6. Results

6.1. Circular dichroism spectroscopy

The conformation of chiral molecules can easily be followed using circular dichroism spectroscopy. Each conformation of a molecule has its own characteristic circular dichroism spectrum. In the case of a molecule that can switch between two conformations the relative amounts of each conformation can be determined if the transition does not involve an intermediate. Furthermore, since in the case of DNA the optically active unit is the individual base pair, long DNA polymers can be used in this experiment without having to take account of the fact that not all bases of a polymer will have the same conformation. The resultant spectrum is the mixture of the spectra of the individual molecules. Since it was intended to measure the effect of Zα peptide on the conformation of a DNA oligomer, it had to be established that the circular dichroism spectrum of the peptide itself does not

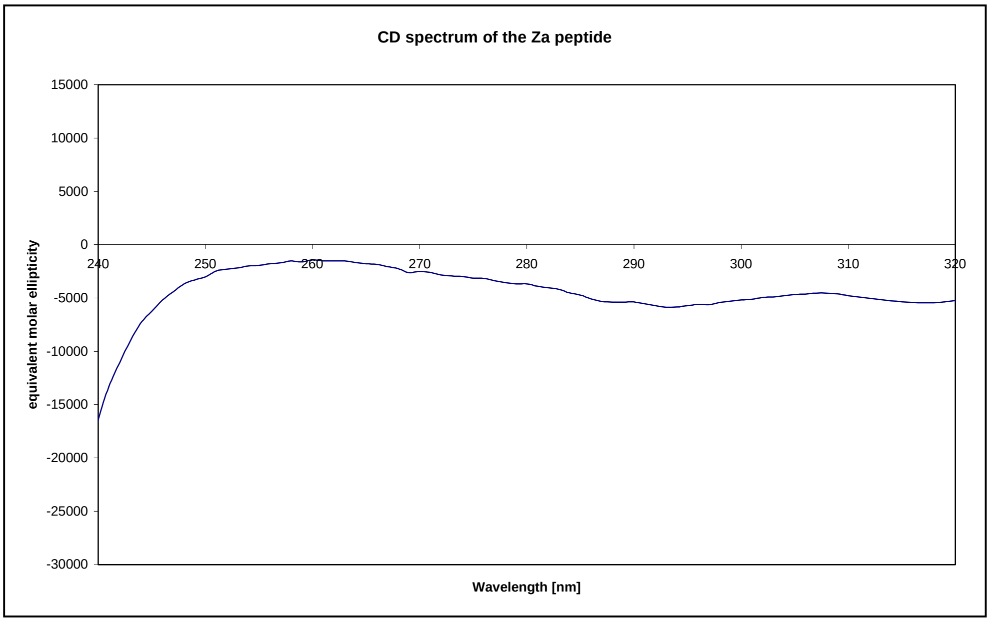

Figure 6.1. Circular dichroism spectrum of 200 µg Zα peptide in 25 mM NaCl, 0.1 mM Na_2·EDTA and 50 mM Tris/HCl pH 7.4 at a temperature of 30 ºC . No significant ellipticity was observed at wavelengths longer than 250 nm .

overlap with the circular dichroism spectrum of the DNA molecule. The circular dichroism spectrum of 200 µg Zα peptide is shown in Figure 6.1 . To allow easy comparison with spectra measured in the presence of DNA the measured Δε values have been converted to molar ellipticities as if DNA had been measured. It its obvious that Zα peptide leads to a significant ellipticity only at wavelengths below 250 nm. As the bands of the circular dichroism spectrum significant for DNA conformation are located at 250 nm and 280 nm, it

$MgCl_2$ are included, serving as models for the spectra of the B-DNA and Z-DNA conformers. Addition of Zα peptide led to the loss of the negative band at 255 nm. A new negative band at 295 nm appeared instead. The observed changes are consistent with the conclusion that Zα peptide induces the DNA polymer to adopt the Z-DNA conformation. In order to assess the amount of peptide needed to induce the B-Z transition the ratios of base pairs DNA per molecule of peptide have been included in Figure 6.3. In calculating these ratios, it was assumed that the peptide used was fully active. The effect of added Zα peptide reached a limit at a calculated ratio of 1 molecule of peptide per 5 basepairs of DNA. The isodichroic point near 278 nm differed from that obtained with $MgCl_2$. The negative band at 295 nm was also less negative.

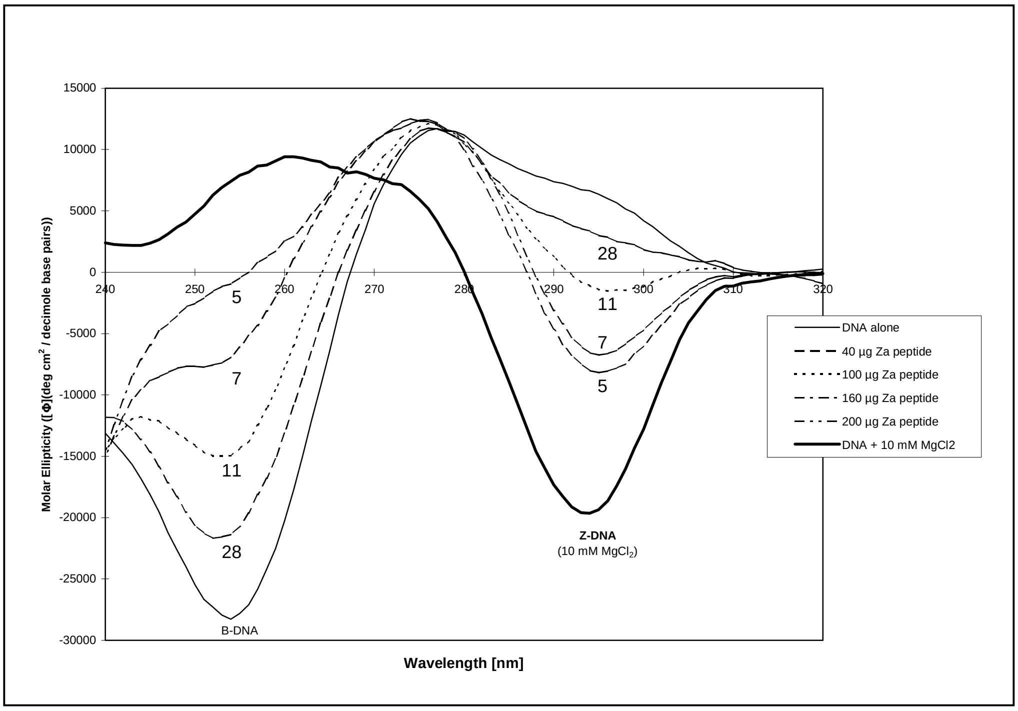

Figure 6.3 Circular dichroism spectra of poly(m^5CG)·poly(m^5CG) in the presence of increasing amounts of Zα peptide. A solution of 46 µM (basepairs) of poly(m^5CG)·poly(m^5CG) was incubated with out or with increasing amounts of Zα peptide up to 200 µg. Addition of the Zα peptide lead to the loss of the negative band at a wavelength of 255 nm and the appearance of a new negative band at 295 nm wavelength.