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$^{15}N-^{15}N$ *J*-Coupling Across Hg^{II}: Direct Observation of Hg^{II}-Mediated T−T Base Pairs in a DNA Duplex

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Recently, nucleic acid-metal interactions are recognized as an important topic to be solved,[1] as they are involved in RNA folding, mechanisms of ribozymes action, gene mutations (Hg^{II}-induced mutation[2]) and the design of biomolecular devices with metal cofactors. Therefore, metal-mediated base pairs with "artificial" bases have been extensively studied for use in nanodevices and as tools for biotechnology.[3] By contrast, we have previously reported that a "natural" base, thymine−thymine (T−T) mismatch in a DNA duplex, specifically binds to Hg^{II} [4] and applied this property to a Hg^{II}-sensor.[5] In addition, these studies revealed that the putative Hg^{II}-mediated T−T base pair (T−Hg^{II}−T pair) is at least as stable as normal Watson−Crick base pairs. Therefore, the chemical structure of the T−Hg^{II}−T pair is an interesting target to understand the Hg^{II}-specificity and the stability of the T−Hg^{II}−T pair.

Although several groups have studied Hg^{II}-thymine binding and a possible structure of T−Hg^{II}−T pairs (Figure 1a),[6] no definitive conclusion has been made until now. In the present study, we determined the chemical structure of the T−Hg^{II}−T pair, on the basis of ^{15}N NMR studies in which novel $^{15}N-^{15}N$ *J*-coupling across Hg^{II} ($^2J_{NN}$) was observed for this T−Hg^{II}−T pair, and this *J*-coupling ($^2J_{NN}$) is a direct evidence for the formation of T−Hg^{II}−T pairs.

Recently, we and other groups have reported that ^{15}N NMR chemical shift changes[7−10] are applicable to the detection of hydrogen-bond formations and RNA metallations, and $^{15}N-^{15}N$ *J*-coupling across a hydrogen bond ($^{2h}J_{NN}$)[11,12] is a definitive method to detect hydrogen bonds (Figure S1 in Supporting Information). Therefore, we postulated that the following NMR techniques with advanced ^{15}N NMR spectroscopy might be applicable for the determination of the chemical structure of the T−Hg^{II}−T pair, in combination with recently developed DNA labeling techniques: (1) Metal ion-binding sites could be specified by ^{15}N NMR chemical shift changes and (2) $^{15}N-^{15}N$ *J*-coupling across a metal center ($^2J_{NN}$) (Figure 1a) might reveal the pairing mode and partners in T−Hg^{II}−T pairs (N−Hg^{II}−N bond connectivity). Therefore, in the current study, we tried to detect $^2J_{NN}$ and chemical shift changes, by using ^{15}N NMR spectroscopy (Figure 2).

For the detection of $^2J_{NN}$, ^{15}N-labeled DNA oligomers **1**−**4** were chemically synthesized (Figure 1c).[12a] We then examined the stability of this duplex in the presence of Hg^{II}, and found that this duplex was stable enough for NMR analyses (T_m = 54 °C: Figures S2−S5 in Supporting Information). As the first trial, we measured the ^{15}N NMR spectrum of duplexes **1•3** with ^{15}N-labeled thymidines (T5 and T16) (Figure 2a). Notably, we clearly observed splitting

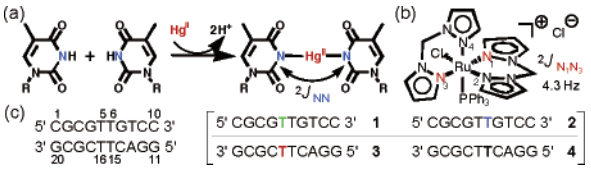

Figure 1. T−Hg^{II}−T pair and DNA oligomers. (a) Suggested base-pairing mode of the T−Hg^{II}-T pair and putative *J*-coupling of $^{15}N-Hg^{II}-^{15}N$ bond ($^2J_{NN}$). (b) Hexacoordination Ru^{II}-complex ($[RuCl(PPh_3)(BPM)_2]^+ \bullet Cl^-$) and *J*-coupling across Ru^{II} ($^2J_{N1N3}$) (see also Figure S1 in Supporting Information). (c) DNA oligomers with a ^{15}N-labeled thymidine at N3. Oligomers are named sequentially. Labeled thymidines are colored in terms of their positions.

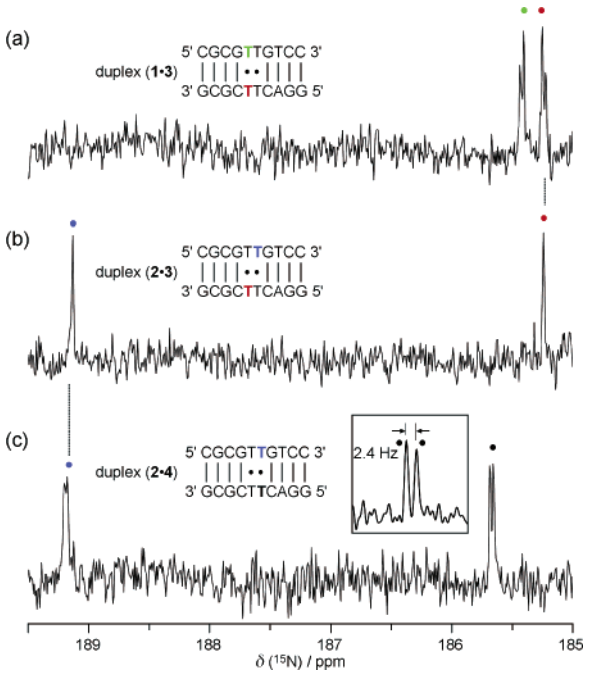

Figure 2. One-dimensional (1D) ^{15}N NMR spectra of duplex−Hg^{II} (1:2) complexes are shown: (a) the spectrum of the duplex (**1•3**)−Hg^{II} complex; (b) the spectrum of the duplex (**2•3**)−Hg^{II} complex; (c) the spectrum of the duplex (**2•4**)−Hg^{II} complex. The inset of the panel c indicates the resolution enhanced spectrum of the N3 resonance of T16 [N3(T16)]. The coupling constant of $^{15}N-Hg^{II}-^{15}N$ ($^2J_{NN}$) is shown in Hz as an absolute value. N3 resonances of thymidines are labeled with colored circles. Each color represents the position of the residue. ^{15}N-frequency (0 ppm) is 81.07646745 MHz.

of ^{15}N resonances for duplex **1•3**, most likely *J*-couplings ($^2J_{NN}$) (Figure 2a) which suggests the formation of a T5−Hg^{II}−T16 pair. Next, in duplex **2•4**, splitting of ^{15}N resonances was observed as well (a possible $^2J_{NN}$ for T6−Hg^{II}−T15 pair) (Figure 2c). By

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Table 1. Table of ^{15}N Chemical Shift Values[a]

atom[b]	metal	free	complex	difference
N3(T5)	Hg^{II}	155.1	185.4	+30.3
N3(T6)	Hg^{II}	153.9	189.2	+35.3
N3(T15)	Hg^{II}	155.8	185.7	+29.9
N3(T16)	Hg^{II}	154.4	185.2	+30.8

[a] Chemical shifts are listed in ppm. The "difference" is ^{15}N chemical shift difference between metal-free and metalated forms. [b] N3(T5), N3(T6), N3(T15) and N3(T16) denote the N3 atoms in the T5, T6, T15, and T16 residues, respectively. See also Tables S2 and S3, and Figure S6 in Supporting Information for further details.

contrast, these splittings disappeared for duplex **2•3** in which labeled thymidines are base-paired with nonlabeled thymidines (Figure 2b). Accordingly, Figure 2b demonstrates that the splitting of ^{15}N resonances observed in duplexes **1•3** and **2•4** (Figure 2a,c) truly rose from *J*-coupling ($^2J_{NN}$). In addition, we can assign all the N3 resonances in T−Hg^{II}−T pairs by using spectra of these combinations of the labeled duplexes. At this stage, we can conclude that T5−Hg^{II}−T16 and T6−Hg^{II}−T15 pairs were surely formed. As a result, our ^{15}N NMR data demonstrate that two imino protons are released upon T−Hg^{II}−T pairing (Figure 1a), which is a novel metal ion-binding manner for DNA and RNA molecules.

For the detailed analysis of the $^2J_{NN}$ value, we compared the $^2J_{NN}$ value with those of other related compounds. As a reference compound, there is a Ru^{II}-complex with a hexa-coordinated octahedral geometry (Figure 1b). In this complex, $^2J_{NN}$ for ^{15}N−Ru^{II}−^{15}N bonds was 4.3 Hz.[13] Unfortunately, there are currently limited data available on $^2J_{NN}$ across a metal center, and it is difficult to determine the relationship between the chemical structure and these *J*-coupling values. However, it is interesting that $^2J_{NN}$ for ^{15}N−Ru^{II}−^{15}N is in the same order to those for the T−Hg^{II}−T pairs (2.4 Hz) (Figure 2, Figure S1, and Table S1 in Supporting Information).

For the characterization of N−Hg^{II}−N bonds, we investigated the ^{15}N chemical shift perturbations upon the N−Hg^{II}−N bond formation (Table 1) (See also Figures S6 and S7 and Tables S2 and S3 in Supporting Information). All N3 resonances in the metal-free form were assigned from ^{1}H-^{15}N HSQC spectra of duplexes **1•3**, **2•4**, and **2•3** (see Figure S7 for assignment details). It was found that huge lower-field shifts of N3 resonances (approximately 30 ppm) were observed upon the N−Hg^{II}−N bond formation (Table 1). Such lower-field shifts cannot be explained without drastic transformations such as proton−Hg^{II} exchanges upon T−Hg^{II}−T pairing. Interestingly, in the cases of other proton−metal exchange systems, such as pyrrole−metal complexations, lower-field shifts of ^{15}N resonances upon metallations were observed (Figures S6 and Table S2 in Supporting Information). Therefore, the ^{15}N chemical shift changes for the T−Hg^{II}−T pairing and the related complexations exhibited lower-field shifts, which is sharp contrast to the higher-field shift of the ^{15}N7(guanosine) upon its innersphere coordination to Cd^{II}, Zn^{II}, and Hg^{II}.[9,10]

Although NMR studies on the Hg^{II}−DNA interaction have been reported,[14] we emphasize that our data are the first definitive determination of the chemical structure of T−Hg^{II}−T pairs, as well as the first ^{15}N NMR data on the direct interaction site (N3 of thymidine) of Hg^{II}. Therefore, our results are the most rigorous and provide a definitive answer for its chemical structure. Furthermore, even in the cases of metal-complexes, ^{15}N NMR data on N-metal bonds are rare and we now provide the first ^{15}N NMR data of N−Hg^{II}−N bonds. Therefore, our observations are not only important data for nucleic acid-metal systems but also provide a fundamental basis for coordination chemistry.

In conclusion, we have demonstrated that $^2J_{NN}$ across a metal center is observable in a biological macromolecule (DNA duplex) and definitely determined the chemical structure of the T−Hg^{II}−T pair by using $^2J_{NN}$. We then found a novel metal ion-binding mode for DNA molecules, which includes the imino proton−metal exchange processes.

Hazardous Information. For safety, it is recommended that $Hg(ClO_4)_2$ should be handled with gloves.

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Supporting Information Available: Experimental section; tables of *J*-coupling values, chemical shifts, and their perturbations; figures of metal-complexes (chemical shift) and their *J*-coupling; ^{1}H−^{15}N HSQC spectra; thermal denaturation profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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