Electrochemically active threading intercalator with high double stranded DNA selectivity

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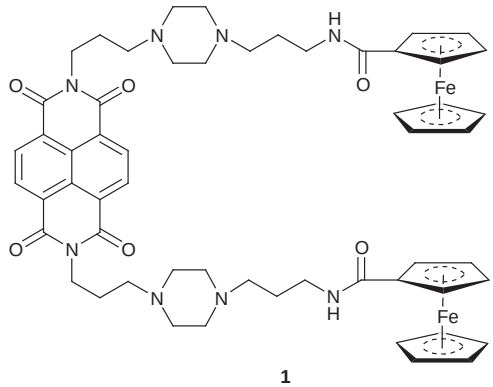
A naphthalene diimide threading intercalator carrying ferrocenyl moieties as its termini can discriminate double stranded DNA from single stranded counterparts owing to a difference in their dissociation rates.

Discrimination of double stranded nucleic acids from single stranded ones is of utmost importance in basic and applied molecular biology. Hybridization of DNA with its complementary DNA or RNA is based on this discrimination and one has to discriminate single stranded RNA of such viruses as HIV from double stranded human chromosomal DNA when targeting the former. Single and double stranded nucleic acids may be discriminated by intercalators but none is known to achieve this feat with a high margin. Recently, threading intercalators were developed in which their substituents can penetrate between the base pairs of double stranded DNA when bound to it.[1,2] This type of intercalator, *e.g.* naphthalene diimides, forms a very stable complex with double stranded DNA, and they dissociate much more slowly from double stranded DNA than do classical intercalators.[1–3] When electrochemically active groups are attached to a threading intercalator, the resulting molecule will serve as a double stranded DNA selective electrochemical probe.[4,5] This idea was substantiated with a naphthalene diimide **1** carrying two ferrocenyl moieties at its ends.



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Naphthalene diimide **1** was prepared by a reaction of the corresponding amino derivative with *N*-hydroxysuccinimide ester of ferrocenecarboxylic acid and purified by silica gel chromatography (MeOH) followed by recrystallization from water (yield, 25%).

Compound **1** undergoes hypochromic and bathochromic shifts of the absorption band of the naphthalene diimide chromophore upon binding to sonicated calf thymus DNA, which is indicative of DNA intercalation. Sodium dodecyl sulfate-driven dissociation experiments[3] carried out with the complex of **1** and intact or denatured calf thymus DNA§ showed that the dissociation rate constant (k_d) of **1** from the intact DNA was 0.06 s^{-1} at 20 °C, whereas that of **1** from the denatured DNA was 4.2 s^{-1}. These magnitudes of dissociation rate constant are one of the smallest for synthetic intercalators. The ratio of 70 in k_d suggests that single and double stranded DNA can be discriminated kinetically by **1**. Note that these magnitudes are minimal estimates, since 'the denatured' DNA is not necessarily composed of single stranded DNA alone. To test whether compound **1** can discriminate single and double stranded DNA on the electrode surface, the following experiments were carried out.

A DNA-immobilized electrode was prepared by a reaction of mercaptohexyl dT_{20} oligonucleotide on a gold electrode with an area of 2.0 mm^2.[7] HPLC experiments revealed that 64 pmol of mercaptohexyl oligonucleotide was immobilized on this electrode. Then 2 μl of a solution containing appropriate amounts (0–70 pmol) of target dA_{20} oligonucleotide were placed on this modified electrode and kept at 25 °C for 20 min to allow hybridization with the immobilized probe dT_{20}. After this reaction, 2 μl of a 1 mM solution of **1** were placed on the

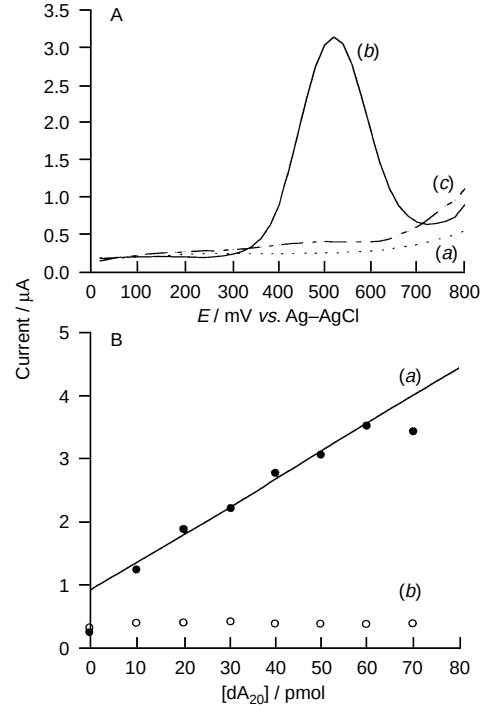

Fig 1 (A) Differential pulse voltammograms (DPVs) of **1** bound to the dT_{20}-immobilized electrode before (*a*) and after hybridization with 60 pmol of dA_{20} (*b*) or dT_{20} (*c*). Differential pulse voltammograms were measured in 50 mM AcOK–AcOH buffer (pH 5.2) with Ag–AgCl and Pt wire as reference and counter electrodes, respectively. Pulse period 200 ms; scan rate 100 mV s^{-1}; pulse amplitude 50 mV; pulse width 50 ms. (B) Correlation of the current response at 521 mV for **1** with the amount of dA_{20} (*a*) and dT_{20} (*b*).

electrode for 5 min. Following washing with 50 mM AcOK–AcOH buffer (pH 5.2) by vortexing for 5 s at 20 °C, cyclic voltammograms (CVs) and differential pulse voltammograms (DPVs) of the complex were measured in the same buffer. Fig. 1(A) shows examples of DPVs for this electrode treated with dA_{20} (target DNA) or dT_{20} (non-target DNA). It is clear that the electrochemical signal is generated only in the presence of complementary oligonucleotide dA_{20} [trace (*b*)] and the current response obtained for dT_{20}, non-complementary to the sequence of dT_{20}, was barely above background [trace (*c*)]. A current response due to the ferrocenyl moieties of **1** increased linearly up to 60 pmol with an increase in the amount of dA_{20} (correlation coefficient 0.990) and then levelled off as shown in Fig. 1(B) [trace (*a*)]. The amount of dA_{20} at the break point (60 pmol) was in agreement with the amount of the probe dT_{20} immobilized on the electrode (64 pmol). The estimated detection limit was 1.0 fmol of target oligonucleotide. This procedure could be repeated several times and the current response per immobilized oligonucleotide was reproducible even with electrodes of different lot samples.

We estimated the amount of the ligand **1** bound on this electrode to be 13 pmol from the area under the CV peak after hybridizing with 60 pmol of dA_{20}. This value is about 30 times smaller than that expected from Scatchard analysis, which revealed that three base pairs of double stranded DNA are required to accommodate a single molecule of **1**. Therefore, *ca*. 400 pmol of **1** ought to be bound to 60 pmol of $dA_{20}dT_{20}$. This discrepancy can arise from several reasons. First, intercalation and redox reaction of **1** on the electrode surface could be less efficient than those in solution. Alternatively, part of **1** could undergo dissociation from DNA during washing of the complexed and intercalated electrode. Despite this low efficiency, the electrochemical responses of the active electrode were stable during the experiment (*ca*. 2 min).

In conclusion, the electrochemically active threading intercalator **1**, coupled with a DNA-carrying electrode, enabled the sensitive detection of target DNA fragments. The whole manipulation was carried out over a short period of time as long as the sensor is prepared and yet the sensitivity of detection is ultra-high, making this sensing system most feasible for practical use in DNA analysis and gene diagnosis.

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Notes and References

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§ Denaturation of DNA was carried out by heating at 100 °C for 10 min and then immediately cooling on ice.

1 S.-F. Yen, E. J. Gabbay and W. D. Wilson, *Biochemistry*, 1982, **21**, 2070.
2 F. A. Tanious, S.-F. Yen and W. D. Wilson *Biochemistry*, 1991, **30**, 1813.
3 W. D. Wilson and F. A. Tanious, in *Molecular Aspects of Anticancer Drug–DNA Interactions*, ed. S. Neidle and M. J. Waring, Macmillan Press, Riverside, NJ, 1994, p. 243.
4 S. Takenaka, Y. Uto, H. Kondo,T. Ihara and M. Takagi, *Anal. Biochem.*, 1994, **218**, 436.
5 Y. Uto, H. Kondo, M. Abe, T. Suzuki and S. Takenaka, *Anal. Biochem.*, 1997, **250**, 122.
6 J. D. McGhee and P. H. von Hippel, *J. Mol. Biol.*, 1974, **86**, 469.
7 M. Maeda, Y. Mitsuhashi, K. Nakano and M. Takagi, *Anal. Sci.*, 1992, **8**, 82.

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