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Protein–DNA interaction: impedance study of MutS binding to a DNA mismatch†

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MutS binding to a double-stranded DNA containing a single nucleotide mismatch can be conveniently monitored by impedance spectroscopy and represents the first step in developing an electrochemical binding essay for single nucleotide mismatch detection.

The development of DNA biosensors for single base mismatch detection is motivated by the potential for applications in modern diagnostic medicine, genetic disease treatment and genome sequencing. Electrochemical detection of base pair mismatches is an attractive alternative to other existing formats[1] providing rapid and sensitive screening and a direct electronic read-out in solid-state electronic devices.[2] Current electrochemical detection systems involve monitoring the difference of the electrochemical or photoelectrochemical signal response as a function of a fully matched or mismatched base pair in DNA monolayers bound to an electrode surface. The redox probe can either be chemically bonded to the DNA, such as ferrocene or quinone[3] or can be intercalated, such as daunomycin, methylene blue, and ferrocenyl naphthalene diimide.[4] However, there are inherent problems with the complex synthetic process, sensitivity and reproducibility of such assays. As part of our research efforts for electronic DNA biosensor development we have focused on the electrochemical characterization of DNA self assembled monolayers (SAMs) on gold surfaces that function as a sensor substrate.[5] Furthermore, electrochemical analyses for DNA–protein reactions have proven to be particularly sensitive.[6]

Here we describe the results of our electrochemical investigation using a new approach involving MutS,[7] a 97 kDa mismatch binding protein, to differentiate between mismatches and perfectly matched ds-DNA (Fig. 1b,d) connected to a gold surface through a 3′-C_3 linker. The results of our impedance study show large differences in the impedance signals between complementary *versus* mismatched ds-DNA in the presence of MutS, and thus demonstrate the utility of MutS in the detection of base-pair mismatches and DNA–protein interactions.

For this study, three DNA sequences were synthesized by standard solid phase techniques (**1**: 5′-AA*C* TAC TGG GCC ATC GTG AC 3′-$(CH_2)_2$-SH; **2**: 5′-GTC ACG ATG GCC CAG TAG TT-3′; **3**: 5′ GTC ACG ATG GCC CAG TA*A* TT-3′). The characterization and purification of the DNA derivatization was performed by RP-HPLC and MALDI-TOF MS. Monolayers of a 20 base-pair ds-DNA were formed by incubating suitable gold substrates for 5 days in solutions of pre-hybridized ds-DNA solution containing equimolar amounts of **1** : **2** and **1** : **3** (0.5 mM in 100 mM Tris-ClO_4 buffer with 800 mM $NaClO_4$, pH 8.5). The **1** : **2** ds-DNA is fully matched, whereas the **1** : **3** hybrid contains a single AC mismatch. The thickness of the monolayer was determined by ellipsometry to be 43 (±5) Å (refractive index = 1.55).

A comparison between dense monolayers of matched DNA and mismatched DNA shows only a small difference in the impedance after incubation of the two surfaces in a solution of MutS. This suggests that a densely packed monolayer of ds-DNA does not provide sufficient space for MutS binding.

To enable effective MutS binding to mismatched DNA, the concentrated ds-DNA monolayer was diluted by incubating it in a 0.1 mM solution of butanethiol for 20 minutes, leading to a surface that has well separated ds-DNA molecules.

The diluted DNA surfaces were used for the interfacial impedance measurements ($E_{\frac{1}{2}}$ = 250 mV *vs.* Ag/AgCl, sinusoidal potential modulation of ±5 mV; ν = 100 kHz–100 mHz) using freshly prepared 4 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1 : 1) as the redox probe. The impedance data were fit to an equivalent circuit (supporting information) that includes a solution resistor R_s, a charge transfer resistance R_{ct}, surface capacitance Q_y and a combination of two parallel diffusion like elements R_{diff} and Q_x, which has the form of a constant phase element (CPE) (using ZsimpWin software, Princeton Applied Research).[8] The dilution of

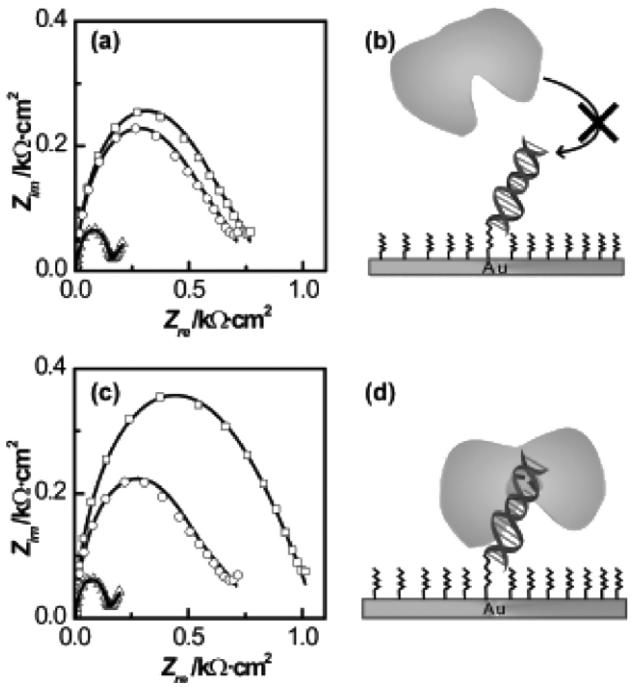

Fig. 1 Nyquist plots (Z_{im} *vs.* Z_{re}) for the Faradaic impedance measurements in the presence of 4 mM $[Fe(CN)_6]^{3-/4-}$ in 20 mM Tris-ClO_4 buffer (pH 8.5) containing 100 mM $NaClO_4$ at (a) **1** : **2** modified electrode; (c) **1** : **3** modified electrode containing a single AC base pair mismatch; (b) and (d) schematically represent MutS interactions with **1** : **2** and **1** : **3**, respectively. In the impedance spectra a) and c), Δ represents the concentrated ds-DNA modified electrode, ○ represents the surface after dilution with 1 mM butanethiol in 20 mM Tris-ClO_4 buffer (pH 8.5) containing 500 mM $NaClO_4$, □ represents the diluted DNA surface after interaction with MutS. The symbols represent the experimental data, and the solid lines are the fitted curves using the equivalent circuit.

† Electronic supplementary information (ESI) available: details of the electrode pretreatment, the preparation of MutS binding solution and the process for DNA–MutS binding. AFM and EIS analysis for bare gold substrate, close-packed ds-DNA monolayer modified substrate. See http://www.rsc.org/suppdata/cc/b3/b314642a/

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Table 1 Values of R_s, R_{ct} and R_{diff} before and after MutS interaction with a diluted DNA SAMs and a diluted mismatch DNA SAMs from the fitting equivalent circuit shown in supporting information. R is in $\Omega \cdot cm^2$

	ds-DNA	MutS ds-DNA	Mismatch ds-DNA	MutS mismatch ds-DNA
R_s	3.89 ± 0.4	3.74 ± 0.5	3.96 ± 0.5	3.55 ± 0.7
R_{ct}	(4.34 ± 0.08) × 10^2	(4.96 ± 0.18) × 10^2	(4.27 ± 0.22) × 10^2	(6.83 ± 0.61) × 10^2
R_{diff}	(1.33 ± 0.05) × 10^3	(1.35 ± 0.07) × 10^3	(1.36 ± 0.17) × 10^3	(1.78 ± 0.13) × 10^3

the densely packed ds-DNA monolayer with butanethiol resulted in a 3 and 1.5 times increase in the resistance elements R_{ct} and R_{diff} of the monolayer for both complementary and mismatched-DNA strands, which is in agreement with the expected higher ordering of the mixed monolayer due to filling any existing pinholes and packing defects.

After incubating with MutS in the binding buffer (supporting information), the association of the protein to the mismatch DNA sensing surface **1** : **3** is accompanied by an increase of the charge-transfer resistance R_{ct} at the electrode from 4.27 (±0.22) × 10^2 $\Omega \cdot cm^2$ to 6.83 (±0.61) × 10^2 $\Omega \cdot cm^2$ (increase by *ca.* 60%), the diffusion resistance R_{diff} from 1.35 (±0.07) × 10^3 $\Omega \cdot cm^2$ to 1.78 (±0.13) × 10^3 $\Omega \cdot cm^2$ (increase by *ca.* 33%), whereas no significant increase in R_{ct} and R_{diff} could be observed for the matched duplex (Table 1). This phenomenon is attributed to the disruption of the electron transfer and diffusion of the solution-based redox probe by the DNA–MutS interaction. The measured averaged ellipsometric thickness of the DNA layer gradually increased from 43 (±5) Å for the ds-DNA SAM to 180 (±20) Å for the MutS bound mismatched DNA. Expectedly, the fully matched DNA surface prepared from **1** : **2** did not show any changes in the thickness.

Additional confirmation of the interaction between the SAM of **1** : **3** and MutS was obtained from atomic force microscopy (AFM) measurements of the dilute DNA substrates before and after immersion in a buffer containing MutS (for experimental details see supporting information). The AFM topographic image of diluted ds-DNA monolayer shows well separated ds-DNA molecules (Fig. 2a) with a diameter of 5 nm, which stand well above the height of the background butanethiol.

After incubation of the mismatch ds-DNA SAM **1** : **3** in a buffer containing MutS, the AFM image (Fig. 2b) shows changes in the surface morphology, which is interpreted as the interaction of MutS with the mismatch ds-DNA SAM. A SAM of **1** : **2** did not show any changes. Measurements made from cross sections along the *c*-axis of single MutS molecules indicate that the full width at half-height of these globular structures ranges from 10 nm to 80 nm and the height was approximately 10 nm, which is in broad agreement with the size of monomeric MutS measured by crystallography (12.5 × 9.0 × 5.5 nm).[9] It is noteworthy that the variation in sizes shown in Fig. 2b is consistent with the fact that MutS–DNA interactions might result in various protein orientations or protein multilayer formation with respect to the surface. Nevertheless, our AFM images demonstrate that the MutS is anchored to mismatch ds-DNA.

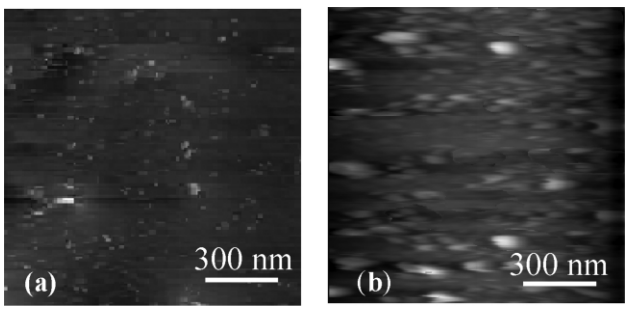

Fig. 2 *In situ* AFM topographic images (1 μm × 1 μm) of (a) ds-DNA immobilized gold surface after exposure to 0.1 mM butanethiol solution for 20 minutes; (b) diluted mismatch ds-DNA modified gold surface after the introduction of MutS. Both images were obtained in 0.2 M Tris buffer solution (pH 7.5).

In conclusion, our results show that the mismatch binding protein MutS enhances the interfacial impedance of mismatched ds-DNA, allowing a good discrimination between a ds-DNA containing a base-pair mismatch and a ds-DNA which is perfectly paired. This is a significant step towards a device capable of rapid and sensitive screening of genetic material and may provide a general method for studying DNA–protein interactions.

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Notes and references

1 (*a*) S. P. A. Fodor, R. P. Rava, X. C. Huang, A. C. Pease, C. P. Holmes and C. L. Adams, *Nature*, 1993, **364**, 555–556; (*b*) M. Schena, D. Shalon, R. W. Davis and P. O. Brown, *Science*, 1995, **270**, 467–470; (*c*) J. Wang, *Nucleic Acids Res.*, 2000, **28**, 3011–3016.

2 (*a*) E. Palecek, M. Fojta, M. Tomschick and J. Wang, *Biosens. Bioelectron.*, 1998, **13**, 621–628; (*b*) J. Zhai, C. Hong and R. Yang, *Biotechnol. Adv.*, 1997, **15**, 43–58; (*c*) L. Alfonfa, A. Bardea, O. Khersonsky, E. Katz and I. Willner, *Biosens. Bioelectron.*, 2001, **16**, 675–687.

3 (*a*) T. Ihara, M. Nakayama, M. Murata, K. Nakano and M. Maeda, *Chem. Commun.*, 1997, 1609–1610; (*b*) G. Hartwich, D. J. Caruana, T. de Lumley-Woodyear, Y. B. Wu, C. N. Campbell and A. Heller, *J. Am. Chem. Soc.*, 1999, **121**, 10803–10812.

4 (*a*) S. O. Kelley, E. M. Boon, J. K. Barton, N. M. Jackson and M. G. Hill, *Nucleic Acids Res.*, 1999, **27**, 4830–4837; (*b*) E. M. Boon, D. M. Ceres, T. G. Drummond, M. G. Hill and J. K. Barton, *Nat. Biotechnol.*, 2000, **18**, 1096–1100; (*c*) 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**, 11155–11161; (*d*) F. Patolsky, A. Lichtenstein and I. Willner, *J. Am. Chem. Soc.*, 2001, **123**, 5194–5205; (*e*) K. Yamashita, M. Takagi, H. Kondo and S. Takenaka, *Anal. Biochem.*, 2002, **306**, 188–196.

5 (*a*) C.-Z. Li, Y.-T. Long, J. S. Lee and H. B. Kraatz, *J. Phys. Chem. B*, 2003, **107**, 2291–2296; (*b*) Y.-T. Long, C.-Z. Li, H. B. Kraatz and J. S. Lee, *Biophys. J.*, 2003, **84**, 3218–3225.

6 (*a*) K. Hashimoto and Y. Ishimori, *Lab on a Chip*, 2001, **1**, 61–63; (*b*) E. M. Boon, J. E. Salas and J. K. Barton, *Nat. Biotechnol.*, 2002, **20**, 282–286; (*c*) L. Alfonta, A. K. Singh and T. Willner, *Anal. Chem.*, 2001, **73**, 91–102; (*d*) J. C. O'Brien, J. T. Stickney and M. D. Porter, *Langmuir*, 2000, **16**, 9559–9567.

7 B. D. Harfe and S. Jinks-Robertson, *Annu. Rev. Genet.*, 2000, **34**, 359–399.

8 M. Dijksma, B. A. Boukamp, B. Kamp and W. P. V. Bennekom, *Langmuir*, 2002, **18**, 3105–3112.

9 M. H. Lamers, A. Perrakis, J. H. Enzlin, H. H. K. Winterwerp, N. D. Wind and T. K. Sixma, *Nature*, 2000, **407**, 711–717.