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Metal-chelating non-canonical amino acids in metalloprotein engineering and design

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The ability to rationally design metalloproteins with desired functions remains a difficult challenge despite many years of effort. Recently, the potential of using genetically encoded metal-chelating non-canonical amino acids (NCAAs) to circumvent longstanding difficulties in this field has begun to be explored. In this review, we describe the development of this approach and its application to the rational design or directed evolution of NCAA-containing metalloproteins in which the bound metal ions serve in structural roles, as catalysts, or as regulators of the assembly or disassembly of protein complexes. These successes highlight the fact that amino acids not found in nature can recapitulate the functions of their naturally occurring counterparts and suggest the promise of this nascent approach for simplifying the metalloprotein design problem.

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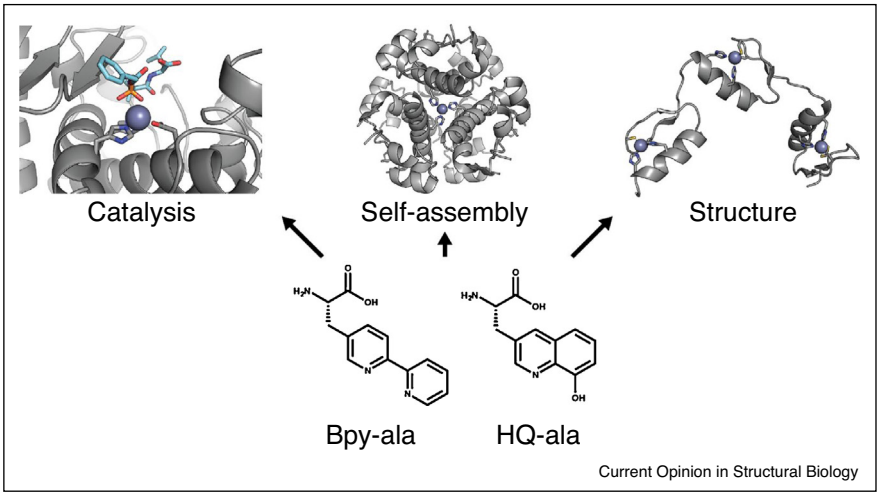
Introduction

The dependence of all living organisms on transition metals is evidence of the diverse functional roles metal ions play in biological systems. In many cases, metals are tightly associated with proteins (termed metalloproteins) whose three-dimensional structures serve to harness the inherent functions of the bound metals in ways that are beneficial to the organism. For example, the chemical reactivities of metal ions (e.g. the ability to serve as Lewis acids or to participate in redox reactions) can be exploited by proteins to generate potent enzymes [1,2]. Moreover, energetically favorably interactions between proteins and metals can drive protein folding [3], increase protein stabilities [4], or mediate protein self-assembly [5]. Given the versatile functions that metal ions carry out in biological systems, it is not surprising that the rational design of new metalloproteins, especially those with properties not found in nature, represents a longstanding goal within the protein engineering field. Despite many notable successes, metalloprotein design remains a challenging problem.

Recent advances in the field of chemical biology have provided tools that may simplify the metalloprotein design problem. Namely, two well-studied organic metal chelators — 2,2′-bipyridine (Bpy) and 8-hydroxyquinoline (8-HQ) — have been genetically encoded as the side chains of the non-canonical amino acids (NCAAs) (2,2′-bipyridyn-5-yl)alanine [6] (Bpy-ala, Figure 1a) and 2-amino-3-(8-hydroxyquinolin-3-yl)propanoic acid [7] (HQ-ala, Figure 1b), respectively. These NCAAs can be directly incorporated within proteins using the well-established amber stop codon suppression technology [8]. Both HQ-ala and Bpy-ala exhibit K_ds in the micromolar to nanomolar range for a number of biologically relevant transition metals [9]. Furthermore, metal complexes of Bpy and 8-HQ have been extensively studied outside of protein contexts and are known to exhibit well-characterized chemistries and unique photophysical properties that can be leveraged to imbue proteins with functions that would be difficult or impossible to achieve with naturally occurring amino acids alone.

The potential of using metal-chelating NCAAs to engineer metalloproteins that both recapitulate and expand upon the structural and catalytic functions of their naturally occurring counterparts is beginning to be explored. Although these efforts have resulted in many notable achievements, they have also highlighted important considerations that must be accounted for when metal-chelating NCAAs are used to design functional metalloproteins. This review will focus on research carried out in the last few years in which metal-chelating NCAAs were used to develop new functional metalloproteins. We will describe notable successes in this nascent field, challenges that were overcome in these studies, and prospects for the future. Because many excellent reviews of metalloprotein engineering efforts exclusively using naturally occurring amino acids have been published recently, [10–12] metalloprotein engineering with canonical amino acids is considered outside the scope of this review.

Figure 1



Metal-chelating non-canonical amino acids in metalloprotein design. Examples of the diverse functions metal ions play in biology including catalytic (thermolysin, PDB ID: 6tmn), control of protein assembly and disassembly (insulin hexamer, PDB ID: 1zeh), and regulation of structure (Zif268, PDB ID: 1aay) are shown. The metal-chelating non-canonical amino acids, (2,2′-bipyridyn-5-yl)alanine (Bpy-ala) and 2-amino-3-(8-hydroxyquinolin-3-yl)propanoic acid (HQ-ala) could facilitate the design of metalloproteins that both recapitulate and expand upon the functions of metalloproteins found in nature.

Potential benefits of using metal-chelating NCAAs in the rational design of metalloproteins

High-affinity metal binding sites in proteins are generally formed through the concerted action of multiple amino acid side chains that interact directly with the metal ion [13] (Figure 2). The ability to simultaneously predict the rotameric preferences of multiple conformationally flexible side chains is a daunting challenge, even for state-of-the-art rational design methods [14]. Furthermore, in many metalloproteins, the orientations of residues that directly interact with the metal (the first coordination sphere) are often stabilized via hydrogen bonding interactions with surrounding amino acid side chains or the peptide backbone [15] (Figure 2). In many cases, these 'second sphere' residues are similarly stabilized through interactions with the remainder of the protein scaffold [16] (Figure 2). Thus, if naturally occurring proteins are to be used as a guide, engineering a high-affinity metal binding site in a protein from scratch would require the ability to design extended hydrogen bonding networks within an existing protein scaffold. Recently developed computational protein design algorithms [17•] suggest that design of native-like metal binding sites may soon be possible. Due to the challenging nature of this problem, most previous efforts focused primarily on the design of first shell residues, although second shell residues have been included in the design process in rare cases [18,19].

Most metalloprotein design strategies are derived from the wealth of information provided by structural characterizations of naturally occurring metal binding sites [20,21]. As the number of metalloprotein structures grew, so too did our ability to classify metal binding sites using the geometric orientations of the residues in the first coordination shell [22,23]. These analyses identified a number of common metal binding motifs in proteins including di-histidines at positions *i* and *i*+4 in an α-helix [24] or *i* and *i*+2 in a β-sheet [25], $(Cys)_2(His)_2$ zinc finger motifs [26], and the calcium-binding EF hand motif [27]. These observations led to the development of an approach for metalloprotein design — first successfully employed almost 30 years ago [28–30] — in which commonly observed metal binding motifs were transplanted into other protein scaffolds.

From a design perspective, genetically encoded, metal-chelating NCAAs possess a number of features that could be used to circumvent challenges associated with more traditional metalloprotein design approaches. First, they provide high-affinity ligands for a number of biologically relevant metals and do so with chemical footprints only slightly larger than the bulkier naturally occurring amino acids. Second, both HQ-ala and Bpy-ala present two 'faces' with distinct chemical properties. Namely, one side of each molecule displays polar nitrogen or oxygen atoms that directly interact with the metal ions while the second 'face' is constructed from hydrophobic carbon atoms. This suggests that both NCAAs can be buried within the cores of proteins and that their orientations can be stabilized through hydrophobic packing interactions rather than the extensive H-bonding networks. Third, the properties of organic ligands including 2,2′-bipyridine and 8-hydroxyquinoline have been thoroughly studied