$[Cu(5,6\text{-dmp})_2]^{2+}$ selectively and reversibly converts calf thymus DNA from right-handed B to left-handed Z conformation

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Circular dichroism spectral studies reveal that $[Cu(5,6\text{-dmp})_2]^{2+}$ (5,6-dmp = 5,6-dimethyl-1,10-phenanthroline) selectively and reversibly induces the conversion of calf thymus DNA from the right-handed B to the left-handed surface-bound Z conformation.

The discovery[1] of left-handed Z-DNA has led to an intense and renewed interest in understanding the polymorphism of DNA and its possible involvement in the regulation of cellular processes.[2,3] The conformational transition from the right-handed B-DNA to the left-handed Z-DNA in solution has been widely studied[4,5] for synthetic polynucleotides *e.g.* poly(dG-dC).poly(dG-dC). In natural systems, however, alternative GC stretches, which are the only sequences capable[1,4,6] of the B ⇔ Z transition are of rather smaller length and so their Z-form would normally escape detection. During our investigation on the interaction of copper(II) phenanthroline complexes† with DNA we have observed for the first time the selective and reversible circular dichroism inversion of CT DNA on the addition of $[(5,6\text{-dmp})_2Cu]^{2+}$ (5,6-dmp = 5,6-dimethyl-1,10-phenanthroline), which is similar to that observed by Pohl and Jovin[1] in 1972 for poly(dG-dC) under high salt concentration.

The observed CD spectrum of calf thymus DNA (Fig. 1) consists of a positive band at 270 nm (UV: λ_{max} = 259 nm) due to base stacking and a negative band at 240 nm due to helicity and is characteristic of DNA in the right-handed B form. Upon the incremental addition of $[(5,6\text{-dmp})_2Cu]^{2+}$ to the DNA, the intensities of both the negative and positive bands decrease and then a nearly inverted CD spectrum with a new positive band around 260 nm and a negative one around 285 nm is obtained. The inverted spectrum is similar to that observed earlier for poly(dG-dC) at high salt concentration,[1] a condition at which the Z conformation is stabilized. Fig. 2 shows that the B to Z transition induced by the complex is highly cooperative. The transition appears to start at $1/R$ (= [Cu complex]/[NP]) value of 0.5 and is complete when $1/R$ reaches unity. Contrastingly, the CD spectra of CT DNA obtained in the presence of copper(II) complexes of all the other phenanthroline ligands‡ show a considerable increase in intensity of the positive band with only a slight perturbation of the negative band and there is no inversion of the CD spectra. Thus $[(5,6\text{-dmp})_2Cu]^{2+}$ appears to be selective in inducing the B to Z transition.

When EDTA was added to DNA incubated with $[(5,6\text{-dmp})_2Cu]^{2+}$, the original CD spectrum typical of native DNA was regenerated; this suggests that the induced B to Z transition is reversible and that the complex binds on the DNA surface to cause the transition. Upon the addition of ethidium bromide (EtBr) to CT DNA to which $[(5,6\text{-dmp})_2Cu]^{2+}$ is bound, the CD peak intensities were slightly reduced and the inverted spectrum did not revert to the original one. Since a strong intercalator like EtBr is well known to convert Z DNA to an intercalated right-handed B form (allosteric effector) under solution conditions that would otherwise favour the Z conformation,[7] the present complex appears to be a strong reverse allosteric effector shifting the equilibrium towards Z conformation even in the presence of an allosteric effector. Further, when the complex is added to CT DNA incubated with EtBr, the inverted CD spectrum characteristic of Z-DNA is obtained. This suggests that EtBr fails to lock the DNA in the B-form and is unable to prevent the B to Z conversion effected by $[(5,6\text{-dmp})_2Cu]^{2+}$.

Competitive ethidium binding studies were undertaken to gain support for the above B to Z conversion. It is well known that the emission of EtBr is enhanced in the presence of DNA. But addition of both $[Cu(phen)_2]^{2+}$ and $[Cu(5,6\text{-dmp})_2]^{2+}$ to CT DNA pretreated with EtBr hinder the expected enhancement in emission (Fig. 3). The effect of the former complex is explicable on the basis of its competition with EtBr for preferential interaction, involving partial intercalation[8] of phen ring into DNA. On the other hand, the substituents at the 5,6-positions in $[Cu(5,6\text{-dmp})_2]^{2+}$ would be expected to prevent the phen ring from similar intercalative interaction with B or any other non-Z conformation as understood from the viscosity studies.§ So the suppression of the expected enhancement in emission in the presence of this complex is consistent with the above conversion from B to Z, rather than any other form capable of

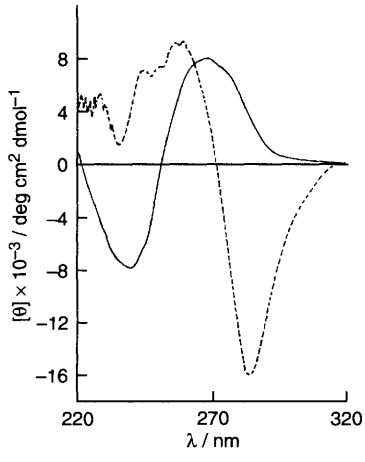

Fig. 1 CD spectra of calf thymus DNA in 5 mmol dm^{-3} Tris–HCl/50 mmol dm^{-3} NaCl buffer–methanol (10 : 1 *v/v*, pH = 7.1) in the absence (—) and in the presence (---) of 0.5 mmol dm^{-3} $[Cu(5,6\text{-dmp})_2]^{2+}$

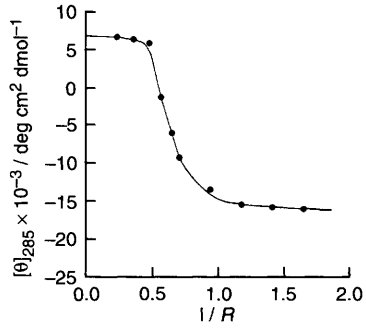

Fig. 2 CD spectral titration of CT DNA with $[Cu(5,6\text{-dmp})_2]^{2+}$; variation of θ_{285} with $1/R$ (= [Cu complex]/[NP])

accommodating the intercalative ligand. Further, the addition of DNA incubated with $[Cu(5,6\text{-dmp})_2]^{2+}$ to EtBr fails to enhance the emission of the latter. So it is clear that the binding of the present complex is extremely strong to resist the reversal of conformational transition by EtBr.

It is reasonable to expect that after the binding of the complex cation possibly to N(7) and O(6) of guanosine,[9] the hydrophobic methyl groups at the 5 and 6 positions act to effectively place themselves between the phosphate groups in close proximity (5.9 Å)[10] with each other leading to the potentiation of Z DNA by decreasing the repulsion otherwise present between them in Z-DNA and changes C_2'-endo, *anti* conformation of sugar pucker of deoxyribose into the C_3'-*endo*, *syn* conformation. Further, the effect appears to be similar to that shown by cytosine 5-methyl in poly(dG-m^{5}dC) in that the 5,6-dimethyl groups may effectively fill the pocket on the external surfaces, that might be hydrated in the major groove of the B form in the absence of the complex, and stabilise dG-dC base pairs in the Z-form.

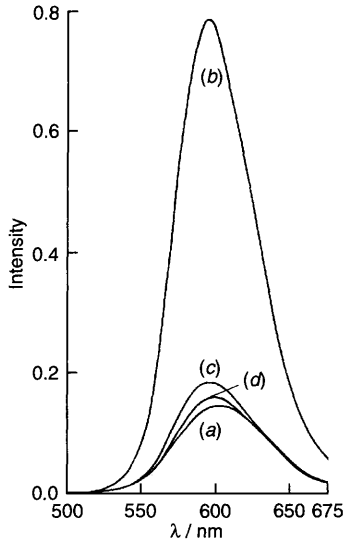

Fig. 3 Emission spectra of ethidium bromide (40 μmol dm^{-3}) (*a*) in the absence and (*b*) in the presence of 40 μmol dm^{-3} NP, (*c*) 40 μmol dm^{-3} NP plus 40 μmol dm^{-3} $[Cu(phen)_2]^{2+}$ and (*d*) 40 μmol dm^{-3} NP plus 40 μmol dm^{-3} $[Cu(5,6\text{-dmp})_2]^{2+}$

Thus $[(5,6\text{-dmp})_2Cu]^{2+}$ is a remarkable reagent which selectively and reversibly effects the complete conversion of B to Z conformation in CT DNA, though the Z-form in such a natural DNA would normally escape detection. The present result constitutes the first indication that mixed sequence DNAs can adopt the Z-conformation. Further, such conformational microheterogeneity, which is necessarily associated with the formation of structural junctions, or interfaces between different DNA conformations has been suggested to play a role in key cellular processes.[2,3]

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Footnotes

† The 2 : 1 1,10-phenanthroline–cuprous complex $[(phen)_2Cu^+]$, with H_2O_2 as a coreactant, is a chemical nuclease that nicks[11] DNA by a reaction mechanism that is sensitive to the conformation of the nucleic acid.

‡ Phenanthroline ligands used for the present studies: 1,10-phenanthroline and 4-methyl-, 4,7-dimethyl-, 5-methyl-, 5,6-dimethyl-, 3,4,7,8-tetramethyl-, 4,7-diphenyl- and 5-nitro-1,10-phenanthroline.

§ An increase in relative specific viscosity is observed for $[Cu(phen)_2]^{2+}$, which is typical and expected of its partial intercalation to DNA; in contrast, no appreciable change for $[Cu(5,6\text{-dmp})_2]^{2+}$ is seen, which is consistent with its non-intercalative possibly surface-binding to DNA.

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