Direct Observation of a Specific Contact in the λ Repressor–O_L1 Complex by Isotope-Edited NMR

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X-ray crystallographic studies have suggested that hydrogen-bonding interactions between DNA bases and amino acid residues are a dominant factor in determining specificity in protein–DNA interactions.[1] However, in many instances, the presence or absence of such hydrogen-bonding contacts cannot be assigned unambiguously on the basis of X-ray data alone. Here, we describe the development of a general technique for probing hydrogen-bonding interactions in protein–DNA complexes and its application to resolving uncertainties regarding contacts in the λ repressor–operator complex.

X-ray studies[2,3] have shown that λ repressor binds its specific 17-base-pair operator sites as a dimer, with each subunit making symmetry-related contacts to the pseudodyad operator (Figure 1).[4] Many of the base contacts made by λ repressor involve amino acid residues that are part of a helix–turn–helix (HTH) structural motif.[1] The recent 1.8-Å X-ray structure of the λ repressor–O_L1 complex[3] revealed, in addition to the HTH contacts, the extensive interaction between the six residues of the protein's N-terminus—the "N-terminal arm"—and base-pairs 6–9 of the consensus[4] operator half-site. In the X-ray studies, electron density for the N-terminal arm is visible only in the consensus half-site, suggesting that either (i) the nonconsensus arm, while ordinarily bound in solution, was not bound in the crystal due to the particular conditions of crystallization or (ii) the nonconsensus arm binds DNA weakly or not at all. Although NMR studies have indicated that the N-terminal arm is flexible in solution,[5] numerous studies have indicated the importance of the arm in the repressor–operator interaction.[6–8]

NMR represents an alternative method for analyzing hydrogen-bonding interactions in the λ–O_L1 complex. X-ray data and interference studies suggest that the exocyclic amino group of C-8 is involved in a hydrogen-bonding interaction with a main-chain carbonyl of the consensus N-terminal arm. Comparison of the chemical shift changes observed for the exocyclic N–H of C-8 and C-8′ could resolve the issue of whether the N-terminal arm contacts the nonconsensus operator half-site.[9] Although the ^1H spectrum of the λ–O_L complex is expected to be too complicated for simple analysis, isotope-edited NMR could be used, in principle, to examine only the ^1H resonances of interest; this was accomplished in the present case by ^{15}N labeling of the exocyclic amines of C-8 and C-8′ in the O_L1 operator.[10,11]

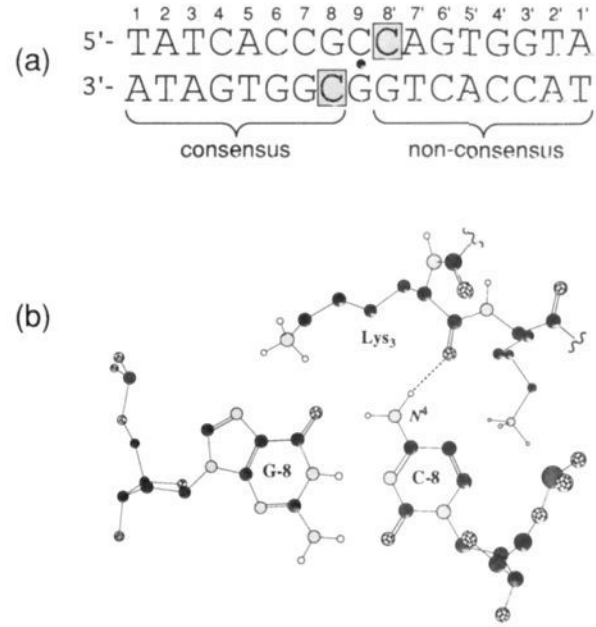

Figure 1. (a) The O_L1 operator sequence showing the symmetry-related C-8 and C-8′ base pairs. The dot represents the center of pseudodyad symmetry. (b) Detail from the X-ray structure[3] of the λ–O_L1 complex showing the proposed hydrogen bond between the main-chain carbonyl of Lys_3 and the exocyclic amine of C-8.

Two 21-mer oligonucleotides containing the O_L1 site were site-specifically labeled with ^{15}N at the C-8 and C-8′ positions, respectively,[12,13] via the convertible nucleoside approach (Figure 1).[11,14] These were annealed to the complementary, nonmodified strands to furnish two duplex 21-mer oligonucleotides which differed only in the position of their ^{15}N label within the O_L1 site.

The 5–9-ppm region of the unedited ^1H NMR spectra[15] for these oligonucleotides (Figure 2 and supplementary material) showed a multitude of resonances arising from the nonexchangeable base protons and the amino protons of dC and dA residues.

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(12) The sequences of the labeled oligonucleotides are 5′-d(AATAC-CACTGGCGGTGATATA)-3′ and 5′-d(TATATCACCGCCAGTGGTATT)-3′, in which C = 4-^{15}N-dC.

(13) Detailed experimental procedures and additional spectra are available in the supplementary material, which may be obtained directly from the authors by FAX [(617) 495-8755].

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(15) NMR sample preparation: all samples were ~1 mM in double-stranded oligonucleotide (with 10% excess unlabeled strand) in NMR buffer (50 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM Tris–HCl, pH 8, 10% D_2O). Samples containing a ~10% excess of λ_{1-102} were concentrated by centrifugation (Centricon-3) in NMR buffer. NMR spectroscopy: NMR spectra were recorded on a Bruker AM-500 instrument equipped with an inverse probe. All experiments were performed at 298 K. In all cases, the ^1H and ^{15}N sweep widths were 10 000 and 4000 Hz, respectively, with the transmitter offset by −14 800 Hz for ^{15}N. Unedited ^1H spectra were recorded using a simple H_2O presaturation in inverse mode. ^{15}N-edited ^1H spectra were recorded in one dimension utilizing the SQC experiment and collecting $2K$ data points. All chemical shifts are referenced to external TMS–propionate (Aldrich) in NMR buffer.

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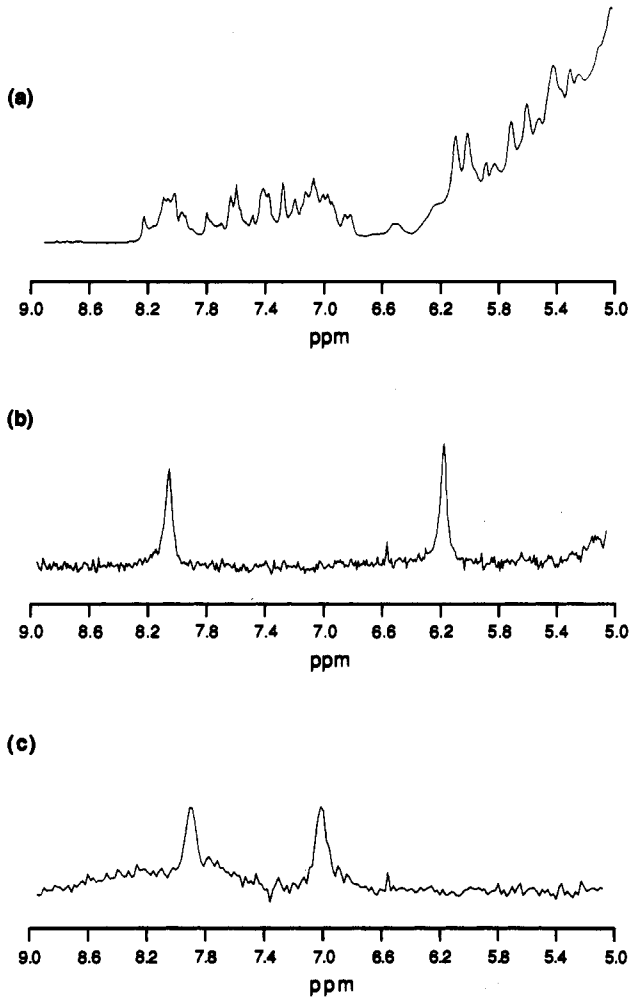

Figure 2. NMR spectra of the consensus-labeled oligonucleotide: (a) ^{1}H NMR spectrum; (b) ^{15}N-edited ^{1}H NMR spectrum; (c) ^{15}N-edited ^{1}H NMR in the presence of λ_{1-102}.

In contrast, the ^{15}N-edited ^{1}H NMR spectrum[16] of the double-stranded oligonucleotides showed only the two protons attached to each ^{15}N (Table I; Figure 2 and supplementary material); the resonances for the Watson–Crick base-paired (WC) protons were observed downfield with respect to the non-base-paired (non-WC) protons.[17]

For the binding studies, we chose to examine an N-terminal DNA-binding fragment of λ repressor, λ_{1-102}, which comprises the first 102 amino acids of the protein.[18] This fragment binds the O_R1 operator site with a K_d of ~0.5 μM and is slightly larger than λ_{1-92}, the fragment used in X-ray studies.

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Table I. Chemical Shifts Observed in the ^{15}N-Edited ^{1}H NMR Spectra of the C-8 (Consensus) and C-8′ (Nonconsensus) ^{15}N-Labeled Oligonucleotides and Their Complexes with λ_{1-102}[a]

	consensus		nonconsensus	
	WC	non-WC	WC	non-WC
free oligonucleotide	8.14	6.21	8.45	6.70
λ_{1-102} complex	7.97	7.06	8.56	6.75
change in δ	–0.17	0.85	0.11	0.05

[a] Chemical shifts are in ppm with respect to TMS–propionate.

Binding of λ_{1-102} to the consensus-labeled oligonucleotide caused the non-WC proton to shift 0.85 ppm downfield, while the WC proton shifted 0.2 ppm upfield (Table I; Figure 2). The 0.85-ppm downfield shift observed for the C-8 WC proton agrees well with the reported 0.87-ppm downfield shift observed for the formation of a WC base-pair with a cytidine residue.[19,20] The observed shifts at C-8 are consistent with a change in the hydrogen-bonding environment of the non-WC proton which presumably results from exchange of the hydrogen-bond acceptor from water to the main-chain carbonyl of Lys_3 (Figure 1). Although similar spectra were recorded for both labeled oligonucleotides alone, there was little change in the chemical shifts of the C-8′ N^4 protons upon binding of the protein (Table I).[13] The lack of a change at C-8′ indicates that this base does not interact with the nonconsensus arm.

A rationale for these observations lies in the obligate asymmetry of O_L1 at base-pair 9, which interacts with Lys_3 of the consensus arm. Since base-pair 9 is not C_2 symmetric, the consensus and nonconsensus arms "see" different contacts at this site; presumably the interaction of base-pair 9 with the consensus arm is matched, whereas that with the nonconsensus arm is mismatched.

The methodology described here may be extended to the labeling of the exocyclic amines of dA[21] and dG[22] residues within DNA and C, A, and G residues in RNA and should serve as a useful tool in the elucidation of nucleic acid–protein and nucleic acid–nucleic acid interactions.[23]

Supplementary Material Available: Experimental procedures for the isotopic labeling of the oligonucleotides along with ^{1}H NMR and ^{15}N-edited ^{1}H NMR spectra of the nonconsensus labeled oligonucleotide (6 pages). Ordering information is available on any current masthead page.

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