

PERTURBATION OF THE CONFORMATION OF POLY(dA-dT). POLY(dA-dT) IN THE PRESENCE OF POLY-L-LYSINE AT LOW IONIC STRENGTH

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ABSTRACT

Poly(dA-dT).poly(dA-dT) in aqueous solution undergoes conformational transition to the X-form in the presence of high concentration of CsF (6.6 M), and 60% alcohol; this new conformation has a characteristic CD spectrum with double minima at 246 and 272 nm. It has been shown that such a transformation can be manifested at low ionic strength (3 mM NaCl) on interaction with poly-L-lysine of varying degrees of polymerization (DP), 14, 110 and 220, at concentrations equivalent to that of the polynucleotide (10^{-5} M). Poly-L-lysine with a DP of 14 was the most effective.

INTRODUCTION

STUDIES on the structural polymorphism of alternating purine-pyrimidine polynucleotides have attracted considerable attention in the recent past. In the case of poly(dG-dC).poly(dG-dC), the transformation from a right-handed B-form to a left-handed Z-form in solution has now been unequivocally established^{1,2}. However, the experimental evidence for the existence of such transformations in the case of other alternating polynucleotides is rare, although theoretical calculations do point to the plausibility of existence of both left- and right-handed conformations^{3,4}. More recently, the conformational flexibility of the duplex of poly(dA-dC). poly(dG-dT), has also been demonstrated⁵.

The structure of the alternating polynucleotide, poly(dA-dT).poly(dA-dT) has been a matter of considerable discussion⁶⁻⁸. Unlike in the case of poly(dG-dC).poly(dG-dC), this polymer does not exhibit any salt-dependent structural transformation. However, an interesting observation was that the CD spectrum of poly(dA-dT).poly(dA-dT) in the presence of $\text{Co}(\text{NH}_3)_6^{3+}$, high concentration of CsF, alcohols and spermidine has a negative 275 nm band⁹⁻¹², as against a positive band around 262 nm in their absence; the other CD bands remain more or less unaffected. No evidence has so far been obtained from other spectral studies to indicate whether the new conformation, referred to as X-form⁹, is a left-handed form. In what follows a transformation of the above kind, mediated by peptides is reported for the first time. Studies using three homopolypeptides of lysine, of varying degree of polymerisation (DP) viz 14, 110 and 220, showed that all of them could reverse the sign of the long wavelength band of

poly(dA-dT).poly(dA-dT) albeit, to different extents; PLL-14 was found to be the most effective.

MATERIALS AND METHODS

Poly(dA-dT).poly(dA-dT) was obtained from Pharmacia P. L. biochemicals (lot No. 317870). The concentration of the polynucleotide in SSC buffer (3 mM, 0.3 mM sodium citrate, pH=7.0) was determined by measurement of the optical density at 262 nm on a Gilford 2600 spectrophotometer using a molar extinction coefficient of $6500 \text{ M}^{-1} \text{ cm}^{-1}$. In all the experiments the concentration of poly(dA-dT).poly(dA-dT) used was $\sim 4.5 \times 10^{-5}$ mol/litre. The three samples of poly-L-lysine (PLL) used were of DP 14, 110 and 220. The lowest molecular weight sample was synthesized in the laboratory by primary amine initiation of polymerization of the appropriate N-carboxy anhydride, and its DP was determined to be 14. Higher DP samples were from Sigma. Circular dichroic (CD) spectra were recorded at $20 \pm 1^\circ\text{C}$ on a JASCO J-500A spectropolarimeter equipped with a data processor (500 N) in thermostated quartz cells of 1.0 cm path length; the unit was routinely calibrated with solutions of Androsterone in dioxane. Increasing amounts of concentrated solutions of poly-L-lysine, made in SSC buffer, were added to the polynucleotide solution with stirring to give the desired peptide to nucleotide (phosphate) (P/N) ratio. All the CD spectra reported are averages of four to eight scans. The ellipticities are expressed in degree $\text{cm}^2 \text{ dmol}^{-1}$ of phosphate. The data presented have been corrected for the peptide in 210-230 region. The peptide does not contribute above 230 nm.

RESULTS AND DISCUSSION

Figure 1 shows the CD spectra of poly(dA-dT).poly(dA-dT)-PLL-14 mixtures. The CD spectrum of poly(dA-dT).poly(dA-dT) in 3 mM NaCl itself has a positive band centred around 262 nm, a characteristic 275 nm shoulder, a negative band at 246 nm and a positive band at 224 nm. The addition of PLL-14, it can be seen from figure 1, profoundly affects the long wavelength band in as much as the intensity decreased by half at $P/N=0.25$, while at a $P/N=0.5$ its identity was lost completely. The shoulder at 275 nm progressively decreased and became a negative sharp band with a blue shift of 3 nm, and at a $P/N=1.25$ had an ellipticity of $24.800 \text{ deg cm}^2 \text{ dmol}^{-1}$. The intensity of the 246 nm band also increased, although, not as much as that of the long wavelength band.

Figures 2 and 3 show the changes observed in circular dichroism of the DNA when increasing amounts of PLL having DP of 110 and 220 were added. The transformation in the CD spectrum is very similar to that observed with PLL-14; however,

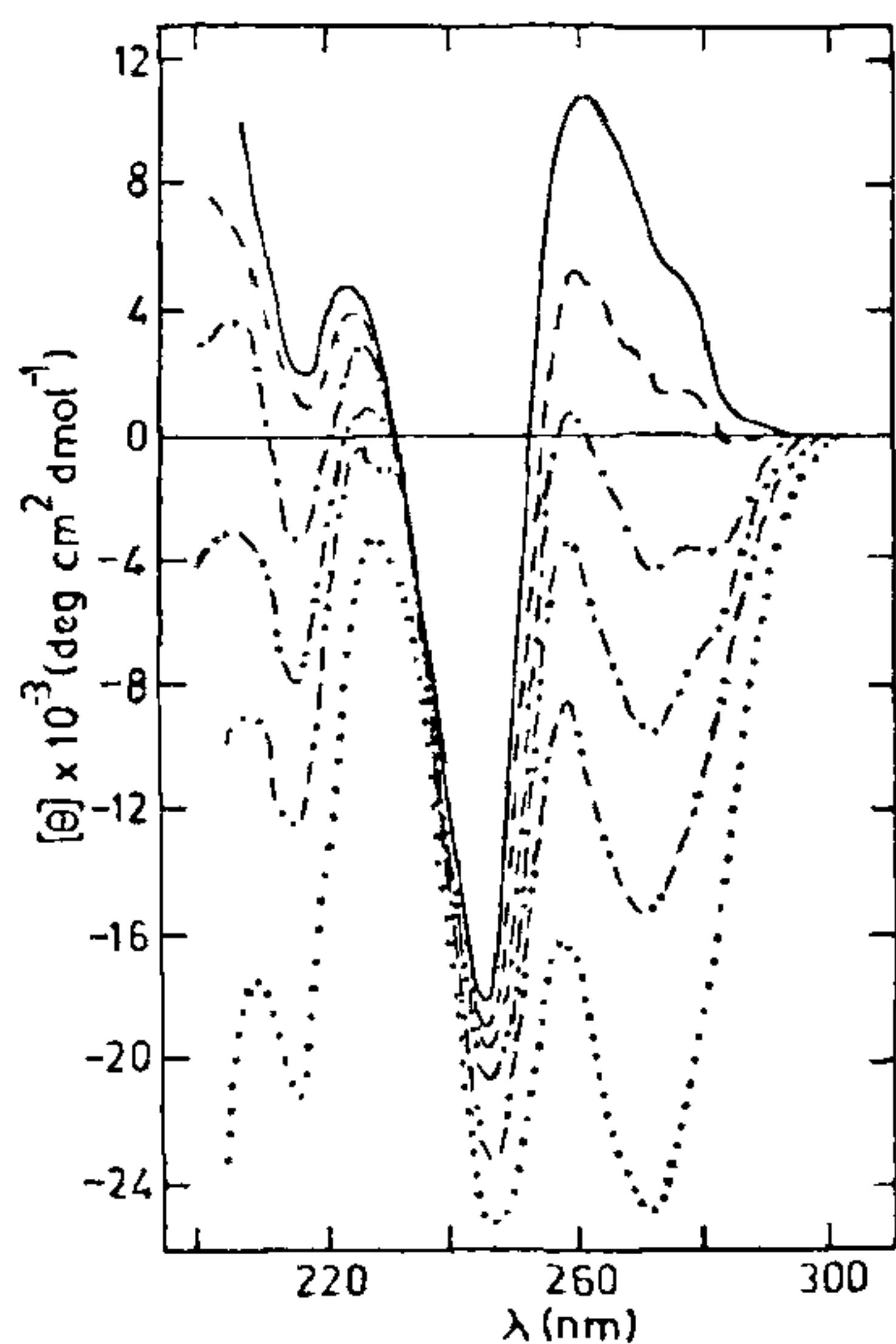


Figure 1. Circular dichroic spectra of poly(dA-dT).poly(dA-dT) as a function of concentration of PLL-14 expressed as peptide residue:nucleotide ratio (P/N): (—), 0.00; (---), 0.25; (- - -), 0.50; (- · - ·), 0.75; (- - · - ·), 1.00; (· · · ·), 1.25.

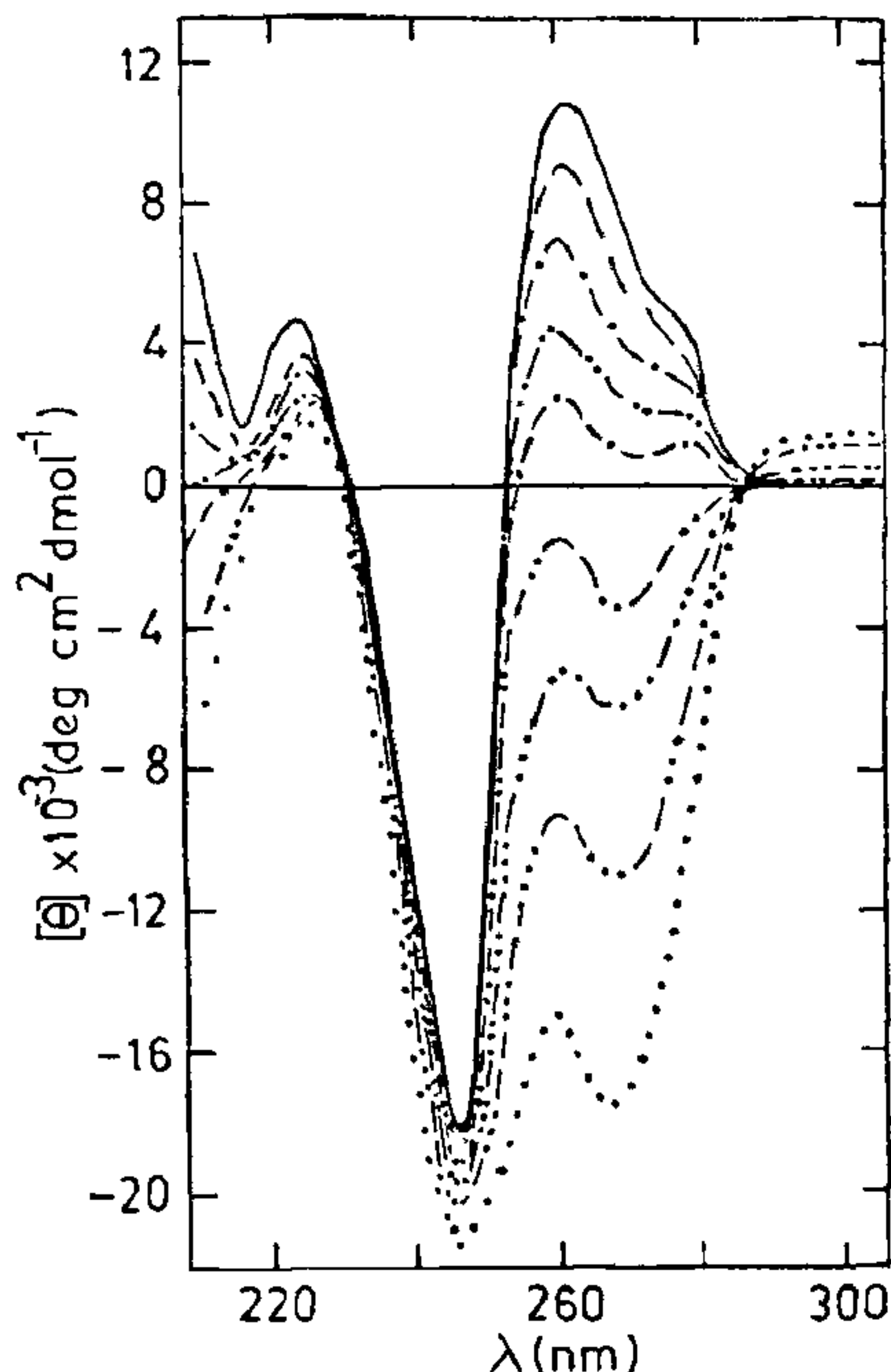


Figure 2. Circular dichroic spectra of poly(dA-dT).poly(dA-dT) as a function of concentration of PLL-110 expressed as P/N ratio: (—), 0.0; (---), 0.2; (- - -), 0.4; (- · - ·), 0.6; (- - · - ·), 0.8; (- - · - ·), 1.0; (- · · · ·), 1.2; (- - - - -), 1.4; (· · · · ·), 1.6.

the magnitude of changes in the intensity of the band is much less. In other words, the addition of lysyl polypeptides to the extent of $P/N=1$ to poly(dA-dT).poly(dA-dT) resulted in a spectrum with double minima at 246 and 272 nm, which could be described as the CD spectrum of the X-form. PLL-14 is the most effective of the three polypeptides to bring about this transformation.

The CD spectrum of PLL-polynucleotide complex resembles that of the poly(dA-dT).poly(dA-dT) in the presence of 6.6 M CsF designated as X-form⁹. The magnitude of ellipticities at both 246 nm as well as 272 nm matches well for the spectra obtained in the presence of PLL-14 at $P/N=1.25$ and in 6.6 M CsF. In other words, the amount of cesium salt required to cause the necessary transformation is about half a million times larger than that of PLL on a molar basis; even the efficient perturbant, $\text{Co}(\text{NH}_3)_6^{3+}$

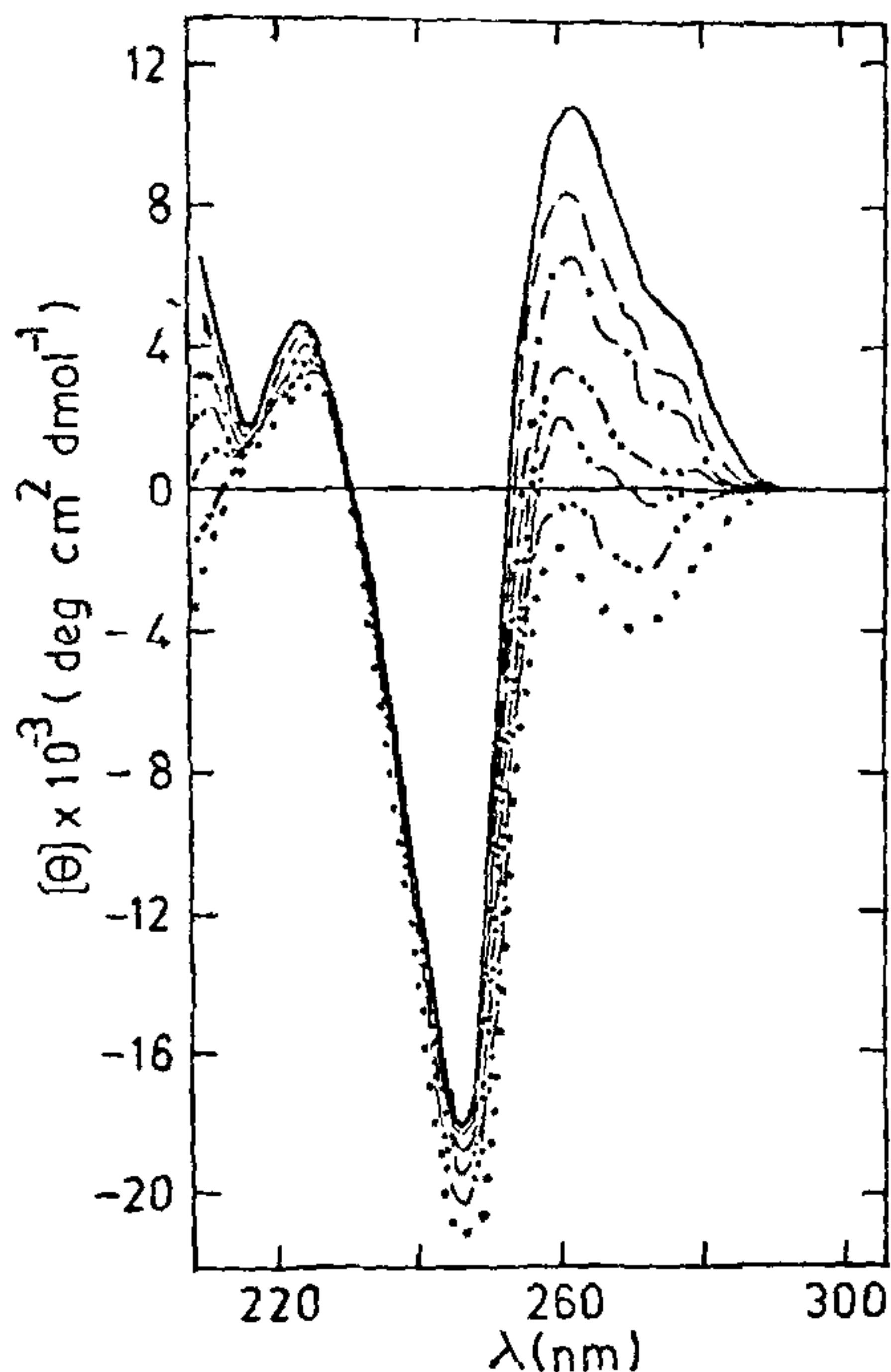


Figure 3. Circular dichroic spectra of poly(dA-dT).poly(dA-dT) as a function of concentration of PLL-220 expressed as P/N ratio: (—), 0.0; (---), 0.2; (- · - ·), 0.4; (- · · · -), 0.6; (- · · · ·), 0.8; (- · · · · ·), 1.0; (·····), 1.2.

needs to be present in thousand-fold excess¹². The conformational transformation of the DNA in presence of 6.6 M CsF or 60% alcohol can be a result of the changes in water structure caused by their presence. The bulk water structure, however, would not be altered in the presence of low concentration ($\sim 10^{-5}$ M) of the oligopeptides. The observed result should therefore arise from an interaction between peptide and nucleotide that is highly specific.

Another interesting feature of the PLL-DNA interaction is that the changes in the CD spectra brought about with increasing quantities of peptide neither show any cooperativity nor do have any isodichroic point indicating that there is a continuous change in conformation and not a shift from an initial to a 'final' state. This leads to the conclusion that only one thermodynamically stable state exists at each addition.

PLL interaction with DNAs has normally been shown to result in a ψ -type conformation¹³, which is essentially an aggregated form of DNA, the aggregation being mediated by the PLL chains. The absence of any CD tails beyond 290 nm in the CD spectra in the present studies (figure 1) precludes any aggregation, up to $P/N=1.25$. At higher P/N ratios, even prior to the appearance of any opalescence, there is evidence of this tailing (data not shown). The lysyl polypeptide has a unique and specific conformational affinity to this DNA; it is not just due to a nonspecific interaction between a polybase and a polyacid.

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