Stereospecific Binding of Chemically Modified Salen-type Schiff Base Complexes of Copper(II) with DNA [salen = bis(salicyclidene)ethylenediamine]

Kousuke Sato,[a] Makoto Chikira,*[a] Yuki Fujii[b] and Atsushi Komatsu[b]

[a] *Department of Applied Chemistry, Chuo University, Kasuga 1-13-27, Bunkyo-ku, Tokyo 112, Japan*
[b] *Department of Chemistry, Ibaraki University, Bunkyo 2-1-1, Mito 310, Japan*

DNA-fibre ESR measurements reveal that the orientation of water-soluble cationic salen-type Schiff base complexes of copper(II) on DNA changes by modification of the bridging group between two imino nitrogens in the complex; the 1,2-phenylenediamine and 2,3-naphthalenediamine bridges enable the complex to intercalate to DNA, while the ethylenediamine bridge induces groove binding.

Stereospecific binding of metal complexes with DNA has been investigated extensively to develop a reagent which can control genetic information and work as an artificial restriction enzyme or an antitumour drug.[1] We report here a series of new intercalators and groove-binders obtained by chemical modifications of a salen-type Schiff base complex of copper(II) [Fig. 1(*a*)]. The complexes were synthesized in a 'one pot' by the reaction of 5-(trialkylammoniomethyl)salicylaldehyde chloride with diamine, and copper(II) acetate in a 2:1.1:1 molar ratio in water at pH 7.5 and isolated as chloride, tetrafluoroborate, or perchlorate salt. Satisfactory elemental analyses (C, H, N and Cu) were obtained for the perchlorates. The 5-(trialkylammoniomethyl)salicylaldehyde chloride was prepared from 5-chloromethylsalicylaldehyde[2] and trialkylamine in THF.

The water-soluble cationis Schiff base complexes of copper(II) bind strongly to DNA. No colour from the complexes remained in the supernatant solution obtained after the ultracentrifugation of the solution of DNA and the complexes. The λ_{max} at 394.9 nm (ε = 17833) of **2** in 5 mmol dm^{-3} HEPES [*N*′-(2-hydroxyethyl)piperazine-*N*-ethanesulfonic acid] and 50 mmol dm^{-3} NaCl aq. (pH 7.2) at 25 °C shifted to 413.5 nm (ε = 13750) with a clear isosbestic point at 424.8 nm, while that at 343.1 nm of **1** did not change after the complex solutions were mixed with calf thymus DNA solution. This result suggests that the binding mode of **1** and **2** are different. The binding constant of **2** on DNA was estimated as 3.14 × 10^4 dm^3 mol^{-1} employing the equation similar to that used by Pyle *et al.*[3]

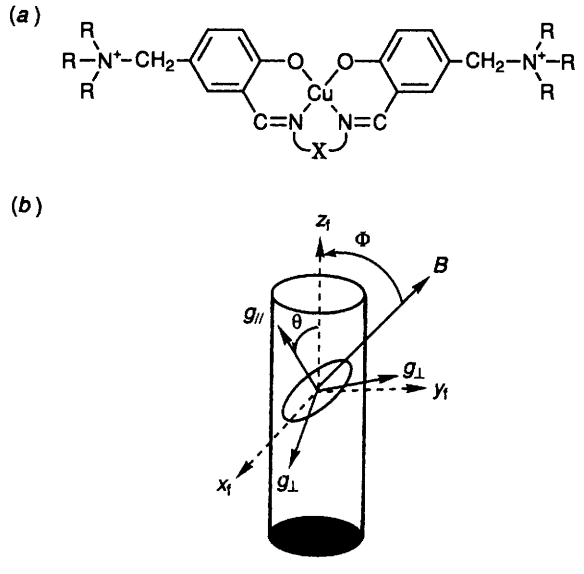

Fig. 1 (*a*) Structure of water-soluble salen-type Schiff-base complexes of copper(II) and (*b*) the relative orientations of the DNA-fibre axis (z_f), *g* tensor axes ($g_{\|}$, $g_{\perp}$), and the static magnetic field (*B*); **1**: R = Me, X = CH_2CH_2 **2**: R = Me, X = *o*-C_6H_4 **3**: R = Et, X = *o*-C_6H_4 **4**: R = Pr^n, X = *o*-C_6H_4 **5**: R = Bu^n, X = *o*-C_6H_4 **6**: R = Me, X = $C(Me)_2C(Me)_2$ **7**: R = Me, X = naphthalene-2,3-diyl

The stereospecific binding of copper(II) complexes was examined by use of the DNA-fibre ESR technique as reported previously.[4–6] As shown in Fig. 2, the ESR spectra of **1** and **2** on B-form salmon testes DNA-fibres have quite different Φ dependency, where Φ denotes the angle between the elongation axis of the DNA-fibres and the direction of static magnetic field [Fig. 1(*b*)]. The angle θ formed by the $g_{\|}$ and the DNA-fibre axes and the fluctuation Δθ were estimated from the computer simulations of the ESR spectra.[4] The weak $g_{\|}$ signals observed in the spectrum of **2** at Φ = 90° [Fig. 2(*b*)] suggest that some of **2** on the DNA is randomly oriented. The observed ESR spectra were reproduced assuming that 25% of both complexes are randomly oriented and that the specifically oriented species of **1** have θ = 30° and Δθ = 15° and those of **2** have θ = 10° and Δθ = 14°. The estimated θ values indicate that the CuN_2O_2 coordination plane of **1** is oriented along the groove of the DNA double helix whereas **2** binds with the coordination plane almost perpendicular to the DNA-fibre axis. This is clear evidence for the intercalation of

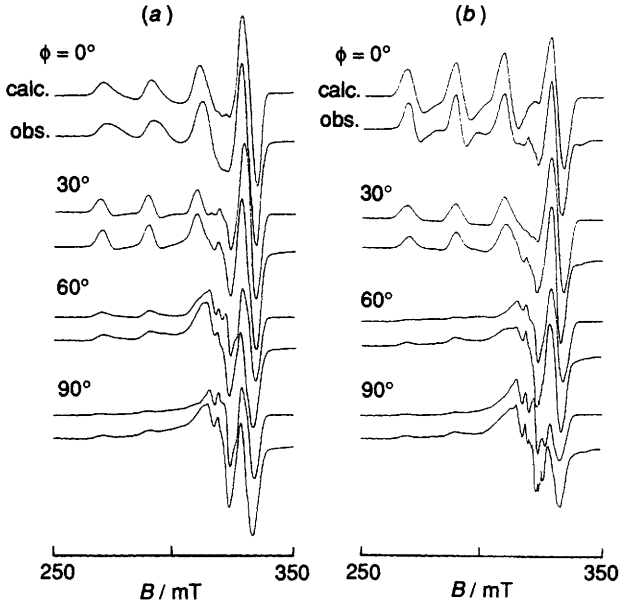

Fig. 2 Observed (lower lines) and calculated (upper lines) ESR spectra of **1** (*a*) and **2** (*b*) on B-form DNA-fibres at room temp. The microwave frequency is 9201 MHz, the magnetic parameters used for the simulations for the oriented species for **1** are $g_{\|}$ = 2.192, $g_{\perp}$ = 2.045, $A_{\|}$ = 0.0203 cm^{-1}, $A_{\perp}$ = 0.0026 cm^{-1}, $A_{N\|}$ = 0.0014 cm^{-1}, $A_{N\perp}$ = 0.0014 cm^{-1}, $\Delta B_{\|}$ = 18.0 G (1 G = 1 × 10^{-4} T), $\Delta B_{\perp}$ = 18.0 G, θ = 30.0°, Δθ = 15°, and for randomly oriented one, $g_{\|}$ = 2.20, $g_{\perp}$ = 2.040, $A_{\|}$ = 0.0198 cm^{-1}, $A_{\perp}$ = 0.0027 cm^{-1}, $A_{N\|}$ = 0.0014 cm^{-1}, $A_{N\perp}$ = 0.0014 cm^{-1}, $\Delta B_{\|}$ = 18.0 G, $\Delta B_{\perp}$ = 18.0 G. For **2**: $g_{\|}$ = 2.196, $g_{\perp}$ = 2.039, $A_{\|}$ = 0.0202 cm^{-1}, $A_{\perp}$ = 0.0027 cm^{-1}, $A_{N\|}$ = 0.0019 cm^{-1}, $A_{N\perp}$ = 0.0016 cm^{-1}, $\Delta B_{\|}$ = 19.0 G, $\Delta B_{\perp}$ = 19.0 G, θ = 10°, Δθ = 14°, and for the randomly oriented one, $g_{\|}$ = 2.196, $g_{\perp}$ = 2.040, $A_{\|}$ = 0.0200 cm^{-1}, $A_{\perp}$ = 0.0027 cm^{-1}, $A_{N\|}$ = 0.0015 cm^{-1}, $A_{N\perp}$ = 0.0014 cm^{-1}, $\Delta B_{\|}$ = 20.0 G, $\Delta B_{\perp}$ = 20.0 G. The ratio of the amount of oriented to non-oriented species was taken as 3:1 in the calculations for both complexes.

2 in DNA. The observed hypochromic shift of the absorption at 394.9 nm is also in accord with the conclusion. A similar orientation dependence in the ESR spectra was observed for the 2,3-naphthalenediamine bridged complex **7**. The substitution of the phenylene or naphthalene group for the ethylene group in the Schiff base increases the flatness of the complex, which enhances the stacking interaction and promotes the intercalation of the complex. The ESR spectrum of **1** reveals the presence of only one species ($g_{\|} = 2.214$, $A_{\|} = 0.0201$ cm^{-1}) in frozen aqueous solution. In the case of **2** and its analogues, strong triplet state signals due to the dimer overlapped with those owing to the monomer. The dimers often occur through π–π stacking which parallels with the ease of intercalation of the complexes between the base-pairs of the double helix DNA.

The binding structures of **3**, **4** and **5** estimated in the same manner revealed that the substituton of ethyl-, *n*-propyl- or more bulky *n*-butyl-groups for the methyl groups on quaternary amine groups in **2** does not change the binding mode of the complexes on DNA, *i.e.* all these complexes intercalate to DNA. However, similar substitutions of the bulky alkyl-groups for methyl groups in **1** which is found to bind in the grooves result in a large increase in the $\Delta\theta$ of the complexes on the DNA fibres. The introduction of methyl groups in the ethylene bridge as in **6** also increased the $\Delta\theta$ of the coordination planes on the DNA fibres considerably.

These results indicate that the orientation of the coordination planes of the complexes on DNA fibres are determined mainly by the bridging group between the imino nitrogens. The substitution of the phenylene or naphthalene bridging group for the ethylene group transforms the complexes from groove binders to intercalators. Once the complexes are bound by the intercalative mode, the fluctuations of the coordination planes of the complexes are not affected so much, whereas the orientations of the complexes bound in the grooves are affected considerably by the alkyl groups on the quaternary amines. Similar trends have been observed on the structural change of the DNA double helix induced by a change in the relative humidity. It is well known that the structure of DNA changes from the B- to A-form when the relative humidity around the DNA decreases below *ca.* 70%.[6] In the A-form DNA, the base pairs are not perpendicular to the helical axis and the shape of the grooves are quite different from those of the B-form DNA. In spite of these structural changes, the ESR spectra of **2** on A-form DNA-fibres are almost the same as those shown in Fig. 2(*b*). On the other hand, **1** on A-form DNA shows an ESR spectra characteristic of the species having a large fluctuation in the orientations.

The present type of salicylaldehyde Schiff bases can form complexes with various metal ions. A change in the central metal ion will bring about a change not only in the binding mode but also in the redox potential. Recently, Muller *et al.* reported that a water-soluble nickel(II) salen acts as a DNA alkylating agent.[7] Thus, the further modification of R or X together with the selection of the central metal ion is an interesting subject in the search for a reagent which interacts with DNA specifically.

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