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Design of a Chemical Nuclease Model with $(Lys)_2Cu$ as the Core Motif

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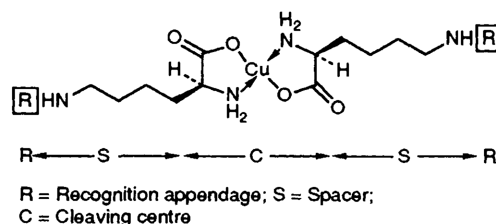
Coded amino acid L-lysine is utilized for the design of a chemical nuclease model wherein the α-amino carboxylate unit of the amino acid is used for the formation of the copper complex acting as the metal cleaving centre, and the ω-amino function of the molecule is exploited to carry the DNA recognition elements; models with duplex purines and quinazolines as the carrier ligands attached at the ω-amino end of the lysine–metal template are synthesized and demonstrated to effect the cleavage of double stranded DNA under physiological conditions.

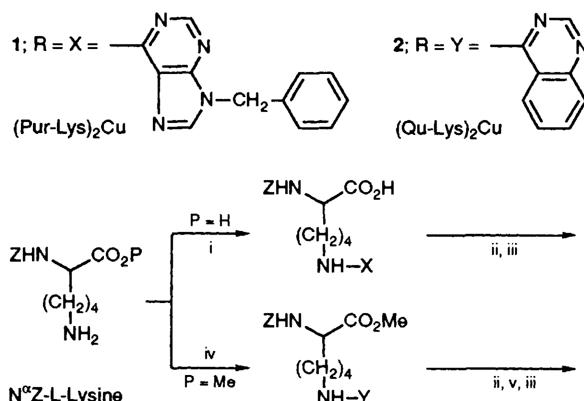
Design of synthetic molecules for sequence specific recognition and cleavage of the DNA double helix continues to be an attractive goal in nucleic acid research.[1] In order to achieve the desirable high sequence specificity† a general approach has been to combine the DNA binding and cleaving capabilities into a single molecule by linking a nucleolytic agent‡ with a known DNA binding drug, protein or oligonucleotide.[2]

We present here, the design of a highly versatile chemical nuclease model (Scheme 1) wherein the DNA cleaving agent is positioned in the centre of a duplex recognition termini linked through an appropriate spacer. This model has similarities with DNA recognition proteins, such as GAL4[3] wherein the recognition takes place at the termini of a symmetric duplex, assembled by a central dimerization element linked *via* a spacer to the end units. The coded amino acid L-lysine, with its α and ω NH_2 groups separated by a four methylene spacer, appeared to us as tailor made for crafting such a design. The strategy envisaged the utilization of the α-amino carboxylate unit of L-lysine to form the desired metal template acting as the cleaving centre on the one hand and the ω-amino group for the attachment of DNA recognition elements on the other, giving rise to a duplex recognition termini harbouring a centrally placed Cu^{II} for potentiation of oxidative scission.

In a preliminary study reported here, models with duplex quinazolines and purines as the carrier ligands at the ω-amino end of the lysine–metal template have been synthesized and demonstrated to effect the cleavage of the double stranded DNA under physiological conditions.

N^{α}Z-L-Lysine[4] (Z = benzyloxycarbonyl) was condensed with 9-benzyl-6-chloropurine[5] in dry Me_2SO in the presence of anhyd. K_2CO_3 to yield 9-benzylpurinyl (6-N^{ω})-N^{α}Z-Lys-OH (85%, m.p. 197–98 °C). Z-Deprotection to 9-benzylpurinyl (6-N^{ω})-Lys-OH (91%, m.p. 245–47 °C) followed by brief heating with aqueous basic copper carbonate (0.5 equiv.) afforded **1** as a light-blue microcrystalline solid (63%, m.p. 215–17 °C).§ Compound **2** was prepared *via* sequence con-





Scheme 1 *Reagents and conditions*: i, X–Cl, anhyd. K_2CO_3, Me_2SO, room temp; ii, Pd/C(10%), H_2, MeOH; AcOH; iii, $CuCO_3{\cdot}Cu(OH)_2$, water, boil, 5 min; iv, Y–Cl, NEt_3, CH_2Cl_2, room temp.; v, NaOH(aq) (1 mol dm^{-3}), room temp.

† A sequence specificity of 12–15 base pairs is needed for mapping the human genome, P. B. Dervan, *Science*, 1986, **232**, 464.

‡ These are also called chemical nucleases—defined as redox active coordination complexes that nick nucleic acids under physiological conditions by oxidative attack on a ribose or a deoxyribose moiety. For recent reviews, see: D. S. Sigman, *Biochemistry*, 1990, **29**, 9097; D. S. Sigman and C. B. Chen, *Annu. Rev. Biochem.*, 1990, **59**, 207.

§ Attempts to prepare **1** by direct treatment of lysine copper complex with 9-benzyl-6-chloropurine proved unsatisfactory due to extensive hydrolysis of chloropurine.

densation of 4-chloroquinazoline[6] with N^{α}Z-L-Lys-OMe to give quinazolyl (4-N^{ω})-N^{α}Z-Lys-OMe (63%, m.p. 113–15 °C), Z-deprotection to the free amino ester (99%, gum), hydrolysis (98%, m.p. 130–32 °C) and metal template formation (tiny blue crystals, 95%, m.p. 208–10 °C)¶ with basic copper carbonate (Scheme 1).||

The DNA binding capability of **1** and **2** was tested by UV absorption spectroscopic studies using calf thymus DNA. A decrease in the molar absoption coefficient combined with the shift of the wavelength of maximum absorbance to the lower energy range (~ 15 nm shift) indicated significant binding of both compounds to DNA double helix (Fig. 1). In binding measurements with poly[d(A-T).d(A-T)] and poly[d(G-C).d(G-C)]** both **1** and **2** exhibited relatively more affinity for poly[d(G-C).d(G-C)].††

The DNA cleaving properties of **1** and **2** were tested with pBR 322. Upon incubation in the presence of 3-mercaptopropionic acid (MPA) as exemplified in Fig. 2, covalently closed supercoiled (form I) DNA is converted largely into an open circle (form II) suggesting nicking of the single strand by both **1** and **2** at the binding site. Although it has been shown that cutting of DNA is associated with binding of **1** and **2**, the scission may be occuring at a single binding site or at one of the several different available sites in DNA.‡‡ The efficiency of cleavage was found to be higher in the case of **2**, probably arising from a better groove binding interaction of the quinazoline ring system.§§ Appearance of linear (form III) DNA upon treatment with a higher concentration of cleaving agents indicates a double strand cleavage.

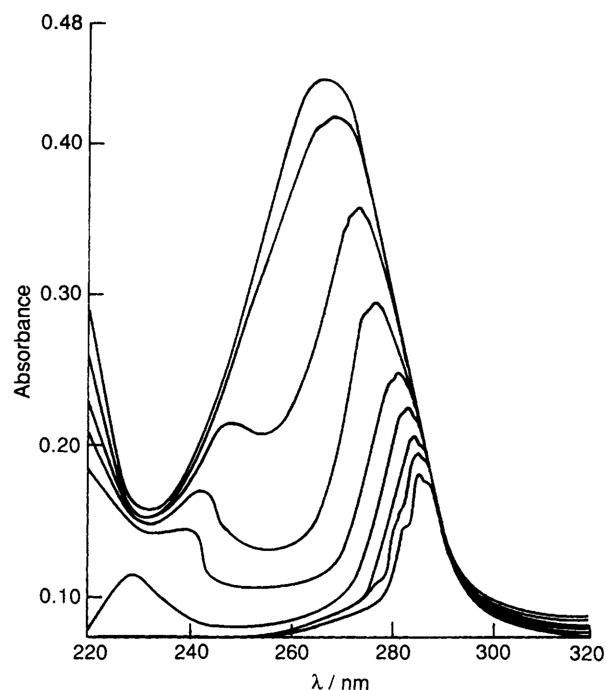


Fig. 1 Spectra for $(Pur\text{-}Lys)_2Cu$ **1** in the presence of varying amounts of calf thymus DNA. The concentration of **1** in each scan was 9.25×10^{-6} mol dm^{-3}. From top to bottom the DNA concentrations (base pair molarity) were 0, 7.00×10^{-5}, 2.60×10^{-4}, 4.60×10^{-4}, 6.3×10^{-4}, 8.20×10^{-4}, 1.20×10^{-3}, 1.21×10^{-3}, 1.37×10^{-3} mol dm^{-3}.

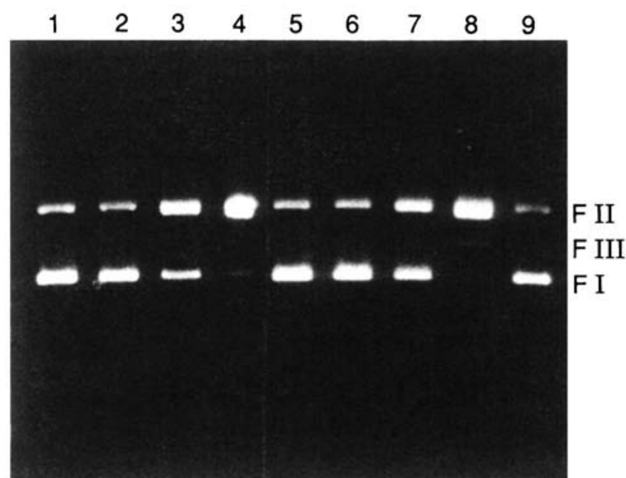


Fig. 2 Cleavage of supercoiled pBR 322 DNA with **1** and **2**. pBr 322 DNA (200 ng. Form I) in a 10 mmol dm^{-3} Tris-HCl, pH 7.5, was incubated (total volume 10 μl) with the cleaving agent in the presence of MPA (2 mmol dm^{-3}). After 10 min of incubation at 25 °C, the reaction was stopped by the addition of 20 μmol dm^{-3} neocuprin and samples were analysed on 1% agarose gel. The electrophoresis was carried out at 5 V cm^{-1} in TBE [89 mmol dm^{-3} Tris-borate, pH 8.3, 2 mmol dm^{-3} ethylenediaminetetraacetic acid (EDTA)]. Lanes 1 and 5: DNA alone; lanes 2, 3 and 4: DNA + 0.37, 0.56 and 0.74 mmol dm^{-3} of **1**; lanes 6, 7 and 8: DNA + 0.17, 0.33 and 0.50 mmol dm^{-3} of **2**; lane 9: Control, DNA + MPA + neocuprin.

¶ Compound **2** was conveniently obtained in >90% yield by direct condensation of 4-chloroquinazoline with lysine copper complex in aqueous acetone in the presence of bicarbonate.

|| All new compounds were fully characterized on the basis of their spectral and analytical data. *Selected spectral data*: 9-Benzylpurinyl (6-N^{ω})-N^{α}Z-Lys-OH: ^{1}H NMR(80 MHz,[$^2H_6$]Me_2SO) δ 1.56 (6H, br), 3.64 (2H, br), 4.45 (1H, m), 5.07 (2H, s). 5.42 (2H, s), 7.04–7.82 (12H, m), 8.17 (1H, s), 8.26 (1H, s); IR(KBr) ν/cm^{-1} 3337, 3288, 2934, 1687, 1628, 1589, 1531; MS (*m/z*) 489 (M + H)$^+$. 9-Benzylpurinyl (6-N^{ω})-Lys-OH: ^{1}H NMR (80 MHz, [$^2H_6$]Me_2SO) δ 1.53 (6H, br), 3.53 (2H, br), 4.46 (1H, m), 5.35 (2H, s), 7.29 (5H, s), 7.82–8.76 (3H, br), 8.23 (1H, s), 8.32 (1H, s); IR (KBr) ν/cm^{-1} 3419, 3055, 1672, 1619; MS (*m/z*) 354 (M$^+$), 355 (M + H)$^+$. $(Pur\text{-}Lys)_2Cu$ **1**: IR(KBr) ν/cm^{-1} 3239, 2930, 1685, 1618; FAB-MS (*m/z*) 770 (M$^+$), 355[1/2(M − Cu) + H]$^+$. Quinazolyl (4-N^{ω})-N^{α}Z-Lys-OMe: ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.68 (6H, m), 3.43–3.84 (5H, s + br), 4.39 (1H, m), 5.07 (2H, s), 5.73 (1H, d, *J* 7.5 Hz), 6.60 (1H, m), 7.04–8.14 (9H, s + m), 8.65 (1H, s); IR(KBr) ν/cm^{-1} 3333, 3175, 2949, 1748, 1701, 1581; MS (*m/z*) 422 (M$^+$). Quinazolyl (4-N^{ω})-Lys-OMe: ^{1}H NMR (80 MHz, $CDCl_3$ + [$^2H_6$]Me_2SO) δ 1.65 (6H, m), 3.50 (2H, m), 3.70 (3H, s), 4.34 (1H, m), 6.50 (3H, br), 7.10–8.00 (4H, m), 8.50 (1H, s); IR(KBr) ν/cm^{-1} 3272, 2951, 1748, 1618, 1586, 1546. Quinazolyl (4-N^{ω})-Lys-OH: ^{1}H NMR (80 MHz, [$^2H_6$]Me_2SO) δ 1.62 (6H, m), 3.62 (2H, m), 4.34 (1H, m), 7.35–8.07 (5H, m), 8.61 (2H, br), 8.71 (1H, s), 9.60 (1H, br); MS (*m/z*) 275 (M + H)$^+$; $(Qu\text{-}Lys)_2Cu$ (**2**): IR (KBr) ν/cm^{-1} 3329, 3280, 3237, 2941, 2856, 1615, 1507, 1469; FAB-MS (*m/z*) 613 (M + H)$^+$.

** The two duplexes used in UV studies were composed of the following alternating sequences:

----ATATAT--- ----CGCGCG----
----TATATA--- and ----GCGCGC----respectively

†† In UV titration of **1** with poly[d(G-C).d(G-C)] and poly-[d(A-T).d(A-T)] the following profile was observed at saturation point.

DNA duplexes	Decrease in absorbance (%)	Red shift /nm
Poly[d(G-C).d(G-C)]	22	10
Poly[d(A-T).d(A-T)]	08	02

Similar results were obtained with **2**.

‡‡ These two possibilities can be distinguished by experiments with high sequencing gels using ^{32}P-end labelled DNA. Such experiments are planned with designs having larger recognition elements (*cf.* footnote¶¶).

§§ Molecular modelling studies have shown the possibility of better groove binding interaction in the case of **2**.

Control experiments verified that neither the cleaving agent alone, nor the reductant (MPA) and Cu^{II} mixture generated any appreciable amount of cleaved products from plasmid DNA.

The Cu^{II} mediated DNA cleavage observed with **1** and **2** appears to be in common with the well studied DNA strand scission with copper complex of bleomycin,[7] haemin,[8] 1,10-phenanthroline,[9] epicatechin and procyanidin B_2.[10]

In conclusion, the present work has shown that the design of chemical nucleases having the cleaving domain flanked by the dimeric recognition elements is feasible, and, by judicious selection may lead to composites with a higher order of sequence specificity.¶¶ It is planned to incorporate in the design presented here motifs that have been shown to recognize specific DNA sequences and patterned from DNA binding proteins like GAL4, MetJ and Trp repressors and other synthetic elements.

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¶¶ Sequence specificity experiments with one of the models—carrying duplex Ala-Gly units at the ω-amino end of $(Lys)_2Cu$ $[(AGK)_2Cu]$— on a ^{32}P 3′-end labelled 117bp restriction fragment (EcoRI/HindIII) of pUC 18 showed almost exclusive attacks at thymidylate residues, in particular, thymines corresponding to the 5′T of the CTAT (3′-5′) box. The most preferred site of attack is found at T of 3′-ATC-5′ at the trinucleotide level. Sequences 3′-GATCT-5′ and 3′-TATCT-5′ are favoured at the pentanucleotide level.

References

1 D. Pei and P. G. Schultz, *J. Am. Chem. Soc.*, 1990, **112**, 4579; D. R. Corey, D. Pei and P. G. Schultz, *Biochemistry*, 1990, **28**, 8277; S. A. Strobel and P. B. Dervan, *Science*, 1990, **249**, 73; E. Uhlmann and A. Peyman, *Chem. Rev.*, 1990, **90**, 543; A. M. Pyle, T. Morii and J. K. Barton, *J. Am. Chem. Soc.*, 1990, **112**, 9432; K. Nagai, B. J. Carter, J. Xu and S. M. Hecht, *J. Am. Chem. Soc.*, 1991, **113**, 5099; A. Sitlani, E. C. Long, A. M. Pyle and J. K. Barton, *J. Am. Chem. Soc.*, 1992, **114**, 2303; M. V. Keck and S. J. Lippard, *J. Am. Chem. Soc.*, 1992, **114**, 3386.

2 J. P. Sluka, J. H. Griffin, J. P. Mack and P. B. Dervan, *J. Am. Chem. Soc.*, 1990, **112**, 6369; D. P. Mack, B. L. Iverson and P. B. Dervan, *J. Am. Chem. Soc.*, 1988, **110**, 7572; D. P. Mack and P. B. Dervan, *J. Am. Chem. Soc.*, 1990, **112**, 4604; M. D. Distefano, J. A. Shin and P. B. Dervan, *J. Am. Chem. Soc.*, 1991, **113**, 5901; J. S. Koh and P. B. Dervan, *J. Am. Chem. Soc.*, 1992, **114**, 1470; P. B. Dervan, *Nature*, 1992, **359**, 87.

3 R. Marmorstein, M. Carey, M. Ptashne and S. C. Harrison, *Nature*, 1992, **356**, 408.

4 A. A. Costapanagiotis, B. O. Handford and B. Weinstein, *J. Org. Chem.*, 1968, **33**, 1261.

5 J. A. Montgomery and C. Temple, *J. Am. Chem. Soc.*, 1961, **83**, 630.

6 W. L. F. Armarego, *J. Appl. Chem.*, 1961, **11**, 70.

7 Y. Sugiura, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 375; N. J. Oppenheimer, C. Chang, L. O. Rodriguez and S. M. Hecht, *J. Biol. Chem.*, 1981, **256**, 1514; G. M. Ehrenfeld, L. O. Rodriguez, S. M. Hecht, C. Chang, V. J. Basus and N. J. Oppenheimer, *Biochemistry* 1985, **24**, 81; G. M. Ehrenfeld, J. B. Shipley, D. C. Heimbrook, H. Sugiyama, E. C. Long, J. H. vanBoom, G. A. Van der Marel, N. J. Oppenheimer and S. M. Hecht, *Biochemistry*, 1987, **26**, 931.

8 R. L. Aft and G. C. Mueller, *J. Biol. Chem.*, 1983, **258**, 12069.

9 B. G. Que, K. M. Downey and A. G. So, *Biochemistry*, 1980, **19**, 5987; L. E. Marshall, D. R. Graham, K. A. Reich and D. S. Sigman, *Biochemistry*, 1981, **20**, 244; D. S. Sigman, *Acc. Chem. Res.*, 1986, **19**, 180; D. S. Sigman, D. R. Graham, V. D'Aurora and A. M. Stern, *J. Biol. Chem.*, 1979, **254**, 12269; S. Goldstein and G. Czapski, *J. Am. Chem. Soc.*, 1986, **108**, 2244 and references cited therein.

10 L. A. Chrisey, G. H. Shahidi Bonzar and S. M. Hecht, *J. Am. Chem. Soc.*, 1988, **110**, 644.