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A surface plasmon resonance study of the intermolecular interaction between *Escherichia coli* topoisomerase I and pBAD/Thio supercoiled plasmid DNA

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ABSTRACT

To date, the bacterial DNA topoisomerases are one of the major target biomolecules for the discovery of new antibacterial drugs. DNA topoisomerase regulates the topological state of DNA, which is very important for replication, transcription and recombination. The relaxation of negatively supercoiled DNA is catalyzed by bacterial DNA topoisomerase I (topoI) and this reaction requires Mg^{2+}. In this report, we first quantitatively studied the intermolecular interactions between *Escherichia coli* topoisomerase I (EctopoI) and pBAD/Thio supercoiled plasmid DNA using surface plasmon resonance (SPR) technique. The equilibrium dissociation constant (K_d) for EctopoI–pBAD/Thio interactions was determined to be about 8 nM. We then studied the effect of Mg^{2+} on the catalysis of EctopoI–pBAD/Thio reaction. A slightly higher equilibrium dissociation constant (~15 nM) was obtained for Mg^{2+} coordinated EctopoI (Mg^{2+}EctopoI)–pBAD/Thio interactions. In addition, we observed a larger dissociation rate constant (k_d) for Mg^{2+}EctopoI–pBAD/Thio interactions (~0.043 s^{-1}), compared to EctopoI–pBAD/Thio interactions (~0.017 s^{-1}). These results suggest that enzyme turnover during plasmid DNA relaxation is enhanced due to the presence of Mg^{2+} and furthers the understanding of importance of the Mg^{2+} ion for bacterial topoisomerase I catalytic activity.

1. Introduction

The drug resistance of bacterial pathogens to available antibacterial drugs is a serious public health issue and needs to be addressed. The bacterial topoisomerase I, a DNA topoisomerase I (topoI), is a novel target biomolecule for the discovery of new antibacterial drugs [1,2]. DNA topoisomerases play important roles on both the supercoiling control of DNA and the resolution of topological barriers during replication, transcription, and recombination [3–5]. The supercoiling tension caused by translocation of RNA polymerase must be relieved by topoisomerases [6,7]. Topoisomerase I cleaves and rejoins a single DNA strand during topoI-DNA reactions [8], which establishes a transient covalent linkage between these two macromolecules. These complexes can be trapped using topoisomerase inhibitors [2,8].

Topoisomerase I can catalyze interconversion of various topological isomers [9] and type IA topoisomerase catalytic activity requires Mg^{2+} [10]. In *Escherichia coli*, EctopoI removes excess negative supercoils in order to regulate DNA supercoiling [11]. EctopoI is a single polypeptide of 865 amino acids and tyrosine 319 is the active site tyrosine in EctopoI that forms a transient covalent linkage to DNA 5′ phosphoryl group during EctopoI-DNA reaction [12]. We quantitatively studied the interactions for EctopoI–pBAD/Thio supercoiled plasmid DNA (hereafter termed as pBAD/Thio) and Mg^{2+} bound EctopoI (Mg^{2+}EctopoI)–pBAD/Thio using the surface plasmon resonance (SPR) technique.

SPR is a widely used label free technique to determine equilibrium dissociation constants and the kinetics of bio-molecular interactions [13]. The sensor surface modification for the SPR assay was confirmed by using electrochemical impedance spectroscopy (EIS). The equilibrium dissociation constant (K_d) for EctopoI binding to pBAD/Thio was determined to be about 8 nM. A slightly higher K_d (~15 nM) value was obtained for Mg^{2+}EctopoI–pBAD/Thio interactions. In addition, the dissociation rate constants (k_d) for the interactions between the enzymes (EctopoI or Mg^{2+}EctopoI)

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and pBAD/Thio were also derived and a larger k_d was obtained for Mg^{2+}EctopoI–pBAD/Thio interactions. These results can help us further understand the important role of Mg^{2+} in the interactions between EctopoI and DNA substrate during catalysis [14].

Mycobacterial tuberculosis topoisomerase I (MttopoI), has a different C-terminal DNA binding domain (CTD) that lacks the three Zn^{2+} binding motifs in the CTD of EctopoI [15]. Binding to pBAD/Thio plasmid DNA by the two enzymes were compared. Under our experimental conditions, weak SPR signals were observed for interactions between MttopoI and pBAD/Thio. Therefore, the K_d value could not be recovered for MttopI–pBAD/Thio interactions.

2. Materials and methods

2.1. Materials

Triethylene glycol mono-11-mercaptoundecyl ether (PEG-thiol), nickel (II) sulfate hexahydrate, sodium, chloride, potassium hexacyanoferrate (III) and ethanolamine HCl were purchased from Sigma–Aldrich, ethanol (200 proof) from Decon Laboratories LLC, 2-{2-[2-(1-mercaptoundec-11-yloxy)-ethoxy]-ethoxy)-ethoxy nitrilotriacetic acid (NTA-thiol) from ProChimia Surfaces, Poland and potassium ferrocyanide trihydrate from Acros Organics. N-hydroxysuccinimide (NHS), N-(3-dimethylaminopropyl)-N′-ethyl-carbodiimide hydrochloride (EDC) and GeneJET Plasmid Maxiprep Kit were received from Thermo-Scientific. All other reagents were purchased from VWR international, Randor, PA, USA. Solutions were prepared using deionized (DI) water (~18 MΩ) (Ultra Purelab system, ELGA/Siemens or Milli-Q Direct 8 water system). The polycrystalline gold chips (50 nm Au over 2.5 nm titanium adhesion layers, coated on 18 mm × 18 mm cover slip glass slides) were purchased from Platypus Technologies, LLC, Madison, WI and each chip was cut into two halves before further processing.

2.2. Methods

2.2.1. Isolation and purification of EctopoI, MttopoI and pBAD/Thio

Recombinant EctopoI and MttopoI were expressed and purified as N-terminal His-tagged proteins according to previously reported procedures [16,17]. For the SPR experiments, the EctopoI was dialyzed against HEPES buffer (10 mM Hepes, pH 7.4 containing 100 mM NaCl and 0.005% (V/V) tween 20 in DI water) overnight at 4 °C. The MttopoI was dialyzed against HEPES buffer with 2.5% (V/V) glycerol overnight at 4 °C. The glycerol was needed to maintain solubility of MttopoI. The pBAD/Thio supercoiled plasmid DNA was purified using Genejet maxiprep kit (Thermo Scientific) according to the manufacturer's protocol.

2.2.2. Sensor preparation and characterization

Gold chips were used to prepare SPR sensors. After rinsing in ethanol for 2–3 min, the chips were cleaned by oxygen plasma (Plasma Cleaner PDC-001, Harric Plasma, Ithaca, NY) for 40 s at an oxygen pressure of 500–600 mTorr and RF power of 10.2 W. The chips were then annealed with hydrogen flame for 20 s to reduce the surface roughness [18]. To form a self-assembled monolayer (SAM), the hydrogen flamed chip was immediately immersed in mixed thiol solution (1:9 V/V mixture of 1 mM NTA-thiol and 1 mM PEG-thiol in ethanol respectively) and incubated overnight. The chip was then copiously rinsed with ethanol and DI water to remove physically adsorbed thiol molecules and dried with argon. The formation of SAM on the cleaned gold surface was confirmed by electrochemical impedance spectroscopy (EIS). A detailed explanation of EIS experiments can be found in Supplementary information (Section S1). Fig. 1A shows the scheme of SAM formation and His-tagged EctopoI or MttopoI immobilization. The modified chip was then mounted inside SPR flow cell. The detail of a similar SPR system can be found in a previous report [19].

2.2.3. Enzyme immobilization and DNA binding

The sensor surface was activated using a 40 mM nickel (II) sulfate solution prepared in DI water for 2 min at a flow rate of 50 μL/min followed by DI water flushing for 2 min. The surface was then equilibrated with the HEPES buffer for 5–10 min. His-tagged EctopoI (2 μM) or MttopoI (2.5 μM) was immobilized on the activated SAM surface at a flow rate of 50 μL/min via the capture covalent method [20]. Sequential treatment of the EctopoI or MttopoI immobilized surface was accomplished with a pulsed injection of NHS-EDC solution (25 mM NHS and 100 mM EDC) in DI water followed by 1 M ethanolamine (pH 8.2) in DI water. This treatment allowed us to achieve the capture covalent immobilization. The experiments with various pBAD/Thio concentrations were accomplished by regeneration of the sensor surface using 1 M NaCl. For the experiments involving Mg^{2+}, 0.5 mM $MgCl_2$ (in Tris buffer, pH 8.0) was passed over EctopoI immobilized surface for 5 min. All the experiments were carried out at 22 °C.

2.2.4. Data analysis

Complex non-linear least square (CNLS) fitting algorithm was used for EIS data using an equivalent circuit model (Supplementary information, Section S1). Equilibrium data analysis methods were used to analyze the SPR data [21]. The simple nonlinear hyperbolic (SNLH) equation (Eq. (1) below) was used to fit the equilibrium SPR response plotted vs DNA concentrations,

$$R = \frac{R_{max}[DNA]}{K_d + [DNA]} \quad (1)$$

where, R is the equilibrium response, [DNA] is the analyte (pBAD/Thio) concentration, K_d is the equilibrium dissociation constant, and R_{max} is the fitting parameter representing the response at very high pBAD/Thio concentration. The dissociation rate constants were

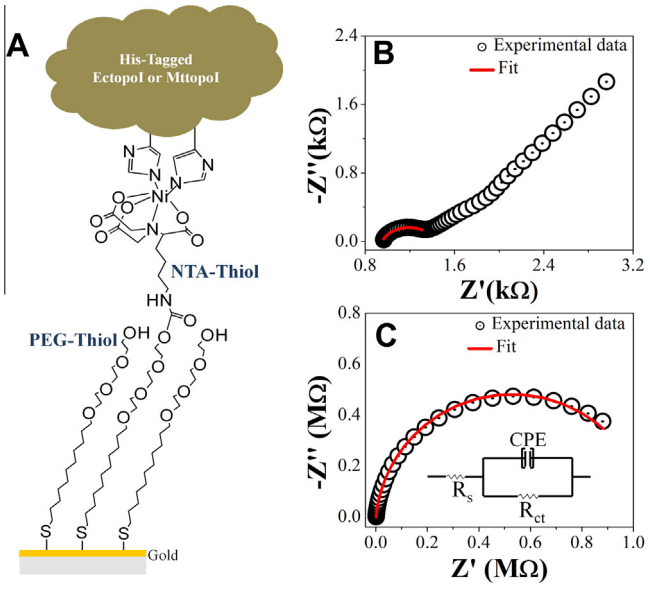

Fig. 1. (A) Scheme showing the sensor surface modification with mixed thiols followed by the His-tagged EctopoI or MttopoI immobilization. (B) electrochemical impedance spectroscopy (EIS) for the bare gold. (C) EIS for SAM modified gold surface, inset: equivalent circuit for EIS data fitting and analysis. CPE is the constant phase element, R_s is the solution resistance and R_{ct} is the charge transfer resistance. A frequency range from 10^{-1} Hz to 10^4 Hz was used during EIS measurements. In both figures B and C, the symbols are experimental data and continuous lines are the CNLS fit (see Supplementary information, Section S1, for details).