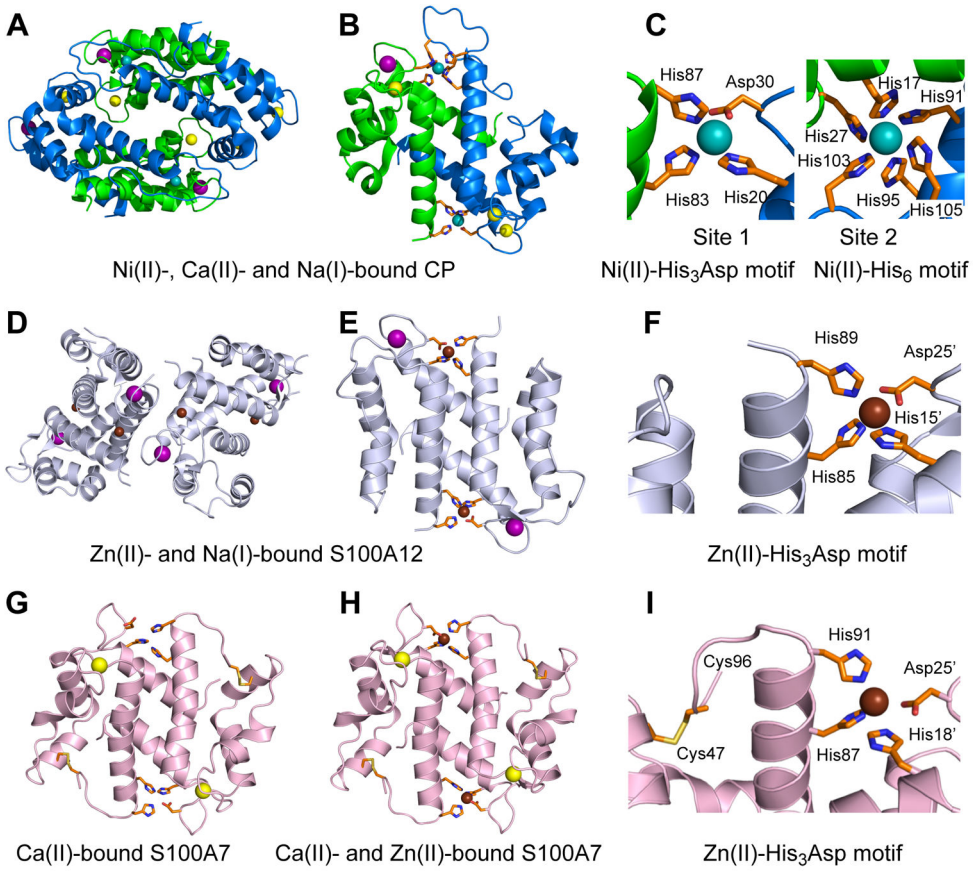

Figure 2.

Crystal structures of human CP, S100A12, and S100A7. (A, B) Structure of Ni(II)-, Ca(II)- and Na(I)-bound CP heterotetramer and a heterodimer unit taken from this structure that shows the transition-metal-binding sites (PDB 5W1F). (C) Zoom-in view showing the His_3Asp (Site 1) and His_6 (Site 2) motifs of CP (PDB 5W1F). (D, E) Structure of Zn(II)- and Na(I)-bound S100A12 tetramer and a dimer unit of this structure that shows the transition-metal-binding sites (PDB 2WC8). (F) Zoom-in view showing the His_3Asp motif of S100A12 (PDB 2WC8). (G) Ca(II)-bound structure of S100A7 dimer (PDB 3PSR). (H) Zn(II)- and Ca(II)-bound structure of S100A7 dimer (PDB 2PSR). (I) Zoom-in view showing the His_3Asp motif of S100A7 (PDB 2PSR). Zn(II), Ni(II), Ca(II), and Na(I) ions are shown as brown, teal, yellow, and purple spheres, respectively.

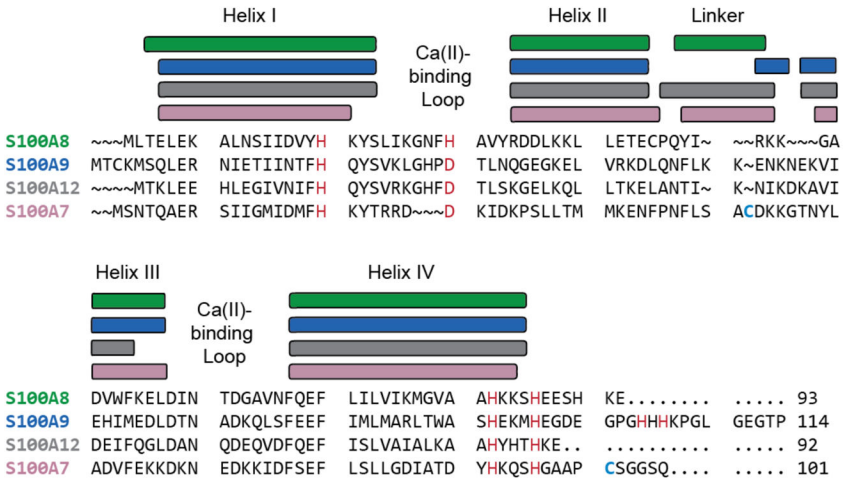

Figure 3.

Sequence alignment of human S100A8, S100A9, S100A12, and S100A7. Secondary structural elements are presented above the alignment. The transition-metal-binding residues are presented in red, and Cys47 and Cys96 of S100A7 are highlighted in blue.

Table 1

Reported Apparent Dissociation Constant Values ($K_{d,Zn(II)}$) for Human S100 Proteins.

Protein	$K_{d,Zn(II)}$	Method	Buffer conditions	Ref.
CP	1.4 nM 5.6 nM	ITC [a]	20 mM Tris, 100 mM NaCl, pH 7.5 stoichiometric Ca(II) [b,c]	35
CP-Ser [d]	133 ± 58 pM 185 ± 219 nM ≥ 10 pM [e] ≥ 240 pM [e]	Competition [f]	75 mM HEPES, 100 mM NaCl, pH 7.5 75 mM HEPES, 100 mM NaCl, pH 7.5, 15 – 45 equivalents Ca(II)	27
	90 ± 366 fM 0.9 ± 1 pM	Competition [g]	75 mM HEPES, 100 mM NaCl, pH 7.0 50 equivalents Ca(II)	28
CP ΔHis_3Asp variant [h]	3.4 ± 1.2 nM	ITC [a]	20 mM HEPES, 100 mM NaCl, pH 7.5 Stoichiometric Ca(II) [b]	68
CP ΔHis_4 Variant [i]	8.2 ± 1.5 nM	ITC [a]	20 mM HEPES, 100 mM NaCl, pH 7.5 Stoichiometric Ca(II) [b]	68
CP-Ser H103A/H104A/H105A variant	4 ± 0.8 pM 22 ± 3 pM	Competition [g]	75 mM HEPES, 100 mM NaCl, pH 7.0 50 equivalents Ca(II)	28
S100A12	16 μM 83 μM	Tyr fluorescence	25 mM Tris-HCl, pH 7.4 [c]	49
S100A12	≥ 9 nM	Competition [j]	75 mM HEPES, 100 mM NaCl, pH 7.0	55
S100A7	100 μM	Equilibrium dialysis	20 mM Tris, 150 mM KCl, pH 7.4 [c]	57
$S100A7_{ox}$	430 ± 13 pM	Competition [f]	75 mM HEPES, 100 mM NaCl, pH 7.0	64
	580 ± 40 pM	Competition [f]	75 mM HEPES, 100 mM NaCl, pH 7.0 100 equivalents Ca(II)	64
$S100A7_{red}$	660 ± 56 pM	Competition [f]	75 mM HEPES, 100 mM NaCl, pH 7.0	64
	420 ± 41 pM	Competition [f]	75 mM HEPES, 100 mM NaCl, pH 7.0 100 equivalents Ca(II)	64
S100A7-Ser [k]	700 ± 58 pM	Competition [f]	75 mM HEPES, 100 mM NaCl, pH 7.0	64
	370 ± 11 pM	Competition [f]	75 mM HEPES, 100 mM NaCl, pH 7.0 100 equivalents Ca(II)	64
S100A7-Ala [l]	500 ± 69 pM	Competition [f]	75 mM HEPES, 100 mM NaCl, pH 7.0	64
	490 ± 21 pM	Competition [f]	75 mM HEPES, 100 mM NaCl, pH 7.0 100 equivalents Ca(II)	64

[a] Isothermal titration calorimetry (ITC) experiments were performed at 30 °C. Stoichiometric Zn(II) binding was observed.

[b] The definition of stoichiometric Ca(II) relative to the CP concentration was not specified.

[c] Metal-buffer equilibrium is a variable when Tris buffer is employed for studies of Zn(II) binding because Tris binds Zn(II) with $K_{d,Zn} = 10^{-4}$ M. 71

[d] CP contains two native Cys residues that were mutated to Ser for these metal-binding studies. CP-Ser is a S100A8(C42S)/S100A9(C3S) variant.

[e] CP-Ser outcompetes ZP4 in the presence of Ca(II), and the $K_{d,Zn(II)}$ values were reported as upper limits.

[f] Competition experiments were performed with Zinpyr-4 (ZP4) at 25 °C.

[g] Competition experiments were performed with HNBO-DPA at 25 °C. These titrations were complicated by outcompetition and error. The fM $K_{d,Zn(II)}$ value is considered to be an upper limit and the pM $K_{d,Zn(II)}$ value is considered to be an estimate. The values were not assigned to particular sites.

[h] A variant of CP that only contains the His_6 site.

[i] A variant of CP that only contains the His_3Asp site.

[j] Competition experiments were performed with FluoZin-3 (FZ3) at 25 °C.

[k] A S100A7(C47S)(C96S) variant.

[l] A S100A7(C47A)(C96A) variant. To determine the $K_{d,Zn(II)}$ values from competition experiments, the averaged data were fit to a two-site model where $K_{d1} \neq K_{d2}$ and $K_{d1} = K_{d2}$ for CP and S100A7, respectively.