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Molecular recognition in protein modification with rhodium metallopeptides

Zachary T. Ball

Department of Chemistry, MS60, Rice University, 6100 Main St., Houston, TX 77005 U.S.A.
1-713-348-6159

Abstract

Chemical manipulation of natural, unengineered proteins is a daunting challenge which tests the limits of reaction design. By combining transition-metal or other catalysts with molecular recognition ideas, it is possible to achieve site-selective protein reactivity without the need for engineered recognition sequences or reactive sites. Some recent examples in this area have used ruthenium photocatalysis, pyridine organocatalysis, and rhodium(II) metallocarbene catalysis, indicating that the fundamental ideas provide opportunities for using diverse reactivity on complex protein substrates and in complex cell-like environments.

Introduction

Chemically altered proteins are becoming increasingly important for chemical biology, biophysical measurements, protein-based therapeutics, and biomaterials studies. While our ability to perform chemical manipulations on protein substrates is crucial, proteins, and biopolymers in general, represent some of the most challenging “substrates” for chemical reactions. Selective chemistries must deal with the twin challenges of broad tolerance of demanding aqueous, functional-group-rich environments and selectivity in a sea of similarly reactive functional groups.

Nature, of course, performs selective post-translational modification as a matter of course. But natural proteins remain complicated and alien species for chemists.[1–3] Natural proteins themselves are often so intractable that engineered approaches to target structures have often been the only way forward. Unnatural amino acids can now be incorporated in many contexts, providing bio-orthogonal handles for subsequent manipulation. Natural “tagging” sequences can be engineered into a protein sequence to achieve similar goals.[1–3] Alternatively, residue-selective methods can and do furnish conjugates of natural proteins by covalently modifying a single residue type (lysine, tyrosine, tryptophan, etc.). But in general, amino acids are repetitive and selective chemistry must rely on local environment,

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zb1@rice.edu.

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secondary structure, or tertiary molecular recognition. Apart from a few cases where unique cysteine or N-terminal modification[4] is possible, residue-selective methods lead to product mixtures and don't allow reactions in lysate, inside living cells, or in other complex environments. Selective catalysis on natural proteins, in any general way, remains an unsolved problem.

One approach to tackle the problem of natural proteins is combining a transition-metal catalyst with ligands that confer sequence- or site-selectivity on a catalytic reaction. In this conception, molecular recognition controls site-selectivity and increases reaction rate or productive reaction rate. Transition-metal catalysis has been a key player in the tremendous advances in small-molecule methodology development in recent decades. And it seemed clear to us that the non-traditional reactivity, non-biological mechanisms, and potentially low concentrations (loading) of transition-metal catalysts could offer significant advantages and opportunities. At the same time, proximity-driven reactivity is a hallmark of enzymatic catalysis—a part of even early lock-and-key models—and forms the basis for many designed protein tags and conjugation reactions. By combining these two concepts, new opportunities were envisioned. Among potential benefits, the molecular recognition motif (protein ligand) could be kept at catalytic concentrations—thus minimally perturbing the system—and could be separated from the tagging reagent—simplifying synthesis.

The prospect of de novo design of enzyme-like reactivity is an alluring one, and a variety of approaches using molecular recognition for catalysis have been pursued. The concepts of molecular recognition in catalysis show up in diverse fields,[5] including catalysis with dendrimers[6–8] and polymers, cyclodextrins,[9–11] and peptides[12–14]. The most well studied and successful examples using biopolymer substrates have been proteolytic and other degradative reactions. Both Ni(II) and Cu(II) complexes induce DNA strand cleavage by oxidative mechanisms. Conjugates with DNA-binding proteins or small molecules allow the creation of DNA- or RNA-targeting probes with sequence-specificity.[15–17] These ideas have also been used for the selective destruction of proteins.[18,19] Kostid [9] has used cyclodextrin-mediated molecular recognition of phenylalanine side chains to achieve proteolysis at Pro-Ser sequences (Figure 1). The result is an intriguing concept, but the selectivity and utility remains little explored. Pro-Ser motifs represent a middle ground between residue-selective and structure-selective approaches. It remains to be seen how useful dipeptide recognition might be in the context of larger biomolecules.

More recently, copper-catalyzed selective oxidative peptide cleavage has been demonstrated with a serine oxidation catalyst. (Figure 1B,C).[20] A presumed initial oxidation of the side-chain hydroxyl is followed by oxidative C–C cleavage to provide an oxalate derivative, which hydrolyzes under the reaction conditions. The work is a significant demonstration of the utility of catalyst design to control reactivity in complex systems. The authors achieve selective oxidation of the serine hydroxyl with O₂, even though oxidation of electron-rich aromatic and sulfur-containing side chains is well studied with other catalysts. The authors do note some side products from reaction at those oxidation-prone side chains, but nonetheless were able to demonstrate oxidative cleavage of the three serine sites in ubiquitin, a chemical analogue of enzymatic protein degradation that delivers completely new selectivity. The authors observed and identified the cleavage fragments (1–19, 21–56,

and 58–64) by protein gel and by mass spec. At this point, the chemistry is residue-selective, targeting all serine side chains, and new approaches would be needed to provide specificity for single sites within larger proteins.

Molecular recognition has been used to wonderful effect in facilitating photoredox chemistry at protein surfaces (Figure 2). Oxidative coupling of tyrosine side chains near the binding site occurred with electron-rich dimethylaniline reagents. Bringing photocatalysis to bear on protein chemistry problems brings with it the potential for spatial and temporal control of reactivity in new and valuable ways. Thus far, model proteins BSA and carbonic anhydrase (CA) have been successfully modified, and future work should provide an understanding of the generality and possibilities of this approach. In a metal-free implementation of these ideas, the Hamachi group, in a series of exciting papers, has demonstrated that “catalysts” as simple as pyridine can efficiently modify lysine near a binding site, via acylation of acylpyridinium intermediates. [21] The work grew out of previous efforts to develop selective modification with traditional electrophiles intermediates, such as tosylates, when it was discovered that catalytic methods delivered improved efficacy. This catalytic approach allow for simple “traceless” modification and the improved efficiency allowed labeling the surface of living cells, an unmet challenge with previous approaches.

To address the problem, we investigated rhodium(II) metallopeptide catalysts.[23] The dirhodium(II) tetracarboxylate center was employed for selective side-chain modification, based on a report of tryptophan modification with stabilized diazo reagents.[24] The peptide ligand could be viewed as a molecular recognition motif, delivering the reactive rhodium(II) complex to specific side chains on the surface of a target protein. Importantly, the dirhodium(II) tetracarboxylate core is stable to hydrolysis, ligand exchange, and decomposition under physiologically relevant conditions, yet contains weakly-held axial ligands (opposite the Rh–Rh bond) that readily dissociate, allowing catalysis in water. We developed general methods to synthesize diverse and complex (we have made complexes up to 30 amino acids) metallopeptides, including, where necessary, a protecting-group strategy for carboxylate deprotection after metalation to enable selective metalation of sequences with multiple carboxylates.[25]

We initially began to explore the topic by studying modification of coiled-coil peptides, which we viewed as a simplistic model for protein-protein interactions (Figure 3). Based on a well-studied model [26], we used rhodium(II) metallopeptides with coil structure to catalyze modification of specific residues flanking the coiled-coil interface. The known tryptophan reactivity[24] could be replicated in this system, and molecular recognition had a profound effect on reactivity: a >1000x increase in reaction rate for template catalysis was seen relative to unstructured peptide substrates.[27]

Tryptophan is uniquely reactive with simple $\text{Rh}_2(\text{OAc})_4$ catalysis. In contrast, proximity-driven catalysis with a metallopeptide allowed selective reactions at many other residues, completely unreactive in reactions with $\text{Rh}_2(\text{OAc})_4$. The phenylalanine benzene ring; terminal carboxylates and carboxamides from asparagine, aspartate, glutamine, and glutamate; and the arginine guanidine group were part of the surprising breadth of side-chain

functionality that could be effectively modified.[23] Recently, rhodium(II) catalysis has also been used for oligonucleotide modification [28], where interesting structure selectivity was observed.

The proximity-driven catalysis could be extended to natural protein substrates, using a proline-rich metallopeptide that binds to SH3 domains (Figure 4a) [29]. Attaching a rhodium complex to S2, L3, or R5 positions on the proline-rich peptide ligand allows efficient modification of W42.

In addition to the breadth of reactivity, rhodium metalloptides are active in cell lysate (Figure 4a) [29,30]. Lysate from *E. coli* producing desired substrate proteins could be directly treated with a biotinylated diazo reagent **1b** and an appropriate catalyst, resulting in orthogonal modification of specific proteins, depending on the structure of the catalyst chosen[30]. A thorough study of rhodium placement at different points on the coil structure was used to optimize reactivity and orthogonality of the designed coils, E3_g2W and E3_c2W [30].

Future directions

Studies into molecular recognition for designed, non-biological transition-metal catalysis have opened up new doors as well. Histidine and methionine residues retard catalysis through coordination with the rhodium center [31,32]. This has opened new opportunities to use rhodium coordination in developing targeted, metal-based protein inhibitors [25,31,32]. Though conceptually straightforward, this approach is surprisingly little-investigated. A cobalt-based approach for targeted inhibition of transcription factors is a noteworthy recent addition to this research area [33,34].

From a fundamental perspective, the catalytic approach outlined here begins to provide approaches for selectivity on polyfunctional substrates and in polyfunctional environments. Small molecule synthesis is traditionally controlled by inherent chemical reactivity. Despite what is now decades of effort to overcome this limitation and develop selective reactions that rival enzymatic transformations, successful examples are rare—such as recent examples with complex natural products [35,36]—and are just beginning to touch the surface of what is possible.

Catalytic, site-specific protein modification, perhaps most importantly, provides a tool to synthesize new biomaterials and bioconjugates quickly and easily. The ideas conveyed here have been used to modify and label specific targets in living systems, to modify structures common to a broad domain class (SH3), or to develop proteolytic activity with specificity rivaling natural enzymes. The future possibilities in this area are immense. Diverse fields and factors are combining to make these studies especially important going forward: Proteins and protein conjugates are increasingly viewed as promising drug candidates; a focus on green and smart materials creates new opportunities for novel biomaterials; and improved methods that allow routine biopolymer analysis (especially mass spectrometry) greatly simplify the task of characterizing reaction “products” that might be 100 kDa or more. Taken together, catalytic chemistry, site-specificity, and efficient, highly selective reactions provide unique and increasing opportunities in diverse fields.

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Highlights

- New ideas are needed for chemical modification of natural proteins.
- Enzyme-like selectivity is possible by combining catalysts with molecular recognition ideas.
- Current examples include complex environments including living systems.
- Rhodium(II) catalysis provides a broad amino-acid substrate scope for implementation of molecular recognition ideas.

Figure 1.

(A) Cyclodextrin–palladium conjugates promote proteolysis at Pro–Ser sequences [9]. (B) Copper-catalyzed peptide cleavage at serine. (C) Sequence of ubiquitin, with serine residues highlighted.

Figure 2.
A catalytic approach for SET-based tyrosine labeling.[22]

Figure 3.
Proximity-driven modification of a designed E3/K3 coiled-coil.

Figure 4.

(A) The Fyn SH3 domain is efficiently modified by diazo reagents specifically at W42 with the catalysts S2E^{Rh}, L3E^{Rh}, and R5E^{Rh}. The 13D^{Rh} serves as an inactive negative control. (B) Modification of proteins in lysate. Proteins containing two different coil sequences (E3_g2W or E3_e2W) are effectively and individually conjugated with the biotin—diazo reagent **1b**, depending on catalyst choice. Biotin-specific blot (at right) demonstrates the potential for designed orthogonal reactivity.