A capture approach for supercoiled plasmid DNA using a triplex-forming oligonucleotide

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Received February 1, 2013; Revised March 7, 2013; Accepted March 15, 2013

ABSTRACT

Proteins that recognize and bind specific sites in DNA are essential for regulation of numerous biological functions. Such proteins often require a negative supercoiled DNA topology to function correctly. In current research, short linear DNA is often used to study DNA–protein interactions. Although linear DNA can easily be modified, for capture on a surface, its relaxed topology does not accurately resemble the natural situation in which DNA is generally negatively supercoiled. Moreover, specific binding sequences are flanked by large stretches of non-target sequence in vivo. Here, we present a straightforward method for capturing negatively supercoiled plasmid DNA on a streptavidin surface. It relies on the formation of a temporary parallel triplex, using a triple helix forming oligonucleotide containing locked nucleic acid nucleotides. All materials required for this method are commercially available. Lac repressor binding to its operator was used as model system. Although the dissociation constants for both the linear and plasmid-based operator are in the range of 4 nM, the association and dissociation rates of Lac repressor binding to the plasmid-based operator are ~18 times slower than on a linear fragment. This difference underscores the importance of using a physiologically relevant DNA topology for studying DNA–protein interactions.

INTRODUCTION

Proteins that recognize and bind specific sites in DNA are essential for controlling a wide range of biological functions at the level of DNA replication (1,2), regulation of gene expression (3), homologous recombination (4) and various other processes. In turn, proteins involved in such processes often require a negative supercoiled (nSC) DNA topology to function correctly (5). Recently, it was also shown that an nSC DNA topology is also required for the specific DNA binding of Cascade, a protein complex involved in the prokaryotic CRISPR-Cas immune system (6). Given the importance of DNA topology, it is not surprising that a lot of effort is made to maintain a correct DNA topology in vivo (7,8).

Proteins that bind specific sites in DNA also face the challenge of finding their specific binding site amongst megabases of non-target DNA. A combination of 1D diffusion (sliding) along the DNA and 3D diffusion (hopping) in the cytoplasm (9) can lead to more rapid targeting, according to the facilitated diffusion model (10–12). Intersegmental transfer can also play a role; however, this is only relevant for proteins containing two distinct DNA binding sites such as for instance the Lac repressor and Cre recombinase (13,14).

DNA–protein interactions can be studied using a variety of techniques, amongst others single-molecule techniques such as total internal reflection fluorescent microscopy (15) and surface plasmon resonance (SPR) (16). Especially in SPR experiments, short linear target DNA is often used to study the kinetics of DNA–protein interactions. Short linear target DNA is convenient for SPR analysis because the 3′ or 5′ ends are easily biotinylated, which allows for stable capturing on a streptavidin surface. However, such linear targets do not accurately mimic the natural situation, in which an nSC DNA topology prevails and where non-target DNA is much more abundant than specific binding sites. This might give rise to distortions in the data.

In that respect, attaching nSC plasmid DNA, containing a specific binding site, would be more appropriate to use in SPR experiments. The lack of 3′ or 5′ ends, however, makes it not straightforward to attach plasmid DNA to a surface. In the present study, we aimed to create an irreversible topological link between an nSC plasmid...
and a biotinylated triplex-forming oligonucleotide (TFOs), which results in a padlock-modified plasmid, or catenane, that can be captured on a surface. The production of padlock-modified plasmids has previously been described for sequence specific labelling double-stranded DNA (17–19), to form such a complex with a good yield, a stable triple helix must be formed.

DNA triplex formation requires stretches of homopurines (A, G) in one strand and homopyrimidines (C, T) on the opposite strand of the double-stranded target DNA (20). Generally, two classes of triplexes can be distinguished, according to the orientation and composition of the third strand: pyrimidine-rich third strands bind parallel to the purine strand of the duplex and form T·AT and C⁺·GC triplets; alternatively, purine-rich third strands bind antiparallel to the purine strand of the duplex and form G·GC, A·AT and T·AT triplets (in A·BC, BC indicates the natural base pair and A the third strand) (21). Parallel triplexes only form at low pH because protonation of the third strand cytosine (C⁺) is required, whereas formation of anti-parallel triplexes is pH independent. Previous studies involving padlock-modified plasmids have mostly relied on the formation of very stable antiparallel triplexes, formed in the presence of a DNA intercalator that is not commercially available (19). However, incorporation of locked nucleic acids (LNA’s) in the pyrimidine third strand of parallel triplexes improves triplex stability and can alleviate the requirement for a low pH to some extent (22).

Here, we present a facile method for capturing of plasmid DNA on a streptavidin surface. A DNA triplex is formed by adding an LNA-modified pyrimidine-rich biotinylated TFO, which is subsequently self-ligated to create a padlock-modified plasmid, or catenane. The Lac repressor has been mutated to exist as a dimer (not a tetramer) that interacts with only one DNA binding site (operator). Plasmids with and without specific Lac repressor operator sequences are used as a model to demonstrate the relevance of this approach in SPR experiments. We observed different binding kinetics to the supercoiled plasmid-based operator compared with a short linear operator. This approach therefore represents a helpful tool to study protein–DNA interactions using a DNA substrate with a physiologically relevant topology.

**MATERIALS AND METHODS**

**Oligonucleotides**

All oligonucleotides used in this study, except for TFO2.0, were obtained from Sigma and ordered without any special requirements. TFO2.0 was ordered from Eurogentec, and was purified by polyacrylamide gel electrophoresis the manufacturer. Details on all oligonucleotides and their sequences are given in Table 1.

**Cloning of Lac repressor coding sequence**

The first 331 residues of the coding sequence of Lac repressor, hence excluding the C-terminal tetramerization domain (residues 340–357), were amplified by polymerase chain reaction (PCR) from a pCDF-1b plasmid.
The coding sequence was amplified in 3 × 100 µl PCR, containing 15 ng of template DNA, 0.2 mM of each deoxyribonucleotide (dNTP), 0.2 mM of primers BG3874 and BG3917 (containing a HRV3C site and an 8× His-tag), 1× Buffer high fidelity (HF) and 3 Units Phusion II (Finnzymes). The PCR program was as follows: 30 s at 98°C, 5 cycles of 10 s at 98°C, 20 s at 60°C, 60 s at 72°C, 25 cycles of 10 s at 98°C, 20 s at 70°C, 60 s at 72°C, followed by 5 min at 72°C after the last cycle. Purified PCR product and destination vector, pWUR533, were subsequently digested with NcoI and SacI restriction enzymes. Both fragments were ligated together, and the resulting plasmid (pWUR533_LacI) was transformed to the Escherichia coli strain XL1-blue for plasmid propagation.

Expression of recombinant Lac repressor

The pWUR533_LacI plasmid was transformed to E. coli BL21(DE3) pSJS1244 for protein expression. Fresh LB medium, containing ampicillin (final concentration: 100 µg/ml) and spectinomycin (final concentration: 50 µg/ml), was inoculated with 1% overnight culture. Expression of recombinant Lac repressor was induced 3 h later by adding isopropyl-β-D-thiogalactopyranoside to a final concentration of 1 mM. After another 3 h, cells were harvested by centrifugation. Cell pellets were either processed immediately or stored at −20°C until further processing.

Purification of recombinant Lac repressor

Cell pellets were resuspended in 150 mM NaCl and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.4) and subsequently lysed using a French press. Clarified cell free extract was passed through a 0.45 µm filter before incubation with His-Select® Nickel affinity gel (Sigma-Aldrich), which was equilibrated with water (3 ml) and 3 column volumes wash buffer [50 mM sodium phosphate buffer (pH 8.0) with 0.3 M sodium chloride and 5 mM imidazole]. After incubation, the column material was washed three times with four column volumes wash buffer. Bound protein was eluted in fractions of 2 column volumes using elution buffer [50 mM sodium phosphate buffer (pH 8.0) with 0.3 M sodium chloride and 250 mM imidazole]. Eluted protein was further purified on a Superdex 200 column (GE Healthcare), using 150 mM NaCl and 10 mM HEPES (pH 7.4) as running buffer; peak fractions corresponding to the dimer protein were collected and used for SPR and microscale thermophoresis (MST) measurements.

Insertion of a Triple Helix Site in pUC19

A purine-rich Triple Helix Site (THS, 19 bp), required for triple helix formation, was inserted between the ampicillin resistance gene and the origin of replication in a pUC19 vector. To do so, 25 ng plasmid was amplified in 50 µl using 0.2 mM dNTPs each, 125 ng of primers BG3534 and BG3535, 1× Buffer HF and 1 Unit Phusion II (Finnzymes). The PCR program was as follows: 90 s at 98°C, 18 cycles of 10 s at 98°C, 30 s at 58°C, 80 s at 72°C, followed by 7 min at 72°C after the last cycle. After PCR, 10 units of DpnI (Fermentas) was added directly to the PCR reaction mixture, for degradation of template DNA, and left at 37°C for 2 h. The resulting plasmid (pUC19_THS) was transformed to chemical competent E. coli DH5α. Insertion of the THS was confirmed by restriction analysis (using DraI) and sequencing (GATC, Constance, Germany) using primer BG3554.

Removal of lac operator sequences

The two lac operator sequences in pUC19_THS, operator 1 (AATTGTGAGCGGATAACAATT) and operator 3 (GGCAGTGAGCGGATAACAATT), were removed to yield two plasmids. The pPAD-ΔO3 plasmid only contains operator 1, whereas pPAD-ΔO1ΔO3 contains no operator at all. The operator sequences were removed by PCR amplification of the whole plasmid, except for the operator region, using primers BG3963 and BG3964 for ΔO3, and BG3962 and BG3963 for ΔO1ΔO3. Plasmid, 35 ng, was amplified in 100 µl using 0.2 mM dNTPs each, 0.2 mM of each primer, 1× Buffer HF and 1 Unit Phusion II (Finnzymes). The PCR program was as follows: 60 s at 98°C, 5 cycles of 10 s at 98°C, 10 s at 60°C, 1 min at 72°C, followed by 25 cycles of 10 s at 98°C, 10 s at 70°C, 1 min at 72°C followed by 7 min at 72°C after the last cycle. Purified PCR product was digested with 10 units of DpnI and NcoI and left at 37°C for 2 h. Fragments were ligated and transformed to electro competent E. coli XL1-blue cells. Successful removal of the operator sequences was confirmed by sequencing (GATC, Constance, Germany) using the standard M13-F primer.

Padlock formation

For padlock formation, approximately 50 nM plasmid (use of more plasmid should be prevented, as this could result in lower yields because of molecular crowding) was mixed with 1 µM TFO2.0, acid buffer (20 mM MgCl₂ and 20 mM ammonium acetate pH 5) was added to a total volume of 10 µl; low pH is needed to protonate cytosine on the third strand (18,21), which is required in this approach. Plasmid and TFO were heated to 80°C and cooled down to 20°C at a rate of −1°C/min in a G-Storm GS1 thermocycler (start at 80°C for 30 s, then step-wise decrease to 20°C, in 350 subsequent steps in which the temperature drops with 0.17°C and stays stable for 8 s). When at 20°C, 2 µM closing probe (BG3812) was added, followed by 1.5 µl 10× T4 DNA ligase buffer (Fermentas), 1 µl of a 5 mM adenosine tri-phosphate solution and 5 Units (1 µl) of T4 DNA ligase (Fermentas). The mixture was incubated overnight at room temperature. Padlock-modified plasmids were purified from a 0.8% agarose gel (Fermentas Kit) to remove excess TFO and closing probe, before capturing them on a Biacore Streptavidin Chip (SA Chip). To yield enough padlock-modified plasmid for capture, padlock formation was routinely performed in three parallel reactions, only to be pooled during the gel purification procedure.
**SPR measurements**

Experiments were performed in a Biacore 3000 system (BIACORE, Uppsala, Sweden) at a constant temperature of 25°C, using 10 mM HEPES at pH 7.4, and 150 mM NaCl as running buffer. Padlock-modified plasmids were captured on an SA chip at a flow of 5 µl/min, pPAD-AO3 to a response of 140 RU and pPAD-AO1ΔO3 to a response of 80 RU. An empty channel served as reference surface. For the padlock-modified plasmids, kinetic measurements were performed by injecting 20 µl Lac repressor at a flow of 10 µl/min, using the kinject command, followed by a 5-min dissociation phase. In total, seven concentrations (2650, 883, 294, 98, 33, 11 and 4 nM) were injected twice.

Linear Operator 1 (O1) DNA, prepared by hybridizing BG4162 and BG4163, was captured on another SA chip to 334 RU. Kinetic measurements were performed by injecting 90 µl Lac repressor at a flow of 90 µl/min, using the kinject command, followed by a 5-min dissociation phase. An empty channel served as reference surface. In total, six concentrations (577, 192, 64, 21, 7 and 2 nM) were injected twice. Data were processed using Scrubber (BioLogic Software, Campbell, Australia), and double referenced data were analysed using BIAevaluation software provided with the Biacore.

**MST**

Plasmids for MST measurements were purified from 100 ml of overnight cultures, using a Jetstar 2.0 maxiprep kit (Genomed). The obtained pellet was re-suspended in 50 µl milli-Q water; DNA concentrations were calculated from gel, by comparing peak intensities of linearized plasmid and the 3000 bp band of a marker (1 kb, Fermentas). Purified Lac repressor was labelled using a protein labelling kit, L003 Monolith™ (NanoTemper. München, Germany). MST measurements were performed in standard capillaries on a Monolith NT.115 machine, using 5% LASER power and 40% light-emitting diode (LED) power. DNA concentrations were varied while keeping the protein concentration constant at 25 nM. Protein was diluted to 50 nM in buffer (20 mM HEPES at pH 7.4, 300 mM NaCl) and subsequently mixed in a 1:1 ratio with dilutions of DNA (in milli-Q). Data were analysed using the software provided with the Monolith NT.115 (NanoTemper. München, Germany).

**RESULTS**

**Strategy and design**

In the present study, we aimed to develop a method for capturing plasmid DNA, which would allow the use of DNA with a physiologically relevant topology in SPR experiments, and in other experiments that require target immobilisation. To allow the approach to be generally applicable, it should be straightforward and easily achievable with general molecular biological techniques and commercially available reagents. A general outline of the method is shown in Figure 1A. A newly designed target site for triple helix formation was inserted in our plasmid of interest. We have adopted a parallel triplex design (Figure 1B) for two main reasons. First, it does not require stabilizing molecules that are not commercially available (19); for stabilisation of the triplex, and to alleviate the requirement for a very low pH for triplex formation, the triplex-forming part of the TFO contains alternating LNA Thymine and DNA Cytosine (22). Second, and inherently related to a parallel triplex, artefacts arising from the presence of a triplex are possibly avoided, at least at neutral pH, because parallel triplexes only form at low pH and are generally expected to dissociate at neutral pH. After addition of the closing probe, the TFO is circularized, and a topological bond is introduced between the circular TFO and the plasmid. At this stage, the triple helical structure could be disrupted, as it is not required for plasmid capture anymore. In additional experiments, padlock-modified plasmids were digested with Dral. The plasmids contain two Dral sites, one of which overlaps with the THS (Figure 1B). One of these sites is not accessible if a triplex is formed, and hence the plasmid will only be linearized. The results suggest that in 40–50% of the analysed padlock-modified plasmids, the triplex is not fully dissociated at neutral pH (Supplementary Methods and Figure 2), most likely due to the length of the triplex and because the TFO is heavily modified. The THS is located between the ampicillin resistance gene and the origin of replication; hence, is it located at great distance of the operator sequence, and therefore we expect the triplex not to intervene with Lac repressor binding. However, the triplex could be shortened, should the presence of the triplex intervene with the study of other binding effects.

**Binding affinity of Lac repressor**

After padlock-modified plasmids were captured on a SA SPR chip, we performed subsequent measurements showing that Lac repressor specifically interacts with these immobilized plasmids. This confirms that the production and capture of padlock-modified plasmids was successful. Double referenced SPR data of Lac repressor binding to both padlock-modified plasmids was fitted with a simple 1:1 binding model (Figure 2A), yielding an overall dissociation constant ($K_D$) of 337 nM for pPAD-AO1ΔO3 (not containing an operator) and 155 nM for pPAD-AO3 (containing operator 1) (Figure 2B). Fits shown in Figure 2A nicely follow the association and equilibrium phase, fits shown in Figure 2B are not matching the data as nicely, although this is to be expected (discussed later in the text). In addition, MST measurements, using the same plasmids, yield dissociation constants in the same range as SPR measurements; 147 nM for pPAD-AO1ΔO3, and 188 nM for pPAD-AO3 (Table 2, Supplementary information).

Double referenced data of Lac repressor binding to linear O1 DNA was fitted with a 1:1 model that takes mass transport limitation into account (23) and resulted in a dissociation constant of 3.9 nM (Figure 2C), whereas a dissociation constant of 4.5 nM was obtained using MST (Table 2, Supplementary information). The association
rate constant \( (k_a) \) as well as the dissociation constant \( (K_D) \) are well in range with values determined earlier (24) (Table 2). Despite the high flow rate used during SPR measurements, mass transport effects are to be expected because of the high association rate of Lac repressor (9); together with the somewhat high ligand density, this feature could account for the deviation between fit and actual data. Affinity data, together with all association rate constants \( (k_a) \) and dissociation rate constants \( (k_d) \), are presented in Table 2. These values show that different methods yield similar affinities and that there are differences in the affinity of Lac repressor binding to both padlock-modified plasmids; this is to be expected because one of these plasmids contains a specific binding site.

Heterogeneous binding kinetics of Lac repressor

Kinetic parameters for the interaction of Lac repressor with the plasmid-based operator were derived by fitting the binding curves of pPAD-ΔO3 with a model that assumes a heterogeneous ligand (plasmid), and that the analyte (A, Lac repressor) can bind independently to two ligand sites, specifically to the operator (C) and non-specifically to the rest of the plasmid (B) (Figure 1C):

\[
A + B \quad \stackrel{k_{a1}}{\rightleftharpoons} \quad AB \quad \stackrel{k_{d1}}{\rightarrow} \quad A + B
\]

The kinetic parameters for the non-target interaction \( (k_{a1} = 1.55 \times 10^5/M/s \text{ and } k_{d1} = 0.052/s) \) were derived from the interaction of Lac repressor with the pPAD-ΔO1ΔO3 plasmid and are considered to be similar for both plasmids. Hence, these values were taken into account during fitting of the data, yielding a dissociation constant of 4.0 nM for the interaction of Lac repressor with the plasmid-based operator. Fits according to the heterogeneous model are shown in Figure 2D. This fit is much better than the fit with a 1:1 binding model as shown in Figure 2B. The association and dissociation rate constants for the specific interaction of Lac repressor with the plasmid-based operator \( (k_{a2} = 6.81 \times 10^4/M/s \text{ and } k_{d2} = 2.74 \times 10^{-4}/s) \) are ~18 times slower in comparison with those obtained for the interaction with linear O1 DNA while the dissociation constants are equal (Table 2).

**DISCUSSION**

In this work, we present a straightforward approach to capture plasmid DNA on a streptavidin surface, and we demonstrate its usefulness by characterizing the interaction between Lac repressor and plasmid DNA. Our strategy requires the insertion of a target site for triple
helix formation in the plasmid of interest. Next to a pUC plasmid, this insertion can be performed in many other commercially available vectors, which also contain the targeted region (see Table 1). Further steps are also easy to implement, making our design a generally applicable method for plasmid capture.

Short linear target DNA is often used to study DNA–protein interactions. However, this does not accurately reflect the natural configuration of DNA because DNA topology as well as non-target DNA are important factors contributing to binding affinity. Moreover, DNA topology has a direct influence on the interaction between DNA and many DNA–binding proteins, as exemplified by the supercoiling-dependent DNA binding of Cascade (6). This is also demonstrated by the binding of DnaA to the origin of replication (oriC) on the E. coli genome: this complex is more stable if oriC has an nSC topology (1,2). In addition, it has been shown that promoters can be stimulated or inhibited by increased negative supercoiling (3), most likely related to the binding efficiency of the RNA polymerase complex.

Here, we have selected the E. coli Lac repressor as a model system because this protein and the three operators it can bind are well studied (25–27). It has been shown that supercoiling has an effect on the dissociation of Lac repressor-operator complexes (28,29) and on Lac repressor-mediated DNA looping (30); however, the proteins used in these studies were all naturally occurring tetramers.

Specifically, the interaction of Lac repressor with O1 has been studied in great detail, and remarkably high association rates have been reported based on equilibrium methods, spanning the range between $1 \times 10^8$–$1 \times 10^{10}$/M/s (31,32). It is, however, more relevant to compare our results to those obtained in a previous SPR analysis of this interaction (24), in which the following kinetic parameters were determined, a $k_a$ of $1.8 \times 10^6$/M/s, a $k_d$ of $3.4 \times 10^{-4}$/s and a $K_D$ of 0.2 nM. In the latter study, the dissociation constant was also determined using an electrophoretic mobility shift assay: a $K_D$ of 4.2 nM. This number is well in range with the values we find, using SPR (3.9 nM and 4.0 nM) and MST (4.5 nM). The major difference between both studies is that Bondeson et al. (24) used a wild-type (tetrameric) Lac repressor, which can bind two operators at the same time, whereas we used a mutated (dimeric) Lac repressor that can bind only one.

Table 2. Kinetic parameters for the interaction of Lac repressor with the various DNA targets, including those found by Bondeson et al. (24)

<table>
<thead>
<tr>
<th>Target DNA</th>
<th>SPR</th>
<th>MST</th>
<th>Electrophoretic mobility shift assay</th>
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</thead>
<tbody>
<tr>
<td>Linear O1 DNA</td>
<td>Fit model</td>
<td>$k_a$ ($\times 10^5$/M/s)</td>
<td>$k_d$ ($\times 10^{-4}$/s)</td>
</tr>
<tr>
<td>pPAD-DeltaO1DeltaO3</td>
<td>1:1 Mass transfer</td>
<td>12</td>
<td>0.48</td>
</tr>
<tr>
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<td>1:1</td>
<td>1.55</td>
<td>5.2</td>
</tr>
<tr>
<td>pPAD-DeltaO3</td>
<td>Heterogeneous</td>
<td>1.55</td>
<td>5.2</td>
</tr>
<tr>
<td>Linear O1 DNA (24)</td>
<td>n.a.</td>
<td>18</td>
<td>0.034</td>
</tr>
</tbody>
</table>

Values in bold refer to interactions with operator DNA, and values in Italic indicate those that were fixed during fitting with the heterogeneous model.
operator at the same time. In general, the presence of multiple binding sites is disadvantageous for SPR analysis; it could give rise to avidity effects and substantial rebounding, which results in a higher apparent affinity (33). This explains why our dissociation rate from the linear O1 DNA ($4.8 \times 10^{-5}$/s) is substantially higher, and hence results in a lower affinity (3.9 nM).

The affinities for interaction with the captured plasmids are considerably lower when compared with the linear target DNA, but Lac repressor still binds with nanomolar affinity to the plasmids. Binding curves of both captured plasmids were initially fitted to a 1:1 binding model. A comparison of these 1:1 fits already shows differences between both plasmids; association rates are in the same range, but the dissociation from the pPAD-$\Delta$O3 plasmid appears to be three times slower. The higher affinity interaction with the pPAD-$\Delta$O3 plasmid is in line with the fact that a specific binding site (operator 1) is present on this plasmid. Affinity values obtained using MST are in the same nanomolar range. They do not follow the trend that the affinity for pPAD-$\Delta$O3 is higher than for pPAD-$\Delta$O1$\Delta$O3. For MST to be accurate, it is essential that the DNA concentrations are precisely known; however, in the course of this project, it has proven difficult to accurately measure concentrations of highly concentrated, and hence viscous DNA preparations. We believe this to be the origin of the discrepancy between these values.

Although previously fitted with a 1:1 binding model, binding of Lac repressor to the pPAD-$\Delta$O3 plasmid should actually be considered as a heterogeneous event. Lac repressor can independently bind to either non-target DNA or operator DNA. We fitted the pPAD-$\Delta$O3 binding data with a model for heterogeneous binding to obtain the kinetic parameters for the secondary, specific, interaction. To do so, we assumed the kinetic parameters for the non-target interaction to be similar for both plasmids and used these as known variables for $k_a1$ and $k_d1$. As such, we found a $K_D$ of 4.0 nM for the interaction between Lac repressor and its plasmid-based operator. This is remarkably close to the values we found using SPR (3.9 nM) and MST (4.5 nM) (Table 2). Interestingly, the actual kinetics are widely different for the interactions of Lac repressor with the plasmid operator and linear O1 DNA. Both the association and dissociation rates are ~18 times slower for binding to the plasmid operator, indicating that the presence of negative supercoiling and non-target DNA has a considerable effect on the actual kinetics of binding.

CONCLUSION

In the work presented here, we demonstrate the feasibility and usefulness of a newly developed plasmid capture approach, by applying it for the characterization of Lac repressor binding. To our knowledge, this is the first time that SPR has been used to determine the affinity and kinetic parameters of the interaction between a protein and its specific target sequence that is located on a supercoiled plasmid. We believe this to be a versatile approach that could be useful in SPR, single molecule and other experiments to expand the range of substrates for DNA–protein interactions beyond the use of short linear target DNA. In addition, the biotin in TFO2.0 could be replaced by other functionalities, such as fluorophores and thus will enable studies requiring plasmid visualization. The use of padlock-modified plasmids provides a useful addition to the molecular biology toolbox, and may be used to uncover properties of supercoiling-dependent proteins, that could not be studied before.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Figures 1 and 2 and Supplementary Methods.

ACKNOWLEDGEMENTS

The authors wish to thank Intan Taufik (Groningen Biomolecular Sciences and Biotechnological Institute and Molecular Microbiology, Groningen) for help with MST measurements and Mitch Lewis (University of Pennsylvania) for helpful discussions on recombinant expression of Lac repressor.

FUNDING

Netherlands Organisation for Scientific Research and the Netherlands Institute for Space Research [ALW-GO-PL-08-08]; NWO Vidi grant [864.11.005 to S.J.I.J.B.]. Funding for open access charge: Microbiology department/Wageningen UR library.

Conflict of interest statement. None declared.

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