shifts were saturated at the ND concentration 5.0 mM. Through the titration experiments, two peak sets were detected. One was originating from free DNA, and another was from complex. These results strongly suggest that only one complex is detected, and it is 1:2 complex. In other wards, the 1:1 complex was not detected at all. The difference of the results between NMR and ESI-TOF mass experiments may be due to the sample concentration differences, 2.5 mM and 20 μM for NMR and ESI-TOF mass, respectively.

The ^{1}H-^{1}H NOESY spectra of the 1:2 complex were recorded with 30, 200 and 300msec mixing times. TOCSY, DQF-COSY and natural abundance ^{1}H-^{13}C spectra were also recorded to complete the resonance assignment. In the imino proton region, sequential walk was completed (Figure 2). Four amide protons of ND (10 ~ 11.5 ppm) were also connected sequentially. Four H1' protons of G in the CGG / CGG region have NOE cross peaks to four corresponding amide protons of NDs. These data show four G bases form hydrogen bonds with four naphthyridine rings of ND. At the base-H1' region, the sequential walk was completed with the aromatic protons of naphtyridine rings (Figure 3). However, two H6 protons of C in the CGG / CGG were not included in this sequential walk. These results suggest the C residues in CGG / CGG region were not stacked; however, four naphthyridine rings were stacked in. Thus two NDs recognize whole CGG / CGG triplet region by hydrogen bonding and stacking.

All the protons of two ND molecules were also completely assigned. All the resonance assignments of aliphatic methylene protons of the linker region were not identical and clearly distinguished each other. This may suggest this linker region were well structured in spite of the intrinsic flexible character.

CONCLUSION

Naphthyridine-dimer (ND) can recognize the G.G mismatch in the DNA duplex. The complex between ND and d(CTAACGGAATG) / d(CATTCGGTTAA) was stable, and the stoichiometry DNA:ND was 1:2. ND intercalates into the base stacking and forms hydrogen bonds with four G residues. These results indicate ND can recognize whole CGG / CGG triplet region as well as G.G mismatch.

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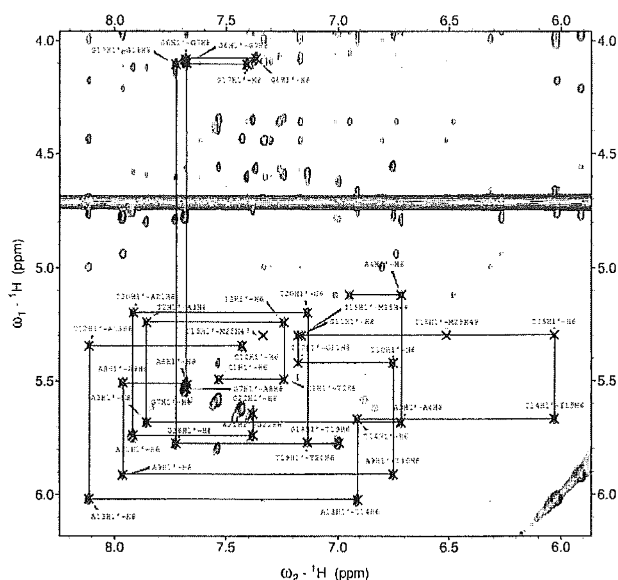

Figure 3. Sequential NOEs of d(CTAACGGAATG) / d(CATTCGGTTAA) complexed with two NDs. Base-sugar H1' region is shown. The NOE cross peaks at 4.1 ppm are from H1' protons of mismatched G residues in CGG / CGG region.

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