Supporting information

**Surface enhanced vibrational spectroscopic evidence for an alternative DNA-independent redox activation of endonuclease III**

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1.1 Cloning, expression and purification of DrEndoIII

The gene DR_0289 from genome of D. radiodurans encoding for Endonuclease III (DrEndoIII) was amplified by PCR from genomic DNA and inserted into pDest14 (Invitrogen) as described for DrAlkA2 by using the following primers (Sigma Aldrich):

FPEndoIIITEV (5'-ATCACCATACCACTCACGAAAACCTGTATTTCAGGAGCAACTCGCAATCTGCTCCTCG3'), RPEndoIII (5'-GGGGACACTTTGTACAGAAAGCTGGGTC TCAAACCTGACATGCTCCAC3') and FRDAll (5'-GGGGACAGTTGTTGACAAAAAGCAAGCTCCTCAGAAGGAGATAGAACC3'). The downstream primers were complementary and reverse to the primers described above. Verification of the mutations was performed by BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

DrEndoIII was expressed in BL21(DE3)pLysS at 20 °C, overnight, in Power Broth medium (Molecular Dimensions). The cells were harvested by centrifugation and re-suspended in an extraction buffer A (50 mM Tris-HCl pH 7.5, 150 mM NaCl), containing lysozyme (Sigma Aldrich), DnaseI (Sigma Aldrich), 1 Complete Mini EDTA-free protein inhibitor cocktail tablet (Roche) and 1 mM MgCl₂. The extract was prepared by disrupting the cells during 3 freeze / thaw cycles (liquid nitrogen and water bath at 20 °C) followed by centrifugation. The purification was performed by applying the cell extract to a 1 mL HisTrap HP column (GE healthcare) by buffer A, followed by washing with 5 % buffer B (buffer A and 0.5 M imidazol) and finally eluted with a gradient of buffer B ranging from 5 – 100 % (Step 1). Fractions containing DrEndoIII were pooled and dialyzed overnight at 4 °C in buffer A in the presence of TEV protease (1:20) (Step 2). Afterwards the TEV treated protein solution was loaded onto a 1 mL HisTrap column in order to remove the 6-His tag and the protease; DrEndoIII was eluted from the column by washing with 10 ml of 10 % buffer B (Step 3). The fractions containing the protein were pooled, concentrated and loaded on a Superdex 75 10 / 300 size exclusion column (GE Healthcare) equilibrated with buffer A (Step 4). The pure DrEndoIII was concentrated and stored at 4 °C. The purity, as judged by SDS-PAGE, was estimated to be greater than 95 %. For the protein with intact 6-His-tag only purification steps 1 and 4 were performed.

1.2 Spectroscopy

For resonance Raman (RR) spectroscopic experiments, about 2 µL of 0.5 mM DrEndoIII in the resting state (20 mM Tris-HCl, pH 7.5, 50 mM NaCl) was introduced into a liquid-nitrogen-cooled cryostat (Linkam), mounted on a microscope stage and cooled down to 77 K. Spectra from the frozen sample were collected in backscattering geometry by a confocal microscope coupled to a Raman spectrometer (Jobin Yvon U1000) that is equipped with 1200 1/mm grating and a liquid nitrogen cooled CCD detector. The 413 nm line from a krypton ion laser (Coherent Innova 302) was used as excitation source. Typically, spectra were accumulated for 60 s with a laser power of 8 mW at the sample. The background scattering was removed by subtraction of a polynomial function. SERR spectra were measured at 77 K, with 1.5 mW laser power and an accumulation times of 60 s; 4 spectra were co-added and analyzed. The immobilized enzyme was subjected to 1-10 mM sodium dithionite (50 mM Tris-HCl, pH 9, 50 mM NaCl) or 1-10 mM K₂IrCl₆.

All FTIR and surface enhanced IR absorption (SEIRA) spectra were recorded from 4000-1000 cm⁻¹ at a spectral resolution of 4 cm⁻¹ on a Bruker Tensor 27 spectrometer equipped with a N₂(l)-cooled MCT detector. All measurements were done at 10 °C while purging the sample compartment with dry air. Typically, each spectrum was composed of around 400 scans, and 10 - 40 spectra were co-added and further analyzed. All FTIR and SEIRA data are presented as difference spectra after subtraction of a reference spectrum. For FTIR measurements, about 8 µL of 0.7 mM protein (in 50 mM Tris HCl, 50 mM NaCl, pH 7.6) was injected into a gas-tight cell for liquids (path length 5 μm; CaF₂ windows). SEIRA measurements were performed in Kretschmann-ATR configuration, using a trapezoidal silicon (Si) crystal, under an IR-beam incident angle of 60°.

Regular double stranded DNA oligos (without the thiol group) were generated by annealing equimolar amounts of Fnothiol oligo (5'-AGTACAGTCTACGCG3') with Rndamage (vide infra) or Rdamage (5'CGCGATGACGTACT3') oligos to a final concentration of 100 µM; an excess of dsDNA free protein inhibitor cocktail tablet (Sigma Aldrich), DnaseI (Sigma Aldrich), 1 Complete Mini EDTA-free protein inhibitor cocktail tablet (Roche) and 1 mM MgCl₂. The downstream primers were pooled, concentrated and loaded on a Superdex 75 10 / 300 size exclusion column (GE Healthcare) equilibrated with buffer A (Step 1). Fractions containing DrEndoIII were pooled and dialyzed overnight at 4 °C in buffer A in the presence of TEV protease (1:20) (Step 2). Afterwards the TEV treated protein solution was loaded onto a 1 mL HisTrap column in order to remove the 6-His tag and the protease; DrEndoIII was eluted from the column by washing with 10 ml of 10 % buffer B (Step 3). The fractions containing the protein were pooled, concentrated and loaded on a Superdex 75 10 / 300 size exclusion column (GE Healthcare) equilibrated with buffer A (Step 4). The pure DrEndoIII was concentrated and stored at 4 °C. The purity, as judged by SDS-PAGE, was estimated to be greater than 95 %. For the protein with intact 6-His-tag only purification steps 1 and 4 were performed.

1.3 Cyclic voltammetry

CV experiments were performed under Ar atmosphere in three electrode electrochemical cell arrangements. Different setups were used for coupling CV with SERR and SEIRA, and for CV experiments only. In each case an Ag/AgCl (3 M, KCl) served as reference electrode (+0.21 V vs. SHE), and a platinum wire as a counter electrode. As the working electrode, Au coated silicon crystal, disk Ag and Au electrodes (BASi) were used in SEIRA, SERR and CV, respectively. Electrode potentials were controlled by a Princeton Applied Research 263A potentiostat. Unless otherwise stated, 50 mM Tris HCl, 50 mM NaCl, pH 7.6 was the measuring buffer. The potentials cited in this work refer to the standard hydrogen electrode (SHE). Electron transfer rate constants were determined from the analysis of scan rate dependent measurements (5-500 mVs⁻¹ range) using Laviron (m-function) method.²

1.4 Electrode cleaning and modification

SEIRA electrodes were prepared by electroless deposition of a gold film on the reflecting layer of the silicon crystal.³ The gold film was cleaned by six voltammetric cycles from +0.2 V to +1.6 V at a scan rate of 50 mVs⁻¹ and using 0.1 M HClO₄ as the electrolyte. Gold electrodes (geometric area ~ 3 mm²) for CV experiments were subjected to piranha solution for ~5 min, polished by alumina and cleaned by ultrasonics in water for 5 min. Flat disc Ag electrodes (geometric area ~ 35 mm²) for SERR experiments were polished and electrochemically roughened by alternative negative (-0.3 V) and positive (+0.3 V)
potential cycles of 60s followed by 30s duration. All electrodes were functionalized by immersion in solutions of ω-substituted mercaptanes for 16–24 h to form a self-assembled monolayer (SAM). The commercially available 11-mercaptoundecanoic acid (MUA) was dissolved in ethanol to yield a 1 mM solution. The following oligonucleotides (Midland Certified, USA) were used in order to construct DNA-terminated SAM: 5’-(5-thiol) AGTACAGTCATCGCG-3’ (F6SH) and 5’CGCGATGACTGACT-3’ (Rnodamage). The 5'-Thiol-Modifier C6 Oligonucleotide was reduced prior to annealing to the reverse and complementary oligonucleotide by using the protocol provided by Midland Certified. In short, the oligonucleotide was dissolved in 0.1 M triethylammonium acetate (TEAA), pH 7.5 (Sigma Aldrich) to a concentration of approximately 100 A260 units/mL, followed by addition of 0.15 volumes of 1 M MgCl₂ and incubated at room temperature for 30 min. Thereafter 0.2 volumes of DTT were added and the mixture was incubated for another 5 min at room temperature. The solution was centrifuged for 5 min to remove the silver-DTT complex. The precipitate was dissolved in 1 volume (1 mL) of 0.1 M TEAA and centrifuged again. The supernatants from both centrifugations were pooled and applied to a PD10-columnm (GE-Healthcare) for removal of excess of DTT. Fractions containing the purified DNA were concentrated to approximately 0.5 mM. Double stranded DNA was generated by combining equimolar amounts of the thiol containing oligo with the reverse and complementary oligonucleotide (dissolved in annealing buffer: 10 mM Tris-HCl pH 7.5, 50 mM NaCl and 1 mM EDTA), heated to 95 °C for 5 min, and slowly cooled down to room temperature. The final concentration of 15 bp thiol-dsDNA was around 0.25 mM. After thorough cleaning (vide supra), Au electrodes were incubated with 100 µM 15 bp thiol-dsDNA overnight (designated as DNA SAMs), and afterwards passivated with 10 mM 6-mercaptohexanol for about 30 min prior to measurements and/or protein immobilization (modified from[4]). The His-tag immobilization, employing nickel chelating 3,3’-Dithiobis[N-(5-amin-5-carboxypentyl)propionamide-N,N'-diacetic acid] dihydrochloride (Ni²⁺-NTA), was performed as previously described.[5]

The protein immobilization on functionalized electrodes was achieved by subjecting the clean electrodes to a protein solution. In CV experiments only (Au disk electrodes, BASi) a droplet of 0.3 – 0.75 mM enzyme was deposited on the electrode surface and left to incubate for ~ 40 min. After protein incubation the electrodes were thoroughly rinsed with buffer to remove unbound protein molecules. Due to a large volume of SEIRA spectroelectrochemical cell (~ 10 mL) Au film electrode was left to incubate in protein solution of 0.1 - 10 µM final concentration, until no further changes in SEIRA protein absorption bands were observed (typically for 40-90 min). Protein solution was afterwards removed and the cell was rinsed with measuring buffer.

1.5 Generation of homology model of DrEndoIII structure

A homology model of DrEndoIII was generated by using the crystal structure of EcEndoIII (PDB: 2ABK) as a template and the SWISS-MODEL Repository. The sequence identity between DrEndoIII and EcEndoIII is 47% and the root-mean-square (rms) deviation between the models was 0.061 Å for 201 aligned atoms. The PDB file generated by homology modeling was further used for visualization of the electrostatic surface potential of DrEndoIII, by using the APBS plugin in PyMOL.[8]
Figure SI 1. Electrostatic surface potential of DrEndoIII. The surface potential (-5 to + 5 kT/e; negative, red, positive, blue) is calculated from the homology model and represented for the DNA binding side (a), and upon 180° rotation (b) of the DrEndoIII molecule; the depicted DNA segment is modelled onto the DrEndoIII structure by superimposing the protein-DNA complex of EndoIII from Bacillus stearothermophilus (PDB: 1ORN); the position of the [4Fe-4S] cluster is shown in the zoom in of the domain A of the helical structure of the homology model of DrEndoIII.

Figure SI 2. CVs of DrEndoIII immobilized on MUA coated disk Ag electrode (v\text{scan} 50 mV/s). Left panel, prior to SERR experiment; right panel, after reduction with sodium dithionite and subsequent SERR measurement.
Figure SI 3

Figure SI 3. CV of DrEndoIII immobilized on DNA-coated Au electrode (ν\text{scan} 50 mV/s) (solid trace) and a control DNA-coated electrode (dotted trace).

Figure SI 4

Figure SI 4. CVs of DrEndoIII immobilized on Au electrode coated with Ni\textsuperscript{2+}-NTA-terminated SAMs (ν\text{scan} 50 mV/s).
Figure SI 5. SEIRA spectra of DrEndoIII immobilized on a Ni$^{2+}$-NTA SAM. Left panel: Spectra before (a) and after (b) DNA binding. Right panel: high frequency region of the SEIRA spectra before (a’) and after (b’) DNA-binding.