COMMUNICATION

ChemComm www.rsc.org/chemcomm

New flavin and deazaflavin oligonucleotide conjugates for the amperometric detection of DNA hybridization

Serge Cosnier,*[a] Chantal Gondran,[a] Cécile Dueymes,[b] Philippe Simon,[bc] Marc Fontecave[b] and Jean-Luc Décout[c]

[a] *Laboratoire d'Electrochimie Organique et de Photochimie Rédox UMR 5630 CNRS Institut de Chimie Moléculaire de Grenoble FR CNRS 2607, Université Joseph Fourier, BP 53, 38041 Grenoble cedex9, France. E-mail: serge.cosnier@ujf-grenoble.fr; Fax: +33 4 76 51 42 67; Tel: +33 4 76 51 49 98*

[b] *Laboratoire de Chimie et Biochimie des Centres Rédox Biologiques, UMR 5047 UJF/CEA/CNRS DRDC-CB, CEA Grenoble, 17 Avenue des Martyrs 38054, Grenoble Cedex 09, France*

[c] *Laboratoire de Chimie Bio-Organique, Département de Pharmacochimie Moléculaire, UMR 5063 CNRS/Université Joseph Fourier, FR CNRS 2607, BP 138, 5 Avenue de Verdun, F-38243 Meylan, France*

Received (in Cambridge, UK) 23rd February 2004, Accepted 28th April 2004
First published as an Advance Article on the web 15th June 2004

The functionalization of an oligonucleotide by flavin and deazaflavin derivatives allowed an amperometric determination of the hybridization process through the disappearance of the electroactivity of the free oligonucleotide and the appearance of a new electrochemical signal characteristic of the resulting duplex.

The intimate combination of the principle of nucleic acid hybridization with the sensitivity of optical, electrochemical or gravimetric transducers has led to the exponential emergence of DNA biosensors (or genosensors) and DNA chips as valuable selective tools in diagnostic laboratories and medical treatment. Among the conventional methods for detecting a DNA hybridization event, the electrochemical transduction ensured attractive advantages such as ease of use in turbid samples, portability, low cost and compatibility with bulk manufacturing procedures. The development of electrochemical DNA biosensors has been recently reviewed.[1,2] In general, the detection of a hybridization reaction between a single strand of DNA (ssDNA) (target) and a complementary oligonucleotide (probe) is performed by the two following electrochemical strategies. The first and most common approach consisted in the use of external electroactive labels such as metal complexes, organic redox markers or enzymes.[3–8] However, it should be noted that non-specific interactions may affect the electrochemical behavior of these labels interfering thus with the detection of the duplex formation. The rather simple and promising second approach is based on the direct electrochemical detection of the hybridization event without addition of an external indicator or label of the oligonucleotide probe. For example, the intrinsic signal of DNA (derived from the direct oxidation of adenine and guanine) and its variation upon hybridization as well as the changes in the electrochemical behavior of conducting polymers functionalized by oligonucleotides in the presence of complementary ssDNA have been exploited.[9,10]

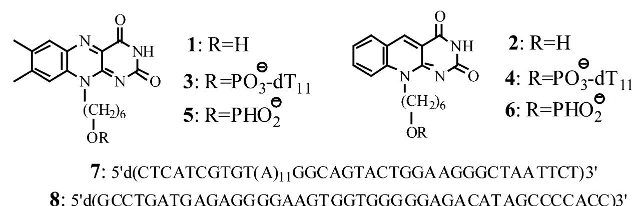
Finally, besides these label-free procedures, an original approach consisted in the use of ssDNA labeled with an electroactive marker. However, only few examples of oligonucleotides bearing redox markers such as ferrocene or anthraquinone were reported.[11–15] As in the case of fluorescent markers, the label is introduced in the target oligonucleotides. Thus hybridization is detected from the appearance of an electrical signal. We recently proposed that a significant improvement could be obtained if the label were to be attached to the probe rather than to the target so that the detected signal is different according to whether the labelled probe is free or hybridized.

In the present paper, we report for the first time, the amperometric detection of DNA hybridization by a double signal *via* the functionalization of an oligonucleotide probe by flavin and deazaflavin derivatives **1** and **2**, respectively (Scheme 1) exhibiting electroactive properties.

The electrochemical behavior of flavin **1** and deazaflavin **2** as model compounds was investigated by differential pulse voltammetry (DPV).[16] Upon reductive scanning up to −1.2 V *vs.* AgCl, a two-electron and a one-electron reduction peak were observed at −0.36 and −0.80 V for **1** and **2** respectively. The current intensity of these well defined peaks was recorded as a function of the concentration of the flavin derivatives providing linear calibration curves. The sensitivity and detection limit were 350 mA M^{-1} and 0.3 μM for **1**, respectively, and 120 mA M^{-1} and 0.4 μM for **2**, respectively.

As previously reported, the flavin and deazaflavin–oligonucleotide conjugates, **3** and **4**, were synthesized from the corresponding H-phosphonates **5** and **6**.[17,18] The influence of the covalent attachment of the oligonucleotide on the electrochemical behavior of the flavin and deazaflavin moities was investigated by DPV. A similar reduction peak was recorded at −0.36 V for **3** indicating the absence of intramolecular interactions between the flavin and the oligonucleotide groups. As expected, the detection sensitivity for **3** (195 mA M^{-1}) was markedly lower than that determined for the free **1**. This difference is probably due to an increase of the diffusional constraints in the case of **3**. In contrast, the attachment of the oligonucleotide to **2** (conjugate 4) markedly shifted the potential of the reduction peak from −0.8 to −1.12 V. It has been previously reported that electrostatic interactions between negatively charged ssDNA and redox markers such as methylene blue or tris (2,2′-bipyridyl) cobalt(III) induced a slight negative shift of their peak potentials.[7,19] In comparison to **3**, it is likely that the absence of methyl groups at positions 7 and 8 may facilitate interactions between the deazaflavin group and the oligonucleotide. Nevertheless, **4** concentration could be monitored through the change in the peak magnitude with a sensitivity of 60 mA M^{-1}.

The electrochemical behavior of **3** was examined in the presence of the complementary DNA target **7** to quantify the hybridization events (Fig. 1). Addition of increasing concentrations of **7** resulted in a decrease in the magnitude of the reduction peak at −0.36 V characteristic of the free oligonucleotide flavin **3**. After addition of about 0.4 equivalents of **7** (Fig. 2) the electrochemical response of **3** reached a pseudo-plateau and corresponded to about 30% of its initial value for a **7**/**3** ratio higher than 1. Interestingly, a novel reduction peak at −1 V appeared and increased with increasing concentrations of **7** . Its intensity reached a plateau for a **7**/**3** ratio higher than 1. In a similar way, the electrochemical behavior of **4** was markedly affected by the presence of increasing amounts of **7**



Scheme 1 Structure of the flavin and deazaflavin derivatives and their corresponding oligonucleotide conjugates.

DOI: 10.1039/b402760d

This journal is © The Royal Society of Chemistry 2004

(Fig. 1). The initial reduction peak at −1.13 V decreased while a new one appeared at −1.2 V, both tending towards stabilized current peak values for a **7**/**4** ratio approximately equimolar (Fig. 3). Similar experiments, carried out with both **3** and **4** and using a non-complementary oligonucleotide target, **8**, did not show any change in the electrochemical behavior of the redox markers. This shows that both the decrease of the signal of the free flavin-oligonucleotide conjugates and the appearance of a new signal upon addition of a target is a specific indication of an hybridization reaction.

The influence of the temperature on the DPV peak signal of **4** was examined after the addition of one equivalent of **7**. The reduction peak potentials due to **4** and the duplex both increased with increasing the temperature while only one peak appeared at 30 °C . Inversely, this unique peak was split into two reduction peaks during temperature reduction from 40 to 8 °C. This reversible phenomenon very well reflects the dissociation of the **4**–**7** complex

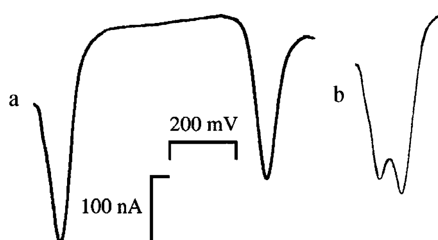


Fig. 1 Differential pulse voltammogram of flavin **3** and deazaflavin **4** oligonucleotide conjugates (2.7 and 8.2 μM) in the presence of 0.7 and 3.9 μM **7** respectively. a) **3** peak at −0.36 V and **3**–**7** duplex at −1.0 V, b) **4** peak and corresponding duplex at −1.13 and −1.20 V, respectively.

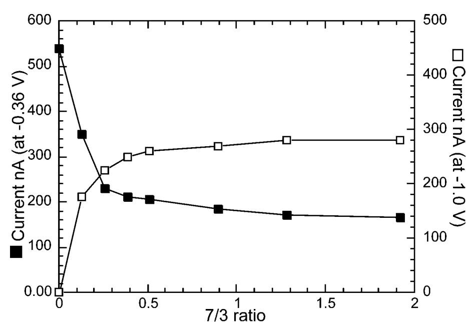


Fig. 2 Dependence of the intensity of the DPV peak of **3** and the corresponding duplex on the **7**/**3** ratio.

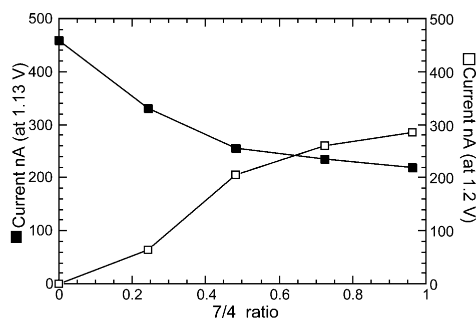


Fig. 3 Dependence of the intensity of the DPV peak of **4** and the corresponding duplex on the **7**/**4** ratio.

(T_m = 30 °C at pH 7, 0.1 M NaCl) and its reformation as a function of temperature).

We have here characterized new, easy to prepare, redox-active oligonucleotides that allow the amperometric determination of DNA hybridization events through the simultaneous disappearance and appearance of reduction peaks corresponding to the free redox-active oligonucleotide and the related hybrid, respectively. Upon hybridization, the flavin moiety of the oligonucleotide–flavin conjugate is likely to enjoy a different electronic environment due to interactions, still undefined, with the adjacent complementary strand, thus explaining the redox potential shift. The previously reported flavin fluorescence quenching upon hybridization supports this hypothesis.[18] In contrast to commonly used ferrocene or quinone tracers, these flavin–oligonucleotide conjugates have the unique ability to provide a double signal, one specific for the free state and one for the hybridized state. As a consequence, their immobilization may be of interest for the development of electrochemical DNA sensors exhibiting an original reagentless detection.

This work was in part financially supported by the "Programme CNRS : Puce à ADN" from the Research Ministry of France.

Notes and references

1 J. Wang, *Anal. Chim. Acta*, 2002, **469**, 63.
2 M. I. Pividori, A. Merkoçi and S. Alegret, *Biosens. Bioelectron.*, 2000, **15**, 291.
3 G. Marrazza, I. Chianella and M. Mascini, *Anal. Chim. Acta*, 1999, **387**, 297.
4 P. M. Armistead and H. H. Thorp, *Anal. Chem.*, 2000, **72**, 3764.
5 L. Alfonta, A. K. Singh and I. Willner, *Anal. Chem.*, 2001, **73**, 91.
6 F. Patolsky, A. Lichtenstein and I. Willner, *J. Am. Chem. Soc.*, 2001, **123**, 5194.
7 J. Yang, Z. Zhang and J. F. Rusling, *Electroanalysis*, 2002, **14**, 1494.
8 M. Dequaire and A. Heller, *Anal. Chem.*, 2002, **74**, 4370.
9 H. Korri-Youssoufi, F. Garnier, P. Srivastava, P. Godillot and A. Yassar, *J. Am. Chem. Soc.*, 1997, **119**, 7388.
10 B. Meric, K. Kerman, D. Ozkan, P. Kara and M. Ozsoz, *Electroanalysis*, 2002, **14**, 1245.
11 T. Ishara, N. Nakayama, M. Murata, K. Nakano and M. Maeda, *J. Chem. Soc., Chem. Commun.*, 1997, 1609.
12 V. Kertesz, N. A. Whittemore, G. B. Inamati, M. Manoharan, P. D. Cook, D. C. Baker and J. Q. Chambers, *Electroanalysis*, 2000, **12**, 889.
13 C. J. Yu, Y. Wan, H. Yowanto, J. Li, C. Tao, M. D. James, C. L. Tan, G. F. Blackburn and T. J. Meade, *J. Am. Chem. Soc.*, 2001, **123**, 11155.
14 H. Cai, C. Xu, P. He and Y. Fang, *J. Electroanal. Chem.*, 2001, **510**, 78.
15 M. Nakayama, T. Ihara, K. Nakano and M. Maeda, *Talanta*, 2002, **56**, 857.
16 Voltammograms were performed with an EG&G PARC Model 264 A Polarographic analyzer/stripping voltammeter and an EG&G PARC Model 303 A SMDE electrode coupled with a Sefram 8340 XY recorder. The differential pulse voltammograms were collected with a pulse amplitude of 50 mV, a width time of 0.2 s at 10 mV s^{-1} scan rate. The working electrode was the hanging mercury drop electrode (HMDE) with a drop area of 2 mm^2. A saturated Ag/AgCl electrode and a platinum wire were used as reference and counter electrodes, respectively. All procedures were performed at 8 °C in a glass cell under a purified argon atmosphere. A 0.1 M NaCl containing 0.05 M phosphate buffer (pH 7) was used as a background electrolyte.
17 C. Frier, J. L. Décout and M. Fontecave, *J. Org. Chem.*, 1997, **62**, 3520.
18 C. Dueymes, J. L. Décout, P. Peltié and M. Fontecave, *Angew. Chem. Int. Ed.*, 2002, **41**, 486.
19 A. Erdem, K. Kerman, B. Meric, U. J. Akarca and M. Ozsoz, *Anal. Chim. Acta*, 2000, **422**, 139.