DALTON COMMUNICATION

Chiral peptide nucleic acids: a new family of ligands for metal ions

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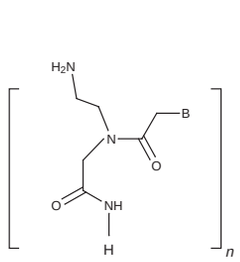
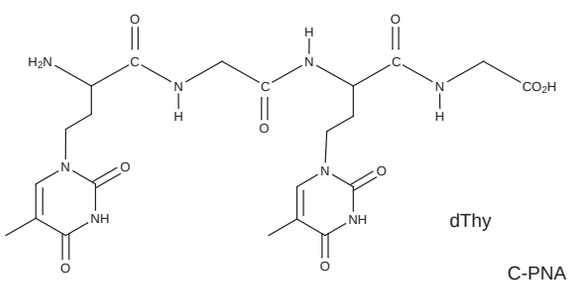
The first studies on peptide nucleic acid co-ordination abilities towards Cu^{2+} ions indicated that when nucleobases were present in the amino acid residue side chain the interactions between the nucleic bases induced very effective metal-ion binding by the peptide backbone donor system.

Peptide nucleic acids (PNA) are synthetic analogues of DNA in which the natural phosphate-deoxyribose backbone has been replaced by a pseudo-peptide chain. There are two types of PNA known at present, one containing a peptide consisting of N-(aminoethyl)glycyl units (PNA, see below)[1] and others in which α-amino acid residues carry DNA nucleobases in their side chains (chiral peptide nucleic acid, C-PNA, see below).[2] The PNA molecules based on glycine are achiral and electrostatically neutral molecules capable of recognising their complementary sequence in DNA and RNA.[3] Both types of PNA molecule are potential metal binders with donor sets centred at both the peptide backbone and the nucleic bases. However, in PNA the peptide-bond nitrogen atom is blocked, making it unable to co-ordinate metal ions and thus the potentially more efficient binding sites are those present in the nucleobase donor system. In this case the attachment of the particular peptides to PNA terminals (*e.g.* the very efficient metal chelator Gly-Gly-His) may form a specific chelator to metal ions *e.g.* it may mediate cleavage of nucleic acid duplexes.[4]

On the other hand, C-PNA are chiral molecules having nucleobases in the amino acid side chain. They are potentially capable of binding metal ions with both the peptide backbone donor system and the nucleobase donor sets. Metal binding by the peptide backbone donor system should be very similar to that of oligoglycine or oligoalanine. However, if the specific interactions (*i.e.* stacking or hydrogen bonding) between the vicinal nucleic bases in the peptide molecule occur the stability of the complex Cu^{2+}–C-PNA could be considerably different to those of simple oligopeptides. To check this possibility we have used two C-PNA molecules: tetrapeptide NH_2-Thy-Gly-Thy-Gly-OH with two thymine molecules (dThy, see above) and octapeptide NH_2-Thy-Gly-Thy-Gly-Thy-Gly-Thy-Gly-OH with four (tThy, see above). The results of the calculations based on the potentiometric titration data, EPR spectra (not shown), absorption (Table 1) and CD (not shown) spectra clearly indicate that the major binding site of the Cu^{2+} ion in both $(PNA)_2$ is the peptide backbone donor system involving the N-terminal amino group and the consecutively deprotonated peptide-bond nitrogens (Table 1). The spectroscopic parameters clearly indicate that below pH 6 the 4N complex is formed by both C-PNAs (Fig. 1).[6] The four nitrogen atom co-ordination is realised by N-terminal NH_2 and three consecutive deprotonated amide nitrogens. The same co-ordination mode in simple tetrapeptides such as tetraAla occurs above pH 8 (Fig. 1). This strongly suggests that nucleic bases present in the amino acid side chains favour very distinctly metal-ion co-ordination to the peptide backbone. Our earlier studies on Cu^{2+} co-ordination to specific ANF (Atrial Natriuretic Factor) pentapeptide: Asn-Ser-Phe-Arg-Tyr-NH_2 having two aromatic rings indicated that the interactions between the aromatic rings may have a strong impact on the stability of the metal–peptide complexes increasing their stability constants by several orders of magnitude[7,8] making the ANF ligand one of the most potent pentapeptide chelators for Cu^{2+} ions amongst the simple peptides. The C-PNA with thymines present in the amino acid side chains having exactly the same binding mode as ANF are even more efficient at Cu^{2+} ion binding. This clearly indicates that nucleobases interact with each other stabilising





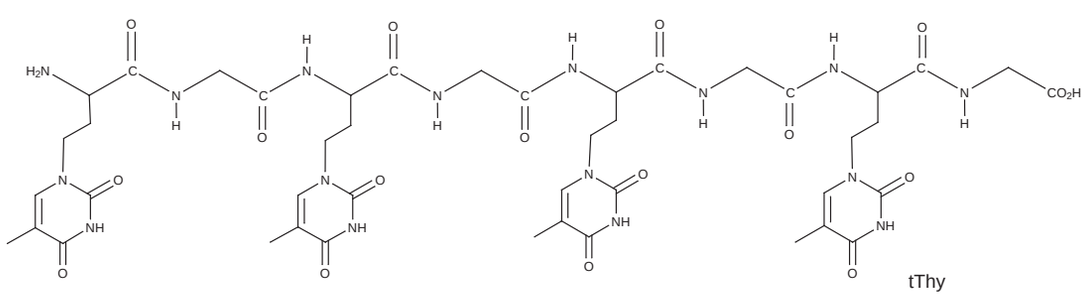


Table 1 Protonation and complex-formation constants of tThy, dThy and tetraAla[a] at 25 °C and $I = 0.1$ mol dm^{-3} KNO_3. The d–d transitions are given for tThy and dThy complexes

logβ					
Species	tThy		dThy		tetraAla
HL	10.69		10.29		8.13
H_2L	21.03		19.92		11.65
H_3L	30.82		26.48		—
H_4L	40.04		28.51		—
H_5L	46.55		—		—
H_6L	49.54		—		—
pK^b	10.69		10.29		—
pK^b	10.34		9.63		—
pK^b	9.79		—		—
pK^b	9.22		—		—
pK (NH_2)	6.51		6.56		8.13
pK (CO_2^-)	2.99		2.03		3.52
Cu^{II} complexes					
logβ {λ_{max}/nm (ε/dm^3 mol^{-1} cm^1)}					
	tThy		dThy		tetraAla
$Cu(H_4L)$	44.44 (1N)	{701.0 (12)}	—	—	—
$Cu(H_3L)$	39.48 (2N)	{652.4 (46)}	—	—	—
$Cu(H_2L)$	32.61 (3N)	{554.8 (63)}	24.98 (1N)	—	—
Cu(HL)	25.79 (4N)	{526.0 (100)}	19.91 (2N)	{647 (55)}	—
CuL	16.40 (4N)[c]	{517.6 (121)}	14.61 (3N)	{570 (86)}	4.77 (1N)
$Cu(H_{-1}L)$	5.85 (4N)[c]	{515.4 (122)}	8.00 (4N)	{504 (127)}	−0.45 (2N)
$Cu(H_{-2}L)$	−4.65 (4N)[c]	{513.6 (135)}	−0.1 (4N)[c]	{504 (127)}	−8.09 (3N)
$Cu(H_{-3}L)$	—	—	−10.68 (4N)[c]	{505 (131)}	−17.33 (4N)
pK_1^{amide}	4.96		5.07		5.22
pK_2^{amide}	6.87		5.30		7.64
pK_3^{amide}	6.82		6.61		9.24
p$K_1^{thymine}$	9.39		8.10		—
p$K_2^{thymine}$	10.55		10.58		—
p$K_3^{thymine}$	10.50		—		—

[a] Data from ref. 5. [b] Protonation constants for individual thymines. [c] 4N complexes with three (CuL), two [$Cu(H_{-1}L)$], one [$Cu(H_{-2}L)$] and none [$Cu(H_{-3}L)$] Thy protonated, respectively.

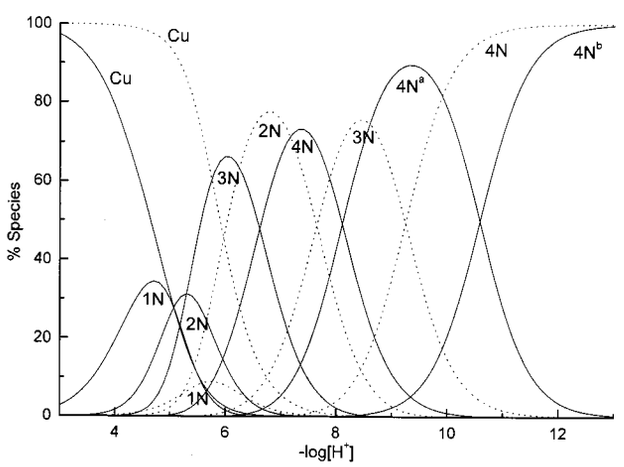


Fig. 1 Species distribution curves for the Cu^{2+}–dThy (solid line) and Cu^{2+}–tetraAla (dotted line) systems as a function of pH. Ligand-to-metal molar ratio 1:1, ligand concentration 1×10^{-3} mol dm^{-3}. 4N, $4N^a$ and $4N^b$ are the complexes with four nitrogens and two, one and none thymine molecules protonated, respectively

very efficiently the metal–peptide bond. It is interesting to note that octapeptides with four thymine molecules induces a much larger steric effect and is almost as effective in metal-ion binding as the tetrapeptide containing two thymine molecules.

Thus, C-PNA type molecules are not only specific in their interactions with nucleic acids but also with metal ions. Therefore, metal ions co-ordinated to the peptide backbone may have a critical impact on the interactions of C-PNA type molecules with nucleic acids. The metal-ion binding may also induce intramolecular interactions between the side-chain nucleobases. Preliminary calculations using HYPERCHEM 4.0[9] indicated the possibility of two thymine rings stacked over the complex plane with one of the thymine oxygens placed close to a Cu^{2+} apical position.

Acknowledgements

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