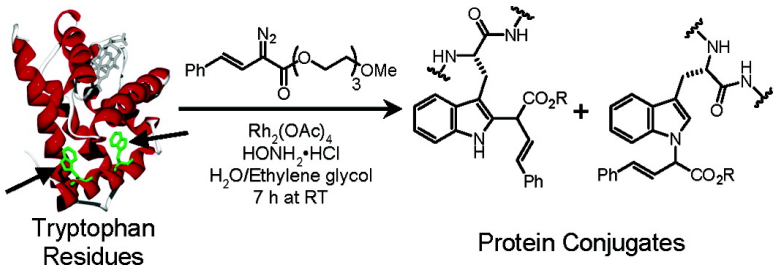
Communication

Selective Tryptophan Modification with Rhodium Carbenoids in Aqueous Solution

John M. Antos, and Matthew B. Francis

J. Am. Chem. Soc., **2004**, 126 (33), 10256-10257• DOI: 10.1021/ja047272c • Publication Date (Web): 30 July 2004



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 7 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 07/30/2004

Selective Tryptophan Modification with Rhodium Carbenoids in Aqueous Solution

John M. Antos and Matthew B. Francis*

Department of Chemistry, University of California, Berkeley, California 94720-1460, and Material Science Division, Lawrence Berkeley National Labs, Berkeley, California 94720

Received May 10, 2004; E-mail: francis@cchem.berkeley.edu

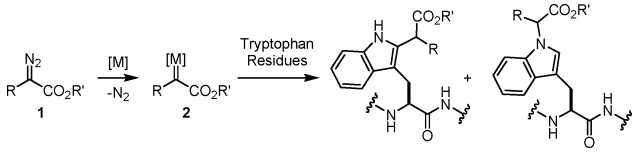
The conjugation of small molecules to protein targets has occupied a central role in the study of biological systems,[1] resulting in an ever increasing need for new reactions that can modify proteins in a selective manner.[2] Transition metal-based methods could provide an exceptionally powerful set of tools for this purpose, as many metal complexes can activate otherwise unreactive functional groups with excellent selectivity. Many of these reactions exhibit virtually complete functional group tolerance and have been used successfully in aqueous solution.[3] A particularly interesting feature for bioconjugation is the ability to influence the reactivity of transition metal complexes through proper selection of the metal ion and ligand sphere. Such tuning could allow selective targeting of one amino acid side chain over another, and the complex steric environments provided by asymmetric ligands could provide a much needed way to distinguish between several occurrences of a particular residue.

To explore these possibilities, we have focused on the use of metallocarbenoids (**2**) for bioconjugation, as these species could react with many of the functional groups that are present on protein surfaces.[4] It was hypothesized that by tuning the reaction parameters, selectivity between these pathways could be achieved. We have found that this is in fact the case, and as a result of these studies a highly selective method for the modification of tryptophan residues has emerged, Scheme 1. This reaction proceeds readily in aqueous solution, and can modify protein substrates at concentrations as low as 10 μM.

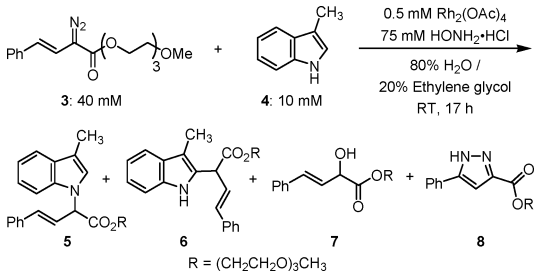
In preliminary studies, it was found that metallocarbenoids derived from stabilized vinyl diazo compound[5] **3** can react with 3-methylindole (**4**) in a highly efficient manner[6] despite the anticipated reaction of the metallocarbene intermediate with water[7] (Scheme 2). With only 4 equiv of diazo compound, a 1:1.4 mixture of *N*-alkylated (**5**) and 2-alkylated (**6**) products was obtained in 51% combined yield, presumably through direct N−H insertion and through the intermediacy of a cyclopropane intermediate, respectively. The remainder of the diazo compound reacted with water to form alcohol **7** as well as trace amounts of pyrazole **8** through a metal-independent electrocyclization pathway.[8] As the organic substrates possess little water solubility, a cosolvent was used to achieve optimal reactivity. With the goal of developing protein-compatible reaction conditions, we selected ethylene glycol for this purpose, as studies have demonstrated that protein structures are resistant to denaturation by this solvent.[9] It was also found that the addition of $HONH_2$·HCl dramatically enhanced the reactivity of the catalyst, presumably by binding to the metal center and stabilizing the reactive intermediates. The origin of this effect is currently under study.

To modify tryptophan residues on protein substrates, two additional criteria needed to be met. First, the reaction must be viable at substrate concentrations of 100 μM and below. Second, both the metallocarbenoid and the rhodium catalyst itself must

Scheme 1. Covalent Modification of Tryptophan Residues on Proteins Using Metallocarbenes



Scheme 2. Modification of 3-Methylindole with Metallocarbenes in Aqueous Media



tolerate the large number of polar spectator groups that are present on the surface of all proteins. The ability of the reaction to meet these criteria was first tested on a 100 μM solution of horse heart myoglobin in a 80:20 water/ethylene glycol mixture. The protein solution was exposed to 100 equiv of **3** and 1 equiv of $Rh_2(OAc)_4$ at room temperature for 7 h, Figure 1a.[10] After removal of the small-molecule byproducts using gel filtration, the protein mixture was analyzed using ESI-MS. Clean conversion to the singly and doubly modified products was observed (Figure 1b), as would be expected by the presence of two tryptophan residues in the amino acid sequence. Based on ESI-MS analysis, approximately 60% conversion was obtained for this reaction. Quantitative SDS-PAGE analyses indicated excellent (>90%) protein recovery after the reaction. Importantly, no appreciable conversion was observed in the absence of the rhodium catalyst (Figure 1c), implicating the intermediacy of a rhodium carbenoid species. Subsequent reactions have indicated that similar conversion can be achieved for 10 μM solutions of myoglobin.[11]

To confirm the site selectivity of the reaction unambiguously, the protein product mixture was digested with trypsin. Analysis of the resulting peptides using MALDI-TOF MS indicated that the reaction occurred only on the segment containing the two tryptophan residues. Both the singly and doubly modified fragments were further subjected to MS/MS analysis, which confirmed modification at W7 and W14. The secondary ion spectrum for the doubly modified fragment is shown in Figure 1d. In addition to the full *y*-ion series expected for the doubly modified peptide, a diagnostic *i*-ion for the modified tryptophan[12] (**9**, *m*/*z* 465) can be observed. Although the low pH of the reaction media (ca. 3.5) caused dissociation of the heme and presumably changes in the tertiary

10.1021/ja047272c CCC: $27.50

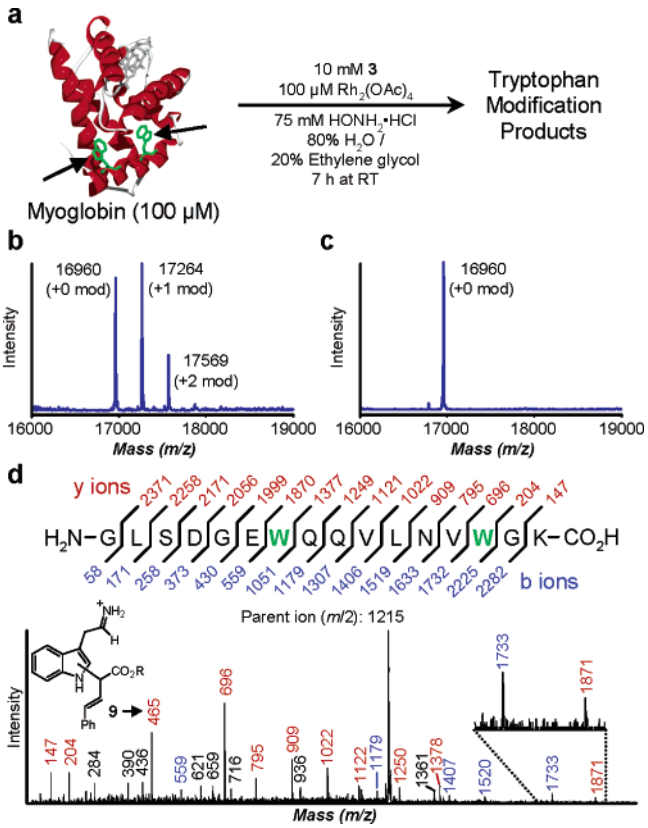


Figure 1. Modification of myoglobin with metallocarbenes. (a) A 100 μM solution of horse heart myoglobin was exposed to **3** and $Rh_2(OAc)_4$ for 7 h. The two tryptophan residues are shown in green. (b) Following removal of the small molecules via gel filtration, the sample was analyzed by ESI-MS. Both singly and doubly modified protein products were identified in the mass reconstruction. (c) In the absence of $Rh_2(OAc)_4$, no products were obtained under otherwise identical conditions. (d) After digestion with trypsin, MS/MS analysis of the doubly modified peptide fragment confirmed modification of only the tryptophan residues. All assigned species agree to within 0.1% of the expected mass values.

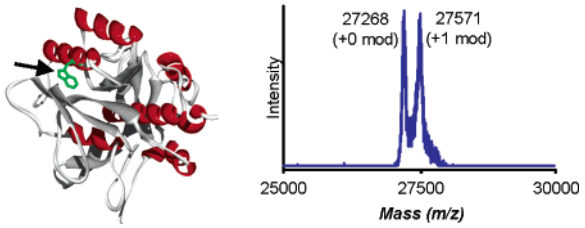


Figure 2. Modification of subtilisin Carlsberg with rhodium carbenoids. Conditions: 100 μM protein, 10 mM **3**, 100 μM $Rh_2(OAc)_4$ and 75 mM $HONH_2$·HCl (pH 1.5) in 80% water/20% ethylene glycol, rt, 7 h. Following removal of the small molecules via gel filtration, the sample was analyzed using MALDI-TOF MS. Only the singly modified protein was observed, as would be expected by the single tryptophan residue (shown in green). In the absence of $Rh_2(OAc)_4$, no reaction occurred under otherwise identical conditions.

protein structure,[13] subsequent reconstitution experiments confirmed that the modified myoglobin was still competent to bind the heme group.[11]

Attempts to modify subtilisin Carlsberg, a protein possessing a single tryptophan residue, were unsuccessful under analogous conditions. However, by lowering the pH of the reaction to 1.5, clean conversion to a singly modified product was observed, Figure 2. Trypsin digest analysis again confirmed that the reaction occurred only on the portion of the protein containing the tryptophan residue, W113. The higher conversion at lower pH presumably results from denaturation of the protein, which increases the solvent accessibility of the tryptophan residue.

These initial studies indicate that transition metal-catalyzed reactions are indeed capable of modifying native protein functional groups with very high selectivity.[14] The reaction described herein represents one of the first methods for selective tryptophan bioconjugation and one of the first examples of a metallocarbene-based reaction in aqueous solution. Current efforts are focused on increasing the pH of the reaction to broaden the substrate scope.

In light of the relatively low abundance of tryptophan residues on protein surfaces, this technique offers a selective bioconjugation strategy that complements more commonly used cys- and lys-based reactions. This possibility is currently being explored for the functionalization of introduced tryptophans on expressed proteins. Furthermore, as tryptophan residues often occur in binding sites and can serve as mediators of electron transfer,[15] this reaction provides a new tool to alter or block their participation in these pathways.

Acknowledgment. We gratefully acknowledge the University of California, Berkeley, the Nanoscale Science, Engineering, and Technology Program (NSET), and the Center for New Directions in Organic Synthesis. CNDOS is supported by Bristol-Myers Squibb as a Sponsoring Member and Novartis Pharma as a Supporting Member. J.M.A. was supported by a Berkeley Fellowship for Graduate Study. We gratefully acknowledge Waters Inc. for access to a Q-TOF Micro mass spectrometer, and Jacob M. Hooker for his analysis expertise.

Supporting Information Available: Full experimental procedures and characterization data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hermanson, G. T. *Bioconjugate Techniques*, 1st ed.; Academic Press: San Diego, 1996.
(2) For recent examples, see: (a) Muir, T. W.; Sondhi, D.; Cole, P. A. *P. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6705−6710. (b) Cotton, G. J.; Muir, T. W. *Chem. Biol.* **1999**, *6*, R247−R256. (c) Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G. *J. Am. Chem. Soc.* **2003**, *125*, 3192−3193. (d) Speers, A. E.; Adam, G. C.; Cravatt, B. F. *J. Am. Chem. Soc.* **2003**, *125*, 4686−4687. (e) Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007−2010. (f) Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett.* **2000**, *2*, 1939−1941. (g) Hooker, J. M.; Kovacs, E. W.; Francis, M. B. *J. Am. Chem. Soc.* **2004**, *126*, 3718−3719.
(3) For examples employed in the context of biomolecules, see: (a) Mortell, K. H.; Gingras, M.; Kiessling, L. L. *J. Am. Chem. Soc.* **1994**, *116*, 12053−12054. (b) Dibowski, H.; Schmidtchen, F. P. *Angew. Chem., Int. Ed.* **1998**, *37*, 476−478. (c) Bong, D. T.; Ghadiri, M. R. *Org. Lett.* **2001**, *3*, 2509−2511.
(4) (a) Ye, T.; McKervey, M. A. *Chem. Rev.* **1994**, *94*, 1091−1160. (b) Doyle, M. P. *Chem. Rev.* **1986**, *86*, 919−939.
(5) (a) Davies, H. M. L.; Clark, T. J.; Church, L. A. *Tetrahedron Lett.* **1989**, *30*, 5057−5060. (b) Davies, H. M. L.; Bruzinski, P. R.; Lake, D. H.; Kong, N.; Fall, M. J. *J. Am. Chem. Soc.* **1996**, *118*, 6897−6907.
(6) Similar reactivity has been observed in CH_2Cl_2: (a) Salim, M.; Capretta, A. *Tetrahedron* **2000**, *56*, 8063−8069. (b) Gibe, R.; Kerr, M. A. *J. Org. Chem.* **2002**, *67*, 6247−6249.
(7) Miller, D. J.; Moody, C. J. *Tetrahedron* **1995**, *51*, 10811−10843.
(8) Davies, H. M. L.; Hutcheson, D. K. *Tetrahedron Lett.* **1993**, *34*, 7243−7246.
(9) Khmelnitsky, Y. L.; Mozhaev, V. V.; Belova, A. B.; Sergeeva, M. V.; Martinek, K. *Eur. J. Biochem.* **1991**, *198*, 31−41.
(10) Horse heart myoglobin structure from Maurus, R.; Bogumil, R.; Nguyen, N. T.; Mauk, A. G.; Brayer, G. *Biochem. J.* **1998**, *332*, 67−74.
(11) See Supporting Information for details and spectra.
(12) These species are commonly observed for tryptophan residues: Falick, A. M.; Hines, W. M.; Medzihradszky, K. F.; Baldwin, M. A.; Gibson, B. W. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 882−893.
(13) Yang, A. Y.; Honig, B. *J. Mol. Biol.* **1994**, *237*, 602−614.
(14) The ability of the reaction to modify cysteine and methionine residues is currently being evaluated.
(15) For examples, see: Stubbe, J.; Nocera, D. G.; Yee, C. S.; Chang, M. C. Y. *Chem. Rev.* **2003**, *103*, 2167−2201.

JA047272C