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NMR Spectroscopic Characterization of Copper(II) and Zinc(II) Complexes of Indomethacin

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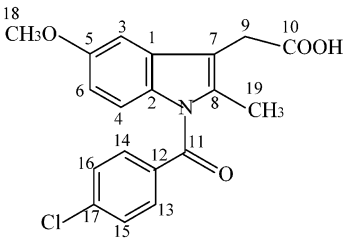
Molecular diffusion constants were studied by NMR spectroscopy to provide information about the solution structures of a variety of Cu(II) and Zn(II) monomeric and dimeric complexes of indomethacin (IndoH). These studies showed that monomeric Zn(II)–Indo complexes substantially dimerize in DMF-d_7 and DMSO-d_6 solutions at room temperature, whereas the Cu(II) and Zn(II) dinuclear complexes remain largely intact in these solutions. There is evidence of an equilibrium between monomers and dimers for the Zn(II) complexes in solution, as shown by a reduced diffusion constant and lower average radius compared to the Cu(II) dimer. Such an equilibrium between monomers and dimers for the Zn(II) complexes is also consistent with previous results obtained from XAFS analysis of DMF solutions of such complexes. The greater lability and lower thermodynamic stability of the Zn(II) dimer complex compared to the Cu(II) analogue, as determined from the NMR experiments, is likely to result in the more ready release of free Indo in the GI tract. This is consistent with the previously observed higher GI toxicities of the Zn–Indo pharmaceutical preparations compared to the Cu(II)–Indo counterparts.

Introduction

Indomethacin [1-(4-chlorobenzoyl)-5-methoxy-2-methyl−1*H*-indole-3-acetic acid = IndoH] is a powerful human antiinflammatory drug that is used in the treatment of a variety of severe inflammatory conditions; however, it is also quite gastrointestinally (GI) toxic in humans and, especially, dogs, which has restricted its applications as human and veterinary pharmaceutics. By contrast, metal complexes of IndoH have greatly reduced GI toxicity, which has allowed their use as veterinary pharmaceutics even in sensitive species, such as dogs, and offers considerable potential for their applications as human pharmaceutics.[1,2]



Indomethacin

Since release of IndoH from the metal complexes in biological fluids can result in all of the side effects of the parent drug, it is essential to develop techniques for the characterization of the complexes in solution and pharmaceutical preparations. In the case of the dimeric Cu(II) complexes, both XAFS and EPR spectroscopy can be used to determine the fate of such complexes in solution and emulsions,[1−4] but EPR spectroscopy is not applicable to Zn(II) and the XAFS is not as sensitive to the presence of dimers because of the larger Zn···Zn distance compared to the Cu−Cu distance.[2,5,6] One-dimensional ^{1}H and ^{13}C NMR spectroscopy was also unsuccessful in determining whether the Zn(II) species were monomers or dimers in solution,[5] but more detailed analyses were not performed until the experiments reported here.

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The use of NMR diffusion experiments, with dimeric Cu(II) complexes as calibration standards, is an alternative method for determining whether Zn(II)−Indo complexes are present in solution as dimers, since EPR experiments have established that the Cu(II) complexes remain as dimers in DMSO and DMF solutions.[1−3] This requires NMR spectroscopic studies on paramagnetic Cu(II) complexes, but the magnetic moment of Cu(II) usually induces very efficient nuclear magnetization relaxation in the neighboring nuclei, giving rise to broad NMR signals. The room-temperature (20 °C) magnetic moment, μ_{eff}, of $[Cu_2(Indo)_4(DMF)_2]$ (*N*,*N*-dimethylformamide = DMF) is 1.55 μ_B/Cu atom,[3] due to an equilibrium mixture between a diamagnetic singlet ground state and a paramagnetic triplet excited state.[1−3] Such a value is relatively high for sharp NMR resonances to be observed, but some dimeric Cu(II) complexes have low enough magnetic moments to allow high-resolution NMR characterization,[7] and even broadened spectra can be used in the determination of diffusion constants by NMR spectroscopy.

The structures of monomeric and dimeric Zn(II) indomethacin complexes in the solid state have been determined by XRD (single-crystal and powder) and XAFS.[5,6] In the current work, dilute solutions of $[Cu_2(Indo)_4(DMF)_2]$, $[Zn_2(Indo)_4(Py)_2]$ (Py = pyridine), $[Zn(Indo)_2(EtOH)_2]$ (EtOH = ethanol), $[Zn(Indo)_2(MeOH)_2]$ (MeOH = methanol), and IndoH were studied by NMR spectroscopy in order to test whether diffusion experiments could provide an alternative to XAFS measurements to determine whether monomers or dimers were present in solution.

Experimental Section

IndoH was obtained from Sigma-Aldrich, $[Cu_2(Indo)_4(DMF)_2]$ was obtained from BVR Pty Ltd (Mittagong, Australia),[3] and monomeric and dimeric Zn(II) compounds were synthesized and characterized according to Zhou et al.[5] Deuterated solvents were obtained from CIL (Cambridge Isotope Laboratories, Inc.). A Bruker (Karlsruhe, Germany) DRX400 NMR spectrometer with a narrow-bore 9.4 T magnet equipped with a Bruker single-axis gradient (*z*) auto-tune-match (ATM) CHP probe was used for all experiments. At full power (10 A) *Z*-gradients (vertical) can deliver a linear gradient of ∼52 G cm^{-1}. The gradient strength was calibrated using the residual water in ^2H_2O,[8] to avoid any radiation damping effects.[9] NMR tubes with a 5-mm internal diameter were used for all experiments. The sample temperature was maintained at 298 K, unless otherwise stated. Residual protonated solvent peak(s) were used to calibrate the spectrum.

Analysis of Data. The diffusion coefficient, *D*,[10−12] was determined by implementing a spin−echo pulse sequence (90−g_i−180−g_i−acquire) or a stimulated spin−echo pulse sequence (90−g_i−90−g_i−90−acquire) where 90 and 180 are hard radio frequency pulses and g_i is an incremented diffusion sensing magnetic field gradient applied along the long axis of the NMR tube. As g_i is incremented, the amplitude of the acquired signal decreases due to diffusion. Equation 1 describes the signal attenuation as a function of g_i:

$$\ln(S_i/S_0) = -\gamma^2 g_i^2 \delta^2 (\Delta - \delta/3) D \quad (1)$$

where S_0 is the echo-signal amplitude with diffusion sensing gradients set to a low value (e.g., 5 G cm^{-1}), S_i is the echo-signal amplitude with diffusion sensing gradients set to g_i (i was incremented from 1 to 8), γ is the magnetogyric ratio of the diffusion spin (^{1}H in the present case), g_i is the diffusion sensing gradient, which was varied from 5 to 50 G cm^{-1}, δ is the duration of g_i, and Δ is the delay between the leading edges of the two diffusion sensing gradients. By plotting $\ln(S_i/S_0)$ versus $-\gamma^2 g_i^2 \delta^2 (\Delta - \delta/3)$ and regressing a straight line onto the data, *D* was obtained from the slope.[11]

Results

One-dimensional ^{1}H NMR spectra of dilute solutions of 1 mM $[Cu_2(Indo)_4(DMF)_2]$, IndoH, $[Zn_2(Indo)_4(Py)_2]$, and $[Zn(Indo)_2(EtOH)_2]$ in DMF-d_7 are shown in Figure 1, and the corresponding Indo(H) ^{1}H NMR chemical shifts are shown in Table 1.

$[Cu_2(Indo)_4(DMF)_2]$ (1 mM) in DMF-d_7 gave broad peaks in the ^{1}H NMR spectrum due to the intramolecular electronic effect of the paramagnetic Cu(II) nucleus on other atoms, whereas the other compounds gave sharp peaks. For Zn(II), the largest difference in ^{1}H chemical shifts was observed for the CH_2 group of Indo(H), which shifted from δ = 3.73 ppm to δ = 3.55 ppm upon coordination. Complexed and free DMF in $[Cu_2(Indo)_4(DMF)_2]$ in DMF-d_7 resonated at slightly different frequencies (an average difference of 1.7 Hz), an effect that was not detected in Zn(II) complexes, where the DMF would have replaced the other solvent in the coordination sphere. The largest ^{1}H chemical shift difference between solutions where the monomeric Zn(II) and dimeric Zn(II) complexes were dissolved was in the CH_2 resonance (0.007 ppm = 2.8 Hz). The protons of the CH_2 group of free IndoH and Indo in Zn(II) complexes also resonated at slightly different frequencies (0.17 ppm). Upon addition of IndoH to 1 mM Zn(II) monomer DMF-d_7 solution, the chemical shift of CH_2 shifted slightly to high frequency, but two separate peaks were not observed indicating fast exchange on the NMR time scale.

Diffusion coefficients of various species, along with their apparent r_a values, are given in Table 2. The *D* values of Indo in monomeric and dimeric Zn(II) complexes in DMF-d_7 were constant and very close to each other. In the solution of the dimeric Zn(II) compound, the *D* value of pyridine was much larger than that of indomethacin in DMF-d_7 and DMSO-d_6. The *D* values of Indo in 1 mM solutions of Zn(II) and Cu(II) dimers were also similar. The *D* value of IndoH in a 1 mM solution in DMF-d_7 was not influenced by the addition of 1 mM tetrabutylammonium hydroxide, which was used to deprotonate IndoH to form the Indo anion.

The Stokes−Einstein equation,[13]

$$D = \frac{kT}{2\pi r_a \mu} \quad (2)$$

where *D* is the diffusion coefficient (cm^2 s^{-1}), *k* is the

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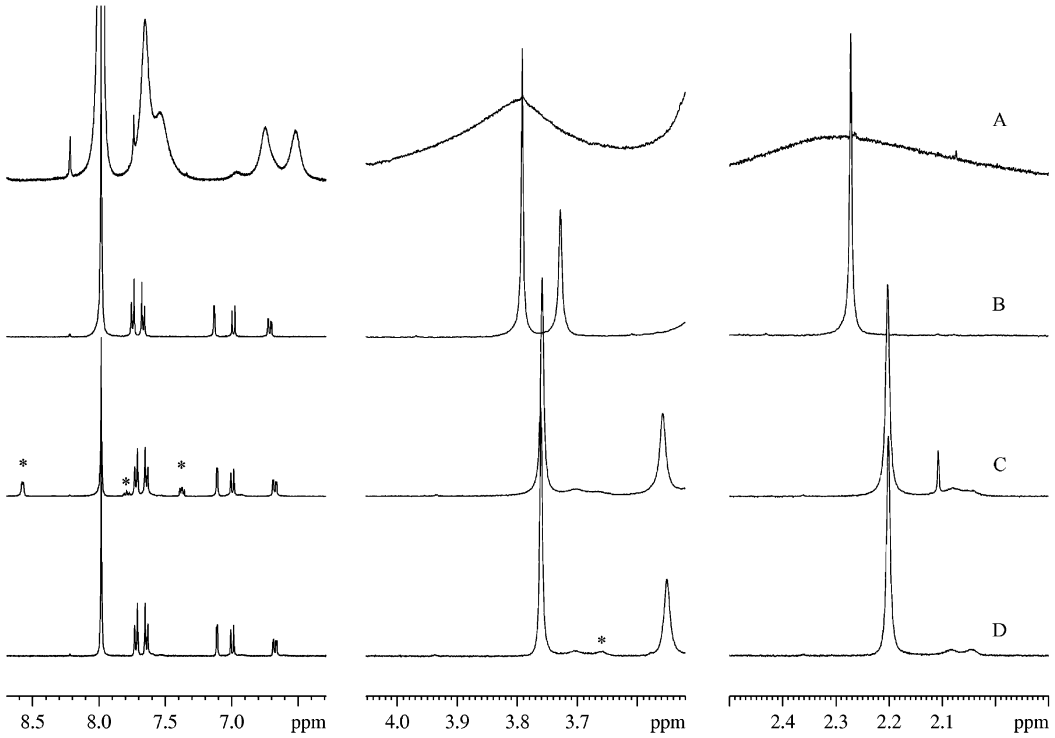

Figure 1. 400-MHz one-dimensional ^{1}H NMR spectra of $[Cu_2(Indo)_4(DMF)_2]$ (A), IndoH (B), $[Zn_2(Indo)_4(Py)_2]$ (C), and $[Zn(Indo)_2(EtOH)_2]$ (D) in DMF-d_7 at 298 K. The sharp peak at 2.12 ppm in C is due to residual acetonitrile used in the synthesis of the complex. Broad peaks at 2.07 and 3.69 ppm are probably due to solvent impurities. The resonances due to the DMF methyl groups (2.91 and 2.70 ppm)[21] of DMF are not shown (for clarity), whereas the sharp peak at 8.01 ppm in all spectra is due to the aldehydic proton of DMF. Resonances due to Py protons and the CH_2 group of EtOH are marked with "*". The CH_3 group (1.06 ppm) and OH (4.34 ppm) of EtOH lie outside the displayed region of interest.

Table 1. ^{1}H NMR Chemical Shifts (ppm) in DMF-d_7 of Indomethacin in Different Compounds[a]

assignment	19 (s)	9 (s)	18 (s)	6 (dd)	4 (d)	3 (d)	13,14 (d)	15,16 (d)
$[Zn(Indo)_2(EtOH)_2]$	2.201	3.551	3.761	6.673	6.995	7.110	7.720	7.642
Zn(II) monomer + IndoH	2.209	3.578	3.765	6.678	6.993	7.112	7.722	7.644
$[Zn_2(Indo)_4(Py)_2]$	2.202	3.558	3.759	6.674	6.994	7.108	7.719	7.641
IndoH	2.272	3.728	3.792	6.712	6.985	7.13	7.674	7.657
$[Cu_2(Indo)_4(DMF)_2]$	2.291	3.790	3.790	6.510	6.750	6.980	7.650	7.540

[a] All solutions were 1 mM in IndoH or Indo complex. The numbers correspond to those in the IndoH structure. Multiplicities for the diamagnetic species are in parantheses: s, singlet; d, doublet. The assignment of the spectrum is based on that of IndoH (Tanaka, M.; Asahi, Y. *Polymer* **1994**, *35*, 1512−1517).

Table 2. Diffusion Data of IndoH and the Corresponding Apparent Hydrodynamic Radius, r_a, of Cu(II) and Zn(II) Indo Complexes at 298 K[a]

	$D \times 10^{10}$ (m^2 s^{-1})	r_a (Å)
1 mM in DMF-d_7		
$[Zn(Indo)_2(EtOH)_2]$	3.82 ± 0.05	21.0
$[Zn(Indo)_2(MeOH)_2]$	3.81 ± 0.01	21.0
$[Zn_2(Indo)_4(Py)_2]$	3.85 ± 0.02	20.8
IndoH	5.45 ± 0.02	14.7
$[Cu_2(Indo)_4(DMF)_2]$	3.57 ± 0.02	22.4
1 mM in DMSO-d_6		
$[Zn(Indo)_2(EtOH)_2]$	1.55 ± 0.02	20.8
$[Zn_2(Indo)_4(Py)_2]$	1.55 ± 0.01	20.8

[a] Diffusion coefficient errors were produced by the Bruker XWINNMR version 3.5 software package,[20] and the variation in the *D* values remained within the experimental error boundaries in replicate experiments.

Boltzmann constant (1.38×10^{-16} g cm^2 s^{-2} K^{-1}), r_a is the radius of the spherical solvated molecular species under consideration, and μ is the viscosity of solution (0.008047 g cm^{-1} s^{-1} for DMF and 0.02 g cm^{-1} s^{-1} for DMSO),[19] has been used to estimate the radii of the solvated species, r_a, which are also reported in Table 2.

Discussion

The *D* values obtained above can provide valuable information about the metal indomethacin complexes in solution. Since the *D* value decreases as molecular weight increases (all other variables being the same, e.g., viscosity,

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solute−solvent interaction), one can draw some conclusions from the *D* values in Table 2. The ratio of *D* values of the Zn(II) complexes in Table 2 is equal to the ratio of viscosities of the two solvents, an indication that the solvated molecular structure of monomeric zinc or dimeric zinc is the same in both solvents. The fact that the *D* values of dimeric Cu(II) and dimeric Zn(II) are close to each other (7%) means that the Zn(II) dimer stays mostly intact in solution, since EPR spectroscopy shows that the Cu(II) dimer is stable in DMF.[1,2,14] The molecular weights of Cu(II) and Zn(II) dimers are 1700 and 1716 g mol^{-1}, respectively, and since pyridine is practically free in solution (the *D* value of pyridine in the Zn(II) dimer solution was 1.60×10^{-9} m^2 s^{-1}), the slight increase in the *D* value of the Zn(II) dimer compared with that of the Cu(II) dimer can be explained by exchange of pyridine with a lighter ligand, such as residual water, or by a lesser extent of complex solvent interaction. However, partial breakdown of the Zn(II) dimer into the monomer, as has been shown elsewhere,[6] is the more likely explanation for the larger *D* value. Relatively small ^{1}H hyperfine shifts[15] were observed in the dimeric Cu(II) complex as well as the Zn(II) complexes, which probably indicate that delocalization of spin density from the metal into molecular orbitals is minimal. Hyperfine shifts can, in favorable cases, be used to extract structural information on the dissolved species.[16,17]

The ^{1}H NMR chemical shifts of Zn(II) complexes were very similar. The largest difference in ^{1}H chemical shifts between the Zn(II) compounds and IndoH (Table 1) involves the CH_2 protons (0.17 ppm), and this is an expected outcome due to the spatial proximity of the CH_2 group to the metal center. This difference is too small, however, to be used as a fingerprint for the Zn(II) compounds. Other indomethacin protons did not show appreciable change in chemical shift in free and complexed states.

The exchange of free and complexed Indo in 1 mM Zn(II) monomer solution was rapid on the NMR time scale. When IndoH was added to the Zn(II) monomer in DMF-d_7 solution, a new peak from free CH_2 was not seen but the ^{1}H resonance frequency of CH_2 shifted to high frequency (+5.73 Hz). This means that the complexation is a dynamic process and that the exchange rate should be larger than the reciprocal of the shift difference (i.e., >0.17 s^{-1}) experienced by any proton of indomethacin between the complexed and free states. The ^{1}H chemical shift of CH_2 moved to high frequency as the amount of free indomethacin increased, which is in accordance with chemical equilibrium principles. The slight difference in ^{1}H chemical shift of complexed and free DMF in 1 mM Cu(II) dimer indicates that free and complexed DMF are in slow exchange on the NMR time scale, and that the DMF exchange rate in the Cu(II) dimer is lower than the Indo exchange rate in the Zn(II) monomer. The similarity of the *D* values of protonated acid (IndoH) and deprotonated acid (Indo), obtained by the addition of an equivalent amount of tetrabutylammonium hydroxide, indicates that the H-bonding dimerization of acid at 1 mM in DMF-d_7 solution is either absent or fast on the NMR time scale.

In the present work, the apparent *r* value, r_a, instead of *r*, has been used since the shape of the solvated species is not known. The dimeric metal compounds have an ellipsoidal shape in the solid phase.[1−6] The value of r_a can be regarded as the corresponding hydrodynamic radius of a spherical species with the same *D* in the same solvent. Use of the Stokes−Einstein equation requires that the diffusing species is moving in a hydrodynamic continuum, and the motion of the diffusing particles is uncorrelated. This environment can be approached when studying dilute large particles in a low molecular weight solvent.[18]

The similarity of the *D* values of monomeric and dimeric Zn(II) compounds when dissolved in a given solvent (DMF-d_7 or DMSO-d_6) means that the structures of the solvated complexes are also very similar. These results indicate that the majority of monomer is oligomerizing to a bulkier and heavier species, which is probably the corresponding dimer. XAFS experiments[6] indicated that the Zn(II) dimer in the equilibrium had the same paddle-wheel structure as observed in the crystal structures,[2,5] since a similar Zn−Zn distance was observed in the solid state and solution by XAFS.[6] As a consequence, the extent of dimerization indicated by the apparent radius that was estimated from the NMR-derived diffusion constant of the Zn(II) complexes is probably an overestimate. This arises since the Zn−Zn distance in the Zn(II) dimers is ∼0.3 Å longer than the corresponding length in the Cu(II) dimers and, hence, the radius of the Zn dimers will be somewhat larger than those for the Cu(II) dimers. Such results are consistent with the XAFS measurements, which indicated that appreciable amounts of both dimer and monomer were present when either the Zn(II) dimer or monomer was dissolved in DMF.[6]

The above findings might also explain why the Cu(II) dimers in their various forms of therapeutic preparations are less GI toxic than the Zn(II) monomers and dimers.[14] The greater lability and lower thermodynamic stability of the Zn(II) dimer complex compared to the Cu(II) analogue is likely to result in the more ready release of the highly GI-toxic free IndoH into the GI tract. This, in turn, would result in the observed higher GI toxicities of the Zn−Indo pharmaceutical preparations.[14]

Conclusions

NMR experiments have been used to determine the diffusion constants and effective radii of Zn(II) and Cu(II) complexes of Indo. These experiments have shown that that Zn(II) complexes are more labile and the dimers less thermodynamically stable than the Cu(II) analogues, which have been shown previously to exist as dimers by EPR spectroscopy and XAS. These results are important in rationalizing the differences in GI toxicities of pharmaceutical preparations involving the various metal−Indo complexes.

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