Backbone and Side Chain Dynamics of *lac* Repressor Headpiece (1−56) and Its Complex with DNA†

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ABSTRACT: The dynamics of the backbone and (some of) the side chains of *lac* headpiece (1−56; *lac* HP56) have been studied for the free protein and for its complex with *lac* half-operator DNA by ^{15}N T_1 and $T_{1\rho}$ relaxation measurements combined with [$^1H-^{15}N$] NOE experiments. For the structurally well-defined part of the free *lac* HP56 (*i.e.*, residues 3−49) a rigid backbone was found for residues in the three α-helices and for the turn of the helix−turn−helix motif. The loop between helices II and III of *lac* headpiece, which was characterized by slight disorder in the structure calculations, shows increased mobility. The detected side chains are very mobile. These data are in full agreement with the rms deviations in the structural data of free *lac* HP56. When *lac* HP56 is complexed with DNA, several changes in mobility take place. The most remarkable change was found for the loop region between helices II and III: residue His-29 within this loop interacts with Thy-3 of the operator DNA. As a result this mobile loop adapts itself to the DNA and becomes more rigid. Moreover, most DNA-contacting side chains show a significant decrease in flexibility, although these side chains do not become as rigid as the backbone. These results suggest that the mobility of the regions within *lac* HP56 important for complexation, *i.e.*, the loop and the DNA-contacting side chains, is essential for a good fit onto the counterparts of the target DNA.

Both structure and dynamics are important in biomolecular interactions, such as protein−DNA, enzyme−substrate, and antibody−antigen interactions, which rely on appropriate recognition of complementary surfaces. A case in point is the gene regulation process of the *lac* operon, in which protein−DNA recognition plays the key role. The *lac* repressor negatively controls the structural genes by binding specifically to the operator sequence. The DNA binding domain of the *lac* repressor comprises the N-terminal 60 amino acids, termed *lac* headpiece (or *lac* HP). An isolated headpiece retains its three dimensional structure and binding specificity for the *lac* operator, which has been demonstrated by structural and biochemical data (Geisler & Weber, 1977; Ogata & Gilbert, 1979; Khoury *et al.*, 1991). The refined NMR solution structures of *lac* HP56[1] in free (Slijper *et al.*, 1996) and DNA-bound states (Chuprina *et al.*, 1993) have been determined. These and earlier NMR studies have revealed that *lac* HP56 consists of three α-helices, located at residues 6−13, 17−24, and 32−45, the first two of which are part of a helix−turn−helix motif. Interestingly, although the structures of the free and DNA-bound *lac* headpiece correspond reasonably well for these regions (Figure 1), the conformation of the loop between helices II and III changes drastically upon binding to the DNA, allowing the side chains of residues Asn-25 and His-29 to contact the DNA. In free *lac* HP56 several less well-defined regions were detected (residues 1−2, 50−56; Slijper *et al.*, 1996), which is either due to lack of structural information or to internal mobility. Moreover, restrained molecular dynamics calculations performed on the *lac* headpiece−DNA complex in aqueous solution suggested that several of the observed protein−DNA interactions are dynamic rather than static in nature (Chuprina *et al.*, 1993).

Protein dynamics can be studied by using a number of experimental approaches, of which the measurement of ^{15}N relaxation parameters is a well-established NMR technique (Kay *et al.*, 1989; Peng & Wagner, 1992a,b). The measurement of the longitudinal (T_1) and transverse ($T_{1\rho}$) relaxation times and the [$^1H-^{15}N$] NOE can provide a comprehensive picture of motion at an atomic level, since the heteronuclear relaxation rates depend mainly on the dynamics of the N−H bond vector with respect to the external magnetic field. This paper reports the measurement of the backbone and side chain ^{15}N T_1, $T_{1\rho}$, and the [$^1H-^{15}N$] NOE of uniformly ^{15}N-labeled *lac* HP56 and the analysis of the internal mobility of *lac* HP56 in the free state and in the complex with the 11 bp *lac* half-operator DNA.

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[1] Abbreviations: HP56, headpiece (1−56); HSQC, heteronuclear single quantum coherence; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser enhancement; ppm, parts per million; RMD, restrained molecular dynamics; SCUBA, stimulated cross-peaks under bleached α's; TOCSY, total correlation spectroscopy; 2D and 3D, two- and three-dimensional; rms, root mean square.

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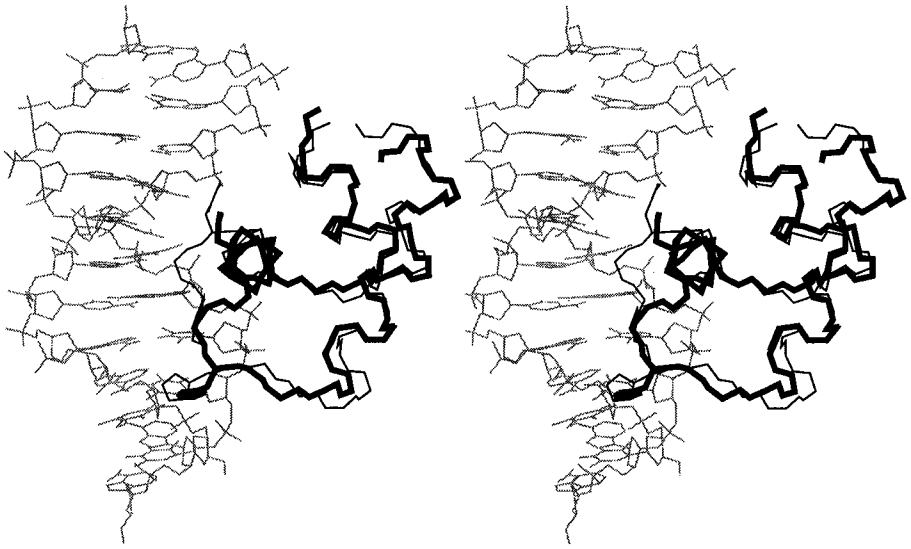

FIGURE 1: Stereoview of the backbone of the *lac* headpiece in free (bold line) and DNA-bound (thin line) states [after Slijper *et al.* (1996)]. The view is through the second or recognition helix of the *lac* headpiece, located in the major groove of the 11 bp half-operator DNA. As can be seen from the figure, the conformation of the loop between helices II and III changes considerably upon binding. The side chains of residues Asn-25 and His-29 are depicted to show that both side chains can only contact the DNA in the conformation of the bound state of the *lac* headpiece.

MATERIALS AND METHODS

Preparation of lac HP56

The expression system is based on the inducible T7 RNA polymerase/promoter system with pET expression vectors, as described by Studier *et al.* (1990). The 168 bp fragment encoding *lac* headpiece (1−56; *lac* HP56) was amplified from the *lacI* gene of pWB100 (Lehming *et al.*, 1987) by performing a polymerase chain reaction using the following primers:

5′-GGAATTCCATATGAAACCGGTAACGTTA-3′
(sense)

5′-GGGATCCTCACAGCTGCTGCGCAACACG-3′
(antisense)

*Eco*RI and *Nde*I sites were added to the 5′ end of the sense primer, and a stop codon followed by a *Bam*HI site were added to the antisense primer. The amplified DNA was digested with *Bam*HI and *Eco*RI, gel purified, inserted into pTZ vectors (Mead *et al.*, 1986), and transformed into *Escherichia coli* JM101. After sequence analysis, the *lac* HP56-encoding *Nde*I/*Bam*HI fragment was inserted into the *Nde*I and *Bam*HI sites of pET-3a (Studier *et al.*, 1990), resulting in the plasmid pET-HP56. The pET-HP56 construct was then transformed into *E. coli* DH9 (F^- *lac* (i^- *o* $^+$ *z* $^+$ y^-) *pro leu rpsL* r_k^- m_k^- *recA*; Hare & Sadler, 1978) containing the pGP1-2 helper plasmid (Tabor & Richardson, 1985). Production of *lac* HP56 is under the control of the T7 RNA polymerase/promoter system. Production of T7 RNA polymerase is regulated by the λ P_L promoter, which is repressed by the temperature sensitive *cI* repressor (*cI* 857).

The *E. coli* DH9 [pET-HP56, pGP1-2] cells were propagated at 30 °C in a fermentor using LB or ^{15}N minimal media [Sambrook *et al.*, 1989; the minimal media contains $^{15}NH_4Cl$ (Isotec Inc.) as sole nitrogen source], with ampicillin and kanamycin (both 100 μg/L), until the OD_{600} was approximately 5 (LB medium) or 1 (minimal medium), respectively. Induction was initiated by shifting the temperature to 42 °C. The fermentor temperature was kept at 42 °C for 30 min, and then the cells were propagated for 1−2 h at 37 °C to accumulate *lac* HP56.

Cells were harvested by centrifugation (10 min at 7500*g*), resuspended in lysis buffer (100 mM Tris, pH 8.0, 40 mM $MgCl_2$, 4 mM $CaCl_2$, 1 mM EDTA, 50 mg/L PMSF and 5 mM DTT), and sonicated (MSE Soniprep 150). Subsequently, the sample ionic strength was increased by adding KCl to a concentration of 500 mM, DNase I was added to 20 μg/mL, and the suspension was stirred for 15−30 min. PEI (polyethyleneimine) was then added to 5% (v/v), and the suspension was stirred again for 15−30 min. After centrifugation (30 min at 20000*g*) the supernatant was loaded on a 500 mL Sephadex G50 (superfine) column (Pharmacia) and eluted in 3.5 mL fractions using HP buffer [60 mM potassium phosphate, pH 7.5, 5% (v/v) glycerol, 1 mM EDTA, 0.3 mM DTT, and 50 mg/mL PMSF]. The *lac* HP56-containing fractions (as detected by measuring OD_{280} and SDS−PAGE) were pooled, and the pH was adjusted to 4.5 with 0.1 N HCl. The *lac* HP56-containing fraction was then loaded on a 10 mL S-Sepharose Fast Flow column. Gradient elution was employed with a range of 0−750 mM KCl in HP buffer, only now the working pH was at 4.5. The *lac* HP56 eluted at approximately 500 mM KCl. The purified *lac* HP56 was concentrated to 3−7 mM (as determined by OD_{280}) in a stirred ultrafiltration cell using YM1 filters (Amicon).

The complementary 11 bp *lac* half-operator DNA fragments (5′-AATTGTGAGCG-3′ and 5′-CGCTCACAATT-3′) were synthesized as described by van der Marel *et al.* (1981) and van Boom *et al.* (1982).

NMR Experiments

All experiments were performed on Bruker AMX500 or AMXT600 spectrometers, operating at a temperature of 25 °C. NMR samples of free [^{15}N]*lac* HP56 contained 4−7 mM protein in buffer consisting of 400 mM KCl and 60

Table 1: Assignments of ^{15}N and Directly Bound ^{1}H in Free and DNA-Complexed *lac* HP56[a]

	free *lac* HP56		*lac* HP56+11 bp DNA			free *lac* HP56		*lac* HP56+11 bp DNA	
residue	^{15}N	$N^{1}H$	^{15}N	$N^{1}H$	residue	^{15}N	$N^{1}H$	^{15}N	$N^{1}H$
Lys-2	126.8	8.72	126.9	8.72	Ser-31	123.8	8.78	–	9.31
Val-4	121.6	8.28	122.5	8.24	Ala-32	127.8	8.91	127.5	8.95
Thr-5	118.6	9.42	118.6	9.45	Lys-33	117.5	8.38	117.4	8.43
Leu-6	120.0	9.05	120.1	9.16	Thr-34	119.1	7.60	119.9	7.64
Tyr-7	117.8	7.66	117.5	7.61	Arg-35	122.5	8.43	122.9	8.56
Asp-8	121.0	7.53	121.0	7.53	$35N^{\epsilon}$	84.5	7.38	84.5	7.46
Val-9	120.5	7.51	120.5	7.50	Glu-36	118.1	8.40	118.1	8.33
Ala-10	122.4	8.04	122.3	8.07	Lys-37	120.6	7.73	120.4	7.73
Glu-11	117.3	7.97	117.4	7.96	Val-38	120.6	8.08	120.8	8.16
Tyr-12	119.9	8.17	121.2	8.07	Glu-39	120.5	8.97	120.9	9.02
Ala-13	118.4	8.52	118.3	8.51	Ala-40	123.4	8.36	123.2	8.32
Gly-14	108.0	8.00	108.1	8.01	Ala-41	122.1	7.60	122.1	7.65
Val-15	112.3	7.81	111.9	7.83	Met-42	116.4	8.37	116.3	8.44
Ser-16	116.2	8.51	115.6	8.60	Ala-43	120.8	7.80	120.7	7.76
Tyr-17	123.7	9.14	–	8.95	Glu-44	120.1	8.28	120.2	8.28
Gln-18	119.2	8.64	–	–	Leu-45	116.1	7.63	116.0	7.67
$18N^{\epsilon 1,2}$	113.2	6.97, 7.61	112.2	6.97, 7.75	Asn-46	116.6	7.76	116.6	7.74
Thr-19	119.1	7.93	120.7	7.94	$46N^{\delta 1,2}$	112.5	6.75, 7.39	112.4	6.75, 7.39
Val-20	121.1	7.67	121.0	7.58	Tyr-47	118.9	8.41	118.8	8.39
Ser-21	114.4	8.18	114.2	8.16	Ile-48	130.0	7.64	130.0	7.64
Arg-22	121.2	7.86	118.6	8.09	Asn-50	119.2	8.39	119.5	8.45
$22N^{\epsilon}$	85.3	7.18	84.9	7.14	$50N^{\delta 1,2}$	113.7	6.92, 7.61	113.6	6.91, 7.62
Val-23	119.6	7.83	119.5	7.85	Arg-51	122.0	8.21	121.9	8.19
Val-24	118.3	8.12	120.6	8.42	$51N^{\epsilon}$	85.7	7.22	85.7	7.23
Asn-25	116.5	8.05	116.1	8.06	Val-52	121.7	8.08	121.7	8.07
$25N^{\delta 1,2}$	114.5	7.43, 6.94	115.2	7.18, 7.42	Ala-53	127.8	8.29	127.8	8.29
Gln-26	119.2	8.16	116.2	8.04	Gln-54	120.0	8.24	119.9	8.23
$26N^{\epsilon 1,2}$	112.9	6.80, 7.48	112.9	6.81, 7.48	$54N^{\epsilon 1,2}$	113.2	6.86, 7.54	113.2	6.87, 7.53
Ala-27	122.6	8.28	122.1	8.36	Gln-55	122.8	8.33	122.7	8.32
Ser-28	113.4	8.17	113.6	8.18	$55N^{\epsilon 1,2}$	113.5	6.85, 7.60	113.5	6.85, 7.60
His-29	118.0	8.61	116.8	8.50	Leu-56	130.5	7.97	130.6	7.95
Val-30	120.7	7.68	120.1	7.64					

[a] The atom identity is only indicated for side chain ^{15}N and directly bound ^{1}H resonances; all other given resonances are from the backbone.

mM KP_i at pH 4.5, dissolved in H_2O/D_2O 95/5% (v/v). The NMR samples of [^{15}N]*lac* HP56−11 bp *lac* half-operator complex contained a 1:1 mixture of protein and DNA with a final concentration of 3−4 mM. This complex was buffered in 200 mM KCl, 5% [D_8]glycerol, and 10 mM KP_i at pH 6.0, and was dissolved in H_2O/D_2O 95%/5% (v/v). ^{15}N assignments of the free and DNA-complexed *lac* HP56 were accomplished using 2D SCUBA−NOESY (150 ms mixing time; Brown, 1988) and Clean−TOCSY (60 ms spin-lock time; Griesinger *et al.*, 1988), ^{15}N-HSQC (Bodenhausen & Ruben, 1980), 3D ^{15}N-NOESY−HSQC (150 and 200 ms mixing time), and 3D ^{15}N-TOCSY−HSQC (60 ms mixing time) experiments (Marion *et al.*, 1989). Water suppression was achieved by irradiation during the relaxation delay. Spectral widths were 10 and 30 ppm for the ^{1}H and ^{15}N directions, respectively.

The heteronuclear relaxation experiments were recorded at 500 and 600 MHz. Pulse sequences for the measurement of the ^{15}N T_1, $T_{1\rho}$, and [$^{1}H-^{15}N$] NOE parameters were based on the sequences developed by Kay *et al.* (1989) and Peng and Wagner (1992b) but were modified to incorporate pulsed field gradients for water suppression (Grzesiek & Bax, 1993a,b; Ross *et al.*, 1993) and sensitivity enhancement (Kay *et al.*, 1992; Dayie & Wagner, 1994). Furthermore, the phase cycle of the ^{15}N T_1 experiments was adapted such that the N_z magnetization will decay to zero and not to the "steady state" ^{15}N magnetization (Torchia *et al.*, 1993). This has the advantages that the exponential fitting procedure can be a two-parameter fit and no measurements with long recovery times are needed. Moreover, selection of the side chain Asn N^{δ} and Gln N^{ϵ} protons was performed similarly as described by Boyd (1995).

^{15}N T_1, $T_{1\rho}$, and [$^{1}H-^{15}N$] NOE experiments typically consisted of 64−128 and 1024 complex points in the t_1 and t_2 directions, respectively. The ^{15}N T_1 experiments were performed with recovery times in the range 0.015−1.0 s. The relaxation delays of the ^{15}N $T_{1\rho}$ experiments ranged from 0.01 to 0.35 s, and the cross-relaxation delays for the [$^{1}H-^{15}N$] NOE experiments ranged from 0 to 2.5 s. For all experimental series six to nine data points were recorded and sampled logarithmically; six for the [$^{1}H-^{15}N$] NOE and eight or nine for the T_1 and $T_{1\rho}$ experiments. The 2D heteronuclear spectra that were used for analysis of the relaxation parameters contained 512 ω_1 × 1024 ω_2 data points.

All spectra were processed using the in-house TRITON software package on Silicon Graphics computers. After linear prediction (Zhu & Bax, 1990) a window multiplication using a sine-bell (shifted with $\pi/3$) was performed, followed by Fourier transformation and zero-filling for each direction. Relaxation rates were extracted by fitting the experimentally found peak intensities to single-exponential functions using the van Wijngaarden−Dekker−Brent algorithm (Press *et al.*, 1992). For the [$^{1}H-^{15}N$] NOE data the three-parameter fit was used, and for the ^{15}N T_1 and $T_{1\rho}$ data a two-parameter fit was used. According to the [$^{1}H-^{15}N$] NOE data, the values of η are calculated from the fitted values $I(0)$ and I_∞, corresponding to the equations used by Peng and Wagner (1992a).

RESULTS AND DISCUSSION

Assignment of the ^{15}N resonances

Virtually all ^{15}N resonances in the HSQC spectra of free and 11 bp *lac* half-operator DNA-complexed *lac* HP56 were assigned unambiguously using 2D and 3D NMR methods; this is summarized in Table 1. No NH cross-peak was found for Met-1; residues 3 and 49 are prolines. About 20% of the assignments were different from those reported by Lancelot *et al.* (1992) for *lac* HP51. Three ^{15}N resonances of DNA-bound *lac* HP56 could not be assigned due to extensive line-broadening (Table 1): these are the cross-peaks of the backbone $N^{\alpha}H$'s of Tyr-17, Gln-18, and Ser-31. The ^{1}H resonances of *lac* headpiece in free and DNA-bound states have been assigned earlier (Zuiderweg *et al.*, 1983, 1985; Lamerichs, 1989, 1990).

Measurement of the relaxation parameters

^{15}N T_1, $T_{1\rho}$, and [$^{1}H-^{15}N$] NOE experiments were performed on uniformly ^{15}N-labeled free and DNA bound *lac* HP56 at 500 and 600 MHz. As an example, the difference spectra of the [$^{1}H-^{15}N$] NOE experiment are presented in Figure 2a and b, for free *lac* HP56 and *lac* HP56 in the DNA bound state, respectively. The backbone ^{15}N relaxation parameters of free and DNA-bound *lac* HP56 measured at 500 MHz are given in Figure 3a−f. No results could be given for residues with overlapping cross-peaks or residues for which the low signal-to-noise ratio (due to line-broadening) gave rise to unreliable peak intensities.

According to Figure 3a, c, and e, the secondary structure elements in free *lac* HP56 have a rigid backbone, whereas the loop between helices II and III is more mobile. The backbone at the N- and C-termini is highly dynamic, which is reflected by the much higher T_1 and $T_{1\rho}$ values and the much lower η values. In free *lac* HP56 the N- and C-terminal residues are not part of secondary structure elements or part of the core (Slijper *et al.*, 1996), and this explains the flexibility of this region of the molecule. The results presented in Figure 3a, c, and e also correlate very well with the C_{α} rms deviations found for the set of refined structures of free *lac* HP56 (Figure 3g), demonstrating that the regions of the protein for which the structure is less well defined are actually dynamic.

For *lac* HP56 bound to 11 bp *lac* operator DNA, it can be seen that the T_1 values of the rigid regions are larger (Figure 3a,b) and the $T_{1\rho}$ values are smaller than those of free *lac* HP56 (Figure 3c,d), which is due to the doubling of the molecular weight. According to Figure 3b, d, and f, the DNA-complexed *lac* HP56 also shows rigid secondary structure elements. However, a remarkable decrease in backbone mobility upon binding of *lac* HP56 to the DNA is detected around residue 30, which is located in the loop between helices II and III (Figure 3). Residue His-29 within this loop contacts the *lac* operator DNA in the bound state of *lac* HP56. As a result the complete loop loses its internal mobility and becomes more rigid. For His-29 only the heteronulear NOE value is shown; due to extensive line-broadening the T_1 and $T_{1\rho}$ values could not be measured.

Structural studies have revealed that the conformation of the loop between helices II and III also changes significantly

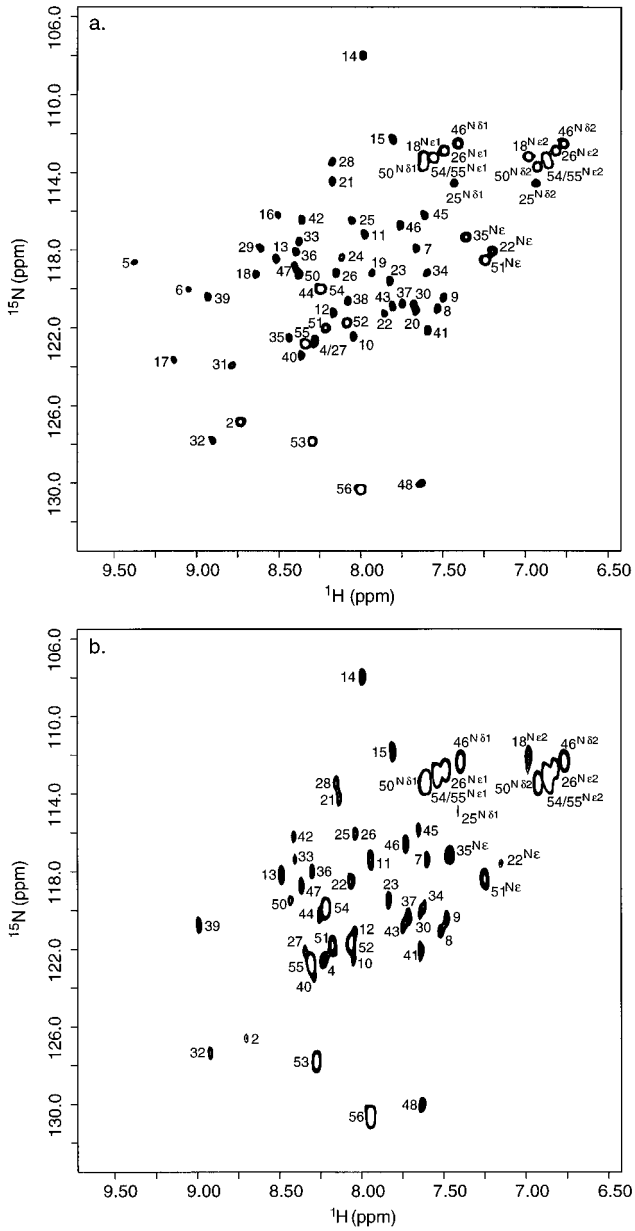

FIGURE 2: Contour plots of the 600 MHz [$^{1}H-^{15}N$] NOE experiments of free (a) and DNA-bound (b) *lac* HP56. Both plots show the difference spectra, *i.e.*, the spectrum of $t = 0$ s was subtracted from the spectrum of $t = 2.5$ s (t is the cross-relaxation time). Several residues with distinct relaxation rates can already be identified from the cross-peak intensity differences; for example Glu-39 (ω_1, ω_2 = 117, 9.0 ppm) is part of the third helix in *lac* HP56, and its backbone is much less mobile than the C-terminal Leu-56 (ω_1, ω_2 = 127, 8.0 ppm).

when the protein binds to operator DNA (Figure 1). Asn-25 and His-29 contact the operator DNA, which is not possible if *lac* HP56 would retain the conformation of the free state. Therefore, the dynamic character of this loop in the free *lac* HP56 is most likely essential for a correct adaptation to the DNA.

The relaxation parameters of most of the ^{15}N-containing side chains in *lac* HP56 were also studied. As an example the results of the $T_{1\rho}$ and the [$^{1}H-^{15}N$] NOE experiments at 600 MHz are presented in Figure 4a,b. The $T_{1\rho}$ and η values of free and DNA complexed *lac* HP56 are indicated with black and hatched boxes, respectively. In free *lac* HP56 all side chain nitrogens are more mobile than the backbone nitrogens. Especially the residues at the C-terminal end of

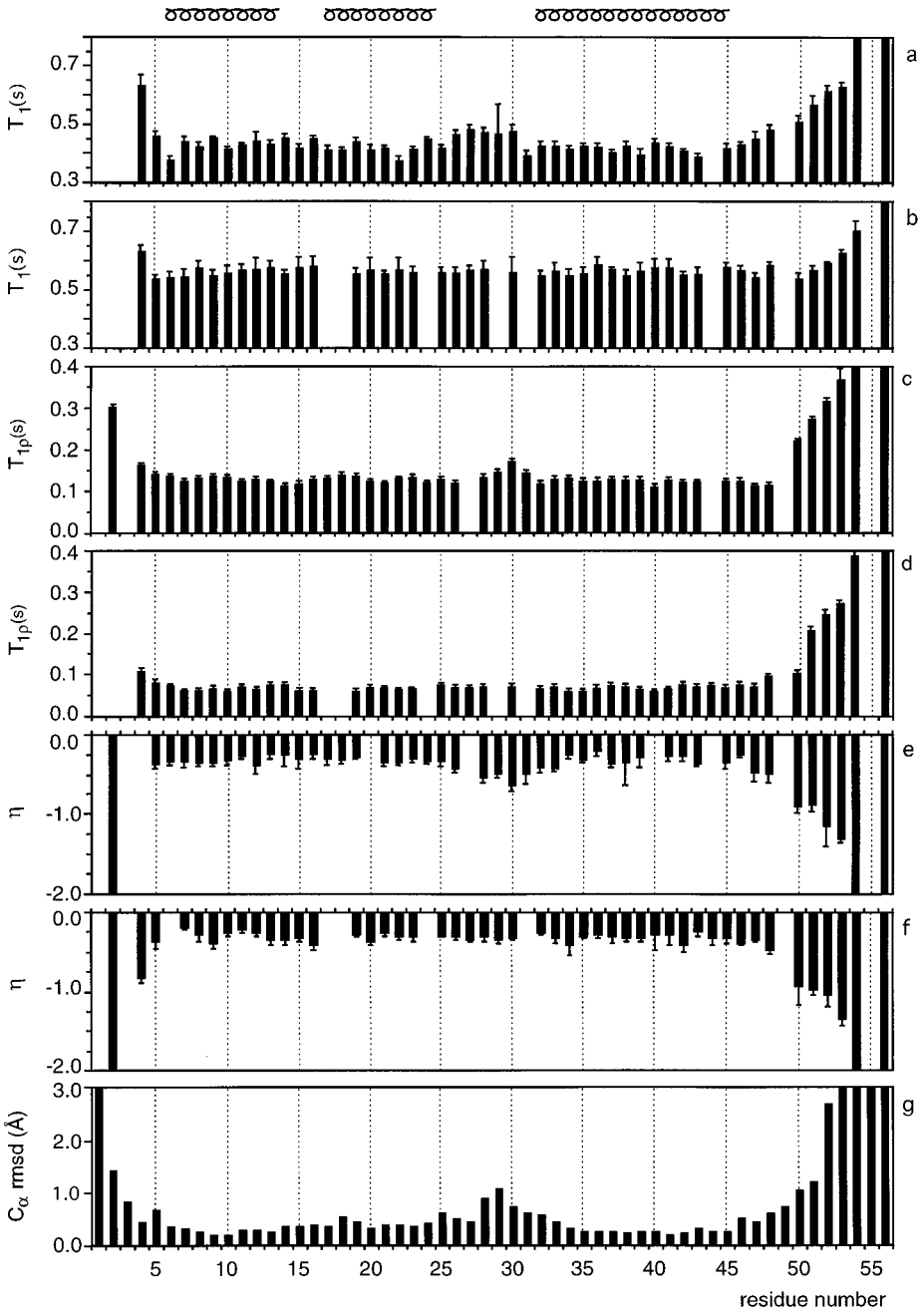

FIGURE 3: Bar graphs of the ^{15}N T_1 (a,b), the ^{15}N $T_{1\rho}$ (c,d), [^{1}H−^{15}N] NOE (e,f), and the Cα rms deviations of free *lac* HP56 (g). The relaxation experiments were performed at 500 MHz. Relaxation parameters for free *lac* HP56 are given in a, c, and e and for DNA-bound *lac* HP56 in b, d, and f. For the results of the [^{1}H−^{15}N] NOE experiments values of η [according to the definitions given by Peng and Wagner (1992a)] are given. On top of the figure the locations of the α-helices in *lac* HP56 are indicated.

the protein and residues with long side chains (*e.g.*, arginines 22, 35, and 51 N^{ϵ}) show high flexibility.

The side chains of some of the DNA-contacting residues in *lac* HP56, Gln-18, Arg-22, Asn-25, Gln-26, Asn-50, and Arg-51, could also be analyzed (Lehming *et al.*, 1988; Sartorius *et al.*, 1991; Chuprina *et al.*, 1993). According to Figure 4, the Gln-18 and Arg-22 side chains show a significant decrease in mobility upon DNA binding, whereas the Asn-25, Gln-26, Asn-50, and Arg-51 side chains are about equally mobile in both states of the protein. The residues Arg-35, Asn-46, and Gln-55 have not been shown to interact with the DNA, and when comparing both states of the *lac* HP56 no change in side chain dynamics is found other than the overall change due to the increase of the molecular weight of the system. The side chain of residue Asn-25 is rather immobile in both the free and the DNA-bound *lac* HP56 and is presumably involved in intra- and intermolecular interactions, respectively. In contrast, the side chains of Gln-26, Asn-50, and Arg-51 remain highly mobile in the complexed *lac* HP56, although for each residue to a different extent. This seems to be correlated with the length of the side chain and the location within the protein; *e.g.*, Arg-51 has a long side chain and is located at the flexible C-terminus. Furthermore, from the η values (Figure 4b) it can be seen that all side chains of the protein which interact with the DNA are still more dynamic than the rigid parts of the backbone of the DNA-complexed *lac* HP56. These results indicate that interactions with the DNA do not necessarily restrict the mobility of the side chains in *lac* HP56; dynamical processes still occur at the protein−DNA interface. Residual mobility in the protein−DNA interface was also suggested by the results of Chuprina *et al.* (1993),

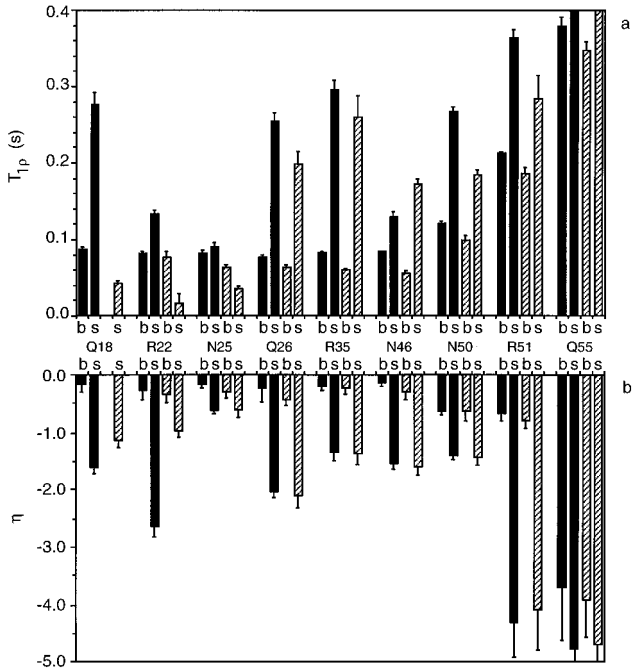

FIGURE 4: Backbone and side chain relaxation parameters of the ^{15}N $T_{1\rho}$ (a) and the [$^1H-^{15}N$] NOE (b) experiments at 600 MHz for the free (black bars) and the DNA-bound (hatched bars) *lac* HP56. The backbone and side chain parameters are indicated with "b" and "s", respectively. For Asn, "side chain" refers to the N^{δ}; for Gln and Arg, this refers to N^{ϵ}. The Gln-18 backbone amide relaxation values of the complexed *lac* HP56 are not given because the cross-peaks were absent due to extensive line-broadening.

who found that several hydrogen bonding patterns between *lac* headpiece side chains and the DNA were possible with similar energies.

Furthermore, it is known that of these three NMR parameters (T_1, $T_{1\rho}$, and [$^1H-^{15}N$] NOE), which are all sensitive to internal motions, the ^{15}N $T_{1\rho}$ values can also be affected by chemical exchange processes. When the parameters of the free and DNA-bound *lac* HP56 are compared, it can be seen from Figure 4 that the Gln-18, Arg-22, and Asn-25 side chain ^{15}N $T_{1\rho}$ values are more affected than the η values. Indeed, intermediate exchange is found for the side chains of these residues (Figure 2), which may indicate that these atoms are involved in hydrogen bonding, interchanging in time. This hydrogen bonding was detected for the $N^{\epsilon}-H^{\epsilon}$ and $N^{\delta}-H^{\delta}$ atoms of the residues Gln-18 and Asn-25, respectively. Moreover, in the RMD simulations several specific hydrogen bonds have been observed for the Arg-22 side chain which alternated in time (Chuprina *et al.*, 1993).

These results suggest that flexibility of the regions within the protein that are important for complexation, i.e., the loop and the DNA-contacting side chains, is essential for a correct fit onto the DNA. However, the additional mobility remaining in all DNA-contacting side chains indicates that protein−DNA recognition is a dynamic and not a static process.

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