J|A|C|S
COMMUNICATIONS

Published on Web 09/08/2005

Reductive Alkylation of Proteins Using Iridium Catalyzed Transfer Hydrogenation

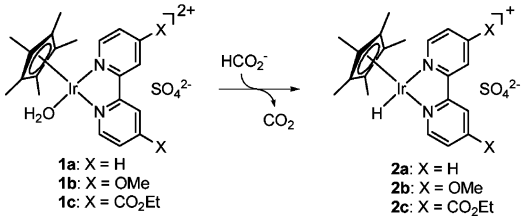
Jesse M. McFarland and Matthew B. Francis*

Department of Chemistry, University of California, Berkeley, California 94720-1460, and Materials Sciences Division, Lawrence Berkeley National Labs, Berkeley, California 94720

Received July 13, 2005; E-mail: francis@cchem.berkeley.edu

Transition metal catalyzed reactions have revolutionized the way that organic chemists synthesize complex molecules. In addition to providing countless new methods for selective bond formation, these reactions often possess excellent functional group tolerance and the potential for reaction tuning through variation of the catalyst coordination sphere. More recently, numerous transition metal catalyzed reactions have been explored in the context of "green chemistry",[1] affording reactions that proceed in aqueous media using minimal quantities of nontoxic reagents. As the field of protein modification similarly relies on highly selective reactions that can be carried out in water under mild conditions, it is likely that transition metal catalysis has much to offer in this context. Recent studies have begun to explore these possibilities, resulting in new tools for the modification of protein targets with excellent chemoselectivity.[2]

Detailed studies by Ogo, Fukuzumi, and co-workers[3] have documented the ability of an easily prepared, water-stable [Cp*Ir(bipy)(H_2O)]SO_4 complex (**1a**)[4] to form hydride species **2a** in the presence of formate ion (eq 1). This complex has been reported to effect the reduction of ketones at pH 2 and can be used as a convenient catalyst for the synthesis of amino acids from α-keto acids, aqueous ammonia, and formic acid at pH 5.[5,6] Although the previously reported reactions are most efficient at elevated temperatures, we identified the water tolerance of this catalyst as an attractive feature for the development of protein modification reactions through reductive pathways. We report herein that this can be achieved, resulting in a convenient transition metal catalyzed method for the reductive alkylation of lysine residues at room temperature and neutral pH.



1a: X = H
1b: X = OMe
1c: X = CO_2Et

2a: X = H
2b: X = OMe
2c: X = CO_2Et

(1)

These studies began with the development of reaction conditions that would meet the stability requirements of most biomolecules. The reaction medium was chosen as 50 mM aqueous phosphate buffer (pH 7.4) containing 25 mM sodium formate as the stoichiometric hydride source. Initial catalyst screening experiments revealed that methoxy-substituted catalyst **1b** was capable of alkylating lysozyme (which has six lysines) with aldehyde **4a** under these buffer conditions at 22 °C (Figure 1a,b and Table 1, entry 5).[7] Presumably, this reaction occurs via the reversible formation of imines with free amino groups on the protein surface, which are subsequently reduced by iridium hydride **2b**. In contrast, previously reported catalyst **1a** afforded only small amounts of reaction product under similar conditions, as did several catalyst analogues bearing substituted pyridines (not shown). Electron-deficient complex **1c** gave no product under any conditions.

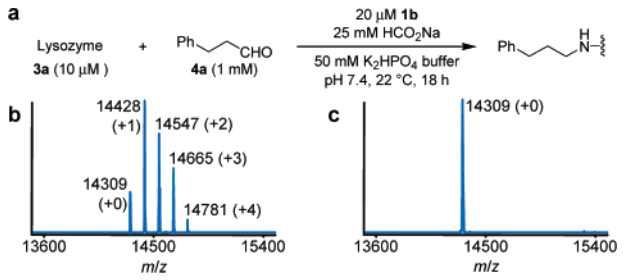


Figure 1. Modification of lysozyme using reductive alkylation. Under the reaction conditions summarized in (a), a distribution of alkylated products results (b). (c) Control experiments lacking catalyst yielded no reaction products. Spectra shown are reconstructed from charge ladders obtained using ESI-MS analysis.

With catalyst **1b**, up to four modifications per protein were observed, as determined by ESI-MS analysis. This ratio can be readily adjusted through alteration of the reaction temperature and the concentrations of the aldehyde and catalyst. The use of elevated temperatures resulted in the successful alkylation of **3a** using concentrations of **1b** as low as 2 μM (Table 1, entries 2−4), and the use of 20 μM **1b** allowed protein concentrations as low as 10 μM to be modified with high conversion at room temperature (entry 5). In general, the reaction has shown little dependence on pH, successfully alkylating proteins between pH 5 and 7.4. Confirmation of the reaction selectivity for amino groups was achieved through model studies using a peptide substrate (H_2N-MYKFARLAND-CO_2H).[8]

The success of this reaction depends on several remarkable features of the catalyst. First, the iridium hydride intermediate can reduce the low equilibrium concentrations of imines faster than aldehydes, although ^{1}H NMR analyses of reaction solutions have shown that the aldehyde components are eventually reduced under these conditions. Even more remarkable is the ability of the catalyst to effect the reaction in the presence of the numerous polar functional groups on the protein surface. UV−vis experiments have

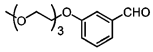
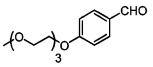
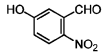
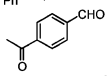
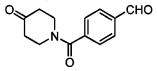
Table 1. Reductive Alkylation of Proteins Using **4a** and **1b**[a]

entry	protein	[**1b**] (μM)	*T* (°C)	Unmod (%)	+1 (%)	+2 (%)	+3 (%)	+4 (%)
1	**3a** (100 μM)	20	22	6	21	37	25	11
2	**3a** (100 μM)	10	37	20	33	29	16	2
3	**3a** (100 μM)	5	37	48	38	31	1	0
4	**3a** (100 μM)	2	37	84	16	0	0	0
5	**3a** (10 μM)	20	22	15	32	34	19	trace
6	**3b** (100 μM)	20	22	11	19	27	22	12[b]
7	**3c** (100 μM)	20	37	24	47	27	2	0
8	**3d** (100 μM)	20	37	85	15	0	0	0
9	**3e** (100 μM)	20	37	59	41	0	0	0
10	**3f** (100 μM)	20	37	70	30	trace	0	0

[a] Additional conditions: 1 mM **4a**, 25 mM HCO_2Na, 50 mM K_2HPO_4, pH 7.4, 18−22 h. Protein substrates: lysozyme (**3a**), cytochrome *c* (**3b**), α-chymotrypsinogen A (**3c**), h. h. myoglobin (**3d**), ribonuclease A (**3e**), and bacteriophage MS2 (**3f**). Product distributions were determined from ESI-MS analyses. [b] **3b** also gave small amounts of +5 (5%) and +6 (3%).

10.1021/ja054686c CCC: $30.25

Table 2. Modification of Lysozyme (**3a**) By Reductive Alkylation[a]

Aldehyde/Ketone		Unmod (%)	+1 (%)	+2 (%)	+3 (%)	+4 (%)
4b	CH_3CH_2CHO	72	28	0	0	0
4c		41	43	16	0	0
4d		6	26	40	23	5
4e		60	34	6	0	0
4f		14	41	36	9	0
4g		100	0	0	0	0
4h		18	46	29	7	0
4i		33	52	15	0	0

[a] Conditions: 100 μM lysozyme, 1 mM **4b**−**i**, 20 μM **1b**, 25 mM sodium formate, 50 mM K_2HPO_4, pH 7.4, 22 °C, 22 h. Product distributions were determined using ESI-MS analysis.

indicated that the catalyst can be completely removed from protein solutions by gel filtration.[8]

To date, the reaction has shown a wide tolerance for both the protein and the aldehyde components. With respect to protein scope, many substrates have been modified successfully, including cytochrome *c* (**3b**, 19 lysines), α-chymotrypsinogen A (**3c**, 14 lysines), myoglobin (**3d**, 19 lysines), ribonuclease A (**3e**, 10 lysines), and an intact virus (bacteriophage MS2, **3f**, 6 lysines per monomer) (Table 1). Although the overall reactivity changes with the structure of the substrate, the reaction conditions can be adjusted in each case to achieve the desired levels of modification. Numerous aldehydes have been screened, a selection of which are summarized in Table 2. Although simple alkyl aldehydes (such as **4b**) generally exhibit low levels of reactivity, substrates possessing aromatic groups have afforded the highest levels of conversion to product. This is likely due to the enhanced stability of the imine intermediates arising from hydrophobic interactions between these groups and the proteins and/or catalyst.[9] Electron-withdrawing substituents generally increase the reactivity of these substrates (e.g., **4d**,**f**,**h**,**i**), although useful modification can still be obtained without these functional groups (**4c**,**e**). No reaction has been observed using ketone substrates, such as **4g** or acetone (not shown). This allows substrates bearing both a ketone and an aldehyde (such as **4h**,**i**) to be coupled chemoselectively, leaving the ketone for further elaboration. After reductive alkylation with these substrates, we have found that proteins can be further modified with alkoxyamines to form oximes.[8]

Compared to lysine acylation with *NHS*-esters, reductive alkylation strategies offer several key advantages. First, the overall charge state of the protein remains unaltered after the modification takes place, thus minimizing changes in protein solubility. This method also avoids competitive hydrolysis pathways that can be problematic for some activated esters, potentially allowing less reagent to be used. Similarly, the aldehyde feedstock materials that are used in this technique are frequently more convenient to prepare and store than the corresponding *NHS*-esters.

As an example of the latter case, we have developed a simple two-step oxidation/reductive alkylation method for the attachment of unfunctionalized poly(ethylene glycol) (PEG) to proteins. Conversion of commercially available PEG alcohol **5** to the corresponding aldehyde **4j** can be accomplished conveniently through exposure to Dess−Martin periodinane (DMP) in CH_2Cl_2 (Figure 2a). The resulting polymer can be isolated by precipitation from ethyl ether, affording an unoptimized 1:1.7 mixture of

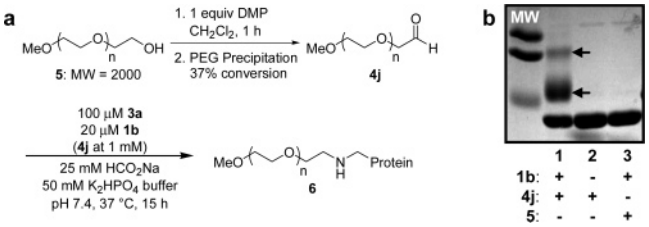


Figure 2. Conjugation of PEG to lysozyme using a simple two-step procedure. (a) Commercially available PEG alcohols can be oxidized to afford aldehydes before conjugation to proteins via reductive alkylation. (b) SDS−PAGE analysis of **6** shows the formation of singly and doubly alkylated conjugates. Control reactions run in the absence of catalyst (lane 2) or aldehyde (lane 3) afforded no conjugates. MW ladder: 25, 20, and 15 kD (from top). Lysozyme MW = 14.3 kD.

aldehyde (**4j**) to remaining alcohol. The crude material was then added directly to aqueous formate/phosphate buffer for protein modification. In the presence of 20 μM **1b** and 1 mM **4j**, lysozyme was modified to afford single and double polymer conjugates with 59% overall conversion, as determined by quantitative SDS−PAGE analysis (Figure 2b). As was observed previously, no modification of the protein was observed in the absence of **1b**. In addition to preparing protein conjugates with PEG polymers that are not available as *NHS*-esters, we are exploring this technique for the attachment of alternative polymers bearing primary hydroxyl groups.

In conclusion, a convenient new method has been described for the reductive alkylation of proteins under mild conditions. The reaction is capable of functionalizing proteins with similar efficiencies to *NHS*-ester- and sodium cyanoborohydride-based techniques, but without the use of water-sensitive materials or harsh reagents. As this reaction represents one of the few transition metal based methods for bioconjugation, it also provides an important design lead for the development of future reductive modification strategies.

Acknowledgment. We would like to thank Dr. Arnold Falick for his assistance with MS/MS experiments. We also acknowledge the NIH (GM072700-01) and the DOE Nanoscale Science, Engineering, and Technology Program for generous financial support, as well as the University of California, Berkeley.

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

References

(1) For several recent examples, see: *Acc. Chem. Res.* **2002**, *35* (9).
(2) (a) Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G. *J. Am. Chem. Soc.* **2003**, *125*, 3192. (b) Speers, A. E.; Adam, G. C.; Cravatt, B. F. *J. Am. Chem. Soc.* **2003**, *125*, 4686. (c) Antos, J. M.; Francis, M. B. *J. Am. Chem. Soc.* **2004**, *126*, 10256. (d) Ojida, A.; Tsutsumi, H.; Kasagi, N.; Hamachi, I. *Tetrahedron Lett.* **2005**, *46*, 3301. For examples on peptides and amino acids, see: (e) Dibowski, H.; Schmidtchen, F. P. *Angew. Chem., Int. Ed.* **1998**, *37*, 476. (f) Bong, D. T.; Ghadiri, M. R. *Org. Lett.* **2001**, *3*, 2509.
(3) Abura, T.; Ogo, S.; Watanabe, Y.; Fukuzumi, S. *J. Am. Chem. Soc.* **2003**, *125*, 4149.
(4) (a) The parent complex was first described by Ziessel, R. *J. Chem. Soc., Chem. Commun.* **1988**, *1*, 16. (b) For a facile synthetic route to these complexes, see: Dadci, L.; Elias, H.; Frey, U.; Hörnig, A.; Koelle, U.; Merbach, A. E.; Paulus, H.; Schneider, J. S. *Inorg. Chem.* **1995**, *34*, 306.
(5) Ogo, S.; Uehara, K.; Abura, T.; Fukuzumi, S. *J. Am. Chem. Soc.* **2004**, *126*, 3020.
(6) For an example of alkyne hydration using **2a**, see: Ogo, S.; Uehara, K.; Abura, T.; Watanabe, Y.; Fukuzumi, S. *J. Am. Chem. Soc.* **2004**, *126*, 16520.
(7) A similar reactivity trend was observed for a related Cp*Ir(phenanthrine)$^{2+}$ complex in the context of CO_2 reduction: Himeda, Y.; Onozawa-Komatsuzaki, N.; Sugihara, H.; Arakawa, H.; Kasuga, K. *Organometallics* **2004**, *23*, 1480.
(8) See Supporting Information for details and spectra.
(9) For examples, see: (a) Breslow, R. *Acc. Chem. Res.* **2004**, *37*, 471. See also: (b) Joshi, N. S.; Whitaker, L.; Francis, M. B. *J. Am. Chem. Soc.* **2004**, *126*, 15942.

JA054686C