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Photo-CIDNP study of the interaction between *lac* repressor headpiece and *lac* operator DNA

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Lac repressor headpiece (HP) and intact *lac* repressor have been studied using the photo-CIDNP method. At neutral pH histidine 29, tyrosines 7, 12 and 17 and methionine 1 are polarised. His-29 polarisations are weaker and broader in HP59 than in HP51 indicating that the C-terminal octapeptide in HP59 adopts a conformation that allows an interaction with His-29. The photo-CIDNP spectra of intact *lac* repressor and HP51 are very similar, showing that the same residues are accessible to the photo-excited flavin. An equimolar mixture of HP51 and a 14 base pair *lac* operator fragment strongly suppresses the photo-CIDNP effect of tyrosines 7 and 17 and abolishes the His-29 polarisations. The results are compared with earlier photo-CIDNP measurements on a complex of headpiece with poly[d(AT)] and with a model derived from a 2D NMR study on a *lac* headpiece-operator complex.

Lac repressor; *Lac* headpiece; *Lac* operator; *Lac* headpiece-operator complex; Photo-CIDNP; ^{1}H-NMR

1. INTRODUCTION

Regulation of gene expression involves the recognition of specific DNA sequences by proteins. In spite of much recent progress in structure determination of DNA-binding proteins by X-ray crystallography and by NMR spectroscopy a detailed theory of protein-DNA recognition is still lacking. For the *lac* repressor of *Escherichia coli*, the determinants for operator recognition presumably reside in the N-terminal domain or headpiece [1,2]. Using 2D NMR methods, the spatial structure of this domain (HP51) was solved [3,4]. Furthermore, based on the observation of NOEs between protein and DNA, a preliminary model of the complex of *lac* headpiece with a 14 bp *lac* operator was proposed [5]. The most striking feature of this model was that the second α-helix of the helix-turn-helix motif of *lac* headpiece binds in the major groove of DNA in an orientation opposite to that found for other repressors [6].

Here we present a photo-CIDNP study of HP51 and HP59 and a comparison with the intact *lac* repressor. Furthermore, the interaction of HP51 with a 14 bp *lac* operator fragment is studied. In the photo-CIDNP method nuclear spin polarisation is created via the interaction of a photo-excited dye with the substrate. Enhanced NMR absorption or emission is observed for a number of amino acid residues (His, Tyr, Trp, Met) if they are accessible to the dye ([7] and Stob and Kaptein, to be published). Using the photo-CIDNP method it was shown previously that, when *lac* headpiece binds non-specifically to poly[d(AT)], the polarisation for His-29 and tyrosines 7 and 17 is quenched, which implies that these residues are in the DNA-binding site [8]. In this study we probe the specific

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Abbreviations: HP51, N-terminal 51 amino acids of *lac* repressor; HP59, N-terminal 59 amino acids of *lac* repressor; FI, *N*-3-carboxymethyl lumiflavin; IPTG, 1-methylethyl 1-thio-β-D-galactopyranoside; ONPF, 2-nitrophenyl-β-D-fucoside; CIDNP, chemically induced dynamic nuclear polarisation; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect

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interaction of headpiece with *lac* operator fragments.

2. MATERIALS AND METHODS

^{1}H photo-CIDNP experiments were performed on a Bruker HX-360 NMR spectrometer as described before [7], except that the light from an argon-ion laser was introduced into the NMR probe using an optical fiber. Difference spectra were obtained by alternately recording 'light' and 'dark' free induction decays (FIDs) and subtracting the resulting spectra. The 'light' FID was acquired after irradiation of the sample for 0.60 s with the argon-ion laser beam prior to data sampling. Chemical shifts were measured relative to internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), from Merck, Sharp and Dohme. All pH values represent uncorrected meter readings.

Lac repressor was isolated and HP51 and HP59 were prepared as described [9,10]. The conditions for the experiments are given in the figure legends. Flavin I was a generous gift of Dr F. Müller (Sandoz, Basel). A 14 bp operator-DNA fragment dGGAATTGTGAGCGG·dCCG CTCACAATTCC was obtained from Dr J.H. van Boom (University of Leiden). IPTG and ONPF were obtained from Sigma. All compounds were dissolved in ^2H_2O from Merck, Sharp and Dohme.

3. RESULTS AND DISCUSSION

3.1. *HP51*

The aromatic region of the photo-CIDNP difference spectrum of HP51 (fig.1) is characterised

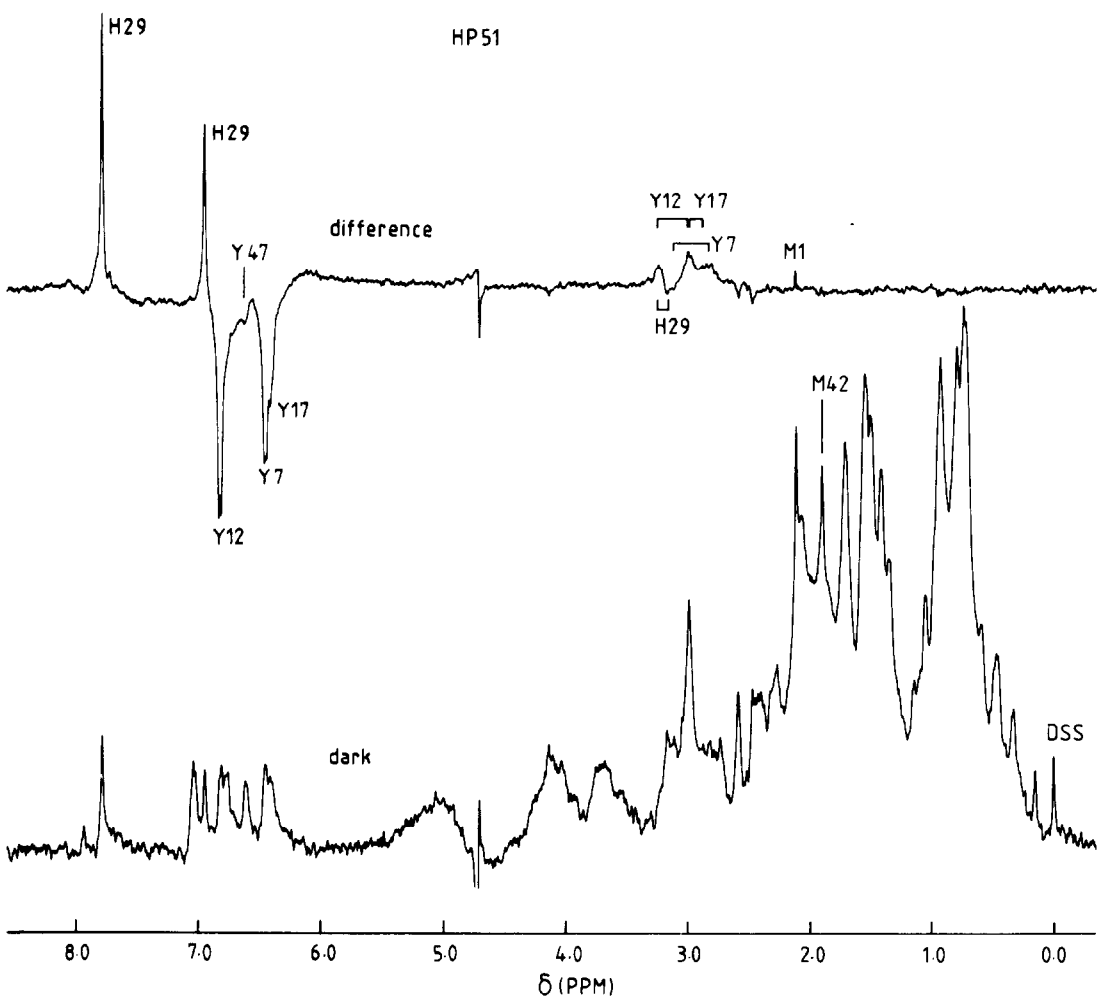

Fig.1. Photo-CIDNP difference spectrum of HP51 (top) obtained with a light pulse of 0.3 s and 5 W laser power (16 scans). The dark spectrum is shown below. The sample contained 50 mM $K^2H_2PO_4$, 0.2 M KCl, 0.4 mM FI and 0.5–0.6 mM HP51 in ^2H_2O and the pH was 7.40. Peak assignments according to [11,15,16] are indicated. H, histidine; Y, tyrosine; M, methionine.

by the polarised C2-H and C4-H proton resonances of His-29 and the emission lines of the ϵ ring protons of tyrosines 7, 12 and 17. Tyr-47 is only weakly polarised and probably much less accessible to the flavin dye. The β-protons of tyrosines 7, 12 and 17 and of His-29 resonating around 3 ppm have been assigned using the data of [11]. Methionine shows only weak CIDNP effects compared to His, Trp and Tyr as was recently shown for the free amino acid (Stob and Kaptein, to be published). Nevertheless it is interesting to note that the methyl group of Met-1 is positively enhanced in fig.1, while that of Met-42 is not polarised and therefore probably inaccessible to the dye.

3.2. *HP59*

The photo-CIDNP difference spectrum of HP59 (fig.2b) is similar to that of HP51 except for the low intensities of the His-29 absorptions. The His-29 lines are also broadened as can be seen from the dark spectrum (fig.2a). Upon prolonged irradiation (16 scans, fig.2c), the His-29 lines become sharper and show more intense CIDNP effects. This somewhat unusual observation must be related to the presence of the amino acid residues 52–59. The conformation of this octapeptide region in HP59 is probably such that a labile interaction exists with His-29 (possibly with the C-terminal carboxyl group) that decreases the accessibility of His-29. This interaction, however, is disrupted upon prolonged laser irradiation, possibly due to a small temperature increase of the sample.

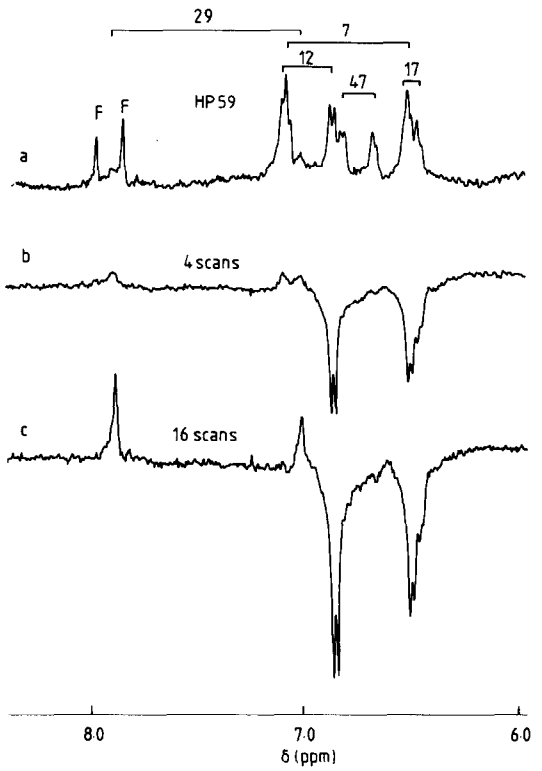

Fig.2. Photo-CIDNP difference spectra of HP59 obtained with a light pulse of 0.3 s and 5 W laser power. The sample contained 50 mM $K^2H_2PO_4$ and 0.4 mM FI in ^2H_2O and 0.5–0.6 mM HP59; (a) dark spectrum, 64 scans; (b) photo-CIDNP difference spectrum, 4 scans and (c) 16 scans.

3.3. Lac *repressor*

Previously it had been shown [12,13] that the aromatic resonances of the headpiece in the NMR spectrum of whole repressor are much sharper than those of the core and have the same chemical shift positions as those of the free headpiece. This was interpreted as showing that headpiece constitutes a separate domain connected to the core with a flexible hinge region, which gives it a higher mobility than the rest of the molecule. Our interpretation of the CIDNP effects of intact *lac* repressor (fig.3) is based on these results. Thus, the polarised signals that have a relatively narrow linewidth are assigned to His-29, tyrosines 7, 12 and 17 and Met-1. The relative CIDNP intensities are somewhat different from those of free headpiece with the polarisation of His-29 being stronger and that of tyrosines 7 and 17 weaker. The accessibility of His-29 and tyrosines 7 and 17 is not surprising, since they are part of the DNA-binding site. The CIDNP effect observed for Tyr-12 indicates that access to this residue is not blocked by the repressor core or the connecting hinge. The strong polarisation of His-29 indicates that the conformation of the 52–59 peptide that blockes His-29 CIDNP in HP59 is not present in the intact *lac* repressor.

The CIDNP spectrum of *lac* repressor does not change in the presence of the inducer IPTG or the anti-inducer ONPF (not shown). Thus, CIDNP is not sensitive to possible conformational changes induced by these molecules.

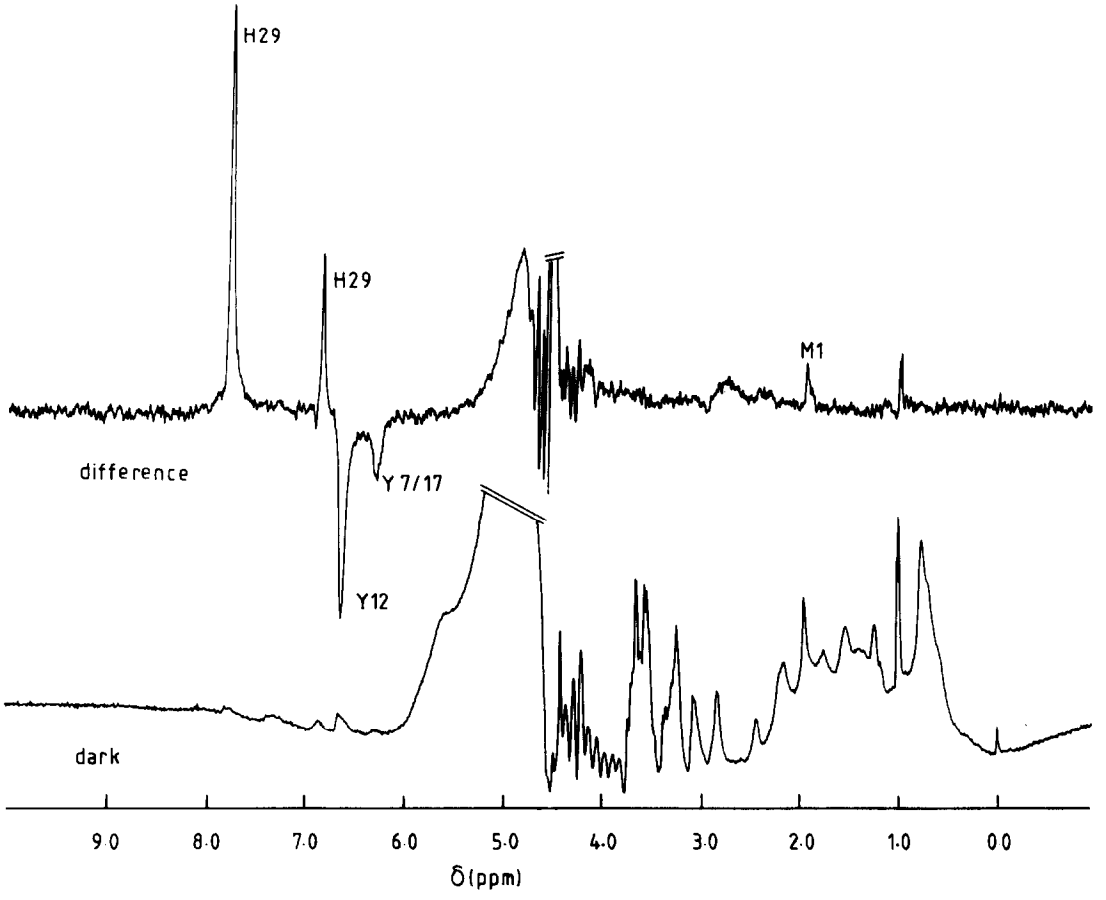

Fig.3. Photo-CIDNP difference and dark spectrum of the *lac* repressor. The difference spectrum was obtained by accumulating 32 light-dark cycles. Between two successive cycles, the sample tube was mixed, to prevent exhaustion of the dye, by increasing the spinner rate, causing a vortex in the sample. The dark spectrum resulted from 1366 scans and the application of an exponential multiplication causing a linebroadening of 5 Hz. The sample contained 0.2 mM *lac* repressor, 0.4 mM FI in ^2H_2O and the pH was 7.0.

3.4. *HP51*-lac *operator complex*

Fig.4 shows the behaviour of the photo-CIDNP effect of HP51 in the presence of a 14 bp *lac* operator half site dGGAATTGTGAGCGG·dC CGCTCACAATTCC. Increasing amounts of operator suppress the polarisation of His-29 and tyrosines 7 and 17, while that of Tyr-12 remains strong (fig.4b,c). Under conditions of high ionic strength the binding between headpiece and operator is disrupted. Consequently, the photo-CIDNP response for His-29 and tyrosines 7 and 17 is restored in the presence of 1.0 M KCl (fig.4d), although the broadening of the His resonances indicates that some residual binding still occurs.

The simplest interpretation of these results is that access of the flavin dye to the side chains of His-29 and tyrosines 7 and 17 is blocked by the presence of DNA while Tyr-12 remains freely accessible. These observations are very similar to the results of a previous photo-CIDNP study of *lac* headpiece with poly[d(AT)] [8], where the same suppression of polarisation of His-29, Tyr-7 and Tyr-17 was found. The binding of *lac* repressor with non-operator DNA is known to be weaker and involves a different binding mode with more ionic contacts than the specific binding to *lac* operator. Thus, it is estimated that 11 ionic contacts are made in non-specific binding, while only 8 ionic contacts are present in the complex with *lac* operator [14]. Yet, the present results show that

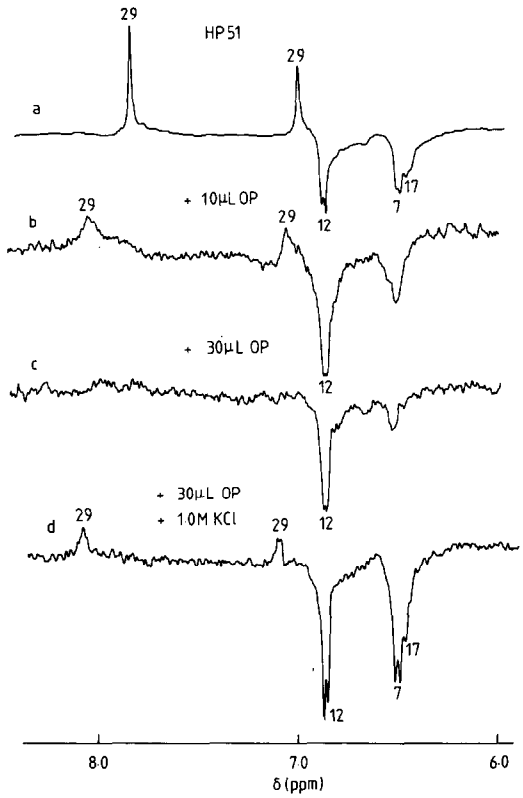

Fig.4. Photo-CIDNP difference spectra of HP51, obtained with a light pulse of 0.3 s and 5 W laser power with varying amounts of operator DNA. The sample was made from 50 mM $K^2H_2PO_4$, 0.4 mM FI and 0.5–0.6 mM HP51 in ^2H_2O. (a) HP51 alone; (b) HP51 and 10 μl operator DNA (0.2 mM); (c) HP51 and 30 μl operator DNA (0.6 mM DNA, pH 7.16); (d) HP51, 30 μl operator DNA and 1.0 M KCl (pH 6.98). For all spectra 16 scans were recorded.

the same side of the headpiece including residues His-29, Tyr-7 and Tyr-17 is interacting with DNA in both specific and non-specific DNA binding.

The present photo-CIDNP results are in complete agreement with the model of the *lac* headpiece-operator complex derived from 2D NOE measurements [5]. This model is shown in fig.5 with the CIDNP-sensitive residues indicated. His-29 is interacting with the phosphate backbone as can be concluded from a pK_a increase of 0.5 unit in the presence of *lac* operator [10] and several NOEs to DNA protons [5]. Tyr-17 probably makes a functional contact with the methyl group of the thymine of base pair AT8 as evidenced by a strong NOE [5]. The side-chain protons of Tyr-7 also

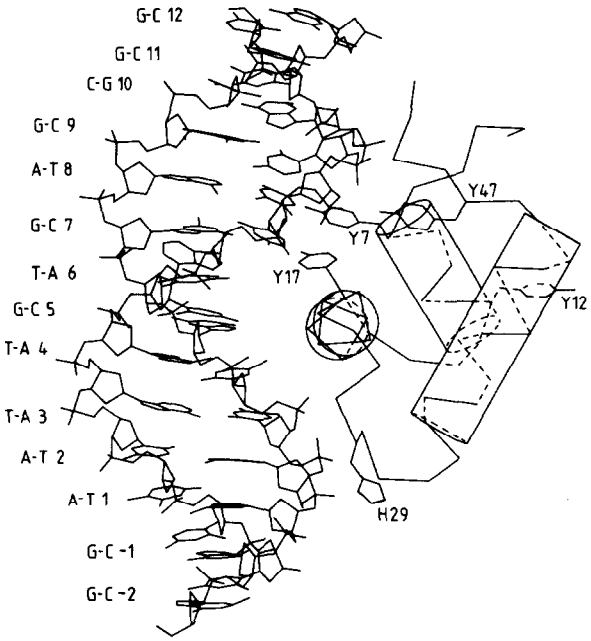

Fig.5. Model of the HP51-*lac* operator complex derived from 2D NOE measurements of a complex between HP56 and *lac* operator [5]. The operator is present in standard B-DNA conformation [17]. The headpiece conformation was taken from [4]. The structure of the complex was further refined by restrained MD calculations (De Vlieg et al., personal communication).

show several NOEs with protons on the DNA, although its functional role in base pair recognition is not yet known. It can be clearly seen in fig.5 that the side chain of Tyr-12 points away from the DNA, which accounts for its accessibility in the complex.

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