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Interactions of diaryl-polyamines with nucleic acids. Allosteric effects with dinuclear copper complexes

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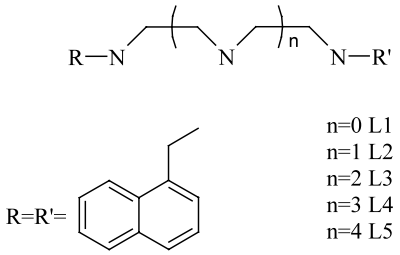
Abstract—A series of α,ω-diarylamines with a variable number of ethylenediamine units between terminal naphthylrings shows dramatic affinity changes towards double-stranded nucleic acids, particularly upon complexation with Cu(II) ions. Metal salts alone have under the applied conditions only a negligible effect. The affinity of the metal-free ligands towards nucleic acids shows significant differences to those of the underlying polyamines, with a reversed stabilization of DNA instead of the usually observed preference for RNA. The affinity changes and preliminary NMR studies are in line with intercalation of naphthylrings into the double-stranded nucleic acid, which is hampered by complex formation with Cu(II). © 2002 Published by Elsevier Science Ltd.

The development of new ligands which can selectively interact with nucleic acids is of much current interest.[1] Ligands that undergo affinity changes under the influence of additional substrates could be used to control the desired effects in a more selective way. Metal ions in combination with bipyridyl-related ligands have been shown earlier to alter binding modes towards nucleic acids.[2] We describe here a new strategy to switch binding modes of polyamines towards double-stranded nucleic acids with the ligands L1–L5, built up by linking naphthyl groups at both ends of different open-chain polyamine compounds containing ethylene bridges between the successive amino groups (Scheme 1). Such ligands are classical chelators for many transition metals; they can be expected to change their conformation significantly upon addition of e.g. Cu(II) salts. As a result of this intercalation, at least one of the aryl groups attached at the ends of the chains should no longer be possible, and by this and/or by other binding distortions the affinity of the ligand could significantly decrease.



R=R'=H, n=3 : L4a

Scheme 1. Ligand structures.

Earlier studies with e.g. large aryl units such as acridines connected by either flexible,[3,4] or rigid [5] spacers did not address metal-induced affinity changes. Such large aryl units are strong intercalators, and for this reason conformational distortions in the spacer induced by allosteric effects may produce only smaller binding changes. Naphthyl derivatives intercalate with moderate strength,[6] are therefore more suited for conformational switching; they were prepared as described before.[7] The derivatives L3 and L4 containing three or four ethylenediamine units (Fig. 1) have 12 or 15 atoms between the aryl units, which in view of literature data[3] makes them promising candidates for bisintercalation.

Addition of Cu(II) to the ligands indeed showed dramatic changes of affinity with these ligands, in particular with L4, thus verifying the switching strategy by

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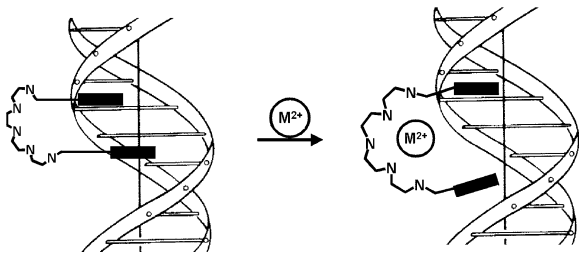

Figure 1. Model for the allosteric effect.

induction of allosteric effects in the ligands (Table 1). This should not be confused with the allosteric changes of DNA itself which occurs upon addition of many ligands.[8]

The affinities were measured by changes of the melting temperature (ΔT_M) of double-stranded polydA–polydT sequences as DNA model, and with polyA–polyU as RNA model, using different ratios r of ligand to nucleic acid base pair. In addition, eight combinations with Cu(II)-free ligands were checked with an ethidiumbromide fluorescence assay, leading to the same affinity variations as observed by the T_M changes.

Addition of Cu(II) salts alone has only a small effect on the melting (with ΔT_M up to +4°C), whereas a T_M decrease by up to 19°C occurred even at low ratios $r=0.1$ of ligand to base pair. The effect is with $\Delta T_M=$ 16°C somewhat smaller with the RNA sequence polyA-polyU, but there it can lead even to beginning **de**stabilization of the double strand. Surprisingly, much larger effects occurred with two instead of one Cu(II) ions per ligand, although potentiometric analysis indicates that as expected all these ligands take up only one copper ion. Speciation studies with AMP, Cu^{2+} and L4 or L5 gave the clue for the need of two copper ions for an optimal conformational switching. Binuclear mixed complexes of stoichiometries $[Cu_2H(AMP)L]^{3+}$, $[Cu_2(AMP)L]^{2+}$ and $[Cu_2(OH)(AMP)L]^{+}$ were found to be formed above pH 6 in the system Cu(II)–AMP–L5; the overall percentages of the species formed as a function of pH are shown in Fig. 2.[9]

For the system Cu(II)–AMP–L4 a complex of stoichiometry $[Cu_2(OH)(AMP)L]^{3+}$ was also found at pH values above 7. Apart from these binuclear complexes, mononuclear ones of stoichiometries $[CuH_j(AMP)L]^{j+}$ ($j=1$–5 for L4, $j=1$–4 for L5) were also found at lower pH. The ternary complexes clearly predominate over the binary ones at the concentrations used for the DNA or RNA affinity measurements. Obviously, even though Cu(II) has only a small influence on the duplex stabilities, the formation of the mixed complexes between the groove phosphates and the polyamines produces the allosteric effect. Melting points with the parent ligand

Table 1. Melting point studies with polydA–polydT, polyA–polyU and L1–L5 and copper complexes (L/Cu^{2+}, 1:1, 1:2)[c]

Ligand	ΔT_M (°C) PolyA PolyT			ΔT_M (°C) PolyA.PolyU		
L	L	L/Cu^{2+} 1:1	L/Cu^{2+} 1:2[a]	L	L/Cu^{2+} 1:1	L/Cu^{2+} 1:2[b]
L1	3.8	[d]	[d]	0.1	[d]	[d]
L2	10.7	10.0	7.7	1.0	0.4	0.4
L3	14.7	12.6	10.4	5.1	3.9	2.7
L4	26.7	20.4	8.1	15.5	14.1	−0.2
L4a	4.5	3.2	3.0	26.0	15.0	10.8
L5	24.2	25.6	12.8	24.3	18.7	7.0

[a] Effect of Cu^{2+} alone: (1) $r=0.1$, $\Delta T_M=4.0$; (2) $r=0.2$, $\Delta T_M=5.2$; (3) $r=0.3$ $\Delta T_M=6.4$.
[b] Effect of Cu^{2+} alone: (1) $r=0.1$, $\Delta T_M=3.5$; (2) $r=0.2$, $\Delta T_M=4.8$; (3) $r=0.3$ $\Delta T_M=6.8$.
[c] Conditions: 0.01 M MES buffer, pH 6.25; molar ratio ligand/nucleic acid phosphate $r=0.1$, error in $\Delta T_M=\pm0.5$°C. Values at ratios of $r=0.2$ and $r=0.3$ showed similar trends and will be reported later.
[d] Precipitation.

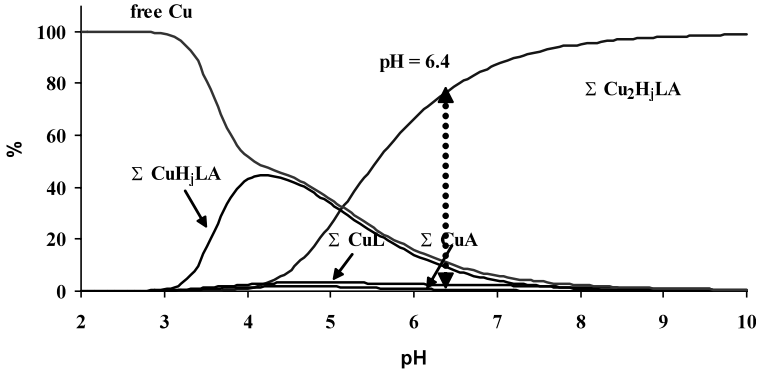

Figure 2. Overall percentages of formation of ternary and binary complexes in the system Cu(II)–L5–AMP at molar ratio 2:1:1. pH 6.4 is indicated with a dotted arrow.

L4a lacking the terminal naphthyl units shows **no** affinity changes with the DNA polymer if one compares the effects with and without copper, but sizeable ones with RNA (Table 1). The T_M values indicate that the effects are not simply due to a competition between the groove phosphates and the ligand for the metal ions;[10] in spite of conformational distortion the copper–ligand complexes do bind to the nucleic acids.

Allosteric ligand–nucleic acid complexes with transition metal ions such as Cu(II) have promising features. They can be redox switched, as not only the charge but also the ligand field and coordination depends on the oxidation state. In particular, such Cu(II) complexes can be used as artificial nucleases,[11] in which interaction between the redox-active metal ion and the nucleic acids can be controlled by ligands related to the polyamines of the present study.

Besides the observed allosteric effects on DNA in presence of copper ions the α,ω diaryl-polyamines exhibit interesting selectivity in binding to DNA or RNA sequences. The ligands offer three binding modes to double-stranded nucleic acids, which can be cooperative or anti-cooperative. The ion pairing between the ammonium centers and the groove phosphates can be distorted by the presence of the large aryl units, and/or these can enhance the affinity by either intercalation or binding to more hydrophobic groove parts. The results in Table 1 show both enhanced and decreased affinity in comparison to the underlying polyamines, which have been characterized before.[12]

The affinities of the polyamines is as usual much higher towards the RNA model, with melting points usually 20–25°C above the DNA sequence even at low ratios $r=0.1$, the only exception being L1 (ethylenediamine) itself with $\Delta\Delta T_M=3.5$°C. Introduction of the terminal aryl groups leads to dramatic changes: with the exception of the very long chains L5 all compounds show the **reversed** larger stabilization of the DNA sequence (Table 1). This reversal is most pronounced for those ligands in which the chain length allows bisintercalation (L4), but is still seen with L2. The affinity is in most cases increased by the presence of the aryl groups in comparison to the underlying 'free' polyamines, which in the case of RNA lead always to T_M values higher by up to 35°C. With DNA the affinity stays about the same for L3 and L2, whereas the longer L4 does show a large increase by 24°C. The strong affinity increase with this ligand upon introduction of the naphthyl end groups (L4 versus L4a, $\Delta\Delta T_M=22.5$°C at $r=0.1$ for DNA) supports additional stabilization by bisintercalation, for which in view of literature data[13] the chain length in L4 is particularly well suited. NMR spectra of a mono-naphthyl compound corresponding to L4 in the presence of CT-DNA gave clear evidence for intercalation by line broadening of up to 15 Hz and upfield shifts of up 0.15 ppm for the naphthalene signals, which is typical for stacking inbetween the nucleobases.[14] Attempts with other ligands failed due to the formation of insoluble material with the nucleic acids. The aryl groups lead to less affinity increase with RNA is likely due to the strong electrostatic effects in the more narrow RNA groove, which by steric constraints can prevent intercalation of the attached naphthalene rings. Available data on related intercalators such as acridines show no uniform preference for alternative RNA or DNA sequences.[15]

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References

1. (a) Wilson, W. D. In *Nucleic Acids in Chemistry and Biology*; Blackburn, G. M.; Gait, M. J., Eds.; 2nd ed.; Oxford University Press: New York, 1996; p. 329; (b) Denison, C.; Kodadek, T. *Chem. Biol.* **1998**, *5*, R129; (c) Hermann, T.; Westhof, W. *Curr. Opin. Biotech.* **1998**, *8*, 278; (d) Afshar, M.; Prescott, C. D.; Varani, G. *Curr. Opin. Biotech.* **1999**, *10*, 59; (e) Chow, C.; Bogdan, F. M. *Chem. Rev.* **1997**, *97*, 148; (f) Chaires, J. B. *Curr. Opin. Struct. Biol.* **1998**, *8*, 314.
2. (a) Sigman, D. S. *Acc. Chem. Res.* **1986**, *19*, 180; (b) Pyle, A. M.; Barton, J. K. *Prog. Inorg. Chem.* **1990**, *38*, 413 and references cited therein.
3. (a) Wakelin, L. P. G. *Med. Res. Rev.* **1986**, *3*, 275; (b) Canellakis, E. S.; Shaw, Y. H.; Hanners, W. E.; Schwartz, R. A. *Biochem. Biophys. Acta* **1976**, *418*, 277.
4. Phenanthridines as bisintercalators: Cory, M.; McKee, D. D.; Kagan, J.; Henry, D. W.; Miller, J. A. *J. Am. Chem. Soc.* **1985**, *107*, 2528.
5. Zimmerman, S. C.; Lamberson, C. R.; Cory, M.; Fairley, T. A. *J. Am. Chem. Soc.* **1989**, *111*, 6805.
6. Schneider, H.-J.; Sartorius, J. *J. Chem. Soc., Perkin Trans. 2* **1997**, 2319.
7. Albelda, M. T.; Bernardo, M. A.; Dïaz, P.; García-España, E.; Seixas de Melo, J.; Pina, F.; Soriano, C.; Luis, S. V. *Chem. Commun.* **2001**, 1520.
8. (a) Kwok, Y.; Zhang, W. T.; Schroth, G. P.; Liang, C. H.; Alexi, N.; Bruice, T. W. *Biochemistry* **2001**, *40*, 12628; (b) Suh, D. C. *Exp. Mol. Med.* **2000**, *32*, 204; (c) Qu, X. G.; Trent, J. O.; Fokt, I.; Priebe, W.; Chaires, J. B. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 12032.
9. Potentiometric studies were carried out in 0.15 mol dm^{-3} NaCl at 298.1 K. The computer program HYPERQUAD (Gans, P.; Sabatini, A.; Vacca, A. *Talanta* **1996**, *43*, 1739) was employed to derive the stability constants of the systems.
10. We thank an earlier reviewer for related questions.
11. Sigman, D. S.; Mazumder, A.; Perrin, D. M. *Chem. Rev.* **1993**, *93*, 2295.
12. Chand, D. K.; Schneider, H.-J.; Aguilar, J. A.; Escartí, F.; García-España, E.; Luis, S. V. *Inorg. Chim. Acta* **2001**, *316*, 71.
13. Laurence, P.; Wakelin, G. *Med. Res. Rev.* **1986**, *6*, 275.
14. Sartorius, J.; Schneider, H.-J. *FEBS Lett.* **1995**, *374*, 387.
15. Wilson, W. D.; Ratmeyer, L.; Zhao, M.; Strekowski, L.; Boykin, D. *Biochemistry* **1993**, *32*, 4098.