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ARTICLE

Sliding and target location of DNA-binding proteins:an NMR view of the lac repressor system

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Abstract In non-specific lac headpiece-DNA complexes selective NMR line broadening is observed that strongly depends on length and composition of the DNA fragments. This broadening involves amide protons found in the non-specific lac-DNA structure to be interacting with the DNA phosphate backbone, and can be ascribed to DNA sliding of the protein along the DNA. This NMR exchange broadening has been used to estimate the 1D diffusion constant for sliding along non-specific DNA. The observed 1D diffusion constant of 4×10^{-12} cm^2/s is two orders of magnitude smaller than derived from previous kinetic experiments, but falls in the range of values determined more recently using single molecule methods. This strongly supports the notion that sliding could play at most a minor role in the association kinetics of binding of lac repressor to lac operator and that other processes such as hopping and intersegment transfer contribute to facilitate the DNA recognition process.

Keywords Protein-DNA interaction · One-dimensional diffusion · NMR relaxation · Exchange broadening · Sliding

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Introduction

Gene regulatory proteins exert their function through binding to sequence-specific DNA regions. Much progress has been made in understanding specific protein-DNA interactions, primarily from X-ray or NMR structures of proteins bound to their DNA targets (Rohs et al. 2010). Another important question is how these proteins find their targets amongst a sea of non-specific DNA sites. For instance, in bacteria such as *E.coli* there are often only a few target sites in 10^7 base-pairs. Riggs et al. (1970) found that the association rate constant of lac repressor toward lac operator in *E.coli* is two or three orders of magnitude larger than expected based on 3D diffusion. This stimulated a lot of research on this so-called facilitated target location. In particular, the classical work by von Hippel, Berg, and coworkers set the stage (Berg et al. 1981; Winter et al. 1981; von Hippel and Berg 1989), but the discussion continues until today. As all sequence-specific DNA binding proteins also have affinity toward non-target DNA, a recurring element in the discussion is the possibility that the protein binds randomly and then slides along the DNA while scanning for the target sequence. As is long known, reducing the dimensionality from 3D to 1D diffusion could greatly speed up the target search (Adam and Delbruck 1968). In this paper, we address the problem of DNA

sliding by lac repressor from the point of view of NMR spectroscopy. Our model is a dimeric lac repressor headpiece that served as a good model in our structural studies on lac repressor-DNA interaction (Kalodimos et al. 2001, 2004).

Specific and non-specific DNA binding by lac repressor

For the *E.coli* lac repressor, X-ray structures have been reported for the free protein and for complexes with lac operator and the inducer IPTG (Lewis et al. 1996). These structures provided a wealth of information on the topology of tetrameric lac repressor (V-shaped dimer of dimers with two DNA-binding sites) and on the conformational differences between its induced and repressed states. However, the resolution was not high enough for a detailed analysis of the interactions that provide specific operator recognition. This information came from NMR studies of the DNA-binding domain (residues 1-62) of lac repressor (lac headpiece or lacHP). The structure of the complex of lacHP with high-affinity symmetrical 22 bp lac operator (Spronk et al. 1999) showed a dense network of hydrogen bonds, and van der Waals and ionic interactions that are in full agreement with the large body of mutagenesis data available for lac repressor (Markiewicz et al. 1994; Suckow et al. 1996). An important observation from both X-ray and NMR structures was that a large part of the so-called hinge region (residues 50–62 connecting the lac repressor core and the headpiece), which is a random coil when free in solution, folds up as an α-helix in the operator complex. The hinge helices from both headpieces interact with each other and with bases in the DNA minor groove, thereby producing a local bend of 45° in the DNA (see Fig. 1a).

NMR studies on complexes with lac operators O1, O2, O3, and with non-operator DNA were not possible with isolated headpieces due to weaker binding. Instead, a dimeric headpiece was used: the disulfide linked dimer formed from lacHP-V52C by oxidation (Kalodimos et al. 2001). The operator affinity of this dimeric headpiece is similar to wild-type repressor. Hinge helix formation and corresponding DNA bending occurred in all operator complexes O1 (Kalodimos et al. 2001) and O2, O3 (Romanuka et al. 2009) and is clearly a signature of specific DNA binding by lac repressor.

To investigate non-specific DNA interaction, the structure of lacHP dimer in complex with an 18 bp non-operator DNA fragment was determined (Kalodimos et al. 2004). In contrast to the specific complex, the hinge helices in the non-specific complex are not formed and DNA remains straight (see Fig. 1b). Importantly, there are no direct interactions with base-pairs. Thus, the non-specific complex is more loosely packed and retains much of the layer of hydration as evidenced by several water-mediated hydrogen bonds. Altogether some 10–11 hydrogen bonds and 5–6 electrostatic interactions are made with DNA phosphates per monomer (Kalodimos et al. 2004). Finally, the protein was found to be highly dynamic in the non-specific complex; backbone dynamics in the μs-ms time range were at least as pronounced as for the protein free in solution. In contrast, the specific complex with lac operator is extremely rigid (Kalodimos et al. 2001).

For complexes of lacHP-dimer with non-operator DNA of length >18 bp no protein-DNA NOEs could be observed. This suggested the possibility of sliding since that would dilute the NOE effects beyond detection (Kalodimos et al. 2004; von Hippel 2004). In this paper, we will explore the sliding phenomenon more quantitatively.

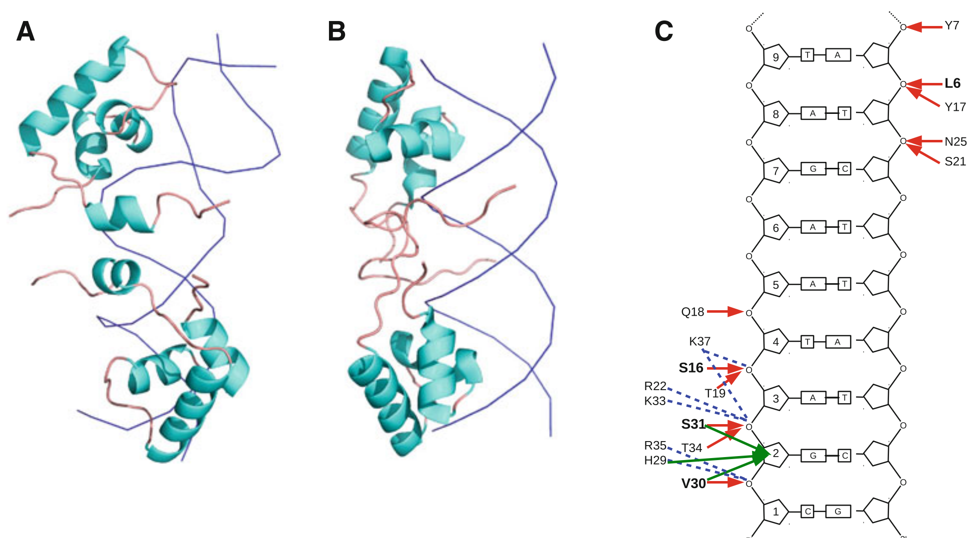

Fig. 1 NMR 3D structures of lacHP **a** bound to a symmetrical lac operator (1CJG.pdb), **b** bound to NOD18 (1OSL.pdb), **c** schematic representation of the protein-NOD18 contacts. *Green arrows* indicate hydrophobic contacts, *dashed blue lines* indicate electrostatic contacts, and *red arrows* indicate hydrogen bonding. Bold residues are involved in H-bonds with DNA through their backbone amide protons

The role of sliding in facilitated target location

The most direct evidence for sliding comes from single molecule fluorescence experiments. Using this technique, Wang et al. (2006) observed a lac repressor-GFP fusion construct sliding along flow-stretched DNA. The 1D diffusion coefficients varied from 2.3×10^{-12} to 1.3×10^{-9} cm^2s^{-1} with an average of 2.1×10^{-10} cm^2s^{-1} (Wang et al. 2006). Several other DNA binding proteins have been studied by this method (Gorman and Greene 2008). Diffusion of lac repressor on DNA has even been seen in living E.coli bacteria (Elf et al. 2007; Hammar et al. 2012).

The mode of diffusion has also been subject of some discussion. Winter et al. (1981) depicted the non-specific lac repressor-DNA complex as purely electrostatic in nature (Winter et al. 1981). The repressor diffuses almost unhindered along a negatively charged DNA cylinder, while only the replacement of counter ions by positively charged amino acid residues provides an energetic barrier. In contrast, Schurr (1979) considered a model whereby the protein follows the major groove of DNA and therefore diffuses in a spiraling fashion. In fact, the NMR structure of the non-specific complex favors the latter model (Kalodimos et al. 2004). In addition to charge–charge interactions, the non-specific interaction involves a substantial number of hydrogen bonds with phosphates. Furthermore, the protein penetrates in the major groove, which would provide a large barrier for linear diffusion. Recently, using single molecule methods evidence for the rotation-coupled mode of sliding has indeed been found for a number of proteins including lac repressor (Blainey et al. 2009).

So sliding does occur, but does it play a role in facilitated target location? Berg et al. (1981) suggested a two-step process for target binding in order to account for the large association rate enhancement reported by Riggs et al. (Riggs et al. 1970). The protein first binds to a random DNA site and then translocates to the target. Several translocation processes were identified: (1) sliding (1D diffusion along the DNA), (2) hopping (series of dissociation-reassociation events; for short times, the probability for rebinding to the same site is high and the process is therefore correlated), and (3) intersegment transfer (the protein binds transiently to two sites of the same DNA chain). Clearly, intersegment transfer can only occur when the protein has at least two binding sites such as lac repressor. Based on the data available for lac repressor in the early 80s, the theory of Berg et al. (1981) could successfully account for the salt dependence of repressor-operator binding. It was found that the enhanced association rate was dominated by a combination of sliding and hopping with intersegment transfer playing, at most, a minor role.

However, several years later, the notion that sliding could play a major role in protein translocation in protein-DNA binding was challenged. The analysis by Winter et al. (1981) was based on kinetic measurements using the so-called filter-binding method, where proteins and protein-DNA complexes are retained on nitrocellulose filters, while free DNA is not. However, Fickert and Müller-Hill (1992) noted that DNA fragments containing the natural lac operon with two or three operators may produce artifacts in filter-binding assays. They reanalyzed the data with DNAs containing a single operator using both filter-binding and gel mobility shift assays and also looked at both tetrameric lac repressor and a dimeric repressor variant containing a single DNA binding site. The dimeric repressor did not show an increased operator association rate and intact repressor showed a modest increase of a factor 9. Moreover, the DNA length dependence of the association rates, which is considered evidence of sliding, was absent. So, their conclusion was that there was no sliding, but that intersegment transfer might explain the results for the tetrameric lac repressor (interestingly, this paper is largely ignored in the recent literature).

Kolomeisky (2011) reviewed the present state of affairs and noted some inconsistencies in the current theories. Firstly, most applications assume that 1D and 3D diffusion constants are similar (Halford and Marko 2004), but this is rather unrealistic. Both the hydrodynamic effect of the spiraling diffusion and the barriers involved in sliding will slow down 1D diffusion considerably. Secondly, in the theory of Berg et al. (1981) and subsequent variations, the optimal times spent at 1D and 3D diffusion are equal. But recent in-cell single molecule experiments have shown that the proteins spend 90 % of their time on non-specific DNA. Kolomeisky's treatment introduces a correlation between 1D and 3D diffusion processes in an attempt to cure these problems.

Here, we present a new approach based on NMR exchange measurements to analyze 1D diffusion of DNA-binding proteins along DNA sequences. Although this problem has been discussed since the pioneering studies of Berg and von Hippel in the early 80s, results of recent single molecule fluorescence measurements of lac repressor and other proteins sliding along DNA have caused a revival in this field (Tafvizi et al. 2011). Our NMR approach shines new light on this matter and has the advantage that it combines kinetics with the possibility to observe the process involved at an atomic level.

Materials and methods

NMR samples

Cloning, expression and purification of the uniformly ^{15}N-labelled lacHP62-V52C mutant were described previously (Kalodimos et al. 2001). The NMR samples contained

Table 1 DNA sequences used for the titration and line broadening experiments

DNA name	Number of base pairs	Sequence
NOD18	18	CGATAAGATATCTTATCG
NOD22	22	CGAGATAAGATATCTTATCTCG
AT9	20	CATATATATATATATATATG
AT10	22	CATATATATATATATATATATG
A2T2	22	CAATTAATTAATTAATTAATTG
A3T3	20	CAAATTTAAATTTAAATTTG
A5T5	22	CAAAAATTTTTAAAAATTTTTG
PAT18	18	CAAAAAAAATTTTTTTTG
PAT22	22	CAAAAAAAAAATTTTTTTTTTG

~100 μM of protein in 0.4 M KCl, 0.06 M potassium phosphate buffer at pH 5.8.

The HPLC purified DNA fragments were purchased commercially (Eurogentec). To form the double-stranded DNA, equimolar amounts of the complementary strands were mixed, heated to 95 °C for 5 min and slow-cooled to anneal over a period of several hours. Next, the lyophilized double stranded DNAs were dissolved in the same buffer as the lacHP62-V52C to obtain stock solutions of known concentrations for the titration experiments (see Table 1). Aliquots of these stock solutions (up to 15–20 fold excess of DNA) were added directly to the protein samples in the NMR tubes.

The NOD16, 18 and 22 sequences were designed to be entirely different from the naturally occurring operators. Table 1 summarizes all DNA fragments used in this study.

NMR spectroscopy

All ^{1}H-^{15}N HSQC spectra were recorded at 298 K on Bruker Avance 750 and 900 MHz spectrometers. The spectral widths were 12 and 40 ppm in the ^{1}H and ^{15}N dimensions respectively, and the numbers of complex data points were 1,024 and 128 in these dimensions. Data were zero-filled in all dimensions and apodized using a squared cosine window. Linear prediction was used in the ^{15}N dimension. All NMR data were processed with the NMRPipe software (Delaglio et al. 1995) and the chemical shift perturbations, the intensity changes, and the line widths of the peaks were analyzed with Sparky (Goddard and Kneller 2008).

HSQC spectra for analysis of the line broadening were measured at 900 MHz with INEPT delays of 5.5 ms.

Chemical shift perturbation (CSP) analysis

CSPs were analyzed using the SAMPLEX program (Krzeminski et al. 2010). As input, we used a set of CSP data and the 3D structure of lac headpiece in a non-specific complex (1OSL.pdb). SAMPLEX calculates the confidence for each residue to be in a perturbed or unperturbed state.

Determination of K_D values

The K_D values have been obtained from the CSP titration data using Eqs. (1) and (2),

$$CSP = f_B \cdot CSP_{\max} \quad (1)$$

where f_B is the fraction protein bound

$$f_B = \frac{[PL]}{[P]_0} = \frac{([L] + [P]_0 + K_D) - \sqrt{([L] + [P]_0 + K_D)^2 - 4[P]_0[L]}}{2[P]_0} \quad (2)$$

using non-linear curve fitting with the program Grace (http://plasma-gate.weizmann.ac.il/Grace/). The errors in K_D values were estimated by fitting the parameters using the program SciLab (http://www.scilab.org) while varying each of the fraction-bound values 1,000 times randomly and independently by stochastic variations with a standard deviation equal to the experimental error (~5 %). Systematic errors of ~10 % on the protein and DNA concentrations were also taken into account.

Results and discussion

Line broadening in non-specific binding DNA complexes

Previously, we determined the structure of the complex of lac repressor headpiece with a non-operator DNA fragment, NOD18 (Kalodimos et al. 2004). At that time, it was already noted that the amide protons resonances in non-specific lac-DNA complexes were affected by exchange broadening. This broadening occurs in particular for the DNA binding residues of lac headpiece and strongly depends on the length of the non-specific sequence. Figure 2a shows that a long non-specific sequence, NOD22, shows significant broadening, even to an extent that would have made the structural analysis of the DNA complex very difficult. However, this broadening reduces significantly when the DNA sequence is shortened, and for NOD16 the exchange broadening is almost negligible. We already hypothesized that this broadening could be related to the sliding of lac headpiece over the NOD sequence. On a long NOD sequence, lacHP can populate several positions, each having a different DNA environment. This

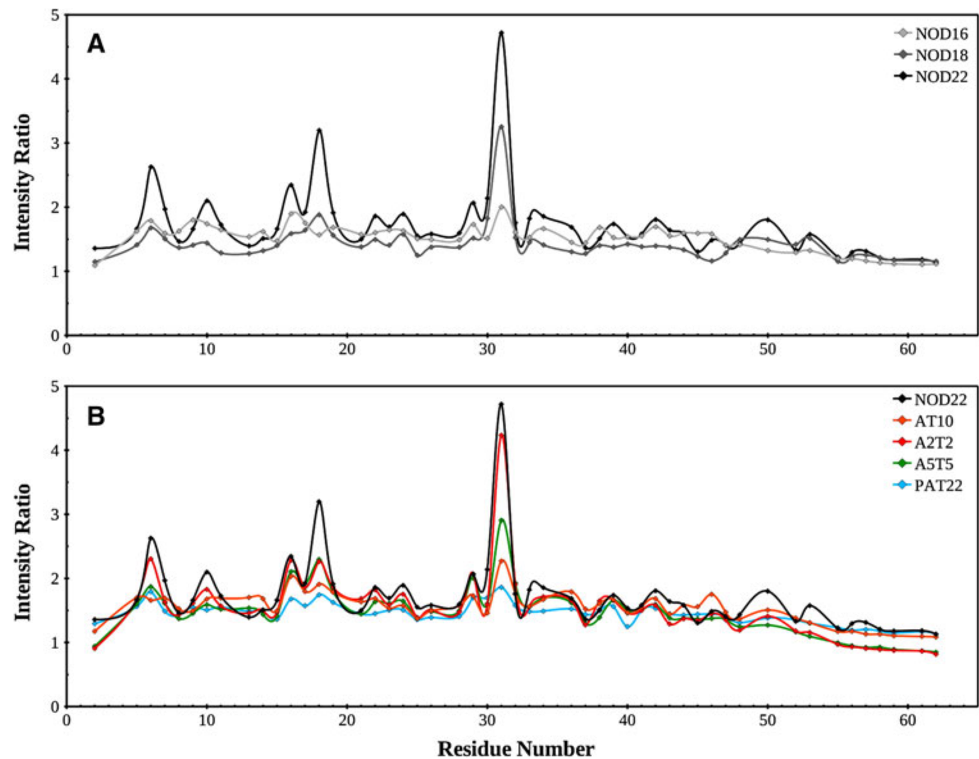

Fig. 2 NMR line broadening of the backbone amide protons of lacHP dimer in the presence of different DNA sequences from HSQC intensity ratio's at 900 MHz. **a** Effect of DNA length. **b** Effect of DNA sequence composition. The different sequences are given in Table 1. The NMR samples contained ~100 μM of protein in 0.4 M KCl, 0.06 M potassium phosphate buffer at pH 5.8. The exchange-broadened spectra were all recorded in the presence of a 15 fold excess of DNA. For calculating the intensity ratios the intensity of the amide cross-peak of DNA-bound LacHP dimer was divided by the cross-peak intensity of free protein

causes amide protons to have different chemical shifts and the exchange of lacHP between the different positions on an intermediate (millisecond) timescale would result in exchange broadening. In this model, the broadening would be directly related to the kinetics of DNA sliding. By reducing the NOD length, the number of different positions would be reduced leading to less exchange broadening. In order to analyze this exchange and use it for studying the DNA sliding kinetics, we extended these initial experiments with a series of experiments using different DNA fragments.

Chemical shift perturbations (CSPs) in DNA titrations

In principle, the amide proton chemical shift broadening can also be caused by exchange between free and DNA-bound lacHP. Figure 3a shows the CSPs that occur upon titrating lacHP with the NOD sequences of different length. The pattern of CSPs across the peptide sequence is distinct from the one of the broadening effect. Whereas the CSPs occur for all amide protons that are close to the protein-DNA interface, the line broadening occurs for a subset of residues (cf. Fig. 2), indicating that this broadening has a different origin. Moreover, at high DNA concentrations with only a small amount of free lacHP present, the strong broadening in long NOD sequences remains, which also shows that the exchange is not related to the binding kinetics.

The largest CSP occurs for Ser31, in the loop between helix 2 and helix 3, which binds to the DNA phosphate backbone in all lac-DNA complexes. Significant chemical shifts changes occur also for other residues contacting the phosphate backbone as well as for the residues at the surface of helix 2, the recognition helix, which binds in the major groove of the DNA. The CSPs are in agreement with the position of lacHP in the structure of the non-specific lacHP-NOD18 complex. The observation that similar CSPs are found in all non-specific complexes (Fig. 3) confirms that the binding mode of lacHP in all complexes is very similar.

The CSP titration data can be used to determine the binding affinities of lacHP62-V52C for the various non-specific DNA sequences (Table 2). As expected, lacHP shows low affinity for all non-specific DNA sequences with apparent dissociation constants K_D ~ 300–400 μM. These K_D's represent significantly weaker binding than the K_D of lacHP to the O1 and O2 operators (K_D ~ 0.05–0.1 nM) determined from electrophoretic mobility shifts assays (Romanuka et al. 2009). The affinity of lacHP for non-specific DNA does not vary much for different non-specific sequences of identical length, but appears to increase slightly with the length of the oligonucleotide (see

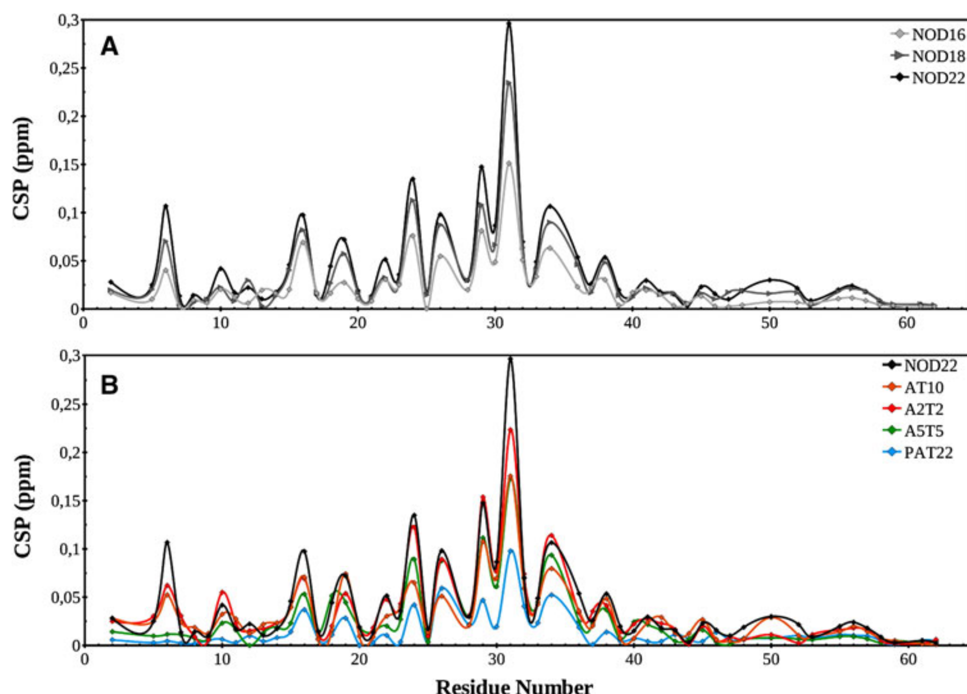

Fig. 3 Chemical shift perturbation of lac headpiece amides for different DNA sequences from HSQC spectra at 900 MHz. **a** Effect of DNA length. **b** Effect of DNA sequence composition. The sample conditions are as in Fig. 2. The indicated CSP values are in the presence of a 15 fold excess of DNA

Tables 1, 2), possibly due to the larger charge of the longer oligonucleotide.

Exchange broadening is due to sliding on DNA

The exchange broadening appears not related to the binding of lacHP to DNA. Indeed if the broadening were due to the free-bound equilibrium, all protein resonances that show CSPs should be broadened proportional to $(CSP)^2$. However, a comparison of Figs. 2 and 3 shows that the exchange broadening is only observed for a subset of residues displaying CSPs in the titrations.

Clear evidence that the exchange contribution is not related to the free-bound equilibrium comes also from measurements where we used different non-specific DNA sequences. A symmetric sequence, PAT22, with the same length as NOD22 and containing identical bases in the headpiece binding site, shows CSPs but negligible exchange broadening (Fig. 3b). When LacHP binds to different sites in the left half of PAT22 it will always be positioned at an ‘A’ site. Since the nucleotide sequence is symmetric the same will hold for binding at the right half of PAT22. Since for this oligonucleotide the amide proton environment does not change during sliding, this supports our model that the exchange is due to DNA sliding. To further analyze this sliding, we measured the exchange broadenings of different AnTn DNA sequences of similar length. In these sequences LacHP would meet different ‘A’ and ‘T’ sites when it would slide along the DNA. In case of fast exchange of LacHP binding to the ‘A’ and ‘T’ sites this would lead to little broadening, but in case of intermediate exchange significant broadening could occur. Figure 2b shows that the broadening for AnTn sequences increases for AT10 (n = 1), reaches a maximum for A2T2 and reduces again for A5T5. Since the affinities for the different AnTn are all similar (Table 2), a simple interpretation would be that the affinities for the A and T sites and thus the occupancies at all sites would be all very similar. The differences in extent of broadening would then be due to either difference in exchange regime or different occupancies at ‘A’ and ‘T’ sites. For n = 1, the exchange between the ‘A’ and ‘T’ sites, which are adjacent to each other on AT10, would then be fast, leading only to a relatively small broadening. When this exchange is due to sliding with a defined 1D diffusion constant, the resulting exchange rate will reduce, since lacHP has to travel longer when the physical distance between the ‘A’ and ‘T’ sites increases. For n = 2, where

Table 2 K_D values and fractions bound of lac headpiece (0.05 mM) for different DNA sequences

DNA name	K_D (μM)	Fraction bound (%)
NOD18	322 ± 30	75 ± 7
NOD22	282 ± 34	76 ± 5
AT9*	469 ± 28	73 ± 10
AT10*	438 ± 32	71 ± 6
A2T2	340 ± 33	72 ± 8
A3T3	450 ± 35	71 ± 10
A5T5	430 ± 29	70 ± 9
PAT18	401 ± 40	65 ± 14
PAT22	370 ± 36	66 ± 15

* K_D and fraction bound obtained for a concentration of 0.1 mM of lac headpiece

the 'A' and 'T sites are separated by two nucleotides the broadening reaches a maximum (Fig. 2b), due to intermediate exchange on a ms timescale for relocation over 2 bases. For n = 2 there are several 'A' and 'T' sites in the left half DNA site and thus one can expect similar probabilities in finding lacHP at the two, 'A' and 'T', binding sites. However, for the AnTn DNA sequences with high n values, the occupancies p_A and p_T to the 'A' and 'T' sites can become very different, which can reduce the exchange broadening effect again. For PAT22 (n = 10), where the second lacHP is occupying the 'T' sites, this could lead to a high occupancy, ~99 %, of state A and low occupancy, ~1 %, of state T. For A5T5, the broadening would still be reduced, due to a different occupancy of the A and T states, of ~90 and 10 % respectively, since the second lacHP is still partially in the way.

The exchange rates involved can be estimated from the change in intensity caused by exchange during the two INEPT periods τ in the different HSQC spectra from

$$\frac{I}{I_0} = e^{-R_{ex}2\tau} \quad (3)$$

For the largest observed effect (at residue Ser 31) for the A2T2 sequence, this would lead to an estimate of R_{ex} of $\sim 10^2\ s^{-1}$. For a model with exchange between two sites A and T with population p_A and p_T, the exchange rate R_{ex} is given by:

$$R_{ex} = p_A p_T (\Delta\omega)^2 \tau_{ex} \quad (4)$$

where Δω is the change in precession frequency when the protein moves from site A to T, $\tau_{ex} = 1/(k_1 + k_{-1})$ is the exchange lifetime, with k_1 and k_{-1} the forward and backward exchange rates respectively.

The value of Δω can be estimated from the difference in CSP for PAT22 and AT10. For PAT22 where lacHP is with almost full occupancy at the 'A' site, the CSP Δδ will be $\sim(\delta_A - \delta_{free})$, whereas for AT10 this will be $\sim(0.5\cdot\delta_A + 0.5\cdot\delta_T - \delta_{free})$. The difference in CSP between PAT22 and AT10 is $\sim(0.5\cdot\delta_A - 0.5\cdot\delta_T)$. With CSP values for PAT22 and AT10 of 0.1 and 0.17 ppm, respectively, a value for $(\nu_A - \nu_T)$ of 125 Hz can be estimated at 900 MHz (the CSPs are mainly for the amide protons). With this estimate for Δν of 125 Hz and $p_A = p_T = 0.5$, we reach an exchange lifetime of ~600 μs and, with $k_1 = k_{-1}$, an exchange (sliding) rate of $\sim 800\ s^{-1}$.

Using the equation for one dimensional diffusion,

$$x^2 = 2D_1\tau_{ex} \quad (5)$$

we can estimate the 1D diffusion rate D_1 along the DNA sequence. For the A2T2 sequence, the exchange displacement x will be 2 base pairs, and with a base pair distance of 3.4 Å, the 1D diffusion rate D_1 becomes $\sim 4\times10^{-12}\ cm^2\ s^{-1}$. Single molecule studies using optical techniques showed a large variation in the D_1 diffusion rates (Wang et al. 2006). The value derived from NMR exchange broadening is close to the lower limit of D_1 in these studies, although much lower than the average of $\sim 10^{-10}\ cm^2\ s^{-1}$. Elf et al. (2007) report an effective diffusion constant of $4\times10^{-9}\ cm^2\ s^{-1}$ for in vivo lac repressor sliding, which is presumably a combination of 1D and 3D diffusion. The in vitro value of D_1 for lac repressor quoted by these authors is $4.6\times10^{-10}\ cm^2\ s^{-1}$.

Exchange broadening is observed for amides H-bonded to the phosphate backbone

CSPs are observed in the DNA titrations for almost all residues of lac headpiece that are close to the DNA interaction surface (represented in green in Fig. 4). However, the resonance broadening is only observed for a subset of those. Analysis of the observed line broadenings reveals that the amino acids can be divided into two groups:

- Residues with significant CSPs and no line broadening (Tyr17, Thr19, Arg22, His29, Gln26, Thr34, Val38). These residues contact DNA mainly through their side chains (hydrophobic interactions with sugar moieties and water-mediated hydrogen bonds with phosphates) and the observed CSPs are due to the difference in environment between free and bound states.
- Residues which exhibit both CSPs and line broadening (Leu6, Ser16, Gln18, Ser31—represented in yellow in Fig. 4). Their amide protons make direct hydrogen bonds to the phosphate backbone of the DNA in the lacHP-NOD18 complex. Of these residues the amide proton of Ser31 showed the largest CSP and line broadening effects in our experiments, probably due to a relatively stable hydrogen bond. We note that, in the lacHP-NOD18 complex, the hydroxyl group of Ser31 is in a position to make an additional hydrogen bond with a phosphate of the DNA backbone.

We have reasoned above that the exchange broadening is not related to the association-dissociation equilibrium of lac headpiece and DNA. The observations (1) that mainly alternating AATT sequences show this broadening, (2) that it is almost absent in homogenous A10T10 stretches and (3) that they occur mainly for amides involved in hydrogen bonds, indicate that there are differences in the chemical environment near A and T bases in the DNA phosphate backbone.

Conclusion

NMR line broadening in non-specific complexes has been taken as evidence for 1D DNA sliding of lac repressor

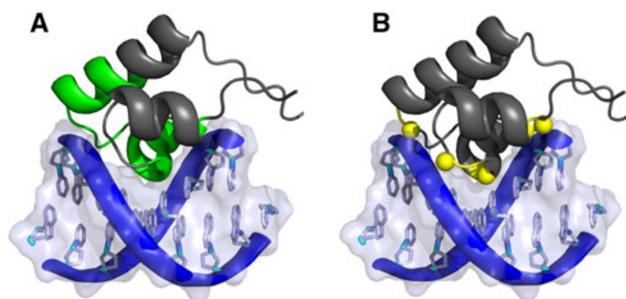

Fig. 4 Structure of lac headpiece bound to non-specific DNA. The figure shows the left side of the non-specific lacHP62-V52C-NOD18 complex. **a** Residues exhibiting CSPs are represented in *green*. **b** Backbone amides exhibiting line broadening are represented with *yellow spheres*

(Kalodimos et al. 2004; von Hippel 2004). The current analysis demonstrates now that for several amide protons this broadening is indeed related to DNA sliding. The structure of the non-specific lacHP-NOD18 DNA complex already showed that most direct protein-DNA interactions were at the phosphate backbone. The current line broadening data show that these hydrogen bonds are in fact highly dynamic and lead to ms exchange lifetimes. The corresponding 1D diffusion constant for sliding of 4×10^{-12} cm^2 s^{-1} is smaller than had been predicted on the basis of association kinetics in the past. Recent single molecule experiments, however, indicated that these rates are non-uniform and vary over a large range from 2×10^{-12} cm^2 s^{-1} to 1.3×10^{-9} cm^2 s^{-1}. Of course these 1D diffusion rates were obtained from two very different techniques, NMR and single molecule optical measurements, and different experimental setups, small headpiece-oligonucleotide complexes and lac repressor dimers on long DNAs. Still our observation clearly demonstrates that the 1D DNA sliding, at least on small DNA stretches, is much slower than previously anticipated. This slow diffusion may well correspond with the low chance that all 20 or so hydrogen bonds of the lac headpiece dimer with non-specific DNA would be simultaneously broken, which would represent a large barrier for rapid diffusion. Such low 1D diffusion rates would not make the lac repressor-DNA association kinetics significantly faster than the 3D diffusion limit. Though this may be unexpected, it should be noted that Fickert and Müller-Hill (1992) already questioned the contribution of 1D sliding to lac repressor DNA association and suggested that intersegment transfer could provide a significantly larger contribution. More recently, Halford and Marko (2004) and Kolomeisky (2011) reevaluated the 1D sliding models and indicated that they may not be sufficient to explain the fast association kinetics of protein-DNA complexes and that significant "hopping" and intersegment transfer may be required. Also NMR exchange studies by Iwahara and Clore of a homeodomain DNA complex using paramagnetic probes stressed the importance of intersegment transfer as a major mechanism for a rapid target search (Iwara and Clore 2006; Clore 2011). Our results would support this notion.

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