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Reversible assembly of protein-DNA nanostructures triggered by mediated electron transfer

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ABSTRACT

Stable protein-DNA nanostructures have been assembled by reconstitution of the multi-ligand binding flavoprotein dodecin on top of flavin-terminated dsDNA monolayers on gold electrodes. These structures could be disassembled by electrochemical flavin reduction *via* mediated electron transfer. For this purpose a negative potential was applied at the Au working electrode in the presence of the redox mediator bis-(ammoniumethyl)-4,4′-bipyridinium tetrabromide. The stepwise formation of the flavin-terminated dsDNA monolayers as well as the binding and electrochemically triggered release of apododecin were monitored by surface plasmon resonance (SPR) and quartz crystal microbalance (QCM) measurements. The assembly and disassembly of the protein-DNA nanostructures were fully reversible processes, which could be carried out multiple times at the same flavin-dsDNA modified surface. When a negative potential was applied in the absence of a redox mediator apododecin could not be released, *i.e.* direct electron transfer was not possible. As alternative redox mediators also methylene blue and phenosafranine were studied, but in the presence of these molecules apododecin was released without applying a potential, probably because the tricyclic aromatic compounds are able to replace the flavins at the binding sites.

1. Introduction

Dodecin from *Halobacterium salinarum* is a dodecameric flavoprotein that comprises six flavin binding pockets with octahedral arrangement [1–9]. As in each binding pocket one or two flavins can be incorporated, dodecin is able to bind up to twelve flavins in total. While dodecin binds not only native but also artificial flavins with high affinity when they are oxidized, flavin reduction induces the dissociation of the holoprotein complex into apododecin and free flavin ligands [3,5,7–9]. This observation can be explained by the structural changes going along with the twofold reduction of a flavin. While an oxidized flavin (a flavoquinone) comprises a single planar aromatic system, a doubly reduced flavin (a flavohydroquinone) is folded by about 20 ° out of plane along the N_5-N_{10} axis [10,11]. As a result, a flavohydroquinone does not fit into the (apo)dodecin binding pocket anymore, and consequently an apododecin-flavin complex dissociates after flavin reduction. For the reconstitution of dodecin on gold surfaces flavin terminated double-stranded DNA (dsDNA) monolayers have been used [3,7–9]. These monolayers were formed by chemisorption of 5′-thiolated single-stranded DNA (ssDNA) comprising 20 bases with subsequent incubation of a mixture of short thiols (mercaptobutanol and mercaptopropionic acid) followed by hybridization with 5′-flavin-modified complementary ssDNA. This multistep protocol involving DNA hybridization at the surface (rather than direct adsorption of flavin- and thiol-modified dsDNA) was chosen to prevent unspecific adsorption of the flavin to the bare gold surface [12]. When dodecin was reconstituted on top of flavin-terminated DNA monolayers it turned out that one apododecin molecule is able to bind two or more surface-grafted flavins [8,9]. By kinetic quartz crystal microbalance (QCM) measurements the binding affinity as well as the binding and unbinding rate constants k_{on} and k_{off} of a single apododecin-flavin complex could be determined [8,9]. These studies revealed that the half-life of a single apododecin-flavin bond is in the range of a few minutes. Nevertheless long-term stable surface modification using dodecin is possible *via* multi-ligand binding. By incubation of bidentate flavin-DNA ligands on top of dodecin-DNA monolayers stable sandwich-like DNA-dodecin-DNA layers could be generated, which could then be disintegrated by chemical flavin reduction before the layers were reprogrammed by reassembly of the dodecin layer followed by binding of a different kind of bidentate flavin-DNA ligand [8]. Hence, dodecin can be used as a key element

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for the formation of redox-switchable[3,7–9,13,14] or, more in general, for stimuli-responsive surfaces [15–24].

However, up to now it was only possible to disassemble dodecin-DNA nanostructures formed on surfaces by chemical but not by electrochemical flavin reduction. Previous investigations (using electrochemistry together with surface plasmon resonance (SPR) measurements) revealed that direct electron transfer (DET) between dodecin and the electrode surface through the DNA linkers used for the reconstitution of dodecin on gold surfaces is not possible [3,7]. Therefore the goal of the current work was to assess the possibility to address dodecin electrochemically by mediated electron transfer (MET) as outlined in Scheme 1.

2. Experimental

2.1. Instruments

For QCM-D measurements a Q-Sense E1 including the QEM 401 electrochemistry module was used in connection with the QSX-301 quartz resonators coated with a chromium adhesion and a gold top layer (Biolin Scientific AB, Stockholm, Sweden). For successful binding and release measurements, brand sparkling new QCM-D crystals were used. For ozone treatment of the QCM-D sensors an ozone chamber (BioForce, Ames, IA, USA) was employed. SPR measurements were performed with a Res-Tec SPR setup (Resonant Technologies GmbH, Framersheim, Germany) using homemade template stripped gold (TSG) chips in a homemade SPR-electrochemistry flow cell. These chips were fabricated as reported previously [8,25]. A 48 nm thick layer of gold was evaporated on "prime" quality silicon wafers which were used as a template. Glass slides were glued on the gold layer by using a two-component adhesive. After drying of the glue, the glass slide was peeled off and used for SPR measurements without further cleaning procedures. Combined QCM-electrochemistry was performed with a Zahner Zennium workstation whereas for combined SPR-electrochemistry a Zahner IM6 workstation was used (Zahner Elektrik GmbH, Kronach, Germany).

2.2. Chemicals

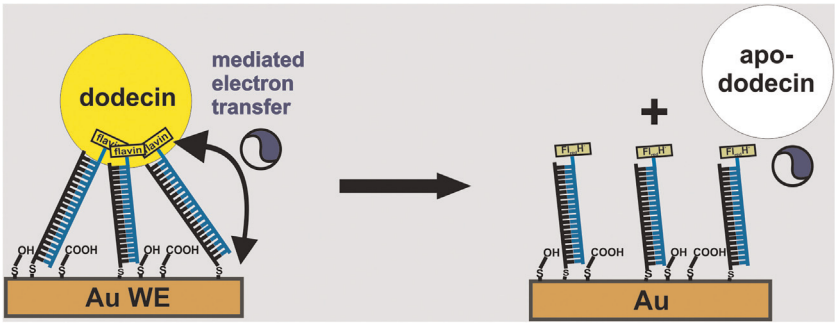
Mercaptobutanol (MCB) was purchased from Sigma-Aldrich, whereas mercaptopropionic acid (MPA) was ordered from AlfaAesar (Karlsruhe, Germany). Sodium dithionite was from Merck KGaA (Darmstadt, Germany). Buffer solutions as well as basic piranha were prepared using reagents from Carl Roth GmbH (Karlsruhe, Germany) and purified water with a resistivity of 18.2 MΩ·cm at 25 °C (Millipore, Merck KGaA, Darmstadt, Germany). Methylene blue and phenosafranine were purchased from Carl Roth GmbH (Karlsruhe, Germany). All oligonucleotides were purchased from Eurogentec S.A. (Seraing, Belgium) or Metabion GmbH (Martinsried, Germany). The capture probe ssDNA comprised 20 bases (5′-AAC-TAC-TGG-GCC-ATC-GTG-AC-3′) and three dithiane groups (6750.6 g·mol^{-1}) at the 5′-end, and complementary ssDNA of 20 bases (5′-GTC-ACG-ATG-GCC-CAG-TAG-TT-3′) modified with a flavin (CofC4) at the 5′-end was used for hybridization (6672.4 g·mol^{-1}). Bis-(ammoniumethyl)-4,4′-bipyridinium tetrabromide, termed aminoethyl viologen (AEV) was synthesized following a modified procedure of Dzaraeva et al. [26]: 5 g of 4,4′-bipyridyl (32 mmol) and 26.22 g of bromoethylamine hydrobromide (128 mmol) were dissolved in 50 mL acetonitrile and stirred for 3 h at 40 °C. Next, the mixture was cooled to room temperature and stirred for additional two days. The precipitate was filtered and washed with cold methanol. The resulting yellow solid was dissolved in 5 mL of water and precipitated as a yellow solid after dropwise addition to 1 L of methanol. The product was filtered and dried under vacuum. Yield: 5.9 g (10.4 mmol; 32.6%). ^{1}H NMR (400 MHz, D_2O): δ (ppm) = 9.17 (m, 4H, Ar-H); 8.60 (m, 4H, Ar-H); 5.05 (t, 4H, $-CH_2$-, J = 6.9 Hz), 3.73 (t, 4H, -CH_2-, J = 6.9 Hz). The redox potential of the viologen was determined by cyclic voltammetry yielding a value of -305 mV vs. NHE (Fig. 1).

2.3. Preparation of dodecin

The dodecameric flavin binding apododecin variant (DtE) from *Halobacterium salinarum* as well as a non-binding variant (in the following referred to as W36A) were expressed and purified as reported previously [1,9,27]. Both protein samples were stored at 4 °C in 20 mM Tris buffer, pH 7.5, containing 1 M NaCl and 5 mM $MgCl_2$ (buffer A). Prior to use, the dodecin solutions were filtered using 0.22 μm syringe filters equipped with polyamide membranes. Subsequently, the protein solution was diluted to a final concentration of 5 μM by adding the corresponding volume of 20 mM phosphate buffer also containing 1 M NaCl and 5 mM $MgCl_2$ (buffer B). The remaining amount of buffer A was about 20%. This phosphate buffer (buffer B) was also used for combined SPR-electrochemistry as well as for QCM-electrochemistry measurements.

2.4. Surface modification

Before each measurement, the quartz crystal resonators were placed in the ozone cleaner for 10 minutes followed by immersion in hot basic piranha ($H_2O/H_2O_2/NH_4OH$ in a ratio of 5/1/1) for 5 minutes at 70 °C (**CAUTION: this solution is highly corrosive and should be handled with special care**). Subsequently, the sensor chip was dried in a nitrogen stream and placed in the ozone cleaner for 10 minutes again. Crystals cleaned by this procedure were subsequently placed in the QCM-D flow cell and left for



Scheme 1. Disassembly of protein-DNA nanostructures by MET A stable dodecin layer is formed by multi-ligand binding of apododecin on top of a flavin-modified dsDNA monolayer. When a negative potential is applied in the presence of a proper redox mediator, this results in the reduction of the flavins followed by the release of apododecin.