Creating Metal-Binding Sites in Proteins: The Antibody Approach

Lynette Cunningham Literature Seminar October 19, 1995

Metal ions play an important role in reactions catalyzed by protein enzymes. Both of the earliest identified zinc enzymes, carbonic anhydrase and carboxypeptidase, take advantage of the Lewis acid character of zinc [1, 2]. In carbonic anhydrase, zinc coordinates water and lowers its pK_a, resulting in a coordinated nucleophilic hydroxo group which attacks the carbon atom in carbon dioxide to yield bicarbonate. In carboxypeptidase A, zinc coordinates the carbonyl oxygen atom of the peptide substrate, making it more susceptible to nucleophilic attack by water.

The identification of metals as key components in catalytic enzymes has sparked interest in building metalloenzymes tailored for specific reactions/substrates from existing proteins. Zinc and other metal sites with K_d values ranging from 10 nM to 0.4 μM have been engineered into three proteins [3, 4, 5]. In each case, certain amino acids in the native protein were mutated to histidine or cysteine which contain good metal-ligating groups. Despite those examples, success has been limited in this area due to a lack of knowledge about how sequence is related to protein structure. It is difficult to predict how a change from one amino acid to another will perturb the protein's structure because protein folding is a complex process.

Antibodies make ideal candidates for remodeling because certain sequence domains, called complementary determining regions (CDRs), can be changed without perturbing the protein's overall structure [6]. The basic antibody structure (Fig. 1) is composed of light and heavy protein chains that form two arms (Fabs) and a tail [7]. Each Fab consists of constant, variable, and hypervariable sequence regions (CDRs). The constant and variable regions make up the framework of the antibody structure while the CDRs form the binding site for antigens such as a toxin or virus. The length and sequence of the CDRs determine the antibody's binding characteristics. Because the CDRs identify the binding location of the antigen (or substrate), they are the logical place in which to create a metal-binding site.

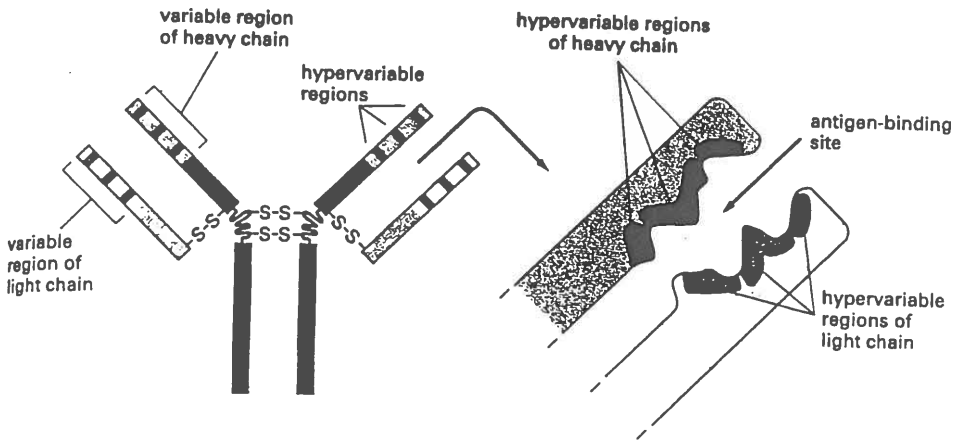

Figure 1. Antibody structure [7]: overall structure (left); Fab domain (right).

Another advantage of using antibodies is the ease with which a substrate-binding site can be generated in the antibody Fab [8]. This is done by immunizing an animal with the substrate and collecting the antibodies generated in response to this molecule. A more recent method involves using PCR (polymerase chain reaction) methods to generate a library of antibodies containing a variety of sequences in the CDR regions. The library is then screened for antibodies that bind the substrate. In theory, the resulting selected antibody could be made catalytic by adding a metal-binding site, thus creating a catalytic metalloantibody. For example, adding a zinc site containing one coordination site available to water could be used to make a metalloenzyme that catalyzes hydrolysis.

Template-based mutagenesis is the most common method used for creating a metal-binding site in an antibody. It consists of using the crystal structure of an existing metalloenzyme as a template for remodeling the antibody CDRs [9]. Once a potential metal-binding site is identified, the amino acids in the target CDRs are replaced with amino acids that coordinate metal in the metalloenzyme.

Another method for introducing metal sites into antibodies, called directed selection, consists of selecting antibodies that can bind metal from large combinatorial libraries. The libraries are composed of antibody Fabs containing randomized heavy chain CDR sequences combined with light chains of defined sequence [10, 11, 12]. The library is then subjected to a selection step such as passage over Sepharose beads containing bound metal (Fig. 2). This method was used successfully by Lerner et al. and resulted in eleven Cu-binding Fab sequences, six Zn-binding Fab sequences as well as Fabs that could bind Pb, Ce, and Fe [10]. Thus, the main advantage of the selection technique is that it provides a number of possibilities rather than just one species.

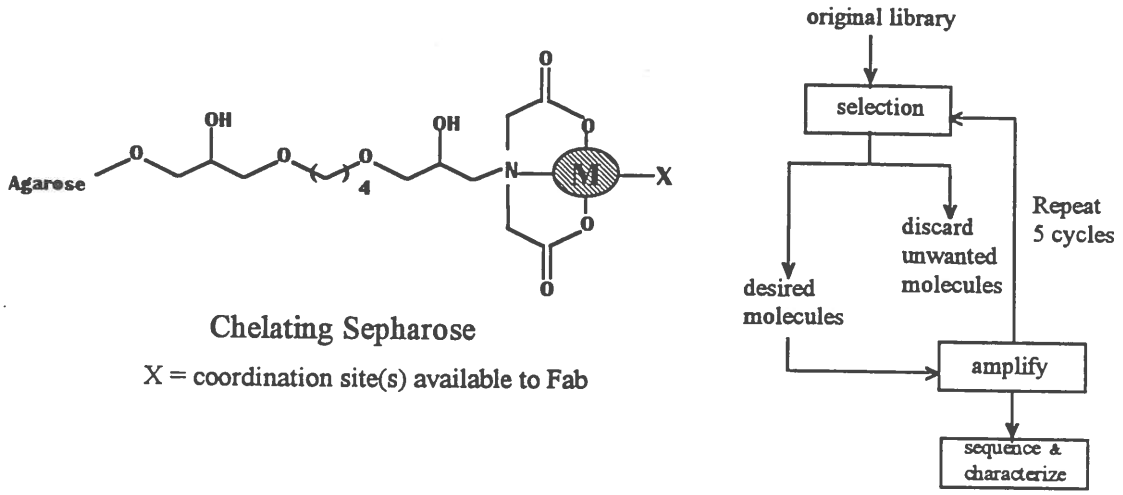

Figure 2. Directed selection [10]: chelating group used to trap metal (left); steps in selection protocol (right).

The binding affinity of created metal-binding sites is measured using fluorescence quenching techniques. These techniques take advantage of the fact that protein tryptophan fluorescence is quenched when metals are bound nearby. A relationship can be established between the amount of metal bound and the extent of fluorescence quenching [13].

Although fluorescence quenching techniques are useful for determining the binding affinity, they cannot identify the coordinating ligands. Benkovic et al. addressed this problem in a recent study in which 43C9 antibody Cu-binding mutants were characterized by electron paramagnetic resonance spectroscopy (EPR), electron spin echo envelope modulation

spectroscopy (ESEEM), and UV-vis spectroscopy [14]. From EPR, the ligands were identified as nitrogen and/or oxygen based on the fact that $A_{||}$ was greater than 160G. The ESEEM spectrum contained features typical of histidine coordination. UV-vis revealed an unusual tyrosine-Cu ligand-to-metal-charge-transfer (LMCT) band at 490 nm.

The use of antibodies in metal-site design has proven successful. Since 1990, there have been five successful studies in which a metal-site was engineered into an antibody [9, 10, 14, 15, 16]. In all studies, a zinc site was produced and in most cases the site also bound copper. The tightest zinc-binding mutant (K_d = 1.5 μM), containing histidine ligands, was made using the catalytic single-chain antibody 43C9 [14].

These results represent an important step in the creation of catalytic metalloantibodies. However, to date, no metalloantibodies have been created which exhibit catalytic activity. Problems include structure perturbation and inadequate interaction between the substrate and metal. This failure points to the need for more work with the directed selection method. Although some molecules will be selected that have inadequate substrate-metal interactions, stable metalloantibodies have a high probability of being present since the selected library is large and contains many molecules with different binding-site ligands. Further application of the directed selection technique would include selecting for a substrate binding site from the previously selected metal-binding Fab library.

Inorganic chemists can make significant contributions to this field which is largely dominated by molecular biologists. New combinations of ligands not seen in naturally-occurring metalloproteins could be identified using directed selection. When combined with inorganic spectral characterization, antibody selection techniques can expand our current knowledge of common metal-ligand interactions.

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