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Comparison of metal-binding strength between methionine and cysteine residues: Implications for the design of metal-binding motifs in proteins

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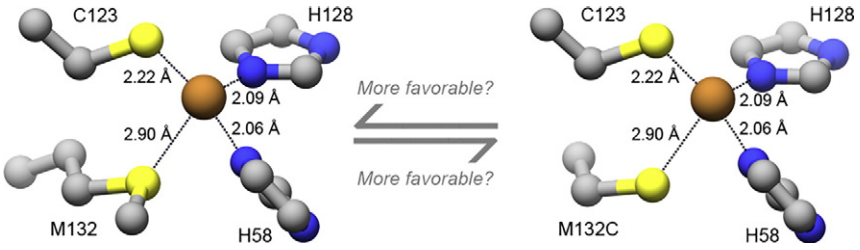
HIGHLIGHTS

- Unlike cysteine, methionine is found only in few examples as a ligand in copper-binding sites of some proteins
- Quantum chemical calculations were carried out to compare the strength of metal-binding between Met-sulfur and Cys-sulfur
- Both neutral Cys (CysH) and its deprotonated form (Cys-) were considered for the calculations
- Met-sulfur interacts strongly with copper than that of CysH while the interaction of cysteinate-sulfur is the strongest
- Met as a ligand can be used to modulate the strength of metal-binding motifs and can aid in designing new motifs

GRAPHICAL ABSTRACT



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ABSTRACT

Metals play vital role in various physiological processes and are bound to biomolecules. Although cysteine sulfur is more frequently found as metal-binding ligand, methionine prefers to occur in copper-binding motifs of some proteins. To address methionine's lower preference in copper-binding sites in comparison to cysteine, we have considered copper-binding motifs (His-Cys-His-Met) from seven different high-resolution protein structures. We performed quantum chemical calculations to find out the strength of interactions between sulfur and metal ion in both Met and Cys residues. In the case of Cys, both neutral (CysH) and the deprotonated form (Cys^-) were considered. We used two different levels of theory (B3LYP and M06-2X) and the model compounds methyl propyl sulfide, ethanethiol and ethanethiolate were used to represent Met, CysH and Cys^- respectively. To compare the metal-binding strength, we mutated Met *in silico* to CysH/Cys^- and performed the calculations. We also carried out calculations with wild-type Cys present in the same metal-binding motif. On average, interactions of Met with copper ion are stronger by 13–35 kcal/mol compared to CysH. However, Cys^- interactions with copper is stronger than that of Met by ~250 kcal/mol. We then considered the entire metal-binding motif with four residues and calculated the interaction energies with the copper ion. We also considered Met → Cys^- mutation in the motif and repeated the calculations. Interaction of the wild-type motif with the copper ion is ~160 kcal/mol weaker than that of mutated motif. Our studies suggest the factors that could explain why Met is not as frequently observed as Cys in the metal-binding motifs. Results of these studies will help in designing metal-binding motifs in proteins with varying interaction strengths.

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1. Introduction

Methionine (Met) and cysteine (Cys) are the only two naturally occurring amino acids containing sulfur atom. However these two amino acids have very different properties. While Met is very often grouped with hydrophobic amino acids that include Leu, Ile, Val, Phe and Trp [1], Cys is particularly recognized for its ability to form disulfide bonds and the formation of such bonds are important for the folding and stability of protein structures [2–3]. Cys is also frequently found in the active site of enzymes such as cysteine proteases [4] and is involved in catalytic activity. Recently, a Met residue has been shown to play a defined catalytic role in the enzyme phosphite dehydrogenase by stabilizing the transition state of the reaction [5]. The sulfur atoms of Cys and Met participate in hydrogen bond interactions. While Cys can take part in hydrogen bond both as an acceptor or donor, Met sulfur can form hydrogen bond only as an acceptor. High-resolution structures of proteins were analyzed to identify and characterize sulfur-containing hydrogen bonds formed by Met and Cys. Zhou et al. [6] used the Top500 protein structure database developed by Jane Richardson and her colleagues [7] and studied the occurrence of six different types of conventional and non-conventional hydrogen bonds involving the sulfur atoms of Met and Cys residues. Majority of Met and Cys residues participate in N-H…S type of hydrogen bonds with the main-chain N-H group. As hydrogen bond donor, large number of examples were found in which Cys sulfur forms S-H…O or S-H…N hydrogen bonds with the backbone functional groups. The strength of hydrogen bonds formed by the sulfur atoms was compared with the classical hydrogen bonds such as N-H…O═C or N-H…O hydrogen bonds [8–10]. Gas phase spectroscopy combined with quantum chemical calculations showed that both Met and Cys sulfur atoms form hydrogen bonds with the backbone N-H group and they should be given importance on par with the classical hydrogen bonds that stabilize the secondary structure of the proteins [9]. The N-H…S hydrogen bond formed by Met and Cys have been compared. It has been shown that N-H…S hydrogen bond formed by Met results in larger red-shift, shorter distance between the hydrogen and sulfur and linear N-H-S angle indicating that the hydrogen bond formed by Met sulfur is stronger [10].

The sulfur atom of a Cys residue has been shown to act as ligands for several metal atoms. Structure analyses of proteins from the Protein Data Bank (PDB) [11] have repeatedly highlighted the role of Cys in metal-binding activity. Zheng et al. [12] analyzed protein structures to characterize the geometry of metal ion-binding sites in proteins and found preferences of certain amino acid residues to bind to certain class of metals. Their analysis showed a strong preference for Cys to bind to Ni, Zn and Cu ions. A similar analysis revealed that Cys is also found to most often coordinate Fe and Cd ions [13]. As part of the zinc finger motif, Cys is known to be important in binding DNA molecules [14–15] and the zinc-binding motifs containing Cys are also found in enzymes such as oxidoreductases [16] and transferases [17]. Although strong preference is exhibited by the thiol/thiolate group of Cys to bind specific metal ions, examples of Met sulfur participating in metal-binding activity seem to be mostly limited to binding Cu ions [12–13]. Proteins in which Cu-binding motifs involving Met sulfur include plastocyanin, pseduoazurin, nitrite reductase and ascorbate oxidase [18]. This gives rise to the following questions. Why is Met sulfur not frequently observed in metal-binding motifs compared to Cys sulfur? Does Cys sulfur bind to the metal strongly compared to Met sulfur? In this paper, we have investigated the strength of sulfur from Met and Cys in metal-binding activity and compared them. We have analyzed high-resolution protein structures from the PDB and found examples of Met-sulfur participating as a metal-binding ligand. We used quantum chemical calculations and calculated the interaction energies between Met/Cys and Cu ion. Since Cys binds to metal ions in its deprotonated cysteinate (Cys^-) form [19], we considered both the neutral Cys (CysH) and Cys^- in our calculations. Our results show that Met sulfur binds strongly to Cu ion compared to CysH. However, when compared to Met sulfur, cysteinate sulfur is the strongest binding ligand. In addition to the energy component, we speculate that the entropy component could also contribute to Met's interaction with metal ions and both contributions could explain why Met sulfur is not frequently found as a metal-binding ligand.

2. Materials and methods

High-resolution protein structures (resolution ≤ 2.5 Å) containing at least one of the metal ions, Cu, Mg, Mo, Co, Zn and Ni, were extracted from the PDB. A non-redundant set of protein structures with redundancy removed at the sequence level of 40% was considered. We identified all the coordinating residues by considering a slightly relaxed distance cut-off of 4.0 Å from the metal ion. Normalized frequencies of metal-binding residues were found out as described in Zheng et al. [12]. We also found out the conservation of metal-coordinating amino acid residues using the same protocol applied in our earlier studies [20]. Briefly, for each metal-containing protein structure, we followed a sequence of steps. (i) The sequence of metal-containing protein was used as a query sequence in Blastp [10]. (ii) The hits obtained from this BLAST search were saved and the redundancy was removed using CD-HIT [21]. (iii) Clustal Ω [22] was used to align these sequences and the conservation of the metal-coordinating residues was found out. Steps (i) to (iii) were repeated for each example of protein containing the metals. An example of a protein structure with a Cu^{2+}-binding motif is shown in Fig. 1A. This protein auracyanin A is a blue-copper protein from a thermophilic bacterium (PDB ID: 2AAN) and the copper-binding motif consists of 2 His, one Met and one Cys residues (Fig. 1B). Met-132 in this motif is 93% conserved when 30 non-redundant homologs were compared (Table 1).

2.1. Quantum chemical calculations

To find out the strength of interactions between Met and Cys sulfur atoms, we performed quantum chemical calculations on model compounds with the metal atoms. Methyl propyl sulfide (MPS) and ethanethiol (ETH) were used as model compounds to represent Met and neutral Cys (CysH) respectively. Since the metal-binding Cys is most frequently found in the deprotonated cysteinate form, the model compound ethanethiolate (ETL) was used to represent cysteinate (Cys^-). Metal ions and the model compounds assumed the coordinates of Met/Cys and the interacting metal ions directly from the PDB structures. Hydrogen positions of the model compounds were fixed using the AddH utility available in the UCSF Chimera software [23]. Positions of hydrogens were optimized using the electronic structure program ORCA v3.0.2. [24] with BP86 density functional theory [25–26] in conjunction with def2-TZVP basis set [27–28]. Interaction energies between the metal ions and the coordinating model compounds were calculated using the following equation:

$$E_{int}{}^{AB} = E^{AB} - E^{A} - E^{B} \quad (1)$$

where E^{AB} represents the single point energy of the model compound A in complex with the metal B. E^{A} and E^{B} correspond to the single point energies of the model compound and metal respectively. Single point energies were calculated using Gaussion09 [29] with two model chemistries. In the first set of calculations, we used B3LYP theory [30–31] with the basis set 6-311 + G*. In the second set, M06-2X [32] was used along with the basis set 6-311 + +G** for the protein atoms and LANL2DZ basis sets [33–36] for the Cu^{2+} ion. Basis set superposition error (BSSE) was applied by employing Boys and Bernardi's standard counterpoise correction method [37].

We have also carried out Natural Bond Orbital (NBO) analysis [38–39] on MPS-metal ion, ETH-metal ion and ETL-metal ion complex systems at B3LYP/6-311 + G* level of theory. We used NBO version 3.1 [40] as implemented in Gaussian 09 [29]. The second order perturbation energies calculated from NBO analyses were further examined to understand the interactions of Met-Sulfur, CysH-Sulfur and Cys-Sulfur with the copper ion.