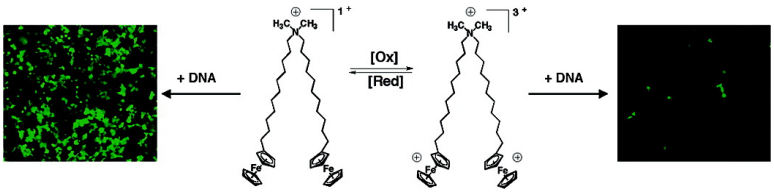
Communication

Ferrocene-Containing Cationic Lipids: Influence of Redox State on Cell Transfection

Nicholas L. Abbott, Christopher M. Jewell, Melissa E. Hays, Yukishige Kondo, and David M. Lynn

J. Am. Chem. Soc., **2005**, 127 (33), 11576-11577• DOI: 10.1021/ja054038t • Publication Date (Web): 29 July 2005



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 07/29/2005

Ferrocene-Containing Cationic Lipids: Influence of Redox State on Cell Transfection

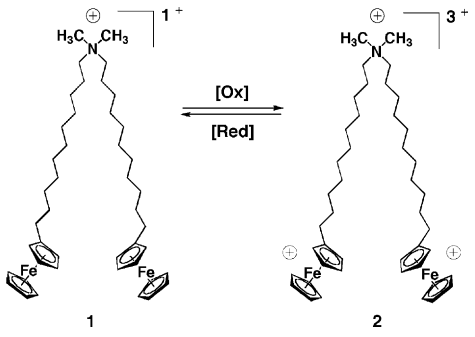
Nicholas L. Abbott,* Christopher M. Jewell, Melissa E. Hays, Yukishige Kondo, and David M. Lynn*

Department of Chemical and Biological Engineering, University of Wisconsin—Madison, 1415 Engineering Drive, Madison, Wisconsin 53706

Received June 17, 2005; E-mail: abbott@engr.wisc.edu; dlynn@engr.wisc.edu

Many cationic lipids form complexes with DNA (lipoplexes) that facilitate the transfer of DNA across cell membranes.[1] As a consequence, cationic lipids have been investigated widely as agents for the delivery of DNA in vitro and in vivo. The development of principles that permit external control over the activation of lipids that otherwise do not mediate transfection would make possible new approaches to achieving active, spatial, and temporal control of DNA delivery.[2,3] We sought to develop these principles by designing DNA lipoplexes incorporating redox-active groups that can be transformed electrochemically to change the charge density of the lipid. We report here that a ferrocene-containing lipid, when used in combination with electrochemical methods, can be used to achieve either high or low (background) levels of gene expression in mammalian cells depending on whether the lipid is present in a reduced or oxidized redox state.

The work reported here builds upon on a series of past studies demonstrating that ferrocene-containing amphiphiles permit electrochemical control over a broad range of surfactant-based properties of aqueous systems.[4−9] Of particular relevance to our current work is a report by Abe and co-workers of the cationic, two-tailed lipid bis-(11-ferrocenylundecyl)dimethylammonium bromide (BFDMA, **1**).[7,8] BFDMA can be cycled reversibly between states having a net charge of +1 (reduced, **1**) and a net charge of +3 (oxidized, **2**) by the electrochemical oxidation or reduction of the ferrocene units located at the end of each aliphatic chain. Whereas reduced BFDMA forms submicrometer vesicles in aqueous media, electrochemical oxidation of reduced BFDMA leads to the formation of smaller, micellar aggregates.[7,8]



The structure of BFDMA is similar to the structure of dimethyldioctadecylammonium bromide and other cationic lipids used conventionally for the delivery of DNA to cells.[1,10] We hypothesized that the redox behavior of BFDMA could be used to influence the nature of the interactions of this lipid with DNA and, consequently, the ability of BFDMA/DNA aggregates to transfect cells.

We used cyclic voltammetry to oxidize and reduce the ferrocenyl groups in BFDMA in aqueous Li_2SO_4 solutions (pH = 5.5) and cell culture medium (OptiMEM, pH = 7.4). The peak anodic and cathodic currents in aqueous Li_2SO_4 were recorded at potentials of 440 and 240 mV, respectively, versus a Ag/AgCl reference electrode (1 mM BFDMA, 1 mM Li_2SO_4, at 25 °C, with an applied potential swept linearly at 30 mV/s). Solutions of completely oxidized BFDMA were prepared by bulk electrolysis using potentials greater than 440 mV. The yellow color characteristic of an aqueous solution of reduced BFDMA changed to bright blue upon oxidation, further permitting the extent of the electrochemical transformation to be monitored by UV/vis spectrophotometry. Both oxidized and reduced BFDMA were measured to be stable upon storage in Li_2SO_4 solutions and cell culture medium over days, as determined by UV/vis spectrophotometry.

We conducted transfection experiments with the COS-7 cell line using BFDMA and a plasmid DNA construct (pEGFP-N1) encoding enhanced green fluorescent protein (EGFP). Lipoplexes were prepared using either reduced or oxidized BFDMA. In a typical experiment, a solution of DNA (600 ng in 25 μL of water) was added to a vortexing solution of 1 mM aqueous Li_2SO_4 (25 μL) containing an amount of reduced or oxidized BFDMA sufficient to give final lipid concentrations of 10, 20, or 40 μM when these formulations were added to cells in 200 μL of culture medium. The lipoplexes were incubated with cells for 4 h, after which time the culture medium was removed and replaced with fresh medium. Gene expression was characterized after 48 h by fluorescence microscopy. Figure 1 shows representative results of an experiment using 10 μM BFDMA. Lipoplexes prepared using reduced BFDMA mediated high levels of EGFP expression (Figure 1a). By contrast, experiments using oxidized lipid resulted in few transfected cells (Figure 1b), similar to background levels observed in control experiments using naked DNA (no lipid, not shown). These results reveal striking differences in the levels of gene expression in cells treated with lipoplexes prepared using reduced BFDMA relative to those prepared using oxidized BFDMA.

Figure 1c shows the quantitative results of experiments using a second plasmid construct (pCMV-Luc) encoding firefly luciferase and either reduced or oxidized BFDMA at lipid concentrations of 10, 20, and 40 μM. Inspection of Figure 1c shows that expression of luciferase using oxidized BFDMA was low and occurred at levels typical of control experiments using naked DNA (no lipid). In contrast, levels of transfection measured using reduced BFDMA were much higher and, at a lipid concentration of 20 μM, were comparable to control experiments using two commercially available cationic lipids (Lipofectamine 2000 and TransIT-LT1; Figure 1c). Experiments performed using a quantitative calcein/ethidium homodimer live/dead assay[11] demonstrated that lipoplexes formed using reduced and oxidized BFDMA are not cytotoxic at lipid concentrations of 10 or 20 μM. Cell viability was significantly reduced at 40 μM BFDMA.

We also conducted dynamic light scattering experiments to determine whether the oxidation state of 10 μM BFDMA substan-

10.1021/ja054038t CCC: $30.25

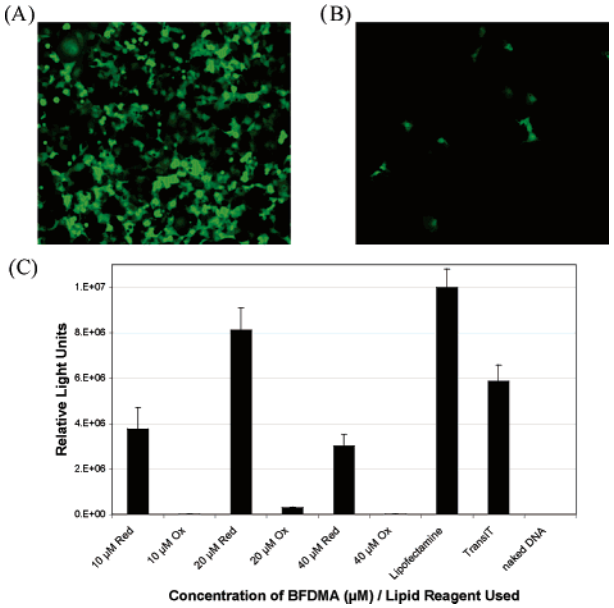


Figure 1. (A and B): Fluorescence microscopy images (10X) showing relative levels of EGFP expression in COS-7 cells transfected with pEGFP-N1 and either (A) reduced BFDMA or (B) oxidized BFDMA at a lipid concentration of 10 μM. (C) Results showing relative levels of luciferase expression in COS-7 cells transfected with pCMV-Luc and either reduced BFDMA or oxidized BFDMA at lipid concentrations of 10, 20, and 40 μM. Experiments performed in the absence of lipid and two commercially available cationic lipid formulations are shown for comparison.

tially influences the interactions of BFDMA with plasmid DNA (2.4 μg/mL) in a reduced-serum cell culture medium (OptiMEM) at 37 °C. Intensity autocorrelation functions measured at 90° were found to be strongly dependent on the oxidation state of BFDMA. Relaxation times measured in the presence of oxidized BFDMA and DNA were an order of magnitude faster than relaxation times measured in solutions of reduced BFDMA and DNA, suggesting the formation of smaller (~300 nm) lipoplexes in the presence of oxidized BFDMA as compared to reduced BFDMA (~1−5 μm). We caution, however, that past studies of polymer−surfactant complexes have established that internal dynamics of these aggregates can influence the relaxation times measured by dynamic light scattering in these solutions, and that unambiguous assignment of aggregate sizes requires measurements of light scattering at multiple angles.[12,13] From the measurements reported here, however, we do conclude that significant differences exist in the nature of the interactions between DNA and either reduced or oxidized BFDMA in cell culture medium. This result supports the hypothesis that the oxidation state of BFDMA affects transfection via its influence on the interactions of BFDMA and DNA.

The development of lipids that are pH- or redox-active,[2] chemically or enzymatically cleavable,[2,14,15] or light-sensitive[3] has been an active area of research. Of particular relevance to our current study is past work on the synthesis and application of redox-active cationic lipids incorporating disulfide bonds into lipid structures.[2] These past studies have focused largely on the intracellular (e.g., cytosolic) and biological reduction of disulfide bonds to promote the intracellular release of DNA. In contrast, the ferrocenyl lipids that we report can be transformed electrochemically and reversibly between active and inactive states. We note that reversible, electrochemical control of the redox equilibrium between thiols and disulfides is not easy to achieve and requires chemically modified electrodes.[16] We also note that ferrocene has been investigated widely as a redox-active functional group in peptide, protein, and oligonucleotide constructs,[17] and the pharmacological evaluation of ferrocene-containing therapeutics represents a rapidly growing field of research.[17−19]

In summary, we have demonstrated that a ferrocene-containing, redox-active cationic lipid can be used to promote either high (on) or very low (off) levels of cell transfection depending on whether the lipid is prepared in a reduced or oxidized state. These results suggest the basis of an approach that may lead to a general and facile method for transforming an inactive lipoplex formulation (prepared from oxidized BFDMA) to an active form (composed of reduced BFDMA) through the application of externally applied electrical potentials. If successfully developed through future studies, the ability to activate lipoplexes toward transfection electrochemically and "on demand" could create new opportunities to deliver DNA in vitro and in vivo with both spatial and temporal control.

Acknowledgment. Financial support was provided by the NSF (CTS-0327489), the NIH (EB 002746), and the Arnold and Mabel Beckman Foundation.

Supporting Information Available: Details of electrochemistry, transfection protocols, and dynamic light scattering experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Kabanov, A. V.; Felgner, P. L.; Seymour, L. W. *Self-Assembling Complexes for Gene Delivery: From Laboratory to Clinical Trial*; John Wiley and Sons: New York, 1998.
(2) Guo, X.; Szoka, F. C. *Acc. Chem. Res.* **2003**, *36*, 335−341.
(3) Shum, P.; Kim, J. M.; Thompson, D. H. *Adv. Drug Delivery Rev.* **2001**, *53*, 273−284.
(4) Saji, T.; Hoshino, K.; Aoyagui, S. *J. Am. Chem. Soc.* **1985**, *107*, 6865−6868.
(5) Saji, T.; Hoshino, K.; Ishii, Y.; Goto, M. *J. Am. Chem. Soc.* **1991**, *113*, 450−456.
(6) Gallardo, B. S.; Gupta, V. K.; Eagerton, F. D.; Jong, L. I.; Craig, V. S.; Shah, R. R.; Abbott, N. L. *Science* **1999**, *283*, 57−60.
(7) Kakizawa, Y.; Sakai, H.; Nishiyama, K.; Abe, M.; Shoji, H.; Kondo, Y.; Yoshino, N. *Langmuir* **1996**, *12*, 921−924.
(8) Kakizawa, Y.; Sakai, H.; Yamaguchi, A.; Kondo, Y.; Yoshino, N.; Abe, M. *Langmuir* **2001**, *17*, 8044−8048.
(9) Bennett, D. E.; Gallardo, B. S.; Abbott, N. L. *J. Am. Chem. Soc.* **1996**, *118*, 6499−6505.
(10) Miller, A. D. *Angew. Chem., Int. Ed.* **1998**, *37*, 1769−1785.
(11) Murphy, C. J.; Campbell, S.; Araki-Sasaki, K.; Marfurt, C. *Cornea* **1998**, *17*, 529−536.
(12) Nystrom, B.; Lindman, B. *Macromolecules* **1995**, *28*, 967−974.
(13) Porsch, B.; Nilsson, S.; Sundelof, L. O. *Macromolecules* **1997**, *30*, 4626−4632.
(14) Meers, P. *Adv. Drug. Delivery Rev.* **2001**, *53*, 265−272.
(15) Prata, C. A.; Zhao, Y.; Barthelemy, P.; Li, Y.; Luo, D.; McIntosh, T. J.; Lee, S. J.; Grinstaff, M. W. *J. Am. Chem. Soc.* **2004**, *126*, 12196−12197.
(16) Zagal, J. H.; Herrera, P. *Electrochim. Acta* **1985**, *30*, 449−454.
(17) van Staveren, D. R.; Metzler-Nolte, N. *Chem. Rev.* **2004**, *104*, 5931−5985.
(18) Domarle, O.; Blampain, G.; Agnaniet, H.; Nzadiyabi, T.; Lebibi, J.; Brocard, J.; Maciejewski, L.; Biot, C.; Georges, A. J.; Millet, P. *Antimicrob. Agents Chemother.* **1998**, *42*, 540−544.
(19) Top, S.; Tang, J.; Vessieres, A.; Carrez, D.; Provot, C.; Jaouen, G. *Chem. Commun.* **1996**, 955−956.

JA054038T