J|A|C|S COMMUNICATIONS

Published on Web 04/26/2010

Structure-Selective Modification of Aromatic Side Chains with Dirhodium Metallopeptide Catalysts

Brian V. Popp and Zachary T. Ball*

Department of Chemistry, Rice University, MS 60, 6100 Main Street, Houston, Texas 77251

Received February 26, 2010; E-mail: zb1@rice.edu

Supramolecular assembly enables reactivity that would otherwise be kinetically impossible. Enzymes routinely exploit this concept to achieve rate acceleration driven by induced reagent proximity, allowing precise chemoselectivity in a complex environment. Efforts to design enzyme-like chemical transformations have used polypeptides[1] or polynucleotides[2] to template organic reactivity, but the extension of these ideas to organometallic catalysis is quite limited, despite the clear opportunities of both biological and chemical organometallic catalysis.[3] In this paper, we describe peptide modification using dirhodium metallopeptide catalysts that combines peptide-based molecular recognition with residue-selective, catalytic side-chain modification. This combination enables selectivity in organometallic catalysis on the basis of molecular shape rather than local environment and extends the scope of dirhodium peptide modification to include previously unreactive tyrosine and phenylalanine residues.

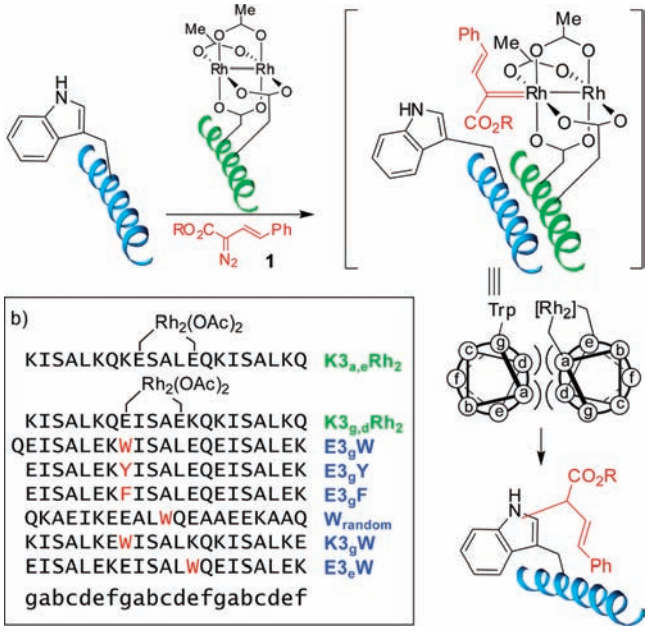


Figure 1. (a) Conceptual representation of structure-selective peptide modification driven by peptide−peptide molecular recognition by a dirhodium metallopeptide catalyst. (b) Peptide sequences used in this study.

Dirhodium tetracarboxylates are an attractive choice for examining peptide-induced selectivity in organometallic catalysis because the dirhodium center is known to catalyze X−H and C−H insertions in water,[4] including those of the indole side chain of tryptophan residues.[5] In addition, dirhodium metallopeptides bound to side-chain carboxylates are stable with respect to ligand exchange in water at pH ≤ 7.[6] We turned to the coiled coil, a simple and robust peptide assembly, to provide molecular recognition.[7,8] Coiled coils mediate essential protein−protein interactions, and peptides that interact with natural coil domains, inhibiting protein−protein interactions, have been described.[9] Coiled-coil sequences feature a heptad repeat (labeled *abcdefg*) with hydrophobic residues in positions *a* and *d* to create a hydrophobic interface along one side of an α-helix, facilitating assembly into dimeric or higher-order structures. The design principles for heterodimeric pairs are well-established, such as the E3/K3 pair, in which complementary charges at the positions that flank the interface, *e* and *g*, select for heterodimerization of the lysine-rich K3 and glutamate-rich E3 peptides.[10]

As shown in Figure 1, we envisioned that the metallopeptide **K3$_{a,e}$Rh$_2$**, in which the peptide is bound to a dirhodium center through two glutamate side chains,[11] might deliver the dirhodium catalyst to the site of a reactive side chain, facilitating covalent modification. Binding the dirhodium center at positions *a* and *e* ensures that the metal will be held proximal to the coil−coil interface, and the *a*, *e* spacing serves to stabilize the necessary helical structure.[6] The E3/K3 parent for the sequences used here forms simple dimers with $K_d \approx 9$ μM. The presence of a bridging dirhodium center in **K3$_{a,e}$Rh$_2$** does not significantly alter these properties, as judged by circular dichroism, Job plot analysis, and thermal denaturation experiments (see the Supporting Information).

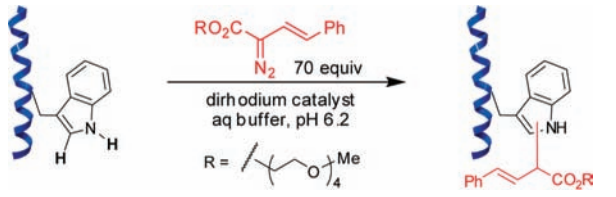
A peptide substrate with a tryptophan positioned so as to flank the hydrophobic interface (**E3$_g$W**, 50 μM) in the presence of 100 mol % $Rh_2(OAc)_4$ reacted sluggishly with the diazo reagent **1**, which had proved optimal in previous studies.[5] Modification of **E3$_g$W** reached 20% conversion after 20 h, as determined by MALDI−TOF MS analysis of the reaction mixture,[12] presumably as a mixture of N-alkylated and 2-alkylated species (Table 1 and Figure 2a).[5] When the same reaction was examined with the metallopeptide catalyst **K3$_{a,e}$Rh$_2$**, covalent modification was dramatically more efficient, reaching >95% conversion in less than 20 min with 10 mol % metallopeptide. When the loading of **K3$_{a,e}$Rh$_2$** was further reduced to 2 mol %, the rate of reaction remained high, and >95% conversion of **E3$_g$W** was observed after 20 h (Table 1 and Figure 2b). Modification of polypeptides with $Rh_2(OAc)_4$ requires large excesses of diazo reagent ($\geq$50 equiv) because of competing unproductive reactivity of the intermediate metallocarbenoid with water. However, the rate acceleration afforded by the metallopeptide catalyst was sufficient to provide 35% conversion within 20 h with only 1 equiv of diazo **1** (Table 1, entry 4). MS/MS analysis established that modification occurs at the tryptophan site (see the Supporting Information), and consistent with previous observations,[5] the doubly labeled product was also observed in some cases. The change in $t_{1/2}$ from >20 h with 100 mol % $Rh_2(OAc)_4$ to <1 h with 2 mol % **K3$_{a,e}$Rh$_2$** allows a conservative estimate of $\geq 10^3$ for the rate enhancement due to substrate binding.

To establish the basis for the observed rate enhancement, a competition experiment was conducted between coil **E3$_g$W** and a shuffled sequence, **W$_{random}$**, having the same overall charge (−3)

10.1021/ja101456c

as **E3$_g$W** (Figure 3). A control experiment demonstrated that the two tryptophan-containing peptides react at similar rates with the nonselective small-molecule catalyst $Rh_2(OAc)_4$ (Figure 3a). In contrast, a competition reaction catalyzed by the metallopeptide **K3$_{a,e}$Rh$_2$** (10 mol %) resulted in extensive (>95%) modification of coil **E3$_g$W** within 2 h, while only traces of modified control peptide were observed (Figure 3b). This result implicates molecular recognition as the basis for selective modification.

Table 1. Covalent Modification of Peptide **E3$_g$W**[a]



		conv. of **E3$_g$W**			
entry	catalyst (mol %)	0.3 h	2 h	20 h	$t_{1/2}$
1	$Rh_2(OAc)_4$ (100)	10	8	17	>20
2	**K3$_{a,e}$Rh$_2$** (10)	64	>95	>95	0.25
3	**K3$_{a,e}$Rh$_2$** (2)	30	74	>95	1
4[b]	**K3$_{a,e}$Rh$_2$** (10)	7	22	35	–

[a] All of the reactions used 50 μM substrate at room temperature. Conversion data are based on MALDI–TOF MS analysis of the reaction mixture. [b] Using 1 equiv of diazo reagent.

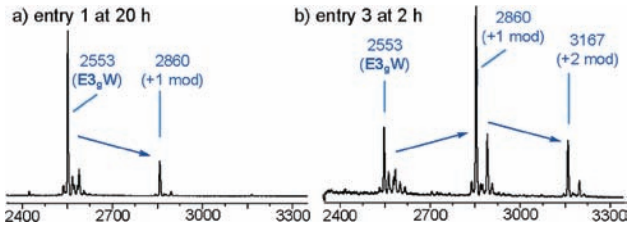


Figure 2. MALDI–TOF MS spectra for modification of peptide **E3$_g$W**. See Table 1 for reaction details.

The dramatic rate enhancement of tryptophan modification led us to examine the reactivity of other aromatic side chains toward modification with a metallocarbenoid (Figure 3c,d). Peptides **E3$_g$Y** and **E3$_g$F** substitute tyrosine and phenylalanine residues for the flanking tryptophan residue in **E3$_g$W**. As anticipated, no modification of either phenylalanine or tyrosine was observed with the $Rh_2(OAc)_4$ catalyst, and only the **W$_{random}$** sequence was modified in competition experiments with peptides **E3$_g$Y** and **E3$_g$F**. In spite of the absence of reactivity with $Rh_2(OAc)_4$, both peptides were modified by the diazo reagent **1** upon treatment with **K3$_{a,e}$Rh$_2$** (20 mol %). The modification of the tyrosine- and phenylalanine-containing coils is slower than that of the tryptophan coil, reaching ~50% conversion in 5 h. Nevertheless, complete conversion was achieved by addition of diazo reagent **1** in two portions. No modification of a control peptide with alanine or serine in place of tyrosine and phenylalanine was observed. In competition experiments, the peptides **E3$_g$Y** and **E3$_g$F** were selectively modified in preference to the tryptophan-containing peptide **W$_{random}$** (Figure 3c,d). That phenylalanine and tyrosine were the sites of covalent modification in these cases was established by MS/MS analysis (see the Supporting Information). While the work of Francis provides good precedent for the structure of modified tryptophan (see above), establishing the structure of modified tyrosine and phenylalanine is more challenging. For tyrosine, significant precedent suggests the likelihood of etherification through O–H insertion.[13] For phenylalanine, the situation is more complicated because the reaction of diazo compounds with simple arene rings in metallocarbene catalysis can result in aromatic C–H insertion, benzylic C–H insertion, or dearomatizing cyclopropanation to form a norcaradiene structure.[14] Efforts to understand the precise structures of these adducts are ongoing.

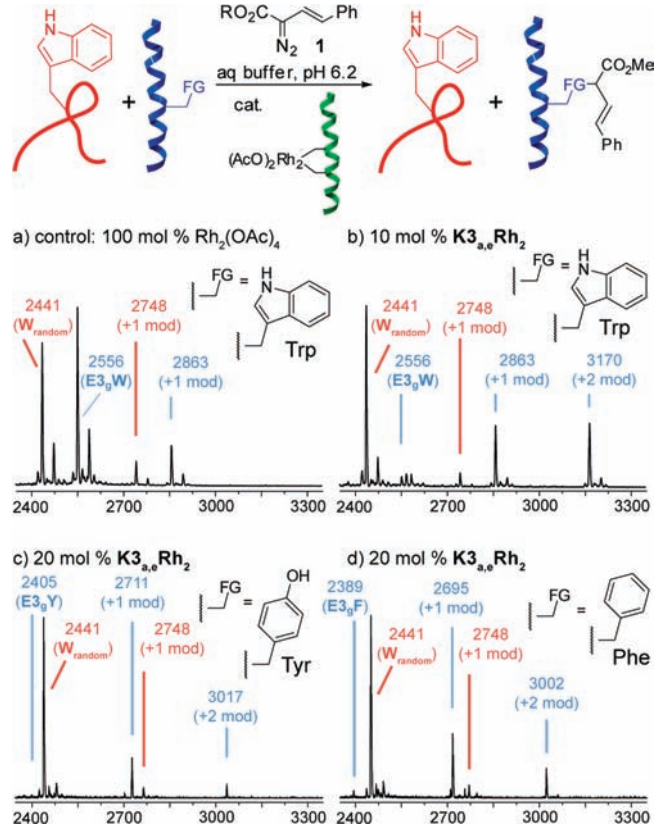


Figure 3. Competitive modification of **E3$_g$X** (X = W, Y, F) peptides (50 μM) in the presence of the control peptide **W$_{random}$** (50 μM). R = $-(CH_2CH_2O)_4Me$. MALDI–TOF MS spectra are shown for (a) **E3$_g$W** with 100 mol % $Rh_2(OAc)_4$ at 20 h, (b) **E3$_g$W** with 10 mol % **K3$_{a,e}$Rh$_2$** at 2 h, (c) **E3$_g$Y** with 20 mol % **K3$_{a,e}$Rh$_2$** at 24 h, and (d) **E3$_g$F** with 20 mol % **K3$_{a,e}$Rh$_2$** at 24 h.

We conducted additional competition experiments to establish the specificity of catalytic modification. Figure 4 illustrates helical wheel representations (looking down the helical axis) that provide insight into the spatial relationships of several competition experiments involving "matched" and "mismatched" peptide targets. In one case, a mixture of the substrate **E3$_g$W** and peptide **K3$_g$W** with noncomplementary charges in the flanking *e* and *g* positions was treated with **K3$_{a,e}$Rh$_2$**. True to expectations, extensive (>95%) conversion of the matched substrate **E3$_g$W** was observed, while the mismatched peptide **K3$_g$W** was modified in only trace amounts (Figure 4a). To explore the specificity of tryptophan placement, a competition was conducted between the substrate **E3$_g$W** and peptide **E3$_e$W** having the tryptophan residue in position *e*, on the opposite flank of the coiled-coil interface (see Figure 4b). In this case, both substrates were expected to form coiled coils with the **K3$_{a,e}$Rh$_2$** catalyst, yet selectivity favoring the correctly positioned tryptophan group was observed. In both cases, control experiments demonstrated that substrates react at similar rates with the small-molecule catalyst $Rh_2(OAc)_4$.

The competition experiment between peptides **E3$_g$W** and **E3$_e$W** is especially enlightening. Because coils can assemble with either parallel or antiparallel orientation, it is possible for the metallopep-

tide $\mathbf{K3_{a,e}Rh_2}$ to localize near either the *g* (parallel orientation, as in Figure 4b) or *e* (antiparallel orientation, not shown) position of the substrate. To better understand the structural basis of the selectivity, we synthesized an alternative metallopeptide, $\mathbf{K3_{g,d}Rh_2}$, having the dirhodium center bound through glutamate residues at positions *g* and *d* on the opposite side of the hydrophobic interface. The selectivity of this new catalyst was opposite that of the catalyst $\mathbf{K3_{a,e}Rh_2}$, providing selective modification of the previously mismatched substrate $\mathbf{E3_eW}$ (Figure 4c). Taken together, the divergent selectivities observed with the two catalysts $\mathbf{K3_{a,e}Rh_2}$ and $\mathbf{K3_{g,d}Rh_2}$ (Figure 4b,c) point to a preference for catalytic modification through parallel rather than antiparallel heterodimerization, in line with expectations for the sequences employed.[8]

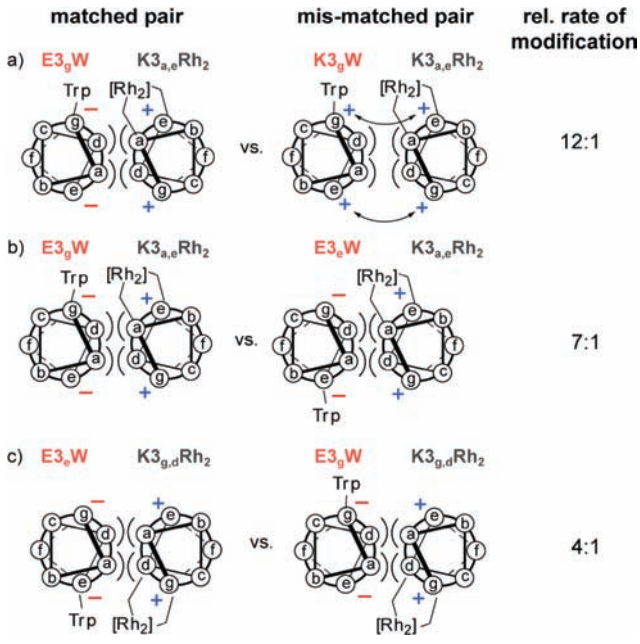


Figure 4. Relative modification rate, based on MALDI−TOF MS analysis of the reaction mixture, for competitive modification with diazo **1** of (a) oppositely charged peptides $\mathbf{E3_gW}$ and $\mathbf{K3_gW}$ with metallopeptide $\mathbf{K3_{a,e}Rh_2}$ and (b, c) peptides $\mathbf{E3_gW}$ and $\mathbf{E3_eW}$ with metallopeptides (b) $\mathbf{K3_{a,e}Rh_2}$ and (c) $\mathbf{K3_{g,d}Rh_2}$. All of the reactions were 50 μM in peptides with 10 mol % metallopeptide and were analyzed after a reaction time of 15 min.

Enzymes employ molecular recognition to allow transformations at specific functional groups in the presence of other inherently more reactive sites. In contrast, selectivity with chemical catalysts is typically governed by intrinsic reactivity; designed ligands that override inherent reactivity are rare. Here we have provided an example of the former selectivity by demonstrating that molecular recognition enables modification of otherwise unreactive tyrosine and phenylalanine residues, even in the presence of a more reactive residue, tryptophan.

This work expands the scope of dirhodium-catalyzed side-chain modification to include tyrosine and phenylalanine residues. While other methods to perform selective tyrosine modification have been described,[15,16] we believe this work describes the first example of selective phenylalanine modification in a polypeptide. Chemical methods for modification of a single natural amino acid type using organic and organometallic reagents, such as electrophiles that modify all cysteines or all lysines, have been described.[15,17] However, a given amino acid occurs frequently enough that selectivity for an amino acid type is usually insufficient to label a single site in a polypeptide mixture or even on a single large protein. By combining residue-selective chemistry with secondary-structure recognition, we have provided a strategy for selective covalent modification of biomolecules. Beyond coiled coils, the principles described here should be amenable to other helix-binding protein domains and to biological molecular recognition generally.

Acknowledgment. We thank Jeffrey Hartgerink and Erica Bakota for assistance with peptide synthesis and acknowledge financial support from a J. Evans Attwell−Welch Postdoctoral Fellowship (B.V.P.), a Robert A. Welch Foundation research grant (C-1680), and Rice University.

Supporting Information Available: Experimental details, characterization data for peptides, and additional MS and HPLC data for modification reactions. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA101456C