Electrochemistry

DOI: 10.1002/ange.200705274

Electrochemically Driven Release of Picomole Amounts of Calcium Ions with Temporal and Spatial Resolution**

Christian Amatore, Damiano Genovese, Emmanuel Maisonhaute,* Noureddine Raouafi, and Bernd Schöllhorn**

In memory of Patrick Allenet

The ability to trigger the delivery or capture of a nanoscale amount of active molecules or ions within a fast (namely, millisecond) controlled time scale and with a micrometric or sub-micrometric spatial resolution is a current challenge. Several cellular functions are indeed controlled by such nanoquantities of molecular and ionic effectors, and their investigation requires the possibility of monitoring their fluxes externally.[1,2] Similarly, the increasing awareness in nanosciences of the necessity of delivering chemicals at the nanoscale level will also demand that new ways are found to perform such operations. Such ability will concern many research areas as well as advanced industrial microfabrication processes.

In this respect, electrochemically commanded delivery ought to offer an interesting solution as a result of the lightness of electrochemical equipment and its easy implementation. Recent studies by Mirkin and co-workers established that electrical control of electrocapillary forces at a polarized liquid/liquid interface allows attoliters of any desired solution to be released following an electrochemical command.[3,4] Despite its seminal value, this method requires the simultaneous flow of a solvent which contains and carries the species to be delivered. Another option consists of finding methods for delivering only the species of concern.

Following the discovery of crown ethers, a great body of research has been, and is currently, focused on the design of ligands for the selective complexation of ions,[5] with the purpose of either sequestrating them from a solution or allowing their dissolution into apolar solvents, in which they could not otherwise be dissolved. The availability of such specific ligands has led to the design of analytical sensors in which a redox center is connected to the ligand. Whenever the standard potential of the coupled redox moiety is modified upon capture of the ion of choice by the ligand, the change in electrochemical behavior demonstrates the presence of the target ion and may even provide information about its concentration.[6–9]

It occurred to us that this strategy could be reversed and used for the fast and precise delivery of picomole amounts of an ion with a spatial scale defined by the dimensions of an electrode bearing a specific ligand attached to it and coupled with an appropriate redox center. Indeed, the electrostatic interaction, which is generally monitored through its action on the potential of the redox reporter upon chelation of the ionic target, could be directed so that a change in the charge of the redox center provoked by its oxidation or reduction may affect sufficiently the complexation ability of the ligand, thus promoting release of a chelated ion.

The validity of the concept has already been demonstrated by using photochemical activation.[10] In that case, the excited state populated on absorption of light involves an intramolecular charge transfer that enforces a drastic change in binding ability. However, deactivation of the excited state of the molecule results in recomplexation occurring at a near-diffusion-limited rate. Since the life time of excited states is generally a few nanoseconds, the ion released initially may only move a few nanometers and be captured again when the molecule returns to its ground state. In contrast, electrochemical activation may induce a permanent change (at least while the electrochemical driving force is applied from the electrode) provided that the redox center is chemically stable. Hence, one may consider that the electrochemical commutation of the complexation ability of a ligand has a similar efficiency as that of photochemical processes, but also has the important advantage of maintaining the commutated status as demanded by the experiment to be carried out. Furthermore, the use of microelectrodes allows the space in which release occurs to be perfectly defined through tailoring the diffusion layer by careful choice of the dimensions of the electrode.[11]

Herein we validate this concept for the direct release of picomole amounts of ions through an electrochemical command. In doing so, we used a system in which the ligand/redox center assembly is anchored as a self-assembled monolayer (SAM) to the electrode surface, since ultimately this is certainly the option which offers the largest synthetic versatility, the most precise control of released quantities,

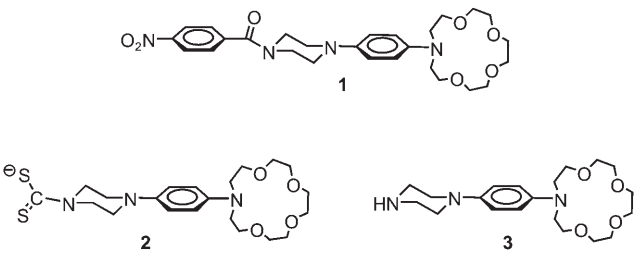
[*] Dr. C. Amatore, D. Genovese, Prof. E. Maisonhaute, Dr. N. Raouafi, Dr. B. Schöllhorn
UMR CNRS 8640 "PASTEUR" and LIA CNRS XiamENS "Nano-BioChem", Département de Chimie, Ecole Normale Supérieure, Université Pierre et Marie Curie-Paris 6, 24 rue Lhomond, 75231 Paris Cedex 05 (France)
Fax: (+33) 1-4432-3863
E-mail: christian.amatore@ens.fr
emmanuel.maisonhaute@ens.fr
bernd.schollhorn@ens.fr

[**] This work was supported by the CNRS (UMR 8640, cooperation CNRS-DGRST, and the LIA XiamENS), the Ecole Normale Supérieure, the Université Pierre et Marie Curie, and the French ministry of research through ANR REEL. We gratefully thank Monique Martin and Pascal Plaza for helpful discussions, and also Alain Fuxa and Guillaume Bertrand for some preliminary experiments. D.G. thanks the University of Bologna for a travel grant.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

and the fastest commutation times (since no significant time constant is involved). Our aim in this study was only to validate this concept, so we selected conditions which allowed the best physicochemical characterization of the system properties. For this reason, we used a microelectrode with sufficiently large dimensions, rather than an ultra-microelectrode, to enable its surface status to be controlled with adequate precision and reproducibility. Similarly, since most crown ethers and similar ligands have been designed to perform in non-aqueous solvents, we carried out this validation study in acetonitrile with Ca^{2+} used as a model ion to be released. We do not view these choices as potential limitations for future applications, such as those envisioned above. Indeed, ultra-microelectrodes with nanometric dimensions may be readily manufactured[12] and their surfaces can be modified by a considerable variety of SAMs or other specific chemicals.[13]

We thus focused the present study on molecules **1** and **2** (Scheme 1). These compounds were synthesized from **3** according to the procedures described in the Supporting Information and initially published by Pearson et al.[14–18]



Scheme 1. Phenylenediamine–aza-crown ether derivatives.

In compounds **1**–**3**, the nitrogen atom of the aza-crown ether ligand also belongs to the paraphenylenediamine redox-active center. Upon oxidation, the coordinating ability of this electron-donating nitrogen atom is expected to be strongly reduced because of the partial positive charge delocalized on it. This charge should induce strong coulombic repulsive forces on any complexed cation, since it is created only a few angstroms away. Our goal was then to optimize the expected square-scheme mechanism (Figure 1), which ultimately controls the efficiency of the release of the calcium ions.

We used cyclic voltammetry to fully characterize the dynamics of the system.[19] The electrochemical behavior of a solution of **1** in acetonitrile containing 0.1 M tetrabutylammonium tetrafluoroborate is very similar to that of the well-characterized tetramethylparaphenylenediamine.[20] Two reversible systems Ox_1/Red_1 and Ox_2/Red_2 are observed, which define the two standard potentials E_1^0 and E_2^0 of the cation-free molecule (Figure 2).

Upon addition of one equivalent of Ca^{2+} ions, both peaks disappear, and an irreversible two-electron peak Ox_3 is observed at much higher potential. The shift from the first oxidation peak is 875 mV, which illustrates that **1** has a very high affinity constant for the complexation of Ca^{2+} ions. On the reverse scan, provided that the Ox_3 wave was scanned over, two reduction waves Red_4 and Red_5 are observed. The

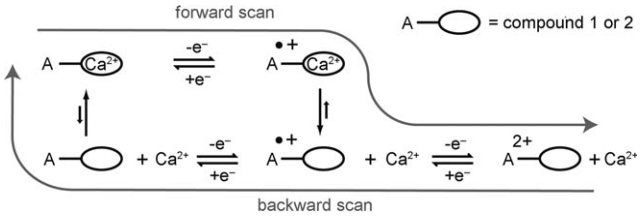


Figure 1. Square scheme illustrating the mechanistic path (gray lines) followed by ligands **1** and **2** in the presence of Ca^{2+} ions during the oxidation (forward scan) and the reduction (reverse scan; see Figures 2 and 3).

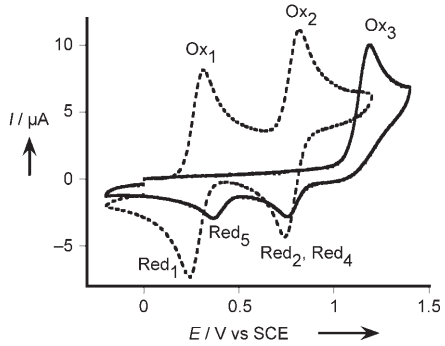


Figure 2. Cyclic voltammograms of **1** (2 mM) in acetonitrile/0.1 M tetrabutylammonium tetrafluoroborate solution in the absence (dashed line) and presence (solid line) of 1.1 equivalents of Ca^{2+} ions. Scan rate: 1 Vs^{-1}; glassy carbon electrode (diameter: 1 mm). The potential scale refers to the saturated calomel electrode.

wave Red_4 occurs nearly at the same potential as Red_2, which indicates that the dication remains almost uncoordinated. Wave Red_5 is more anodic than wave Red_1, thus showing that its electrochemical process is facilitated. This finding shows that only when the cation radical is reduced to the neutral ligand form, namely, at Red_5, can a fast back-complexation of Ca^{2+} ions take place, which drives the reduction process (EC system,[19] Figure 1). This proposal was confirmed since inverting the reverse scan potential after scanning over wave Red_2 (data not shown) resulted in a wave very close to that of the Ox_2 wave, which shows that the monocation was formed and not significantly coordinated on the voltammetric timescale. Since wave Ox_3 evolves after Ox_2, two electrons are transferred at the same potential (ECE mechanism).[19,21]

This study of the parent compound **1** kinetics and mechanism in solution clearly demonstrates that efficient release of a Ca^{2+} ion occurs upon oxidation of the $[Ca(\mathbf{1})]^{2+}$ complex. However, the use of a system in solution is certainly not adequate for applications such as those envisioned above. Anchoring the $[Ca(\mathbf{1})]^{2+}$ complex onto the electrode surface would meet such requirements, and the dimensions of the electrode would define those of the space in which release occurs through precise definition of its diffusion layer.[11,22]

A common strategy for such purpose consists of exploiting the high affinity of sulfur for gold to produce self-assembled monolayers (SAMs). We chose to adapt a recent technique based on the formation of dithiocarbamate SAMs to anchor our system (**2**),[23–26] since this should allow synthetic flexibility

in the future. Dithiocarbamates have been used only recently for the preparation of SAMs. Their affinity to gold is equal or even higher than for structurally comparable thiols. Reported procedures imply that prior to adsorption onto the gold suface, a dithiocarbamate is formed in the presence of carbon disulfide and a strong base, such as sodium hydroxide, in water or ethanol, which is accompanied by a partial precipitation of the corresponding dithiocarbamate salt.

This experimental protocol was slightly modified and a solution of **3** and carbon disulfide in chloroform was prepared without additional base. We did not observe the formation of any precipitate. In our case, since the aliphatic amine is a weak base and the dithiocarbamic acid is thermodynamically unstable,[27] it is likely that the chemisorption on the gold electrode is sufficient to promote the conversion of the acid into the dithiocarbamate, with the formation of the sulfur–gold bond being the driving force of the reaction. Avoiding the generation of a precipitate allows easier control of the system during the formation of the SAMs and in particular for diluting the active ligand on the gold surface with inert thiols (see below).[28,29]

Monolayers of **2** formed spontaneously on gold electrodes. After rinsing the electrodes with chloroform and acetonitrile, the electrochemical properties were evaluated in acetonitrile (Figure 3). They were very similar to those reported above for **1** in solution, but the shape of both voltammetric waves was characteristic of surface-confined redox molecules. Hence, they strongly depend on the procedure used to construct the monolayer. This behavior is typical for SAMs because of lateral interactions between electroactive molecules.[19,30] By competitive adsorption of *n*-hexanethiol, which dilutes the amount of active molecule in the SAM, such lateral interactions may be minimized, and the coverage may be adjusted at will between zero and maximum coverage.[28,29,31]

The gradual addition of Ca^{2+} ions to the solution results in the two reversible oxidation waves of the free ligand (Ox_1*/Red_1* and Ox_2*/Red_2* in Figure 3) progressively disappearing, and a broader and larger irreversible band (Ox_3*) developing at a much higher potential.

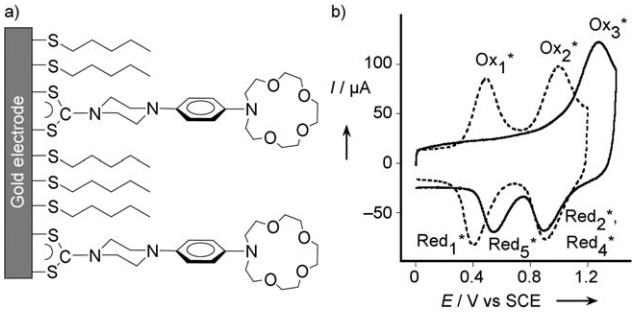


Figure 3. a) Schematic representation of the mixed self-assembled monolayer formed by **2** upon dipping a gold electrode (area: 4.8 mm^2) in chloroform containing CS_2 (0.124 mM), **3** (0.24 mM), and hexanethiol (0.71 mM) as a dilutant. b) Cyclic voltammograms of the mixed monolayer in (a) in acetonitrile/0.1 M tetraethylammonium tetrafluoroborate in the absence (dashed line) and in the presence (solid line) of Ca^{2+} ions (0.92 mM). Scan rate: 50 $V s^{-1}$. The surface coverage of **2** is 6.5×10^{-11} mol cm^{-2}. Note, that in the presence of Ca^{2+} ions the anodic scan was reversed before the electrolysis at wave Ox_3* was complete.

Two reduction waves (Red_4* and Red_5*) were observed on the reverse voltammogram upon scanning over wave Ox_3*. Reduction Red_4* was close to the former wave Red_2* observed for the free SAM, while Red_5* developed before Red_1*. This observation is similar to that described and rationalized above for **1** in solution, and shows that confinement of the system within a SAM assembly does not alter the overall process depicted in Scheme 1. This was again evident while scanning over wave Ox_3*, that is, in less than 1 ms. The quantity of released calcium ions is given by the half of the charge corresponding to the oxidation at Ox_3* (two-electron process versus one calcium ion per center), namely, 2.3×10^{-12} mol for the system in Figure 3. Since the electrode surface area is 4.8 mm^2, this value corresponds to the release of 4.7×10^{-11} mol cm^{-2} ions per unit of surface area. Such a value is compatible with the expected SAM coverage of the electrode surface and with the coverage of 6.5×10^{-11} mol cm^{-2} deduced by integration of the voltammetric signal in the absence of calcium ions (waves Ox_1* or Ox_2* in Figure 3). The difference of 27 % stems from the fact that the anodic potential scan in the presence of calcium ions was interrupted before the end of the wave Ox_3* to avoid damaging the SAM. This value shows also that the amount of calcium ions to be released may be adjusted broadly by using ultra-microelectrodes (which have much smaller surface areas than the microelectrode used in the present study) and finely by the potential excursion range and the dilution of the active SAM component with inert ones.[31]

Furthermore, although this is not the property that we aimed for in this study, this result establishes that our system also behaves as an extremely good electroanalytical sensor by drastically modifying its electrochemical properties upon complexation.[6–9]

In conclusion, the validity of the principle of electrochemically driven release of picomole amounts of a specific ion stored in a complexing self-assembled monolayer has been established. The results demonstrate that release occurred on a sub-millisecond time scale, and was irreversible provided that the two-electron-oxidized redox center was not returned electrochemically to its neutral initial state. Further work will involve a full kinetic characterization of the process through the use of ultrafast cyclic voltammetry.[22] The system may be incorporated on ultra-microelectrodes of various sizes, and this will allow the nanometric shape and dimensions of the space into which ions are delivered to be selected.[12] Finally, the present syntheses have been developed for a system in non-aqueous media. Our future goal concerns the ability of provoking concentration jumps of cation effectors near a living cell,[1,2] to extend our current studies on vesicular release of neurotransmitters and of oxidative stress by triggering the response of single cells with precise temporal and spatial resolution.[1,2,32–34] This is the fundamental reason for our choice of evaluating the performance of the system with calcium ions. Experiments not reported here established that using aqueous electrolytes is not a problem for the stability of the redox center. However, the complexing ability of the present ligand in its the neutral state is clearly not high

enough for impeding spontaneous leaking of Ca^{2+} ions into a calcium-free aqueous medium. Therefore, fulfilling our ultimate goal requires the synthesis of redox-center-coupled ligands with better complexing ability.[6–9]

Received: November 16, 2007
Revised: March 10, 2008
Published online: June 2, 2008

Keywords: calcium · crown compounds · electrochemistry · monolayers · self-assembly

[1] Y. M. Tang, E. R. Travis, R. M. Wightman, *J. Neurochem.* **2000**, *74*, 702.
[2] C. L. Haynes, L. A. Buhler, R. M. Wightman, *Biophys. Chem.* **2006**, *123*, 20.
[3] F. O. Laforge, J. Carpino, S. A. Rotenberg, M. V. Mirkin, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 11895.
[4] K. T. Rodolfa, A. Bruckbauer, D. Zhou, A. I. Shevchuk, Y. E. Korchev, D. Klenerman, *Nano Lett.* **2006**, *6*, 252.
[5] C. J. Pedersen, *J. Am. Chem. Soc.* **1967**, *89*, 7017.
[6] L. Zhang, C. M. Cardona, L. Echegoyen, *Chem. Commun.* **2006**, 4461.
[7] A. E. Kaifer, *Acc. Chem. Res.* **1999**, *32*, 62.
[8] G. W. Gokel, W. M. Leevy, M. E. Weber, *Chem. Rev.* **2004**, *104*, 2723.
[9] P. D. Beer, P. A. Gale, G. Z. Chen, *Coord. Chem. Rev.* **1999**, *185*, 3.
[10] M. M. Martin, P. Plaza, Y. H. Meyer, F. Badaoui, J. Bourson, J.-P. Lefevre, B. Valeur, *J. Phys. Chem.* **1996**, *100*, 6879.
[11] C. Amatore in *Physical Electrochemistry* (Ed.: I. Rubinstein), Marcel Dekker, New York, **1995**.
[12] N. Baltes, L. Thouin, C. Amatore, *Angew. Chem.* **2004**, *116*, 1455; *Angew. Chem. Int. Ed.* **2004**, *43*, 1431.
[13] J. J. Watkins, J. Chen, H. S. White, H. D. Abruna, E. Maisonhaute, C. Amatore, *Anal. Chem.* **2003**, *75*, 3962.
[14] A. J. Pearson, W. J. Xiao, *J. Org. Chem.* **2003**, *68*, 5361.
[15] A. J. Pearson, W. J. Xiao, *J. Org. Chem.* **2003**, *68*, 2161.
[16] A. J. Pearson, J. J. Hwang, M. E. Ignatov, *Tetrahedron Lett.* **2001**, *42*, 3537.
[17] A. J. Pearson, J. J. Hwang, *Tetrahedron Lett.* **2001**, *42*, 3541.
[18] A. J. Pearson, J. J. Hwang, *Tetrahedron Lett.* **2001**, *42*, 3533.
[19] A. J. Bard, L. R. Faulkner, *Electrochemical Methods*, Wiley, New York, **2001**.
[20] R. G. Evans, O. V. Klymenko, C. Hardacre, K. R. Seddon, R. G. Compton, *J. Electroanal. Chem.* **2003**, *556*, 179.
[21] In Figure 2 the current peak value for wave Ox_3 is about the same as those of waves Ox_1 or Ox_2, although one would expect a twofold increase to account for the two-electron process which occurs at this wave. Conversely, the correct result is obtained in Figure 3, when the system is immobilized and diluted on the electrode surface. Except for this peculiar observation, and the consequences of confinement on the wave shapes, all voltammetric features of the two calcium-bound systems are in exact correspondence ($Ox_3 \leftrightarrow Ox_3$*, $Red_4 \leftrightarrow Red_4$*, and $Red_5 \leftrightarrow Red_5$*) in terms of relative magnitudes and potential locations. This finding shows that the small magnitude of wave Ox_3 (hence, those of Red_4 and Red_5) reflects a drastic reduction (by a factor ca. 2.6) of the diffusion coefficient D of the electroactive system in solution when a calcium ion is bound. This result suggests a change in diffusion coefficient, which is in full agreement with the fact that this behavior is not observed in the adsorbed phase where no molecular diffusion is involved. We have no concrete rationalization for a reduction of the solution diffusion coefficient by ca. 2.6, since this would correspond to a drastic change in the size of individual molecules which cannot be accounted for by any realistic change in the conformation upon calcium binding. However, it is possible that calcium binding in free solution involves some calcium-mediated molecular clustering so as to produce a complex aggregate $[Ca(\mathbf{1})]^{2+}{}_n$. Such aggregates, because of their size, would experience a drastic decrease in their diffusion coefficient(s) D_n compared to that (D_0) of the unbound **1** or of an isolated $[Ca(\mathbf{1})]^{2+}$ complex. However, oxidation would lead to the correct electron stoichiometry (since $2nF$/mol of aggregate is equivalent to $2F$/mol of each $[Ca(\mathbf{1})]^{2+}$ moiety) although the current peak height of wave Ox_3* (hence, those of Red_4* and Red_5*) would decrease as $(D_n/D_0)1/2$.
[22] C. Amatore, E. Maisonhaute, *Anal. Chem.* **2005**, *77*, 303 A.
[23] Y. Zhao, W. Perez-Segarra, Q. Shi, A. Wei, *J. Am. Chem. Soc.* **2005**, *127*, 7328.
[24] J. M. Wessels, H. G. Nothofer, W. E. Ford, F. von Wrochen, F. Scholz, T. Vessmeyer, T. Schroedter, H. Weller, A. Yasuda, *J. Am. Chem. Soc.* **2004**, *126*, 3349.
[25] M. Lieder, *Electrochim. Acta* **2004**, *49*, 1813.
[26] E. Almirall, A. Fragoso, R. Cao, *Electrochem. Commun.* **1999**, *1*, 10.
[27] P. Morf, F. Raimondi, H.-G. Nothofer, B. Schnyder, A. Yasuda, J. M. Wessels, T. A. Jung, *Langmuir* **2006**, *22*, 658.
[28] C. Amatore, E. Maisonhaute, B. Schöllhorn, J. Wadhawan, *ChemPhysChem* **2007**, *8*, 1321.
[29] C. Amatore, S. Gazard, E. Maisonhaute, C. Pebay, B. Schöllhorn, J.-L. Syssa-Magalé, J. Wadhawan, *Eur. J. Inorg. Chem.* **2007**, 4035.
[30] C. P. Smith, H. S. White, *Anal. Chem.* **1992**, *64*, 2398.
[31] H. O. Finklea in *Encyclopédia of Electrochemistry*, *Vol. 10* (Eds.: M. Fujihira, I. Rubinstein, J. F. Rusling), Wiley-VCH, Weinheim, **2005**.
[32] Q. Xin, R. M. Wightman, *Anal. Chem.* **1998**, *70*, 1677.
[33] C. Amatore, S. Arbault, M. Guille, F. Lemaître, *ChemPhysChem* **2007**, *8*, 1597.
[34] C. Amatore, S. Arbault, C. Bouton, K. Coffi, J.-C. Drapier, H. Ghandour, Y. Tong, *ChemBioChem* **2006**, *7*, 653.

