ON THE INTERACTION OF THE *lac* REPRESSOR HEADPIECE WITH NUCLEIC ACIDS

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1. Introduction

The *lac* repressor protein has been extensively studied in an attempt to understand the interactions which allow recognition of and extremely tight binding to the *lac* operator region of DNA [1]. Genetic experiments have suggested that an important DNA binding region is in the N-terminal region of the repressor, e. g., changes of tyrosine 17 and to a lesser extent tyrosine 7 result in loss of DNA binding capability. A 51 amino acid headpiece (HP) can be cleaved from the repressor's N-terminus [2]. This fragment retains the capability to bind both specifically to operator DNA and non-specifically, though the binding constants are reduced from those for the intact repressor [3]. NMR experiments have shown that this headpiece exists as a mobile domain in the intact repressor which has the same structure as the isolated peptide [4,5]. NMR has also shown that this segment has extensive secondary and perhaps tertiary structure [6]. Photo-CIDNP experiments on HP have shown that tyrosines 7 and 17 and histidine 29 have reduced accessibility for reaction with the flavin dye when DNA is bound [7]. We have examined the binding of HP to a short synthetic DNA segment by high resolution NMR in both H_2O and ^2H_2O solution.

2. Experimental

The 51 amino acid *lac* repressor headpiece (HP) was prepared as in [2]. Poly(dA) and poly(dT) with lengths of 12–18 basepairs were purchased from Collaborative Research. These were purified using a DEAE–Sephadex column then renatured to form poly[d(A · T)]. All experiments were performed using H_2O or ^2H_2O with 5 mM $MgCl_2$, and 2 mM Tris at pH 7.0. In this buffer the midpoint of the DNA melting transition, monitored optically, was 33°C. Binding of HP raised the helix–coil transition of poly[d(A · T)] by ~5°C. To ensure complete binding for the NMR experiments the nucleic acid was present in excess, $[HP]/[d(A \cdot T)_{15}] = 0.3$ and maintained at 7°C. The complex could be dissociated at will by addition of 0.5 M NaCl. HP was ~0.1 mM.

NMR spectra were taken on the modified Bruker HXS-360 spectrometer at the Stanford Magnetic Resonance Laboratory. Spectra in ^2H_2O solution were taken using normal FT methods with a 70° pulse and delay of 2.84 s between pulses. Spectra in H_2O solution were taken using the Redfield 21412 method [8] with an offset of 2628 Hz from water and a delay of 2.84 s between pulses. Spectra were taken of HP, poly[d(A · T)], the HP–[d(A · T)] complex and salt-dissociated complex.

3. Results and discussion

Several spectral changes are observed upon addition of poly[d(A · T)] to a HP solution. The most obvious of these is a significant broadening of all HP lines, due to the increase in 'size' of the protein and the concomitant increase in rotational correlation time. This effect is evidence of complex formation and is reversed upon addition of 0.5 M salt, which dissociates the complex. Although broadened, no significant shifts of the aromatic HP resonances are observed. This is consistent with [7], and indicates that full intercalation of the tyrosines into the DNA helix does not occur, at least for this non-specific binding, since intercalation should lead to strong upfield shifts due to ring currents from the DNA basepairs.

The exchangeable resonances of HP and DNA observable in H_2O solution provide further probes of the interaction between protein and nucleic acid. Since these resonances stem from protons which are necessarily involved in hydrogen bonds of the secondary

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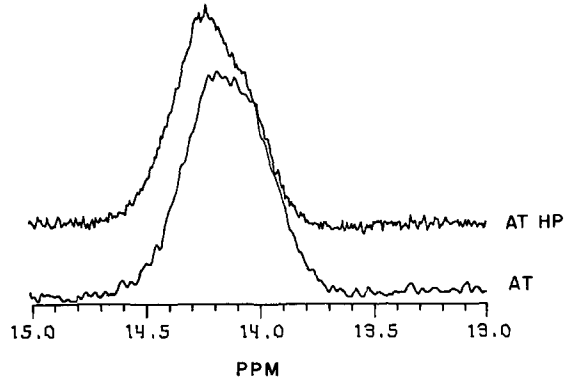

Fig.1. NMR spectra in H_2O of the hydrogen-bonded protons of poly[d(A · T)] alone and the HP–poly[d(A · T)] complex (ratio 0.3).

structure, they provide a probe of this structure. The resonances from protons in the hydrogen bonds of the nucleic acids are shifted strongly downfield, to ~13 ppm. Fig.1 shows this region for poly[d(A · T)] alone and poly[d(A · T)]–HP complex at a ratio of 3.3. Although the nucleic acid is present in excess, separate lines are not seen for bound and unbound species, rather only a small shift of the line is observed. This may arise from small shifts upon binding and/or rapid exchange between bound and unbound forms. The area of this peak is conserved upon binding, a good indication that binding does not disturb the double-helical DNA structure. Disruption of this structure should lead to large shifts and/or loss of protons in the resonance due to rapid exchange with solvent.

Resonances from the amide protons of HP are observable in the 8–10 ppm region. For HP alone many resonances are observed in this region (fig.2), and upon DNA binding, shifts are observed for many of these resonances and there is a large loss of intensity near 8 ppm. These changes represent a change in structure of some segment of the HP or a rather direct binding of the DNA to it. However, since assignments of the amide resonances have not yet been made, the segment involved cannot be identified. Due to interference of the DNA peaks it cannot be determined if these resonances have been shifted upfield, or if they are lost through increased rate of exchange with solvent.

From these studies one may reach several conclusions about the binding of the *lac* repressor headpiece to poly[d(A · T)]:

(1) The tyrosines of HP do not fully intercalate into the DNA helix;

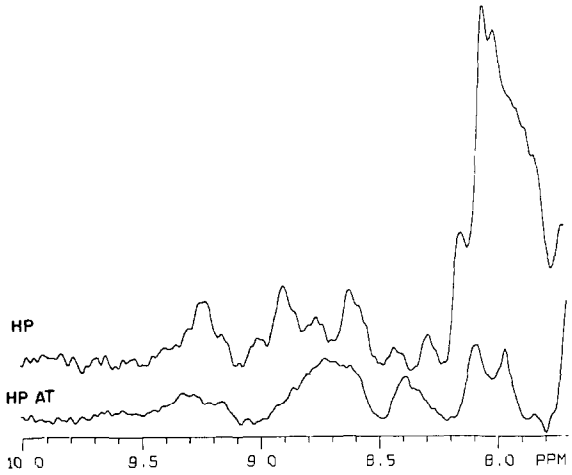

Fig.2. NMR spectra in H_2O solution of the amide protons of HP alone and the HP–poly[d(A · T)] complex (ratio 0.3).

(2) The double helical DNA structure is not strongly perturbed by binding;

(3) There is a rearrangement of some segment of the HP upon binding, or a direct binding of DNA near several hydrogen-bonded protons in the backbone.

As assignments of amide residues become available the changes can be interpreted more specifically in terms of molecular structure.

Acknowledgements

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