Use of 1,3-dithiane combined with aryldiazonium cation for immobilization of biomolecules based on electrochemical addressing†

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We report the use of 1,3-dithiane combined with aryldiazonium cation for the immobilization of biomolecules based on electrochemical addressing.

Electrochemically assisted modification (EAM) of electrode surfaces with specific chemical end functionalities for the immobilization of biomolecules has been extensively studied during the past two decades because the modification can be completed within a very short time and allows selective functionalization of closely-spaced electrode surfaces by application of potential bias.[1–9]

There are two types of EAMs. The first is a direct electrodeposition of functional molecules, in which the electrochemical reduction of aryldiazonium salts introduced by Delamar *et al.*[2] became one of the most interesting methods that offers the advantages of stable covalent attachment of useful functional groups to various substrates such as carbon,[2,3] silicon,[4] metals[5] and even indium-tin-oxide (ITO).[6] The second is an electrochemical reaction of electroactive molecules pre-coated on electrodes. For example, hydroquinone moieties of self-assembled monolayers on gold surfaces are electrochemically modified to their corresponding chemically reactive functions[7–9] and nitro groups of nitrophenyl derivatized surfaces are electrochemically reduced to amines.[2b] Among them, on-demand formation of an aldehyde group by electrochemical deprotection is a fairly attractive technique because not only can it be used for the direct immobilization of amine-terminated molecules, including proteins, without additional linkers or chemical activation steps, but also can ensure the stability of the aldehyde during storage and surface modification.[8,10]

Here we report a combination approach of the two EAMs as a useful method for the immobilization of proteins. The combination comprises the aryldiazonium function and the electroactive function, producing aldehyde groups as their respective most promising alternatives of the two EAMs. In this work, we employed *in situ* generated 4-(1,3-dithian-2-yl)benzenediazonium (DTD) cations from 4-(1,3-dithian-2-yl)aniline *via* diazotation (Scheme 1A). The new aniline was synthesized in this work. Electrochemical oxidation of 1,3-dithiane as an aliphatic electroactive group affords carbonyl functionality in high yield.[11] Scheme 1B displays a reductive electrodeposition of a DTD cation on ITO electrode surfaces and a suggested mechanism for the electrochemical deprotection of aldehydes. Applied anodic potential oxidizes monosubstituted 1,3-dithiane to a positively charged substituent which is attacked by water present in solution and subsequently leads to the formation of an aldehyde group by removing the disulfide as a protecting group. To the best of our knowledge, this is the first use of 1,3-dithiane for surface modification although it is well-known as a protecting group of carbonyl functionality in organic synthesis. An alternative to the aromatic groups, including hydroquinone moieties commonly employed as electroactive groups,[7–9] the use of aliphatic 1,3-dithiane is expected to have several merits: a simpler procedure for synthesis; less change of electrochemical potential on chemically derivatizing electroactive parts; lower nonspecific binding of biomolecules on the surfaces. Differently from the common use of aryldiazonium cation based on electro-addressing for the selective immobilization, the electrodeposition of the cation in our work was used only for the attachment of dithiane functionality.

The electrodeposition of the DTD cation on the ITO electrode surface was conducted by using cyclic voltammetry (CV), which produces a large irreversible reduction current wave at −0.2 V during the first cycle followed by greatly diminished currents on even the second cycle, as shown in Fig. 1A. The result exhibits very fast saturation of the electrode surface, suggesting that the electrodeposition is more efficient for the molecular layer formation than the previous reports for other aryldiazonium cations.[6] Electrochemical deprotection of the 1,3-dithiane group on the surface was investigated with CV and X-ray photoelectron spectroscopy (XPS). Fig. 1B shows CV for anodic oxidation of the DTD-modified electrode. An irreversible anodic current peak was observed at 1.5 V on the first scan, which then disappeared on the next scans. The surface density calculated from the area

Scheme 1 (A) *In situ* preparation of the DTD cation from its aniline precursor *via* diazotation reaction. (B) Electrodeposition of DTD cation on the ITO electrode surface, followed by electrochemical conversion of the 1,3-dithiane part on the electrodeposited ITO surface.

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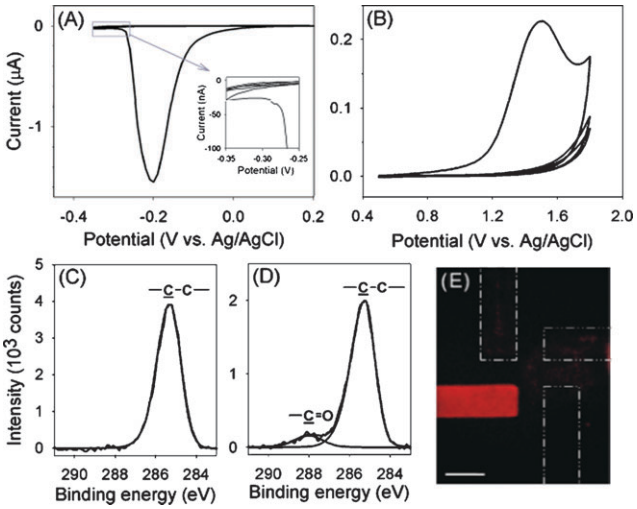

Fig. 1 (A) CV for the electrodeposition of the DTD cation (1.5 mM in 0.1 M HCl) on an ITO microelectrode. Scan rate is 50 mV s^{-1}. (B) CV for the electrochemical deprotection of the DTD modified surface. CH_3CN : H_2O (95 : 5) solution with 0.1 M Bu_4NBF_4 was used in the CV. Scan rate is 50 mV s^{-1}. C1s XPS results for DTD-modified surfaces (C) before and (D) after electrochemical deprotection. (E) Fluorescence microscopic image obtained from the spatially selective immobilization of antibody. The broken lines indicate the position of ITO electrodes. Scale bar is 150 μm.

of the peak current with an approximation of the 2-electron process was found to be 1.0×10^{-9} mol cm^{-2}. Considering the reaction is not quantitative, the value is greater than the expected surface density for the monolayer film formed by electrodeposition of common aryldiazonium cations, implying partial formation of multilayers due to polymerization of DTD on the surface.[12]

XPS for carbon (C1s) atoms was used to examine aldehyde formation after the electrochemical deprotection of DTD-modified surfaces. While before the deprotection the spectra exhibited a peak at 285.3 eV due to the carbon atoms of DTD (Fig. 1C), after the deprotection a new peak was detected at 288.0 eV (Fig. 1D) corresponding to the carbonyl group that might be attributed to the aldehyde.[10] Direct antibody immobilization on the deprotected surfaces was conducted to verify the aldehyde functionality, reacting with a primary amine on the protein, producing an imine.[10] As an antibody, anti-rabbit IgG labeled with tetramethylrhodamine isothiocyanate (TRITC) was employed. We prepared and used an ITO microelectrode array for this work as previously reported.[13] Following simultaneous electrodeposition of DTD on all of the electrodes, the resulting surface was subjected to the electrochemical deprotection of only one electrode. After that, the whole surface was exposed to phosphate-buffered saline with a 0.05% Tween (PBST) solution of the antibody during 1 h. Fig. 1E displays a fluorescence microscopic image obtained from the exposure. Extremely contrasted red color is seen exclusively in the oxidized surfaces. XPS and immobilization results clearly support that the electrochemical deprotection produces aldehydes that can directly react with amine containing proteins. Moreover, Fig. 1E demonstrates that our combined

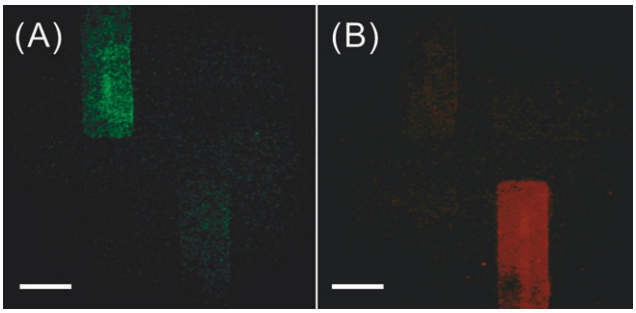

Fig. 2 Fluorescence microscopic image results from a sandwich-type multianalyte immunoassay for the serial detection of (a) mouse IgG and (b) rabbit IgG. Scale bar is 150 μm.

EAMs are greatly efficient for the spatially selective immobilization of proteins on the closely spaced microelectrode array.

The immobilization result was applied for a multianalyte sandwich-type immunoassay based on fluorescence observation. Two electrodes among four electrodes of the array were selectively functionalized with two different kinds of antibodies as probes, respectively. The protocol to prepare the platform for the immunoassay is summarized as below. Following the simultaneous electrodeposition of DTD on the entire electrode, the upper electrode only was addressed for the electrochemical deprotection. The resulting array surface was exposed to a drop of anti-mouse IgG solution of PBST for 2 h. After washing the exposed surface, the same procedure was repeated for the lower electrode and anti-rabbit IgG. For the immunoassay, the platform of the antibody-immobilized array was incubated with a drop of 2 μg mL^{-1} mouse IgG in PBST solution for 1 h. After washing, the platform surface was exposed to a drop of a solution mixture of anti-mouse IgG labeled with FITC and anti-rabbit IgG labeled with TRITC in PBST for 3 h.

Fig. 2A shows a fluorescence image obtained from the immunoassay, where the upper electrode only gives bright fluorescence due to FITC, indicating that mouse IgG could be detected with a high specificity and a negligible nonspecific binding of proteins using our method. To examine multianalyte detection, the platform used in mouse IgG detection was reused for the detection of another antigen, rabbit-IgG (2 μg mL^{-1}). The second immunoassay led also to a highly contrasted fluorescence image due to TRITC being observed for the lower electrode (Fig. 2B). Two differently colored detection images with a high signal to noise ratio imply that not only cross reaction of two antigens is negligible but also multianalyte detection is successfully achieved.

In conclusion, we have demonstrated for the first time use of the combination of 1,3-dithiane with an aryldiazonium cation for immobilization of proteins. Our work using DTD cations might present general and promising tools for the covalent immobilization of biomolecules including DNA, peptides as well as proteins,[14] because it could offer advantages over the conventional self-assembly process of electroactive thiols or silanes: (i) saving time to produce an electroactive film due to rapid electrodeposition of aryldiazonium; (ii) providing the flexibility of electrode materials for on-demand formation of aldehyde; (iii) allowing multiple electro-addressing due to the dually electroactive functionality of the DTD cation.

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Notes and references

1 (*a*) M. N. Yousaf, B. T. Houseman and M. Mrksich, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 5992–5996; (*b*) Y. L. Bunimovich, G. Ge, D. C. Beverly, R. S. Ries, L. Hood and J. R. Heath, *Langmuir*, 2004, **20**, 10630–10638; (*c*) N. K. Devaraj, P. H. Dinolfo, C. E. D. Chidsey and J. P. Collman, *J. Am. Chem. Soc.*, 2006, **128**, 1794–1795.

2 (*a*) M. Delamar, R. Hitmi, J. Pinson and J. M. Savéant, *J. Am. Chem. Soc.*, 1992, **114**, 5883–5884; (*b*) P. Allongue, M. Delamar, B. Desbat, O. Fagebaume, R. Hitmi, J. Pinson and J. M. Savéant, *J. Am. Chem. Soc.*, 1997, **119**, 201–207.

3 (*a*) B. P. Corgier, C. A. Marquette and L. J. Blum, *J. Am. Chem. Soc.*, 2005, **127**, 18328–18332; (*b*) B. P. Corgier, A. Laurent, P. Perriat, L. J. Blum and C. A. Marquette, *Angew. Chem.*, 2007, **119**, 4186–4188.

4 P. Allongue, C. Henry de Villeneuve, G. Cherouvrier, R. Cortès and M. C. Bernard, *J. Electroanal. Chem.*, 2003, **161**, 550–551.

5 A. Adenier, M. C. Bernard, M. M. Chehimi, E. Cabet-Deliry, B. Desbat, O. Fagebaume, J. Pinson and F. Podvorica, *J. Am. Chem. Soc.*, 2001, **123**, 4541–4549.

6 S. Maldonado, T. J. Smith, R. D. Williams, S. Morin, E. Barton and K. J. Stevenson, *Langmuir*, 2006, **22**, 2884–2891.

7 M. N. Yousaf and M. Mrksich, *J. Am. Chem. Soc.*, 1999, **121**, 4286–4287.

8 W. S. Yeo and M. Mrksich, *Adv. Mater.*, 2004, **16**, 1352–1356.

9 K. Kim, H. Yang, E. Kim, Y. B. Han, Y. T. Kim, S. H. Kang and J. Kwak, *Langmuir*, 2002, **18**, 1460–1462.

10 R. E. Ducker, S. J. Janusz, S. Sun and G. J. Leggett, *J. Am. Chem. Soc.*, 2007, **129**, 14842–14843.

11 M. Martre, G. Mousset, R. B. Rhlid and H. Veschambre, *Tetrahedron Lett.*, 1990, **31**, 2599–2602.

12 Combellas, F. Kanoufi, J. Pinson and F. I. Podvorica, *J. Am. Chem. Soc.*, 2008, **130**, 8576–8577.

13 E. J. Kim, H. U. Shin, S. Park, D. Sung, S. Jon, S. G. Sampathkumar, K. J. Yarema, S. Y. Choi and K. Kim, *Chem. Commun.*, 2008, 3543–3545.

14 T. Govindaraju, P. Jonkheijm, L. Gogolin, H. Schroeder, C. F. W. Becker, C. M. Niemeyer and H. Waldmann, *Chem. Commun.*, 2008, 3723–3275.