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Interaction of two *LAC* repressor protein segments with polynucleotides

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Abstract. The interaction of the oligopeptides Ala-Gln-GIn-Leu-Ala-Gly-OH and Gln-Leu-Ala-Gly-OMe corresponding, respectively, to the sequence 53–58 and 55–58 of *lac* repressor protein with four polynucleotides was studied. The two peptides did not interact with poly dA. poly dT, poly d(A-T).poly d(A-T) or poly d(A-G).poly d(C-T). But they interacted in a characteristic way with poly d(A-C). poly d (G-T), the sequences of which are in abundance in the *lac* operator region. Both the peptides stabilised the melting of poly d (A-C). poly d (G-T) at a peptide to nucleotide ratio (P/N) of 4; at lower ratios, they destabilised the DNA slightly. The circular dichroism of the alternating polynucleotide with CAC/GTG sequences was perturbed by both the oligopeptides. The hexapeptide at a P/N of 4 caused the transformation of the B-form circular dichroism spectrum to a new state, characterised by strong 220 and 240 nm bands, and a rather weak long wavelength spectrum.

Keywords. *Lac* repressor protein; hexapeptide; poly d(A-C)· poly d(G-T); circular dichroism spectra; protein–nucleic acid interactions.

Introduction

The interaction of *lac* repressor with *lac* operator has been extensively studied and reviewed (Muller-Hill, 1975; Bourgeois and Pfal, 1976; Butler *et al.*, 1977; Caruthers, 1980; Helene and Lancelot, 1982). The site for specific binding of *lac* repressor protein to *lac* operator has been shown to reside in the N-terminal region, the so called head piece. Limited protease digestion of the repressor yields a 51,56 or 59 residue fragment, and these head pieces were shown to bind specifically to *lac* operator (Ogata and Gilbert, 1979; Ribeiro *et al.*, 1981; Nick *et al.*, 1982). Based on the crystal structure of *cro* repressor protein and the homology between *cro* and *lac* proteins, the fragment 17–26 of *lac* repressor was ascribed a primary role in binding to the operator (Mathews *et al.*, 1982). However, genetic and biochemical studies indicated that the region 50–58 is also important for operator recognition and that the glutamine residues in this sequence at 54 and 55 positions are essential for operator binding (Muller-Hill, 1975; Miller *et al.*, 1975). Gieiseler and Weber (1977) suggested that the fragment 50–59 acts only as a flexible hinge between the head piece and the core of the repressor. According to Ogata and Gilbert (1979), the function of the fragment 50–58 is to maintain the operator binding structure of the repressor. Gursky *et al.* (1976) suggested that the fragment 53–57 forms part of a inter-subunit ß-sheet in the tetrameric repressor, and further

Abbreviations used: CD, Circular dichroism; T_m, melting temperature.

implicated that this region is involved in operatorbinding. The importance of Gin 54 in the binding was brought out also by Rein and coworkers from theoretical modeling (Garduno *et al.*,1981).

There is, so far, no consensus on the mode of interaction between the tetrameric repressor and the *lac* operator. A study of interaction of a hexa- (53–58) and a tetra-eptide (55–58) fragment of the head piece of *lac* repressor with synthetic poly-ucleotides with defined sequence was undertaken, using melting curves and circular dichroism as probes.

Materials and methods

The hexapeptide fragment 53–58, Ala-Gln-Gln-Leu-Ala-Gly-OH and the tetrapeptide fragment 55–58, Gin-Leu-Ala-Gly-OMe, (Rao *et al.*,1984) were synthesised, stepwise, in solution. Poly-nucleotides were purchased from P. L. Biochemicals. All other reagents used were of analytical grade.

The following extinction coefficients at 260 nm (Wells *et al.*, 1970) were used to estimate the concentration of the polynucleotides: Poly d(A-C) · poly d(G-T): 6500; poly d(A-G)-poly d(C-T): 5700; dA-poly dT: 6000; and poly d(A-T)-poly d(A-T): 6800 M^{-1} cm^{-1}. The measurements were made in a buffer containing 3 mM NaCl and 0·3 mM trisodium citrate (pH: 7·0).

Circular dichroic (CD) spectra were recorded with a JASCO J-500A Spectropolarimeter, equipped with a data processor. The CD unit was standardised with a 0·05 % solution of androsterone in dioxane. Cylindrical quartz cells of 0·5 cm and 1·0 cm path length were used. Concentration of the polynucleotide solutions were ~ 2×10^{-4} M/lit. of phosphate. Increasing quantities of concentrated solutions of the peptides made in the same buffer was added to obtain the desired P/N ratios. CD spectra reported are averages of 4–8 scans.

Melting profiles were recorded with a Gilford 2600 UV-VIS spectrophotometer equipped with a thermal analyser and a HP-7225A printer-plotter. The concentration of DNA solutions were $0{\cdot}8 \times 10^{-4}$ M/lit. of phosphate and the melting curves were recorded with a heating rate of 0·5°C/min.

The melting curves of oligopeptide-polynucleotide mixtures were found to be reversible in that after cooling the denatured systems, the melting profiles were reproduced.

Results

The hexapeptide stabilised the AT rich (69%) *Clostridium perfringens* DNA in that its melting temperature was slightly enhanced (4°C) at a peptide to nucleotide ratio (P/N) of 2; the *lac* operator is rich in AT base pairs (~ 70 %). But, surprisingly, the peptide did not affect the CD or melting profile of either poly dA · poly dT or the alternating polymer poly d(A-T) · poly d(A-T) (Rao *et al.*, 1984).

The interaction of the hexapeptide poly d(A-C) · poly (G-T) was followed by CD measurements. The titration of this alternating polynucleotide against the hexapeptide is depicted in figure 1. The initial CD trace is typical of the B-form DNA

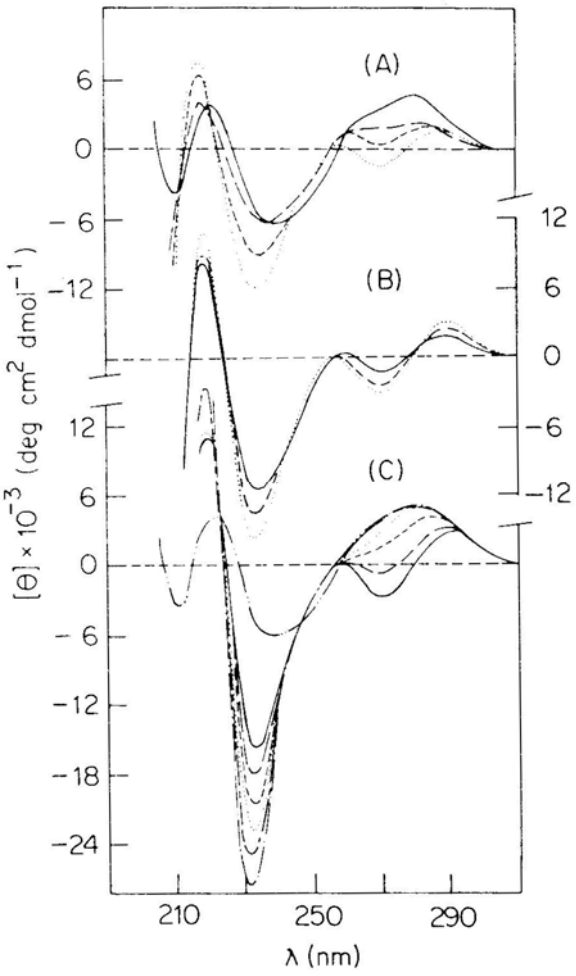

Figure 1. Circular dichroic spectra of poly d(A-C). poly d (G-T) as a function of hexapeptide concentration expressed as peptide: nucleotide ratio (P/N)
(A) (—), O; (– –), 0·8; (- - - -), 1·6; (· · ·),2·4
(B) (—), 2·4; (– –), 3·2; (· · ·), 4·0
(C) (— · · —), 0; (——), 4; (– – –), 4·8; (– – – –), 5·6; (· · ·), 6·4; (— —), 7·2; (— —), 8–0.

and the spectrum was remarkably perturbed on addition of the peptide. The long wavelength band was dramatically altered on progressive addition of the peptide. At a P/N of 4, the positive band became conservative in that the spectrum had a positive component, with a maximum at 288 nm, and a negative component of nearly equal intensity, with a minimum at 270 nm, and a cross over at 279 nm (figure 1A and B); this spectrum can be designated as spectrum II of the polynucleotide. On further addition of the peptide, the original band trace was more or less recovered at a P/N $\geqslant$ 6·4 (figure 1 C). There was a redshift of the maximum at 278 nm by 10 nm to 288 nm at a P/N of 4, which moved back to original location at a P/N $\geqslant$ 6·4 (figure 2).

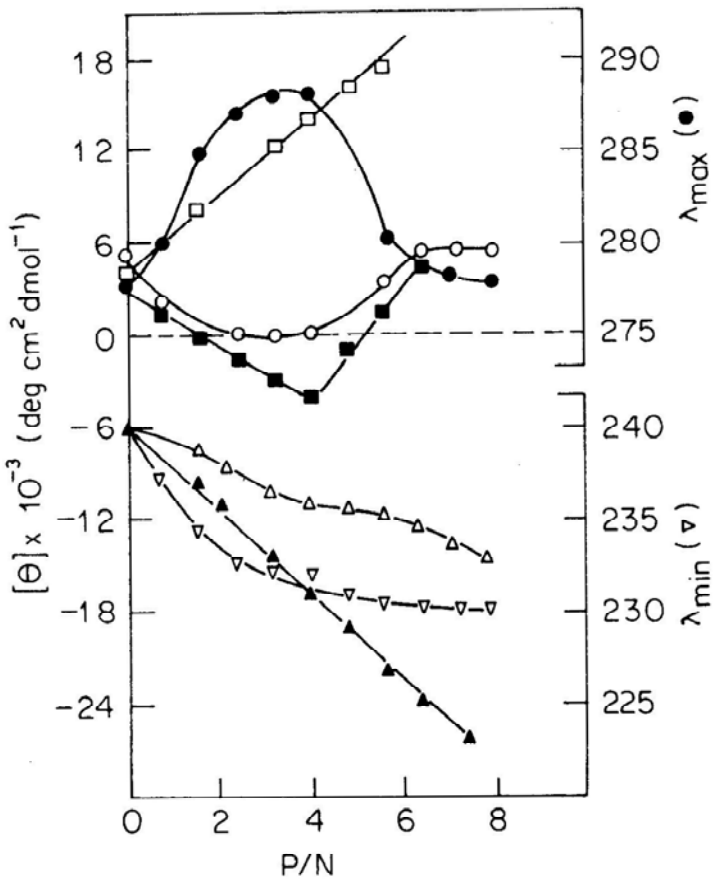

Figure 2. CD data for the addition of hexapeptide to poly d(A-C). Poly d(G-T). Variation of λ_{max} of long wavelength (278) band (●) and λ_{min} of short wavelength (240) band (◇)Variation of 278 nm (O); $\theta_{240\ nm}$ (■); $\theta_{240\ nm}$ p ; θ_{min} (Δ); θ_{220} after subtracting of peptide (▭).

The positive ellipticity at 270 nm sharply decreased initially, then crossed over to a negative value which reached the most negative value of – 3880 deg cm^2 d mole^{-1} at P/N of 4, and there after rose sharply back to the original positive value, with a cross over at a P/N of 5 (figure 2); this CD trace is designated as Spectrum III of the alternating polynucleotide.

The changes in the ellipticity of the 240 nm band surprisingly did not parallel those of the long wave length band. There was a sharp blueshift of the minimum initially (P/N:2) and later it stabilised around 230 nm (figure 2). However, the ellipticity of this negative band did not attain a limiting value, but increased linearly. (figure 2) and had a value of 29,600 deg cm^2 decimole $^{-1}$ at 230 nm at a P/N of 8, almost five times that of the initial value (6000 deg cm^2 decimolee $^{-1}$) . Concomitant with increase in the rotational strength, this band became much sharper, indicating a higher level of organisation.

The ellipticity of the positive 220 nm band also increased on addition of the peptide.

At this wave length, the hexapeptide also contributes to the dichroism, as is evident from its CD spectrum shown in figure 3. Therefore, a titration of the peptide in the absence of the polynucleotide was done; the features of the CD trace at higher concentration were found to remain unchanged and the ellipticity per decimole of the peptide remained constant indicating no qualitative change in the conformation of the peptide and no intermolecular β-sheet formation, on increasing the concentration. The increase in the ellipticity of the 220 nm band in the peptide—DNA mixtures, after substracting the contribution of the peptide chromphore, depicted in figure 2 should then be attributed to changes in the CD of only the polynucleotide, and this is reasonable in view of the fact that peptidic systems do not have large positive 220 nm bands.

The melting data of poly d(A-C) · poly d(G-T) in the presence of increasing amounts of the hexapeptide also were remarkable; initially, the melting temperature(T_m) slightly decreased, as can be seen from figure 4, indicating a marginal destabilisation of the DNA structure. But at a P/N = 4, a strong stabilisation, implicated by a 11°C raise in the T_m, was observed. Beyond a P/N of 5, there was again a destabilisation of the DNA structure.

The 55–58 tetfapeptide fragment also did not interact with poly dA-poly dT or poly d(A-T)·poly d(A-T). However, it did affect the CD of poly d(A-C)·poly d(G-T), as can be seen from the CD titration data depicted in figure 5. Upto a P/N of 3, there were only minor alterations in the CD profile. But at a P/N of 4, the intensity of the long wave-length band sharply decreased and concomittantly, the ellipticity of the 240 nm band became less negative (~ 70 %); the intensities of the negative and positive bands in this spectrum were more equal when compared to the initial spectrum.

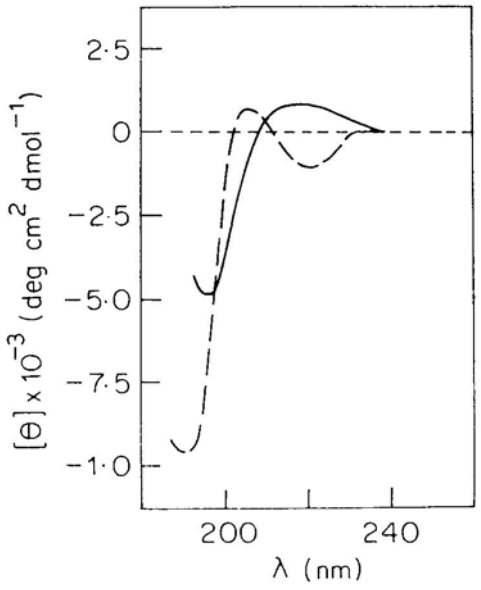

Figure 3. Circular dichroic spectra of hexapeptide (– – – –) and tetrapeptide (——).

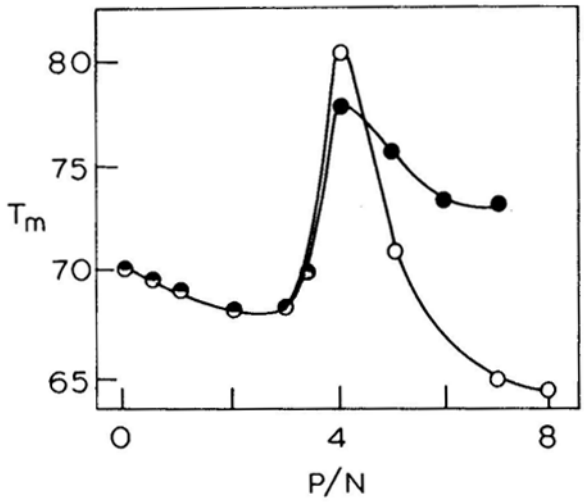

Figure 4. Variation of T_m of poly d(A-C). poly d(G-T) as a function of peptide: nucleotide ratio (P/N) for hexapeptide (O) and tetrapeptide (●).

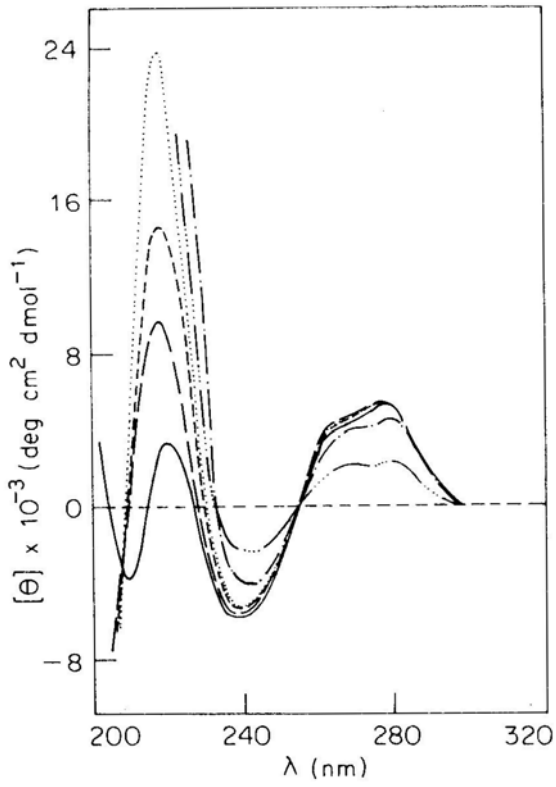

Figure 5. Circular dichroic spectra of poly d(A-C). poly d(G-T) in presence of tetrapeptide at various P/N ratios. (——), 0; (— —), 1; (– – –), 2; (· · ·), 3; (— · · · · · —), 4; (— · —), 5.

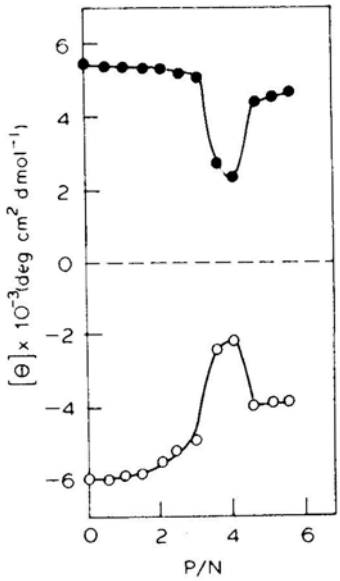

Figure 6. Variation in the ellipticity of the 278 nm (●)and 240 nm(O) bands of poly d(A-C). poly d(G-T) as a function of addition of tetrapeptide.

Significantly, on further addition of the tetrapeptide (P/N > 4), the CD regained its initial features (figure 6) but with a some what slightly reduced ellipticity.

The observed enhancement in the 220 nm band intensity (figure 5) were apparently due to the contribution of the peptide; the CD spectrum of the tetrapeptide (figure 3, curve 2) has a small positive band in the 220 nm region, unlike the hexapeptide; CD data on concentration dependence of the tetrapeptide itself in the absence of DNA indicated that the recorded changes in the intensity of the 220 nm band in the presence of the polynucleotide should be attributed to the peptide component alone.

The variation of T_m of poly d(A-C)-poly d(G-T) on addition of the tetrapeptide (figure 4), substantiated the CD data. Initially, the curves for the hexa- and tetra-peptides overlap, but at a P/N of 4, a sharp increase in T_m was observed and at P/N > 4 also there was some extent of stabilisation, unlike the case with the hexapeptide.

The tetra- and hexa-peptides did not surprisingly affect the melting temperature of poly d(A-G) · poly d(C-T) even at a P/N ratio of 6. This is a very significant observation in that in this polynucleotide the GC base pairs are transversed in comparison to poly d(A-C)·poly d(G-T) and this suggests a specificity in the recognition of CAC/GTG sequences in preference to CTC/GAG sequences.

Discussion

The base pair sequence of *lac* operator region is shown in figure 7. There is conflicting evidence regarding the binding site of *lac* repressor with the *lac* operator. The

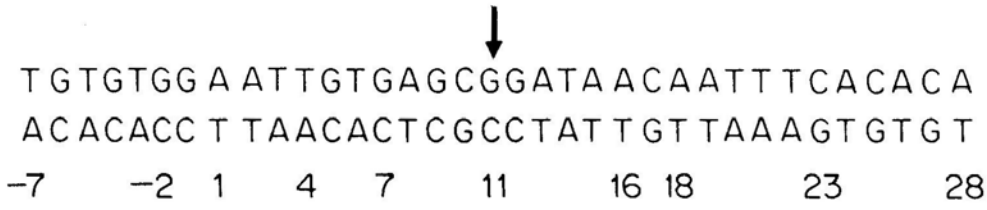

Figure 7. The base pair sequence of *lac* operator region. The pseudo two fold axis is located at position 11.

tetrameric repressor protects base pairs – 4 to +20 from DNAsel digestion (Schmitz and Galos, 1979). Methylation experiments of Ogata and Gilbert (1979) led them to postulate the binding site to be 1–23 base pairs; the outer boundaries of the repressor-operator interaction, according to Caruthers (1980), are not defined by these experiments. An alternate suggestion for the binding site was base pairs 3–19 (Bahl *et al.*,1977).

An examination of the *lac* operator region reveals the significant feature that CAC/GTG sequences are in abundance. Actually, there are two palindromic sequences of 6 bp each at – 2 to – 7 and 23–28 positions. Further, the four base pairs at 4–7 and the three at 16-18 are also CAC/GTG sequences.

Theoretical modelling of Rein and coworkers implicated that Gin 54 especially interacts with a GC base pair of the *lac* operator at position 7, which interestingly is also part of a CAC/GTG sequence (Rein *et al.*, 1983).

Our present results clearly show that CAC/GTG, and not CTC/GAG, sequences interact with the hexa- and tetra-peptides which has implications for the repressor-perator interaction. Base pairs in these sequences have apparently special dynamical features. Lu and colleagues showed from NMR study of the kinetics of the imino hydrogen exchange, that a faster opening kinetics exists at a GTG/CAC site of the *lac* operator (Cheng *et al.*, 1984). These sequences have been found in active sites for specific interaction with proteins in a variety of eukaryotic and prokaryotic DNAs and sometimes occur as large blocks (Pangaaniban and Temin, 1983; Tonegawa, 1983). In circular DNA, their ability to form Z DNA, when subjected to unwinding torsion, has been demonstrated (Nordheim and Rich, 1983). It is likely then, that the solution conformation of poly d(A-C) · poly d(G-T) has dynamic structures which the hexapep-ide apparently is well suited to perturb, and lead to two new structures characterised by the CD traces II and III (figure 1B and C), the form II in particular is markedly stabilised by the peptide at P/N = 4.

The functional groups that could interact with DNA in the two peptides are limited to the amide group of glutamine(s) and the α-NH_3^+ group apart from the hydrophobic side chains of alanine and leucine; there are no charge centres in the side chains. Glutamines are implicated by Rich to interact specifically in the major groove with AT and in the minor groove with GC (Seeman *et al.*, 1976). We observed a kind of specificity in our data in that the alternating AG/CT system was not favoured for interaction as compared to AC/GT system. We have performed melting experiments in the presence of Gln as well as the dipeptide, Gln-Gln, and these were found to be singularly

ineffective in stabilising or perturbing the structure of poly d(A-C) · poly d(G-T). It appears then that the sequences under study have the seeds of specificity of interaction with poly d(A-C) · poly d(G-T) and not with the polymer with transversed GC base pairs. It seems that $CONH_2$ of glutamine, properly positioned, forms hydrogen bonds, as for example suggested by Helene, with adjacent base pairs (Helene and Lancelot, 1982).

It is perhaps pertinent to comment on the circular dichroic changes brought about by the hexapeptide, as CD is the most sensitive optical tool available to follow changes in the solution structure of DNA. Tinoco and coworkers (Johnson *et al.*, 1981) showed, from extensive calculations of CD of a double stranded dodecanucleotide, that the intensity of the long wavelength circular dichroic band decreases as the winding angle increases (from B-C-D form) and the base pair twist becomes negative; simultaneously the intensity of the 240 nm band enhances. The above feature were observed in the titration of poly d(A-C) · poly (G-T) with the hexapeptide in the P/N range 0–4. At higher hexapeptide concentrations, a new set of CD features emerged which are indicative of a transformation to another form. Experiments to understand these changes using NMR are under way.

Acknowledgements

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