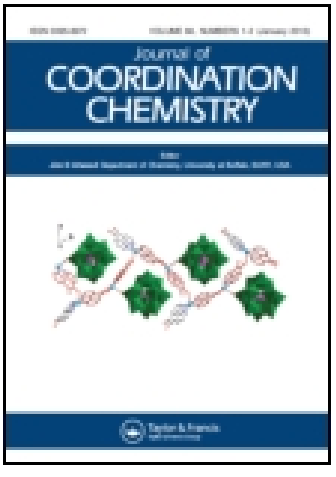

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Binding site residues in β-lactamases: role in non-classical interactions and metal binding

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Binding site residues in β-lactamases: role in non-classical interactions and metal binding

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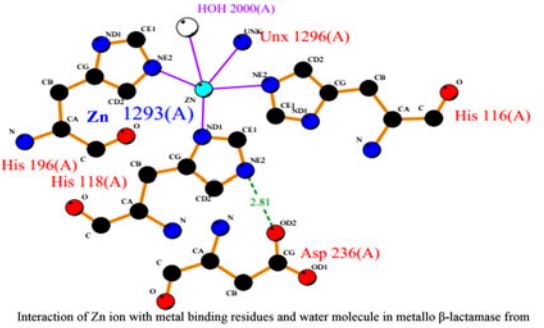

Interaction of Zn ion with metal binding residues and water molecule in metallo β-lactamase from *Pseudomonas aeruginosa* (PDB ID 2Y87)

Proteins bind with one or more metal ions in their native state to facilitate the biological function of the protein. The study of critical interactions between the biological molecules and metals is an important field of study. In this work, we focus on the functional specificity of residues coordinating with the metals commonly found in β-lactamases, Zn, Mg, Na, Cu, Mn, Ni, Fe, K, and Cd through non-classical interactions. All the residues located in the metal-binding site of β-lactamases are involved in non-classical interactions. The data obtained from this study will be useful to understand the functional role of metal-coordinating residues in the specificity of β-lactamases, thus offering promising strategy to design effective β-lactamase inhibitors.

Keywords: β-Lactamase; Metal-coordinating residues; Non-classical interactions; Hydrophobicity; Protein functional site

1. Introduction

Metal ions play an indispensable role in the structure and function of enzymes. Apart from their participation in electron transfer processes and in conformational changes, they are critical in preserving the structural integrity of proteins [1]. Metal ions provide appropriate

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conformation to the enzymes which are requisite for biological function [2]. Metals with distinguished role in biological processes include zinc, iron, manganese, cadmium, copper, potassium, sodium, and calcium. The occurrence of molybdenum and tungsten is less than the above-listed metals. Metals play an important role in the regulatory function of proteins such as signal transduction and maintaining the integrity of protein complexes [3]. Since metals play a significant role in biological processes, it is necessary to predict the metal-coordinating residues in proteins based on structural information.

Production of hydrolytic enzymes is the foremost mechanism of resistance exhibited by bacteria against β-lactam antibiotic therapy [4]. Even though the first bacterial enzyme was reported in 1940, the importance of resistance was not discerned until first clinical failure associated with penicillinase was reported in 1944 [5]. These bacterial enzymes hydrolyze the amide bond of β-lactam rings and render them ineffective, hence called β-lactamase. Current approaches involve the use of β-lactam inhibitors in combination with β-lactam antibiotics to combat β-lactamase resistance.

Hydrogen bonds between the electronegative atom of one molecule and hydrogen of another confers significantly to the stability of proteins [6]. Hydrogen bonds in the stability and conformation of proteins have been emphasized by several investigators [7–13]. In addition to classical H-bonds, non-classical H-bonds have been the subject of theoretical scrutiny [14] with several investigators recognizing the importance of these non-classical interactions [15–23]. To understand the role of metal-coordinating residues in the functional specificity of β-lactamases, we examined the geometric characteristics of non-classical hydrogen bonds, donor and acceptor role of metal-binding residues, hydrophobicity of metal-binding residues, stabilizing role of metal-coordinating residues and inter-residue contacts.

2. Materials and methods

2.1. *Data-set of β-lactamases*

A non-redundant set of 96 β-lactamases were selected from the Protein Data Bank (PDB) [24]. The criteria used for the selection are as follows:

(1) Only crystal structures with 2.5 Å or better resolution were selected.
(2) The sequence identity was less than 40%.
(3) If the protein contains more than one chain, then chain A was considered for analysis.
(4) Structures were optimized by adding hydrogens using the program REDUCE [25] with default settings.

Metal binding information was available only for 35 β-lactamases, and the PDB codes for the selected proteins are listed below: 2Y87-A [26], 2ZJ9-A [27], 1WUP-A [28], 2DKF-A [29], 3H3E-A [30], 1FOF-A [31], 1L9Y-A [32], 2WYM-A [33], 1DDK-A [34], 2V20-A [35], 2WRS-A [36], 1JJE-A [37], 1M2X-A [38], 1DD6-A [39], 1BMC-A [40], 2BG8-A [41], 1ZKJ-A [42], 2P4Z-A [43], 2YZ3-A [44], 1BC2-A [45], 2ZO4-A [46], 2GMN-A [47], 3ZR9-A [48], 4ZNB-A [49], 3LVZ-A [50], 2FHX-A [51], 1I2S-A [52], 3L6N-A [53], 3S1Y-A [54], 1JTD-A [55], 3R2U-A [56], 1E25-A [57], 1ZKP-A [58], 3S0Z-A [59], and 3SPU-A [60].

2.2. *Metal-binding site*

In the present study, we employed the use of Ligplot tool to identify the residues coordinating with metals ions that are commonly found in β-lactamases [61].

2.3. *Non-classical interaction analysis*

The analyses of non-classical interactions were performed using the program Hydrogen Bond Analysis Tool (HBAT) [62]. The criteria used for C–H···O interactions were d (H–O) ≤ 3.0 Å and θ (C–H···O) ≥ 90°, where d was the distance between the H and the acceptor O; θ was the angle between C–H bond and center of the acceptor atom. The parameter used for the analysis is shown in figure 1. The criteria for X–H···π (where X = C, N) were P1 ≤ 5.0 Å, P2 ≤ 4.0 Å, P3 ≥ 90 Å, P4 ≤ 40°. In π-electron acceptor, the distances were usually measured to the centroid (M) of multiple bonds or phenyl rings [63]. P1 and P2 were distances from X and H, respectively, to M, P3 was the angle between vectors C–H and H–M, and P4 was the angle between CM and MN. To understand the percentage contribution of each amino acid to the stability of β-lactamases, the ratio between the particular residue involved in the interactions and the total number of residues present in protein was computed and denoted as S,

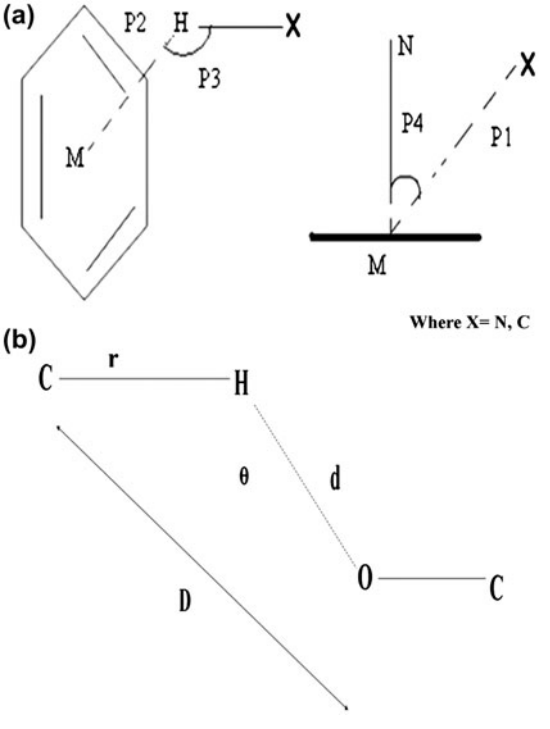

Figure 1. Parameters of non-classical interactions in HBAT. (a) X–H···π interactions [M – centroid, P1 and P2 are distances from X and H, respectively, to M, P3 is the angle between vectors X–H and H–M, and P4 is the angle between XM and MN]; (b) parameters of C–H···O interacting pairs [parameters are: r, distance between C and H atom; d, distance between the H atom and the O atom; D, distance between C and O atom; θ, defined as the angle between the C–H bond and the center of the acceptor atom].

$$S = \frac{\text{Number of particular residue involved in interactions}}{\text{Total number of residues present in interactions.}} \times 100$$

2.4. *Analysis of preferential contacts of metal-binding residues*

The α-carbons were generally used to represent the amino acid residues in protein. The distances between the metal-coordinating residues involved in non-classical interactions were computed by calculating the distance between the α-carbon of the first residue and the corresponding residues in protein. Composition of the amino acids correlated with this residue was calculated within a sphere of 8 Å, the range which was adequate for local and non-local interactions [64]. The contribution from <±4 with respect to Cα atom was represented as short-range contact, ±4 to ±10 as medium-range contacts, and >±10 were treated as long-range contacts [65].

2.5. *Computation of hydrophobicity and secondary structure preference of metal-binding residues*

Analysis of hydrophobicity of metal-coordinating residues was indispensable to predict the conformation of amino acid residues on a protein backbone [66]. Solvent accessibilities of metal-coordinating residues have been calculated using the program ASA-View [67]. Secondary structure assignment of metal-coordinating residues was done based on the information available from PDB. Proteins were categorized according to the manually assigned classes of α-helix, β-strand, and coil.

2.6. *Conservation score of metal-coordinating residues*

Importance of metal-coordinating residues in the function of β-lactamases was calculated based on ConSurf algorithm [68].

(1) Generation of MSA followed by the construction of phylogenetic tree.
(2) Calculate the ConSurf score for each site as defined by phylogenetic tree analysis.
(3) Physiological conservation score P_k at position K was calculated as follows:

$$P_k = \sum_{m-1}^{N} \left(A_{ij}^{m}(k) M_{ij} \right)$$

where A_{ij}^{m} is a matrix describing amino acid substitutions, assigned 0 if there is no substitution and 1 when there is a replacement, N represents the number of sequence in the alignment, M_{ij} indicates the replacement values.

(4) Finally, conservation score for each position is generated: Highest score represents more conserved regions, whereas lowest score indicates variable region [69].

2.7. *Structural importance of metal-coordinating residues*

Structural importance of residues located in the metal-binding site of β-lactamases was examined using the server SCide [70].

3. Results

3.1. *Metal-binding site*

Identification of metals covalently bound to β-lactamases and the importance of metal-coordinating residues in the structural and functional characterization of proteins is analyzed using Ligplot. On analyzing the number of occurrences of different kinds of metals in metal coordination groups, we found that Zn plays a dominant role followed by Cu, Cd, and Mn, due to the well-defined coordination geometries of these metal ions [71]. Percentage contribution of different metal ions to the functional specificity of β-lactamases is shown in figure 2. Among the proteins studied, 79% are from class-B β-lactamase, 11% are from class-A β-lactamase, 9% from class-C β-lactamase, and 1% from class-D β-lactamase. Further, the frequency of each residue involved in coordination with metal ions is shown in figure 3. As a representative picture, PyMol view of β-lactamase PDB ID 3ZR9 with more than one metal ion is shown in figure 4.

3.2. *Influence of metal-coordinating residues in the catalytic activity of β-lactamase*

The preference of metals for specific amino acid is analyzed, and the results are tabulated in table 1. Interestingly, the contribution of Cys, Asp, and His residues are higher in all the

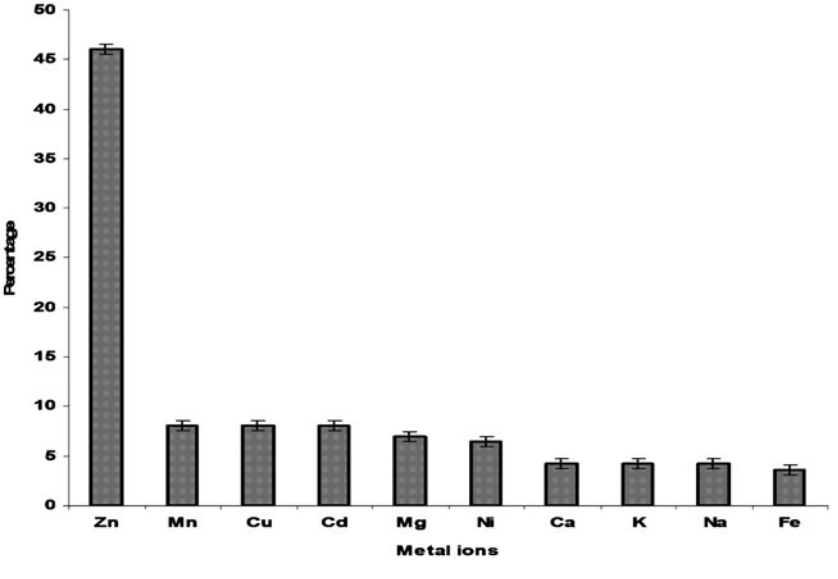

Figure 2. Percentage contribution of metal ions to the functional specificity of β-lactamases.

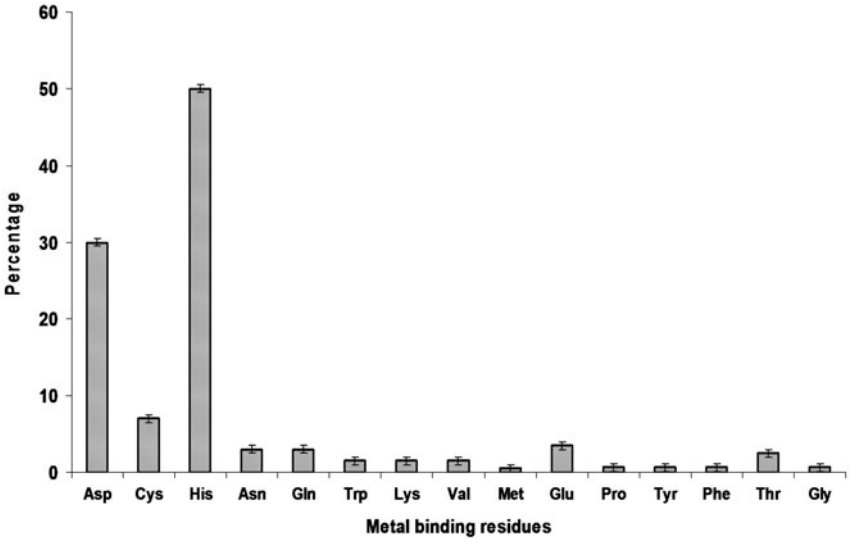

Figure 3. Percentage of metal-binding residues involved in non-classical interactions.

metal ions when compared to other amino acid residues. Our results are also supported by mutagenesis studies that state the importance of His, Asp, and Cys residues in the catalytic activity of β-lactamases [72]. Other metals such as Mg, Ni, and Ca also show significant contribution to the enzymatic activity of β-lactamase and are found to be frequently stabilized by Lys, Val, and Thr residues. No significant contribution is observed with Phe, Pro, and Met residues. The most common mechanism of β-lactamase in cleaving the β-lactam antibiotics involves the coordination of metal-binding residues with metal ion and water molecules [73]. As a representative picture, the interaction of metal-binding residues with metal ion and water is shown in figure 5.

3.3. *Non-classical interactions*

The energetic contributions of metal-coordinating residues involved in non-classical interactions are computed using HBAT. All the residues located in metal-binding sites are involved in non-classical interactions.

3.4. *Analysis of preferential contacts of metal-binding residues*

Preferential contacts of metal-coordinating residues are analyzed, and the results are shown in figure 6. From our results, we find that 55% of metal-coordinating residues prefer to be in short-range contacts, 25% in long-range contacts, and 20% in medium-range contacts.

3.5. *Hydrophobicity and secondary structure analysis of metal-binding residues*

Our finding reveals that 47.8% of metal-coordinating residues involved in non-classical hydrogen bonds stabilize the core region of β-lactamases, whereas 23.8% stabilize the

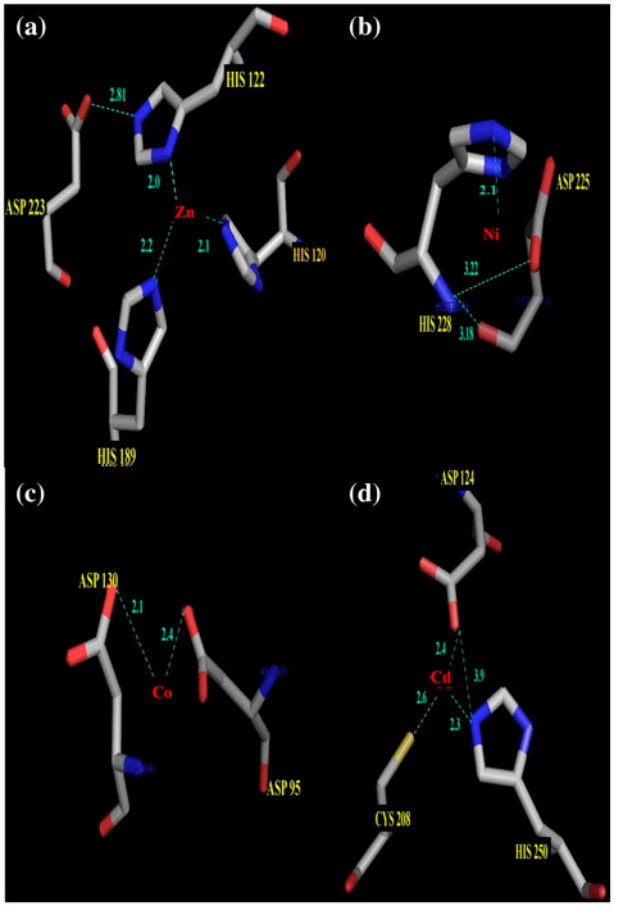

Figure 4. PyMol view of β-lactamase PDB ID 3ZR9 with more than one metal ion. (a) Interaction of metal-coordinating residues with Zn, (b) with Ni, (c) with Co, and (d) with Cd.

Table 1. Percentage occurrence of amino acid residues for specific metal ions.

	Asp (%)	Cys (%)	His (%)	Asn (%)	Gln (%)	Trp (%)	Lys (%)	Val (%)	Met (%)	Pro (%)	Phe (%)	Gly (%)	Thr (%)	Tyr (%)	Glu (%)
Zn	32	16	44.8	0	0	0.8	1.6	1.6	0.8	0	0	0.8	0.8	0	0.8
Mn	33.3	0	58.3	0	0	0	0	8.4	0	0	0	0	0	0	0
Cu	36.8	18.1	18	0	0	9	0	9	0	9	0	0	0	0	0
Cd	33.3	25	33.3	12.5	0	0	0	8.4	0	0	0	0	0	0	0
Mg	12.5	25	25	12.5	25	0	0	12.5	0	0	0	0	12.5	0	0
Ni	25	0	0	0	0	0	12.5	12.5	0	0	0	0	12.5	0	0
Ca	0	0	0	0	0	0	0	50	0	0	0	0	0	0	50
K	20	0	20	0	0	20	20	0	0	0	0	0	0	20	0
Na	33.3	0	33.3	0	0	0	0	0	0	0	9	0	0	0	24.4
Fe	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0

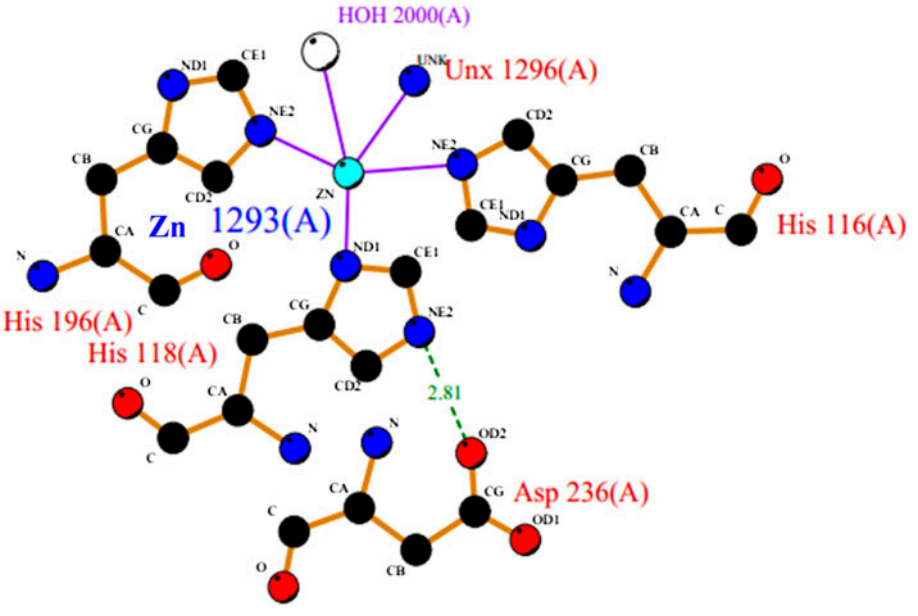

Figure 5. Pictorial representation of interaction between metal ion with metal-binding residues and water molecule.

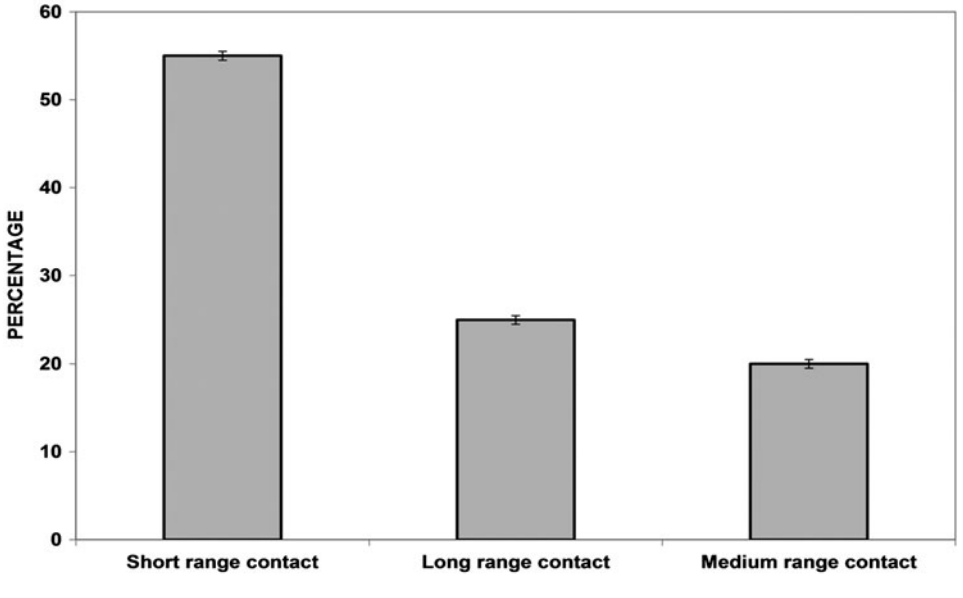

Figure 6. Preferential contacts of metal-coordinating residues.

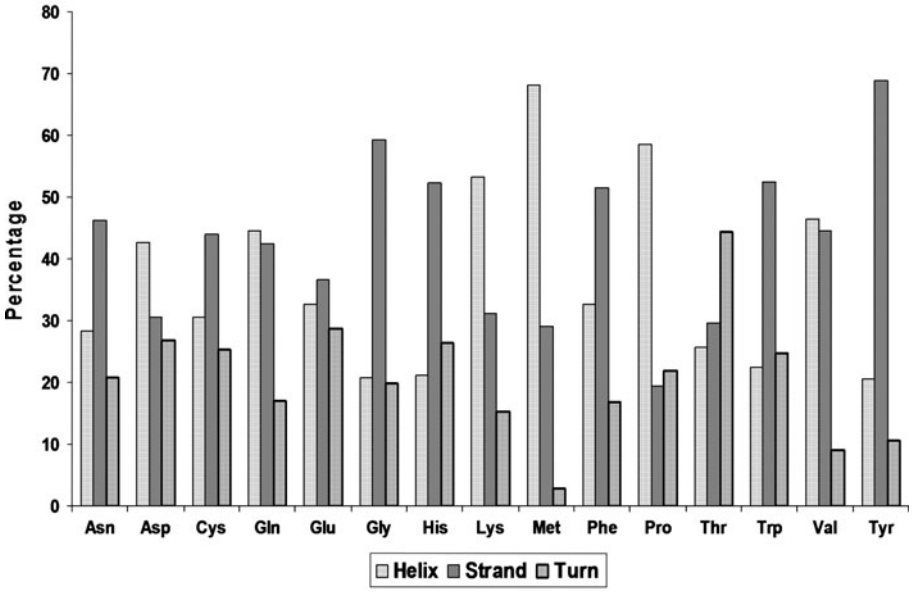

Figure 7. Secondary structural preference of metal-coordinating residues.

terminus region. Earlier analysis proves that non-classical interactions play a critical role in supporting the secondary structural scaffold of proteins [15]. Secondary structure preference of metal-coordinating residues is analyzed, and the results are shown in figure 7.

3.6. *Conservation score of metal-coordinating residues*

Functional importance of residues located in the metal-binding site of β-lactamases is analyzed. Our analysis shows that a considerable number of residues located in metal-binding sites of β-lactamases show higher conservation score, revealing the functional importance of these residues.

3.7. *Structural importance of metal-binding residues*

Stabilization centers are residues which mostly locate at the core region of protein providing stability by resisting the propensity to unfolding through long-range contacts [74]. From our analysis, we found that only a minimal number of metal-coordinating residues have more than one stabilization center, as most of the metal-coordinating residues are in short-range contact.

4. Discussion

Metals play an important role in biological reactions with flexible coordination geometry that permits proteins to attain the required conformation essential to perform the biological activity. Binding sites in proteins are designed to accept particular metal ion to perform

specific biological reactions. Hence, in the present study, the functional specificity of residues coordinating with metals commonly found in β-lactamases is analyzed through non-classical interactions. From our analysis, we observed that Zn ion plays a major role in the functional specificity of β-lactamases followed by Cu, Cd, and Mn ions. Since the metal ions are positively charged, they act as electrophiles and share electrons in order to form the interaction with amino acids [75]. From our data, we observed that the interaction of metal ions with the most abundant amino acid is achieved by sharing of electrons with the carboxylate group of Asp, imidazolyl nitrogen of His, and the sulfhydryl group of Cys. Our result correlates with the earlier literature which states that Zn ion is frequently stabilized by Asp, Cys, and His residues and thus shows significant involvement in the metal-binding site of proteins [76, 77]. Mutagenesis analysis of metal-coordinating residues shows that any changes in the interacting residues show dramatic decrease in the catalytic activity toward β-lactam antibiotics [78]. These studies reveal the functional importance of metal-coordinating residues in enhancing the catalytic activity of β-lactamase. The next abundant metals found in our data-set are Cu, Cd, and Mn; the enzymes that form metal–enzyme complexes with Cu, Cd, and Mn have significant beta-lactamase activity although much lower than that of Zn enzymes. There are reports which support the catalytic activity of Cu and Mn [31], but ours is the first report in which Cd is found to have significant enzymatic activity. As proved in kinetic and hydrodynamic evidence [79], dimer formation and enzyme activity of β-lactamases is promoted optimally by Zn, Cu, and Cd ions, whereas minimal activity is observed for Ca, Na, and K ions [31]. In metallo-β-lactamases, these ions are required for hydrolyzing the amide bond of beta-lactam antibiotics. Here, the metal ion coordinates with metal-binding residues and facilitates nucleophilic attack over the carbonyl carbon of β-lactam antibiotics leading to the disruption of antibiotics. These studies prove the influence of metal ions on the functional specificity of β-lactamases [80]. In our study, no interaction is observed between metal ions and Ala, Arg, Leu, Ile, and Ser residues. The major activity of β-lactamase is to cleave the β-lactam antibiotics making them ineffective. Many β-lactamases have a water molecule that directly coordinates with the metal ion, which acts as a nucleophile to attack the carbonyl carbon of β-lactam antibiotics. Catalytic activity of β-lactamase is mainly promoted by the interaction of metal ions with the metal-binding residues and the water molecule. There is strong support from earlier reports [81] that states that zinc complex with a coordinated water exhibits high β-lactamase activity, whereas a zinc complex with no water molecules shows lower activity. The water molecule coordinated with metal ion may act as a nucleophile to attack the carbonyl carbon of β-lactam antibiotics. Here, the role of metal ion is to lower the pKa of the coordinated water, thereby facilitating nucleophilic attack, leading to the disruption of β-lactam antibiotics. It is also commonly suggested that metal ions act as Lewis acids by coordination to the β-lactam oxygen, facilitating the nucleophilic attack [82]. The characteristics that allow metal ion to act as Lewis acid are the positive charge of metal ion that attracts electrons and the empty orbital that can accommodate an electron pair, which clearly indicates the influence of the charge of metal ion over the catalytic activity of β-lactamase. Even though the energetic contribution of these interactions is in the range of 2–3 kcal M^{-1} [83], these interactions occur more frequently compared to the classical hydrogen bonds, therefore contributing significant energy to the catalytic activity of β-lactamases. Hydrophobicity of metal-coordinating residues shows that most of the residues involved in non-classical hydrogen bonds are in buried regions of β-lactamases. Earlier reports suggest that enzymes have more significant stability from the hydrogen bonding of buried polar amino acids than non polar groups [84–86]. Substantial numbers of metal-binding polar residues involved are

located in the core region of proteins, revealing the importance of these residues in stability of β-lactamases. Investigations on evolutionarily conserved residues provide considerable information in understanding the functionally important sites that signify the high selection pressure of evolutionarily conserved residues [87]. As reported in previous studies [88], our results are also supplemented with the fact that residues with higher conservation values are likely to engage in coordination with metal ion. Conservation of buried polar residues and the hydrogen bond interaction formed by them plays an important role in preserving structure of proteins [89]. Our analysis on the prediction of protein functional sites shows that majority of the polar residues that stabilizes the core region of β-lactamases are highly conserved, providing additional support for the importance of buried polar residues. Propensity of amino acid to form particular secondary structure depends on the physicochemical properties. The most common secondary structure is α-helix followed by β-strand [90]. Secondary structure preferences of metal-coordinating residues show that most of the residues prefer to be in strand conformation. Metal-coordinating residues such as Asn, Cys, Glu, Gly, His, Phe, Thr, Trp, and Tyr favor strand conformation, whereas the rest favor helix conformation. Stabilization analysis of metal-coordinating residues shows that Thr, Asp, and His residues contribute significantly to the stabilization of β-lactamases with more than one stabilization centers. Intra-protein interaction of metal-coordinating residues is analyzed within a sphere of 8 Å. From our analysis, we find that short-range interactions have the highest contribution. This result specifies the crucial role of short-range interactions in forming metal coordination. On the whole, results obtained in this study provide valuable information on the catalytic role of metal-coordinating residues in β-lactamases.

5. Conclusion

As β-lactam resistance has emerged as a serious health threat, the coordination chemistry of metal ion–residue complex in enhancing the catalytic activity of β-lactamase is analyzed. All the residues coordinating with the metal ions are involved in non-canonical interactions. Results are obtained from stabilization centers and higher conservation score proves the influence of these residues in the enzymatic activity of β-lactamase. As the metal-coordinating residues plays a crucial role in the activity of β-lactamase, data obtained from this study will be crucial in designing effective β-lactamase inhibitors.

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