

Creating regular arrangements of nucleobases through metal ion coordination and H bond formation

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Abstract: If interactions between nucleobases are extended beyond the normal H bonding schemes by also allowing covalent metal ion cross-linking, complexes of varying topologies are created, which open the field of metal-nucleic acid chemistry from biologically relevant model compounds to molecular recognition and supramolecular chemistry.

1. INTRODUCTION

Since the proposal of the double-helical structure of DNA by Watson and Crick in 1953 [1] and its confirmation by the X-ray analysis of a B-DNA dodecamer in 1980 [2] it has been recognized that DNA can adopt also many unusual secondary structures [3] which violate the general picture of a double-stranded, antiparallel structure with Watson-Crick pairing between guanine (G) and cytosine (C), as well as adenine (A) and thymine (T). For tRNA's nonstandard base pairs, base triplets, loops and hairpins have been known since the late sixties, when the first tRNA molecules were crystallized and subsequently characterized by single crystal X-ray diffraction [4]. It was from this work that the role of metal ions for stabilization of particular three-dimensional structural elements became evident and details of the hydrolytic cleavage of the RNA backbone by M-OH emerged [5]. The discovery of the antitumor agent Cisplatin [6] and subsequent findings of DNA being its target molecule [7] led to a strong interest in metal-nucleic acid interactions [8]. The awareness that metal-caused mutagenesis and carcinogenicity might involve metal-nucleic acid interactions and that, for example, coordinatively saturated metal complexes can induce a switch from right-handed B-DNA to left-handed Z-DNA at very low concentrations, further spurred this development.

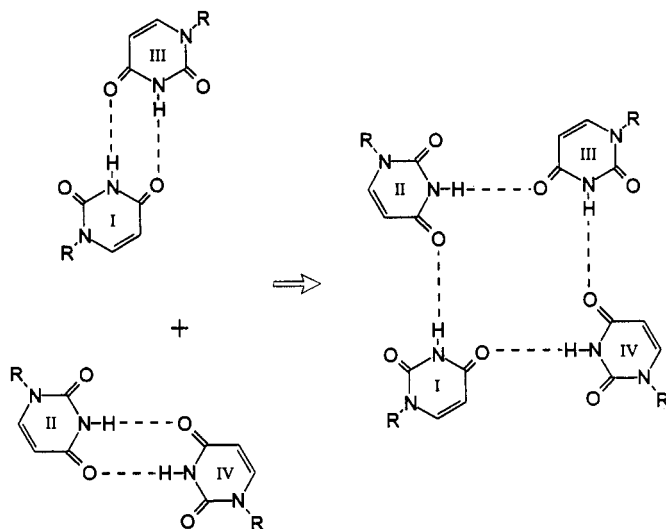
We are interested in all aspects of metal-nucleic acid interactions and are trying to model, whenever possible and meaningful, such interactions on a molecular level [9]. Understanding the role of the metal ion is a major goal of this work. The use of kinetically inert metal ion complexes, especially of Pt(II), has proved advantageous in that isolation and structural characterization of compounds in many cases is possible, while hampered or impossible with kinetically labile complexes. Results of this work have led to a merging of biological aspects and such relevant to molecular self assembly, molecular recognition and supramolecular chemistry.

2. NUCLEOBASE QUARTETS AND MODELS THEREOF

The existence of nucleobase quartets, present in tetrastranded DNA or RNA, has been proposed as early as 1970 [10,11]. X-ray crystallography had shown already in 1963 that pairs of 9-ethylguanine and 1-methyl-5-bromocytosine, H bonded in the Watson-Crick fashion, can associate to larger aggregates as a result of crystal packing [12], a situation meanwhile also confirmed for DNA fragments [13]. Dimers of Watson-Crick adenine, thymine pairs are likewise feasible. Four-stranded DNA structures have been implicated in mechanisms leading to strand exchange during genetic recombination [14,15], and base quartets occur at the ends of chromosomes, the so called telomeres [16]. Telomeres contain guanine quartets associated through eight cyclic H bonds and stabilized by a cation (usually K^+ , but also by Na^+ or occasionally divalent cations) in the center of the quartet or halfway between two layers of G quartets.

Fold-back structures of single-stranded deoxyoligonucleotides binding strongly to certain proteins ("aptamers" [17]) also contain G quartets and a metal cation for additional stabilization. Combined with several layers of guanine quartets, quartets of T [18] are likewise possible, as are quartets of uracil (U) in tetra-stranded RNA when layered on top of G quartets [19].

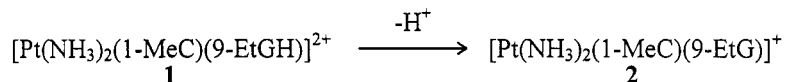
Uracil and its N1 substituted derivatives usually form H bonded dimers in the solid state, involving N(3)H and O(4) sites. On paper, it is quite easy to produce a cyclic quartet (Scheme 1) from two dimers. We were surprised to find out how readily quartet structures indeed form in the presence of Na[AuCl₄] [20] and *trans*-Pt(NH₃)₂Cl₄ [21]. In [Na(1-MeUH)₄][AuCl₄] four almost coplanar 1-methyluracil nucleobases



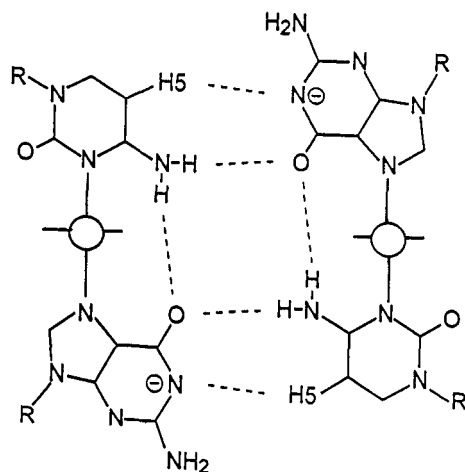
Scheme 1

(1-MeUH) are joined via four cyclic H bonds (2.83 Å, N(3) ... O(4)), thereby producing a square of O(4) oxygen atoms (3.28 Å side length) with a Na⁺ in the center (Na ... O(4), 2.336(7) and 2.301(8) Å). In the second example, *trans*-Pt(NH₃)₂Cl₄ · 2(1-MeUH), the four 1-MeUH rings form also N(3)H ... O(4) H bonds (3.06 Å), but the nucleobases are arranged saddle-shaped and the arrangement additionally is stabilized by H bonds between the protons of the NH₃ ligands and the O(4) oxygen atoms (2.95 - 3.05 Å). The NH₃ groups are above and below the uracil quartet (1.75 Å), with the three NH protons disordered over four O(4) sites. The ease of formation of these uracil quartet structures tentatively suggests that they may be much more inherently stable than has been anticipated for long.

A nucleobase quartet of very much different composition is obtained, when the mixed-nucleobase complex *trans*-[Pt(NH₃)₂(1-MeC-*N*3)(9-EtGH-*N*7)]²⁺ (**1**) is deprotonated at the guanine N(1) position (1-MeC = 1-methylcytosine; 9-EtGH = 9-ethylguanine) [22]:



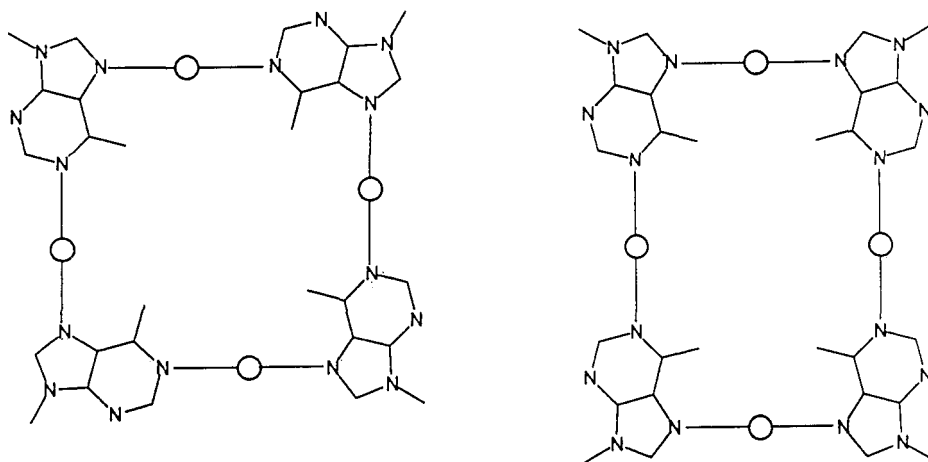
1 represents a structural model of the most abundant interstrand cross-link of *trans*-Pt(NH₃)₂Cl₂ with DNA [23]. According to ¹H NMR spectroscopy (DMSO-*d*₆), the two complementary ends of **2** associate in solution via four intermolecular H bonds (in addition to the intramolecular one between cytosine-N(4)H₂ and guanine-O(6)) as shown in Scheme 2. The most unusual aspect of this H bonding scheme is involvement of the aromatic H(5) proton of the cytosine model nucleobase in H bonding with guanine-



Scheme 2

N(1). The association constant of **2** is unexpectedly high ($K = 59.1 \text{ l mol}^{-1}$ in DMSO). The quartet structure is even maintained under the conditions of an ESI mass spectrometry experiment.

Finally, metal cross-linked purine quartets of yet different composition are the goal of ongoing efforts in our laboratory. A previous observation made with N(7), N(1) dimetalated adenine [24] and meanwhile confirmed in several more cases, according to which the M-N vectors are at right angles, has led us to pursue the preparation of molecular squares and rectangles of the type given in Scheme 3. For metal ions adopting linear coordination geometries, only two combinations are expected to give closed rings; alternatively, meander or helix formation is to be expected [25]. The square should be particularly favourable as far as intramolecular H bonding between the exocyclic 6-positions is concerned, if A and G alternate, hence in a metalated AGAG quartet. Di- and trinuclear precursor complexes of *trans*-(amine)₂Pt(II) have meanwhile been prepared and structurally characterized [26].



Scheme 3

3. METAL IONS AND TRIPLEXES

Formation of triple-stranded DNA is known since 1957 [27]. In the simplest case a DNA triplex consists of a purine and two pyrimidine base strands. The oligopurine strand forms H bonds with a complementary oligopyrimidine strand according to the Watson-Crick scheme to give a normal, antiparallel DNA duplex and in addition the purine strand pairs in the Hoogsteen fashion with the third pyrimidine strand. In that case any cytosine base in the third strand needs to be protonated at N(3). Many variants of this theme are possible, including such containing purine bases in the third strand. Directions of the third strand are either parallel or antiparallel to the purine strand of the duplex [28]. Both inter- and intramolecular triplexes are possible. In the latter case, back-folding of one strand of a duplex DNA is required, leaving a single-stranded loop as well. Folding of a single stranded DNA can likewise result in triplex formation, with loops at the points of changes of strand direction.

Metal ions appear to influence triplex formation significantly, not just for charge balancing reasons [29]. Divalent cations, including those of transition metals, have been found to induce triplex formation from regular duplex structures [30]. It has been proposed [31] that the formation of purine, purine, pyrimidine triplex structures is facilitated by metal ion coordination to N(7) of the purine base present in the third strand.

Our interest in the role of metal ions in DNA triplexes relates, among others, to the idea to covalently cross-link the third strand with the DNA duplex [32]. There appears to be some potential in possible applications of this concept in the field of "antigene" therapy [32]. In fact, it has been shown that a *trans*-Pt(II) modified oligonucleotide can be directed specifically to a target sequence in a DNA molecule consisting of almost 2500 base pairs [34]. As far as model compounds for metalated DNA triplexes are concerned, both single- and twofold-metalated base triplets have been synthesized and structurally and/or spectroscopically investigated [24,25,32].

4. H BONDING OF METALATED NUCLEOBASES

It is obvious that metal binding to nucleobase sites usually involved in H bonding with other nucleobases prevents normal H bonding patterns. It is less obvious, however, that alternative H bonding schemes, even between complementary bases such as G and C, can be realized: For example, blockage of the Watson-Crick site N(1) and the Hoogsteen site N(7) of guanine simultaneously makes cytosine to dock to the N(2)H₂ and N(3) sites of guanine [35].

If a Pt(II) entity resides at the N(7) position of guanine, the following scenarios have been verified on the nucleobase level: (i) "Normal" Watson-Crick H bonding with cytosine. This feature, qualitatively established earlier by ¹H NMR spectroscopy [36] and confirmed by X-ray crystallography [32], has now been quantified [37] and further supported by additional X-ray structural data [38]. (ii) Pairing between a N(7) metalated, N(1) deprotonated guanine and neutral guanine. This situation corresponds to a mispair between two guanine bases, brought about by initial binding of a metal ion, followed by ionization of the proton at N(1) [39]. (iii) Pairing between two N(7) platinated G's. As with (ii), this H bonding pattern involves the O(6), N(1) and N(2) sites of the two guanine nucleobases, with one of the two bases deprotonated. In that case the two guanine residues are self-complementary, recognizing each other. Although this pattern is expected to be favoured at a pH corresponding to the pK_a of the platinated G (≈ 8), there is evidence that it is realized even at considerably lower pH [38,40].

H bonded supramolecular assemblies of a different kind are feasible when H bonding between N(1) metalated, unsubstituted cytosine and free or N(7) metalated guanine is considered [41]. Watson-Crick H bonding is realized, but due to differences in pK_a (ca 4-5 for cytosine; ca. 7 for N(1) platinated cytosine) the pH range available for this H bonding pattern is shifted to somewhat higher values as compared to normal G,C pairing. Similarly, cytosine self-pairing analogous to that between hemiprotonated cytosine

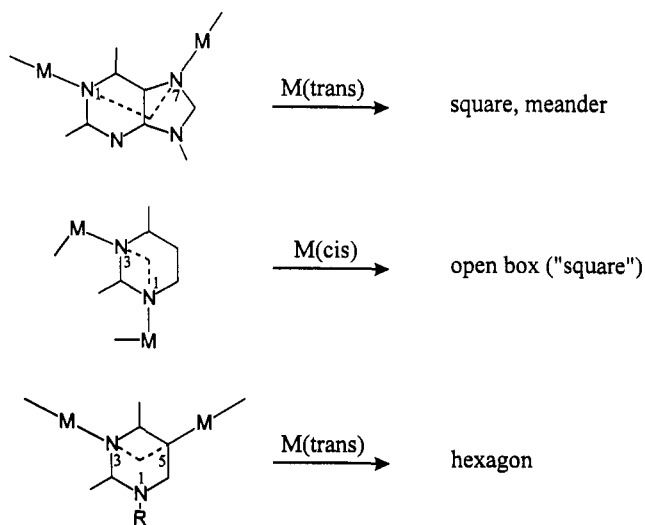
nucleobases is possible also for N(1) metalated cytosine, although not at moderately acidic pH, as common for the free base, but rather at neutral pH.

5. MOLECULAR ARCHITECTURE WITH METAL-NUCLEOBASE COMPLEXES

As outlined above (c.f. (1)), the right angle between M-N(1) and M-N(7) vectors in dimetalated purine bases permit cyclic or open structures with the organic ligand providing the 90° angle. This feature makes any closed square or rectangle qualitatively different from those "molecular squares" that utilize the right angle of a Pt(II) or Pd(II) entity in *cis*-geometry and an organic ligand with two donor atoms in a transoid orientation (frequently 4,4'-bipyridine) [42]. Compared to the purine squares or rectangles, the roles of metal ion and organic ligand are reversed in the two types of compounds. The possibility to take advantage of G,G pairing (c.f. (4)) provides an additional point of interest for open structures containing metal purine complexes [25]. Finally, combining *cis*- and *trans*-geometries of metal ions in a defined fashion [43] may add yet another twist to this theme.

As has been demonstrated by us, diplatinated (N(1) and N(3)) uracil nucleobases can form a "square" when cross-linked by *cis*-a₂Pt(II) [44]. It shows a remarkable affinity for additional metal ions, forming readily octanuclear complexes eventually [45]. In contrast, the utilization of a *trans*-geometry about the metal, realized in a compound of 1-methylcytosine with N(3) and C(5) metal binding, leads to the expected flat hexagon structure [45], although open structures are likewise feasible.

The three examples (Scheme 4) are to be considered representatives of a large class of compounds to be



potentially available from the combination of nucleobases with metal ions. Whether or not such complexes are of any usefulness (e.g. for molecular recognition) remains to be investigated.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

1. J. D. Watson and F. H. C. Crick, *Nature* **171**, 737 (1953).
2. R. Wing, H. R. Drew, T. Takano, C. Broka, S. Tanaka, K. Itakura and R. E. Dickerson, *Nature* **287**, 755 (1980).
3. See, e.g.: R. D. Wells and S. C. Harvey, eds., "Unusual DNA Structures", Springer, New York (1988).
4. S.-H. Kim and A. Rich, *Science* **162**, 1381 (1968).
5. R. S. Brown, B. E. Hingerty, J. C. Dewan and A. Klug, *Nature* **303**, 543 (1983).
6. B. Rosenberg, L. VanCamp, J. E. Trosko and V. H. Mansour, *Nature* **222**, 385 (1969).
7. H. C. Harder and B. Rosenberg, *Int. J. Cancer* **6**, 207 (1970).
8. See, e.g.: Series H. Sigel and A. Sigel, eds., "Met. Ions Biol. Syst.", M. Dekker Inc., New York and Basel.
9. (a) B. Lippert, *Biometals* **5**, 195 (1992). (b) B. Lippert, *Prog. Inorg. Chem.* **37**, 1 (1989). (c) E. Zangrando, F. Pichierri, L. Randaccio and B. Lippert, *Coord. Chem. Rev.* **156**, 275 (1996).
10. A. R. Morgan, *Nature* **227**, 1310 (1970).
11. S. Mc Gavin, *J. Mol. Biol.* **55**, 293 (1971).
12. H. M. Sobell, K. Tomita and A. Rich, *Proc. Natl. Acad. Sci. USA* **49**, 885 (1963).
13. C. Ban, B. Ramakrishnan and M. Sundaralingam, *J. Mol. Biol.* **236**, 275 (1994).
14. C. Gaillard and F. Strauss, *Science* **264**, 433 (1994).
15. A. Lebrun and R. Lavery, *J. Biomol. Struct. Dyn.* **13**, 459 (1995).
16. See, e.g.: (a) W. I. Sundquist, *Nucl. Acids Mol. Biol.* **5**, 1 (1991). (b) A. I. H. Murchie, F. Aboul-Ela, G. Laughlan, B. Luisi and D. M. J. Lilley, *Nucl. Acids Mol. Biol.* **9**, 143 (1995).
17. See e.g.: K. Y. Wang, S. H. Krawczyk, N. Bischofberger, S. Swaminathan and P. H. Bolton, *Biochemistry* **32**, 11285 (1993).
18. (a) G. Gupta, A. E. Garcia, Q. Guo, M. Lu and N. R. Kallenbach, *Biochemistry* **32**, 7098 (1993). (b) M. H. Sarma, J. Luo, K. Umamoto, R.-d. Yuan and R. H. Sarma, *J. Biomol. Struct. Dyn.* **9**, 1131 (1992).
19. C. Cheong and P. B. Moore, *Biochemistry* **31**, 8406 (1992).
20. B. Fischer, H. Preut, B. Lippert, H. Schöllhorn and U. Thewalt, *Polyhedron* **9**, 2199 (1990).
21. H. Witkowski, E. Freisinger and B. Lippert, *J. Chem. Soc., Chem. Commun.*, 1315 (1997).
22. S. Metzger and B. Lippert, *J. Am. Chem. Soc.* **118**, 12467 (1996).
23. V. Brabec, M. Šíp and M. Leng, *Biochemistry* **32**, 11676 (1993).
24. A. Schreiber E. C. Hillgeris and B. Lippert, *Z. Naturforsch.* **48b**, 1603 (1993).
25. A. Schreiber, M.-S. Lüth, A. Erxleben, E. C. Fusch and B. Lippert, *J. Am. Chem. Soc.* **118**, 4142 (1996).
26. M.-S. Lüth and B. Lippert, to be published.
27. G. Felsenfeld, D. R. Davies and A. Rich, *J. Am. Chem. Soc.* **79**, 2023 (1957).
28. (a) V. N. Soyfer and V. N. Potaman, *Triple-Helical Nucleic Acids*, Springer, New York (1996). (b) D. G. Knorre, V. V. Vlassov, V. F. Zarytova, A. V. Lebedev and O. S. Federova, *Design and Targeted Reactions of Oligonucleotide Derivatives*, CRC Press, Boca Raton (1994).
29. M. Sabat and B. Lippert, *Met. Ions Biol. Syst.* **33**, 143, (1996).
30. (a) J. Bernues, R. Beltran, J. M. Casasnovas and F. Azorin, *Nucl. Acids Res.* **18**, 4067 (1990). (b) V. A. Malkov, O. N. Voloshin, V. N. Soyfer and M. D. Frank-Kamenetskii, *Nucl. Acids Res.* **21**, 585 (1993).
31. V. N. Potaman and V. N. Soyfer, *J. Biomol. Struct. Dyn.* **11**, 1035 (1994).
32. I. Dieter-Wurm, M. Sabat and B. Lippert, *J. Am. Chem. Soc.* **114**, 357 (1992).
33. See, e.g.: N. T. Thuong and C. Hélène, *Angew. Chem. Int. Ed. Engl.* **32**, 666 (1993).
34. C. Colombier, B. Lippert and M. Leng, *Nucl. Acids Res.* **24**, 4519 (1996).
35. R. K. O. Sigel, E. Feisinger and B. Lippert, submitted for publication.
36. B. Lippert, *J. Am. Chem. Soc.* **103**, 5691 (1981).
37. R. K. O. Sigel and B. Lippert, to be submitted.

38. S. Meier, E. Freisinger and B. Lippert, to be submitted.
39. G. Schröder, B. Lippert, M. Sabat, C. J. L. Lock, R. Faggiani, B. Song and H. Sigel, *J. Chem. Soc., Dalton Trans.*, 3767 (1995).
40. C. Meiser, E. Freisinger and B. Lippert, to be submitted.
41. W. Brüning, E. Freisinger, M. Sabat and B. Lippert, to be submitted.
42. (a) M. Fujita, J. Yazaki and K. Ogura, *J. Am. Chem. Soc.* **112**, 5645 (1990). (b) P. J. Stang and D. H. Cao, *J. Am. Chem. Soc.* **116**, 4981 (1994). (c) C. A. Hunter, *Angew. Chem. Int. Ed. Engl.* **34**, 1079 (1995).
43. S. Jaworski, S. Menzer, B. Lippert and M. Sabat, *Inorg. Chim. Acta* **205**, 31 (1993).
44. (a) H. Rauter, E. C. Hillgeris and B. Lippert, *J. Chem. Soc., Chem. Commun.*, 1385 (1992). (b) H. Rauter, E. C. Hillgeris, A. Erxleben and B. Lippert, *J. Am. Chem. Soc.* **116**, 616 (1994).
45. H. Rauter, I. Mutikainen, M. Blomberg, C. J. L. Lock, P. Amo-Ochoa, E. Freisinger, L. Randaccio, E. Zangrando, E. Chiarparin and B. Lippert, *Angew. Chem. Int. Ed. Engl.* **36**, 1296 (1997).