An Ytterbium(III) Complex of Duanomycin, a Model Metal Complex of Anthracycline Antibiotics

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The anthracycline antibiotics isolated from several *Streptomyces* species have currently been widely used, despite their severe side effects, as chemotherapeutic agents with high efficacy in the treatment of human cancers, including dose-dependent cardiomyopthy.[1] These antibiotics consist of a 4-ring anthraquinone chromophore and an amino sugar substituent at the 7-position. (See structure in Figure 1.) The biological activity of these antibiotics is largely due to their DNA binding capability (via intercalation of the 4-ring moiety) and the redox activity of the quinone functional group, which engender significant perturbation of nucleic acid metabolism and electron transfer reactions in the cells and cause oxidative damage of the membrane of cells and organelles.[2] Several reports have suggested the participation of metal ions (such as Mg^{2+}, Ca^{2+}, and transition metal ions) in the interactions of these antibiotics with biomolecules (e.g. DNA and phospholipids) and subsequent damage of these biomolecules.[3] Iron has further been postulated to take part in the cardiotoxicity of these antibiotics via free radical damage of the inner membrane of cardiac mitochondria.[3] However, the metal-binding properties and the structure of the metal complexes were still not clearly defined. To avoid the redox complicity of the Fe–anthracycline system, we first investigated the system using non-redox-active lanthanide(III) ions (Ln^{3+}) as probes, which can be the working models to gain further insights into the role of metal ions (especially the abundant alkaline earth metal ions) in the biological actions of these antibiotics. Although Ln^{3+} ions bind ligands with much less covalency than does Fe^{3+}, they have been used successfully as substitutes for the Fe in the O-rich binding sites in transferrin.[4] We report in this communication the study of a prototypic anthracycline antibiotic, daunomycin, and the determination of the structure of a Yb^{3+}–daunomycin complex by the use of optical and 2D ^1H NMR techniques (EXSY and COSY). This primary study provides us with the tools for future studies of the interaction of metal–anthracycline complexes with biomolecules.

The fast electronic relaxation rates of Yb^{3+} and several other Ln^{3+} ions give rise to relatively sharp isotropically shifted ^1H NMR features, which can be studied by means of two-dimensional NMR techniques for detailed signal assignment.[5] The proton NMR spectrum of a Yb^{3+}–daunomycin (1:3 ratio) methanol-d_4 solution exhibits 13 isotropically shifted signals clearly detected in the upfield region of 0 to −40 ppm, with signals from the free antibiotic still well resolved in the diamagnetic region (Figure 1), suggesting that the drug is in slow exchange with its metal-bound form on the NMR time scale. While a Yb^{3+}–adriamycin (1 to 2 ratio) methanol solution exhibited a mixture of several unidentified species in a previous study (thus the ^1H NMR spectrum was not assigned),[6] we have observed that the Yb^{3+}–daunomycin methanol solutions with various metal to ligand ratios of 1:5 to 3:1 gave rise to only a single 1:1 complex during the time of the experiments.[7] Although the formation of a 1:1 Yb^{3+} complex of adriamycin was suggested by a Job plot, the observation of a mixture in the ^1H NMR spectrum was not consistent with such a conclusion.[6,8] We have found that the discrepancy was due to the change of proton activity in the solution, which was not addressed in the previous study. A precise determination of the structure of the metal–drug complex is crucial for the understanding of the role of metal ions in the action of anthracyclines and for future study of the interaction of the metal–drug complexes with biomolecules.

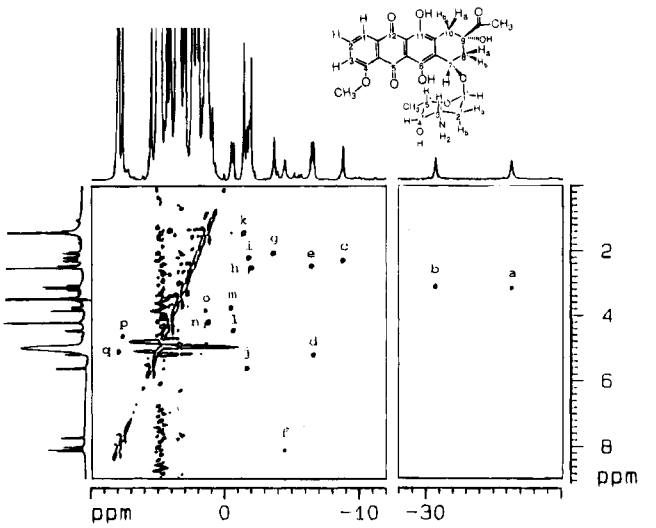

Figure 1. Proton EXSY spectrum (Bruker AMX360 at 360.13 MHz and 298 K) of Yb^{3+}:drug = 1:3 in methanol-d_4. A standard phase-sensitive NOESY with a mixing time of 30 ms, 1024 (fs) × 512 (f1) data points, and bandwidths of 20 (f2) × 7.2 (f1) kHz, were applied for data acquisition. A 45°-shifted sine-squared apodization function was applied to both dimensions prior to Fourier transformation.

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(7) A 1:1 Yb^{3+}–daunomycin complex is formed on the basis of a Job plot with absorptions at 498 (sh), 532, and 570 nm (ϵ_{570} = 15.2 mM^{-1} cm^{-1} and $K_a' = K_a/a_H$ = 82.1 mM^{-1} with a_H being proton activity), despite the fact that Ln^{3+} ions can easily form 8-coordinate complexes (Figure S). However, a 1:3 Yb^{3+}–daunomycin complex is formed in methanol in the presence of a base (triethylamine or NaOH) with a shift of the 570-nm absorption to 576 nm and an increase in absorptivity (Figure S). Similar results have also been observed in aqueous solution: a 1:1 complex (λ_{max} = 580 nm) was fomred at pH 5.5, and a 1:3 complex (λ_{max} = 586 nm), at pH >6.5. Precipitation of Ln^{3+} as hydroxides at higher pH prevents further pH-dependent study in aqueous solution.
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The proton NMR spectrum of daunomycin can be assigned via conventional bond-correlated COSY spectroscopy. We have thus taken the advantage of the fully assigned free drug for the assignment of the isotropically shifted features of the 1:1 Yb^{3+}–daunomycin complex by the use of 2D magnetization transfer techniques (EXSY) owing to the presence of chemical exchange between the free drug and its complexed form in the methanol solution. Figure 1 shows the EXSY spectrum of a 6 mM daunomycin methanol-d_4 solution in the presence of $^1/_3$ equiv of $YbCl_3$. All the 17 nonexchangeable protons of the metal complex can be correlated to the free drug and unambiguously assigned. Thus, signals f, p, and q are associated with protons 1, 3, and 2, respectively; a and b are due to the geminal protons $C_{10}H_2$; d and (c, e) are due to the protons in the 7 and 8 positions, respectively; h, k, and n are associated with the methyl protons in positions 14, 5′, and 4, respectively; and j, (i, g), m, o, and l are associated with the sugar protons 1′–5′, respectively. The much shorter T_1 relaxation times of the signals at −30.6 and −36.2 ppm (22.6 and 16.5 ms, respectively) due to the geminal $C_{10}H_2$ protons as compared to that of the C_7H proton at −6.5 ppm (215 ms) suggest that the Yb^{3+} ion is coordinated to one of the two β-ketophenolate metal-binding sites on the aglycon rings in positions 11 and 12 close to the $C_{10}H_2$ protons. The much longer relaxation times of the sugar protons (185–335 ms) indicate that Yb^{3+} does not interact with the amino sugar to a great extent, as opposed to an early study of a Pd^{2+}–anthracycline complex where the metal had been proposed to bind with the sugar from a second complex.[9]

The sharpness of the isotropically shifted features also allows the use of coherence transfer techniques for signal assignment and for the elucidation of the configuration of vicinal proton pairs which correlates with their scalar coupling constants. Figure 2 shows the magnitude-mode ^1H COSY spectrum of the isotropically shifted features of the 1:1 Yb^{3+}–daunomycin complex. The $C_{10}H_2$ protons (−36.2 and −30.6 ppm) can be clearly distinguished from those protons in positions 7 (−6.5 ppm) and 8 (−6.3 and −8.7 ppm) in the COSY spectrum, where a three-spin system can be established in the latter proton pairs with the geminal C_8H_2 protons showing intense cross signals and a vicinal C_7H–C_8H pair showing weak cross signals as shoulders. The bond correlation of the sugar moiety can also be observed, where the connectivities of $C_{2'}H_2$–$C_{3'}H$ and $HC_{5'}$–CH_3 can be established. The crystal structure of daunomycin shows that the $C_{3'}$–NH_2 and the $C_{5'}$–CH_3 groups are in the equatorial positions, leaving the $C_{3'}H$ and $C_{5'}H$ protons in the axial positions and the $C_{1'}H$ and $C_{4'}H$ protons in the equatorial positions.[10] On the basis of the intensity of the coherence-transfer cross signals, one of the $C_{2'}H_2$ signals at −1.75 ppm, which shows an intense cross signal with the vicinal axial $C_{3'}H$ proton at −0.4 ppm, can be further assigned to the axial $C_{2'}H$ proton. The vicinal proton pairs with anti configuration afford large coupling constants, thus more intense cross signals. That the cross signals of the $C_{3'}H$–$C_{4'}H$ and $C_{4'}H$–$C_{5'}H$ pairs were not detected could be attributable to the smaller coupling constants of these axial–equatorial proton pairs. We have shown here by means of NMR that the configuration of the sugar moiety in the metal–daunomycin complex in solution is similar to that of the crystal structure of the free drug.[10]

Metal ions have previously been reported to facilitate the reduction of quinones to form semiquinones by lowering their

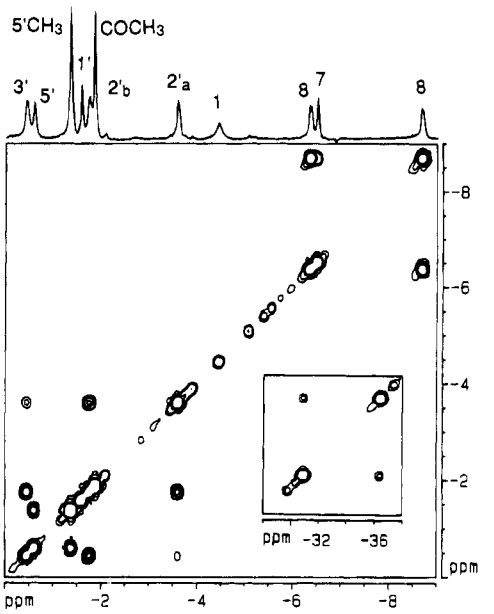

Figure 2. Proton COSY spectrum (at 360.13 MHz and 298 K) of the 1:1 Yb^{3+}–daunomycin complex in methanol-d_4. A bandwidth of 11.6 kHz was applied to both dimensions with 1024 (fs) × 512 (f1) data points. The inset was obtained by the use of a 3.8-kHz bandwidth centered between the two signals with 80 (f2) × 40 (f1) data points and zero-filled to 128 × 128 data points. A 0°-shifted sine-squared apodization was applied to both dimensions of the spectra prior to Fourier transformation, without executing spectral symmetrization.

electrode potential via the formation of more stable metal–semiquinone complexes with an extra charge on the semiquinones.[11,12] Thus, the influence of metal ions in the action of anthracycline antibiotics toward the damage of biomolecules can be partially due to a fine-tuning of the redox potential of these antibiotics via metal ion binding, which may change their dioxygen-activation and free radical-generation capability. Although these antibiotics show formidable side effects, they will still be used as chemotherapeutic agents for cancer treatments owing to their broad spectrum of antitumor activity.[13] The successful assignment of the ^1H NMR spectrum and determination of the structure of the Yb^{3+}–daunomycin complex allow us to study further the interaction of metal–anthracycline complexes with other biomolecules, by the use of Ln^{3+} ions as spectroscopic probes for better understanding of the antitumor and cardiotoxic mechanism of the anthracycline antibiotics. Moreover, the NMR techniques in this report can also be applied routinely for future studies of other metal–biomolecular interactions.

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Supplementary Material Available: Electronic spectra of daunomycin and complexes and Job plots (Figure S) (1 page). Ordering information is given on any current masthead page.

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