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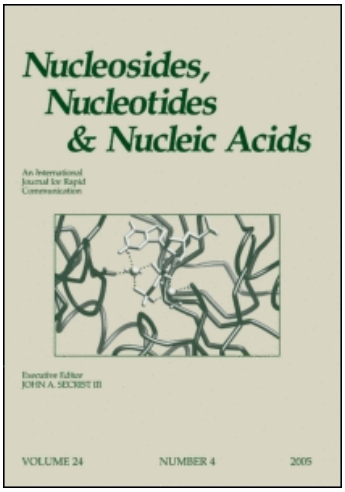


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# NMR Investigation of Palindromic LAC Operator DNA Sequences of Varying Size: Influences of Complex Formation with the LAC Repressor Headpiece on the DNA $^1$H Resonances

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# NMR INVESTIGATION OF PALINDROMIC *LAC* OPERATOR DNA SEQUENCES OF VARYING SIZE: INFLUENCES OF COMPLEX FORMATION WITH THE *LAC* REPRESSOR HEADPIECE ON THE DNA $^{1}H$ RESONANCES

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**Abstract.** $^{1}H$-NMR studies on symmetrical *lac* operators were performed to determine the minimum size of a *lac* operator for tight complex formation with the *lac* repressor headpiece. Four operators of varying size from 18 base pairs to 24 base pairs were chemically synthesized. The data obtained suggest that for a synthetic *lac* operator to form a tight complex with the *lac* headpiece it should be at least 22 base pairs long.

**Introduction.** The minimum size of an oligonucleotide required for the formation of a specific protein DNA complex is an important parameter in biophysical studies of protein DNA interactions. Owing to steric effects, the area protected by a specific binding protein against nuclease digestion is generally larger than the sequence element within which mutations (1) and chemical modifications of the DNA can be shown to affect the affinity of the protein. Since the formation of a specific protein-DNA complex is usually accompanied by changes in the imino proton NMR resonances we used this effect in order to determine the minimum size of a symmetric operator required for an "intact" specific complex with *lac* repressor headpiece. The *lac* repressor is a tetrameric protein which controls the transcription of the structural genes Z, Y, and A in *E. coli*. The *lac* repressor headpiece is the DNA-binding domain of the *lac* repressor. It was shown in previous studies that the structure of the isolated headpiece stays intact (2). A headpiece of a length of 51, 56, or 59 amino acids is obtained depending on the protease used for the digestion of the repressor. Binding of the *lac* repressor protects about 27 base pairs (bp) against nuclease digestion (3), while for an operator core sequence as short as 17 bp specific binding has been demonstrated (4). The symmetric operator sequence used in this study has a higher affinity for the *lac* repressor than the wild type operator (5).



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```
  -2          +10
5'-GGAATTGTGAGCGCTCACAATTCC-3'          24 bp
3'-CCTTAACACTCGCGAGAGTTAAGG-5'

  -1          +10
5'-GAATTGTGAGCGCTCACAATTC-3'            22 bp
3'-CTTAACACTCGCGAGAGTTAAG-5'

  +1          +10
5'-AATTGTGAGCGCTCACAATT-3'              20 bp
3'-TTAACACTCGCGAGAGTTAA-5'

  +2          +10
5'-ATTGTGAGCGCTCACAAT-3'                18 bp
3'-TAACACTCGCGAGAGTTA-5'
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FIG. 1. The lac operators used for the investigation of protein-DNA interaction. Notation is according to Gilbert (11). The central $GC_{10}$ base pair is underscored.

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Material and Methods. A headpiece of 56 amino acids in length (HP56) was used for this study. The limited proteolysis of the repressor with chymotrypsin was performed according to (6) and isolated as published previously (7). Four *lac* operators (Fig. 1) were synthesized by simultaneous DNA synthesis (8, 9, 10). They consist in one half of 9 to 12 bp sequences from the left half of the native *lac* operator. In each of the other halves the bases were repeated so as to obtain palindromes. Analogously to the left half of the native operator, the synthesized operators have two binding sites for the *lac* repressor headpiece. The synthesized palindromes differ from the wild type operators by the absence of the $GC_{11}$ and an AT to GC transversion in positions 13 and 15.

NMR measurements. For the NMR experiments the *lac* operator duplexes were dissolved in 0.2 M NaCl (pH 7.1). The NMR measurements on the *lac* operator-*lac* repressor headpiece complexes were carried out at the same salt concentration and pH value. All spectra were taken at 303 K and 500 MHz, on a Bruker AM 500 spectrometer equipped with an Aspect 3000 computer. The concentration of the DNA duplexes was 1.5 mM before, and 1.0 mM after the addition of HP56. The chemical shifts were determined relative to internal sodium 3-trimethyl-(2,2,3,3-$^2H_4$)-propionate with an accuracy of ±0.01 ppm. The assignments of the imino proton resonances are based on 1D-NOE measurements in $H_2O$ (data not shown). The spectra for the free operators and the corresponding complexes are shown in Fig. 2.

Results. Among all imino proton resonances obtained only the $AT_6$ imino proton resonance showed a shift of about 0.11 ppm upon complex formation between the 18 bp operator and the headpiece. This large shift was also described in earlier studies (12). In the case of the 20 bp operator one additional imino proton resonance ($AT_3$ or $AT_4$) is

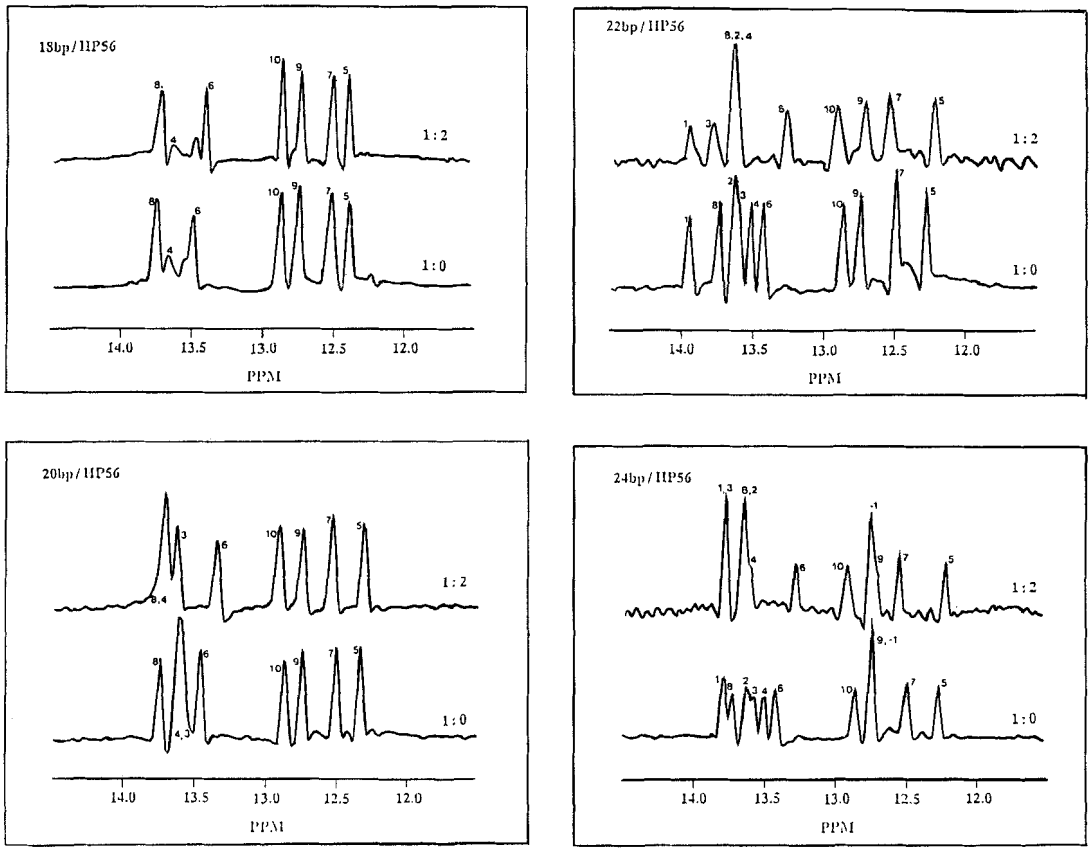


FIG. 2. Resolution-enhanced spectra for the free operators and the corresponding HP56 complexes in a 2:1 molar ratio.

shifted by the addition of HP56. Comparison of this spectrum with the spectra of the other operators indicates that the shifted signal most likely could be assigned to $AT_4$. The spectrum of the HP56 complex with the 22 bp operator shows shifts for nearly all of the imino proton resonances compared to the free DNA. The imino proton resonances of the 24 bp operator are shifted very similarly to those of the 22 bp operator in the HP56 complexes. The differences between the spectral positions of imino proton resonances for the free operators and the spectral positions of the imino proton in the corresponding complexes are shown in Fig. 3.

Conclusions. All four operators investigated most likely form specific complexes. In the case of the 18 bp and 20 bp operators some interactions necessary for a tight complex are not present. This may be caused by a modification of the B-DNA structure owing to fraying of the

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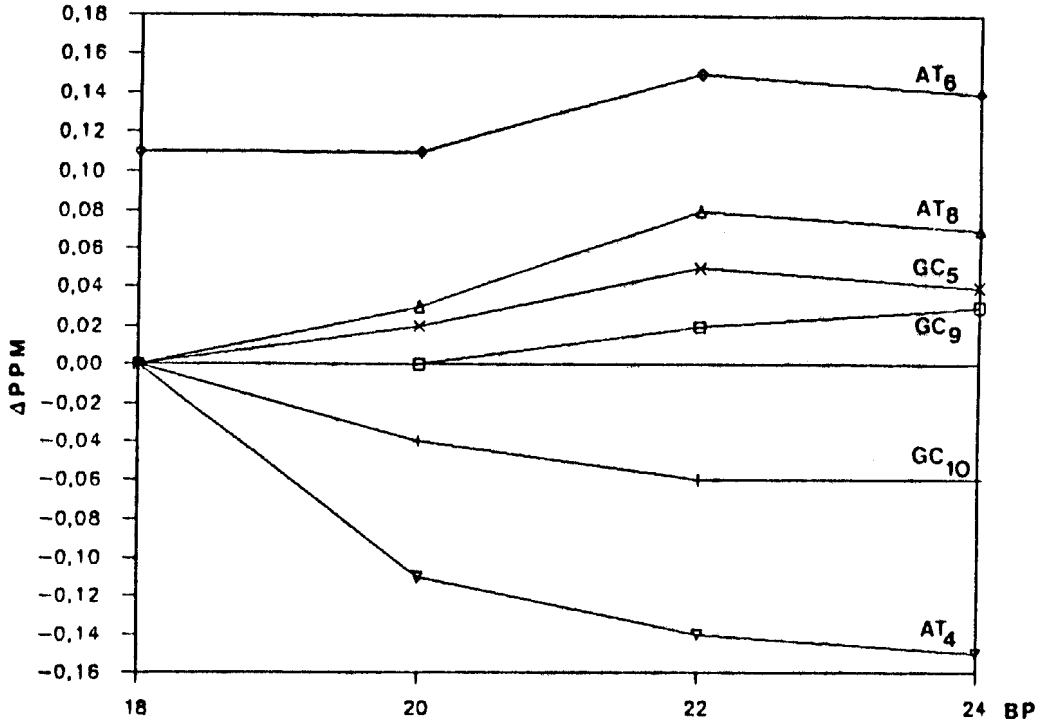


FIG. 3. Differences between chemical shifts of the free DNA imino protons and the corresponding complexes.

one or two terminal base pairs. In addition, unspecific interactions between the -1 and +1 DNA backbone region and the protein could be necessary for tight complex formation.

From the present data it can be concluded that an operator with two binding sites for the headpiece, when used in studies of *lac* operator headpiece complexes, has to be at least 22 bp long to enable a "intact" specific complex to be formed. It was thus demonstrated that the minimum size of a specific recognition sequence can be acccurately determind from imino proton NMR measurements.

## REFERENCES

(1) Betz, J.L., Sasmore, H.M., Buck, F., Insley, M.Y., Caruthers, M.H., Gene 50, 123 (1986).
(2) Buck, F., Rüterjans, H., Beyreuther, K., FEBS Lett. 96, 335 (1978).
(3) Gilbert, W., Maxam, A., Proc. Natl. Acad. Sci. USA 70, 3581 (1973).
(4) Bahl, C. P., Wu, R., Narang, S. A., Methods in Enzymolgy 65, 877 (1980).
(5) Sadler, J. R., Sasmore, H., Betz, J. L., Proc. Natl. Acad. Sci. USA 80, 6785 (1983).
(6) Arndt, K.T., Boschelli, F., Lu, F., Miller, J.H., Biochemistry 20, 6109 (1981).
(7) Buck, F., Hahn, K.-D., Zemann, W., Rüterjans, H., Sadler, J. R., Beyreuther, K., Kaptein, R., Scheek, R., Hull, W. E., Eur. J. Biochem. 132, 321 (1983).
(8) Seliger, H., Herold, A., Kotschi, U., Lyons, J., Schmidt, G., Eisenbeiß, F., Nucleosides & Nucleotides 6, 137 (1987).

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(9) Fera, B., Singrün, B., Kupferschmitt, G., Schmidt, J., Buck, F., Rüterjans, H., Nucleosides & Nucleotides 6, 477 (1987).

(10) Fera, B., Rüterjans, H., German patent application P 3724604.0-41 (1987).

(11) Ogata, R., Gilbert, W., Proc. Natl. Acad. Sci. USA 75, 5851 (1978).

(12) Scheek, R.M., Zuiderweg, E.R.P., Klappe, K.J.M, van Boom, J.H., Kaptein, R., Rüterjans, H., Beyreuther, K., Biochemistry 22, 228-235 (1983).

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