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A survey of methionine-aromatic interaction geometries in the oxidoreductase class of enzymes: What could Met-aromatic interactions be doing near metal sites?

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ABSTRACT

Redox reactions of the aromatic amino acids tyrosine (Tyr) and tryptophan (Trp) are crucial for the biological functions of many metalloproteins. An important question is how biological systems can use the protein environment to move electrons through proteins in a controlled manner. Methionine (Met)-aromatic interactions are common in proteins, but little is known about redox reactions of such motifs. Here, we explore methionine sulfur-aromatic interactions in the oxidoreductase (EC 1) class of proteins and their proximity to metal sites. We also propose a new metric for classifying Met-aromatic interactions called "interaction order." Over 12,000 protein structures from the Protein Data Bank were analyzed. A linear algebraic heuristic was used to classify the interaction of Met-sulfur with tyrosine, tryptophan, and phenylalanine. We found that 83% of oxidoreductase proteins contained aromatic interactions meeting our criteria, with a preferential angle of about 60° between Met-sulfur lone pairs and aromatic planes. A total of 41% of Met-aromatic interactions meeting our criteria were found to be within 20 Å of a metal site, and 6% were found within 10 Å. A surprising number of "bridging" interactions, involving two aromatic residues and one Met also were identified. Finally, selected examples of potentially important Met-aromatic redox motifs are outlined. On the basis of our results, we suggest that Met-aromatic interactions should be considered as mediators of electron transfer reactions, as well as their more widely recognized roles as structural motifs.

1. Introduction

The intramolecular forces that hold proteins in their native states and promote specific peptide or ligand binding interactions have long been of great interest [1]. Fundamental concepts in protein folding have been established and several modern theoretical treatments can describe features of protein structure at early times, and at later times, during the folding process [2,3]. In addition, the last 20 years have seen the emergence of a wealth of data about protein structure [4]. Yearly submissions to the Protein Data Bank (PDB) have increased by almost a factor of 10 and the total number of structures available has increased 20-fold since 1997. The growth of this database has allowed for the development of new ideas about how protein structures form and function [5–7]. For instance, analysis of PDB data reveals a great many trends in spatial arrangement of amino acids relative to each other, including H-bonding, salt bridges, π-stacking, cation-π, and other interactions of aromatic residues. Of particular interest are interactions of the aromatic amino acids phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp). Some notable examples involve interactions between one of those residues and proline (Pro) [8], alanine (Ala), [9] or methionine (Met) [10]. Of interest in this manuscript are Met-aromatic interactions. Here, we use a statistical analysis of structural data to explore whether or not Met-aromatic interactions could be involved in metalloprotein redox reactions.

The aromatic amino acids Tyr and Trp play central roles in protein electron transfer (ET) chemistry [11,12]. Canonical examples include the functions of photosystem II, [13] DNA photolyase, [14] and ribonucleotide reductase [15]. Oxidized amino acids, such as Tyr and Trp, also are discussed in the context of oxidative stress [16]. The biological redox reactions required in many of the above examples can involve hole/electron transport over distances greater than 20 Å. Single step ET, even at high driving force, cannot deliver holes/electrons fast enough to active sites that require the input of electrons to catalyze reactions on timescales shorter than milliseconds [17]. A common bypass to single step ET is the presence of intermediate redox moieties, often Tyr or Trp, that can promote multistep ET (or hopping) [11,18]. Along with those functional roles, a recent proposal suggests that chains of closely spaced Tyr/Trp residues could act as hole conduits stretching from

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metallocofactors to protein surfaces [19]. In such a case, hole transport through these chains is proposed to provide a protective mechanism when metal sites are activated in the absence of substrate (or in other oxidative malfunctions). This idea is supported by theory and experiment [20]. All of the above examples are central to biological functions. In this context, it is remarkable that two residues (Tyr and Trp) can carry out such an array of functions in vivo. This also leads to the natural question of how the microenvironment of an amino acid aids in steering electrons through peptides.

Pioneering work, when fewer than 100 structures were in the PDB, demonstrated a propensity for Met to localize its sulfur atom near the aromatic ring of Phe, Tyr, or Trp at a higher-than-expected frequency in a survey of X-ray structures [10,21]. Since those initial studies, this localization has been formally termed an "interaction," where sulfur-aromatic distances of under about 5 Å are considered the most significant. More recent analyses of PDB data showed that about 1/3 of all structures contain at least one Met-aromatic interaction [22]. Other informatics surveys of PDB data support the structural importance of Met-aromatic interactions (e.g., in membrane proteins [23]) and highlight that other residues, such as histidine, can interact with Met [24]. Related surveys are available for Cys-aromatic interactions [25,26]. Other literature supports the idea that Met-aromatic motifs are associated with protein stability/folding [27,28]. Structurally, the Met-aromatic interaction is estimated to add an additional stabilization energy of 1–1.5 kcal mol^{-1} in comparison to a purely hydrophobic interaction [22,25].

The structural roles of Met are well established, but the single electron redox chemistry of Met is much less clear [29]. The most common redox reactions to Met involve 2 electron oxidation to Met-sulfoxide, which has been implicated as an antioxidant and a marker for oxidative stress [30–32]. However, when Met sulfur atoms interact with an aromatic residue, they tend to be less prone to such oxidation [33]. A contrasting report highlights how the oxidation state of a Met-sulfur can influence its interactions with other aromatic amino acids, with oxidized Met sulfoxide interacting more strongly [34]. Computational studies of sulfur-aromatic interactions in amino acid fragments [35] and in other model compounds [36] demonstrate the importance of sulfur-aromatic interactions in modifying the redox behavior of both the sulfur and the aromatic group. In particular, studies of models underscore the attractive two-center, three-electron bond that forms between an oxidized sulfur and a nearby π system [36]. In this study, we survey protein structural data to address the question of the importance of Met-aromatic interactions in modulating ET reactions in metalloproteins. The work presented here analyzes PDB data for selected redox proteins to identify and classify Met-aromatic interactions and their relationship with metal sites.

2. Experimental

2.1. Module construction

A Python module (Python 3.5) was developed in conjunction with the Python Pandas Data Analysis Library (www.pandas.pydata.org) for mining and downstream processing of all .pdb files. The in-house module is composed of 4 major routines and associated subroutines (see Supporting Information). The entire module was imported into a separate namespace and the routines were called sequentially within a loop that iterated over .pdb files of the oxidoreductase class. A base routine was used to fetch .pdb files directly from the Protein Data Bank (www.rcsb.org/pdb). We developed an algorithm (*Met-aromatic*) and used it to process imported coordinate data for every iteration. The *Met-aromatic* algorithm followed methods utilized by Zauhar et al. with the exception of a difference in the distance between the Met δ-sulfur and the aromatic plane [37]. All linear algebraic manipulations were performed using the Python Numpy package (www.numpy.org). All plotting was done using the Matplotlib Python 2D plotting library (https://matplotlib.org). A total of 12,186 PDB entries (available 15 January 2017) were imported into and processed using our library.

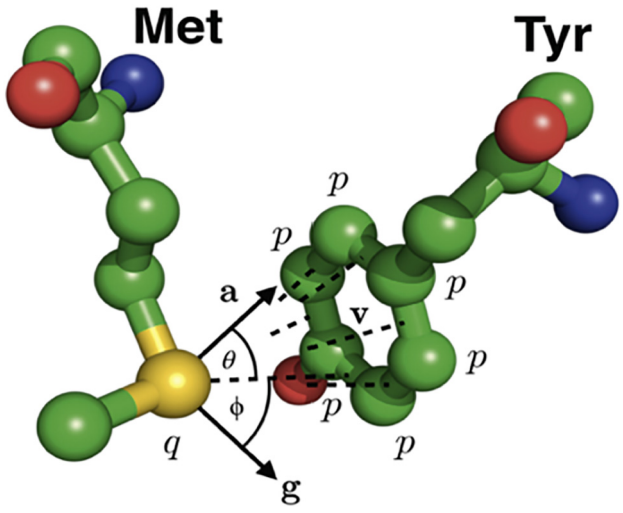

Fig. 1. The methionine interaction feature space. An example Met 224/Tyr 314 aromatic interaction for 1-deoxy-D-xylulose 5-phosphate reductoisomerase (PDB ID 3ANL). Solid arrows represent vectors parallel to lone pair axes of symmetry (**a** and **g**) and dashed lines represent vectors spanning from Met-SD to midpoints between aromatic carbon atoms. Some vectors (**v**, dashed lines) are trimmed for clarity.

2.2. Heuristic for distance classification of aromatic interactions

Imported .pdb files were preprocessed in order to eliminate spurious data and to format the data set for downstream processing. Preprocessing was also used to eliminate duplicate data for each protein. Specifically, we only analyzed atomic coordinates with chain identifier "A" for each PDB file in addition to only processing the first model for multi-model entries. The looping procedure passed over entries not containing residues. Banked coordinate data meeting such requirements were augmented into a feature space where Met δ-sulfur (SD) coordinates were considered points q. Midpoints between aromatic carbon atoms in Phe, Tyr, and Trp were considered members of a set P. Midpoints were chosen to select for those interactions involving the aromatic π system rather than those involving a single aromatic carbon atom. As such, each aromatic residue was associated with one set P. A vector **v** (Fig. 1) was assigned from each SD coordinate to every aromatic midpoint contained within the feature space. This first step of the algorithm was inclusive of very long vectors however, particularly if a point q was located on one end of a protein and a corresponding set P was located on the opposing end. To find closely spaced S-aromatic moieties, our algorithm selected in coordinates q and sets P where the Euclidean norm $\|\mathbf{v}\| = \|p\text{-}q\|$ ($p \in P$) was found to be less than or equal to 4.9 Å. The distance cut-off for Met-aromatic interactions (despite a 3.5 Å sum of the C and S Van der Waals radii) is somewhat more strict than related literature surveys, which demonstrated maxima in the distribution of sulfur-aromatic interactions at approximately 5.3 Å [10] or 5.5 Å [22].

2.3. Heuristic for angular classifications

Two vectors, **a** and **g**, were used to approximate the position and orientation of Met SD lone pairs. These vectors were found by completing the last two vertices of a regular tetrahedron, where our input was the known position of the first two vertices (CE and CG atomic coordinates) and the origin (SD). We then mapped vectors **a**, **g**, and **v** to the origin of the frame defining all the PDB coordinates and found the angles between **a** and **v** (Met-θ) and between **g** and **v** (Met-ϕ), as shown