Exogenous Ligand Binding Property of a Heme–DNA Coordination Complex

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(Received October 21, 2005; CL-051337)

Imidazole was found to be coordinated, as an exogenous ligand, to Fe^{3+} in a coordination complex formed between heme, the iron(III)–protoporphyrin IX complex, and a parallel G-quadruplexed DNA assembled from a single repeat sequence of the human telomere, d(TTAGGG), the stoichiometric ratio being 1:1 and the binding constant 19 M^{-1}.

A G-quadruplexed DNA is composed of stacked G-tetrads, each of which involves the planar association of four guanine bases.[1] The size and planarity of a G-tetrad are well-suited for interacting with a porphyrin ring through π–π stacking. The complexation of G-quadruplexed DNAs with porphyrin or metal–porphyrin derivatives has been studied to characterize their molecular recognition of each other[2] as well as to create catalytic DNAs that exhibit hemoprotein functions.[3,4] Knowledge of heme coordination structures in these complexes is indispensable for designing heme–DNA complexes that mimic hemoprotein function. We have demonstrated the formation of a stable coordination complex between a parallel G-quadruplexed DNA assembled from a single repeat sequence of the human telomere, d(TTAGGG), and heme, the iron(III)–protoporphyrin IX complex.[4] We report herein the results of a ligand binding study of the "heme–DNA" complex, which demonstrate that imidazole (Im) is coordinated to Fe^{3+} in the heme–DNA complex as an exogenous ligand, in a stoichiometric ratio of 1:1, with a binding affinity similar to that of metmyoglobin (metMb). The absorption and NMR spectroscopic properties of the Im adduct of the heme–DNA complex (heme–DNA(Im)) were remarkably similar to those of the same adduct of metMb (metMb(Im)), indicating that the coordination structures around heme Fe^{3+} in the two systems are similar. These findings provide novel insights as to molecular design for creating artificial heme enzymes using spontaneous assembly between G-quadruplexed DNAs and heme.

We first determined the solution structure of the heme–DNA complex in the presence of 0.08 w/v% Triton X-100 by means of absorption and NMR spectroscopies.[5] The Soret absorption of the heme exhibited a red-shift from 398 to 404 nm, together with 180% hyperchromisity, and isosbestic points at 382 and 420 nm, with increasing DNA concentration (Supporting Information). Scatchard plots of the absorbance at 404 nm observed upon titration of heme with the DNA were represented as a straight line and yielded the association constant of 2.0×10^7 M^{-1}, with the stoichiometric ratio of 1:1 between the quadruplexed DNA and heme (Supporting Information). The parallel G-quadruplex formed from the d(TTAGGG) sequence[6] has been shown to dimerize through end-to-end stacking of the 3′-terminal G-tetrads.[7] The obtained stoichiometric ratio between the quadruplexed DNA and heme indicated that heme is not sandwiched between the 3′-terminal G-tetrads of two quadruplexed DNAs, but is probably stacked on the 5′-terminal G-tetrad, i.e., the G-tetrad formed by guanine bases at the 4th position from the 5′-terminus. These results are somewhat different from those reported previously[4] and we have realized that the results of Scatchard plotting are highly affected by the concentration of Triton X-100.

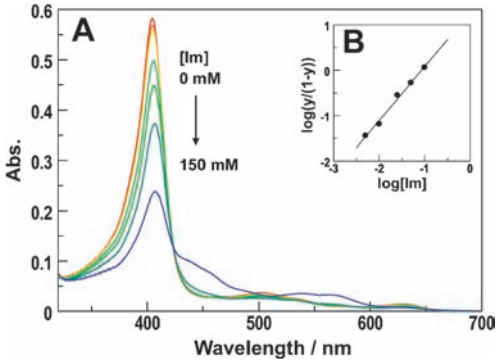

Figure 1. Absorption spectra, 320–700, nm of the heme–DNA complex in the presence of various concentrations of imidazole (Im) at 25 °C, [heme–DNA] = 5.0 μM (A). The molar ratio of [Im] to [heme–DNA] was varied from 0 to 3×10^4. Hill plots of the Soret absorbance at 404 nm (B).

We then examined the binding of Im to the heme–DNA complex by absorption spectroscopy (Figure 1). In the heme–DNA complex, a DNA base is possibly coordinated to Fe^{3+} as axial ligands,[4] and hence the addition of an exogenous ligand with high binding affinity toward heme Fe^{3+} results in the replacement of the axial ligand. The binding affinity of Im toward heme Fe^{3+} is low enough to prepare heme–DNA(Im). The spectrum of the heme–DNA complex exhibited Soret absorption at 404 nm (molar extinction coefficient $\varepsilon = 116\,mM^{-1}\cdot cm^{-1}$), together with absorption maxima at 504 nm ($\varepsilon = 7.6$) and 630 nm ($\varepsilon = 3.0$). With increasing Im concentration, the Soret absorption red-shifted to 407 nm, with isosbestic points (at 425, 468, 549, and 585 nm; Figure 1a). Hill plot analysis of the absorbance at 404 nm provided the binding constant (K_b) of 19 M^{-1}, and a 1:1 stoichiometric ratio between the heme–DNA complex and Im (Figure 1b). The K_b value obtained for the heme–DNA complex is almost the same as that for metMb, i.e., $K_b = 45\,M^{-1}$.[8] These results unequivocally demonstrated that Im can be accommodated in the heme–DNA complex, as in the case of metMb. Furthermore, the absorption spectrum of the heme–DNA complex in the presence of a 2×10^4-fold excess of Im exhibited Soret absorption at 407 nm ($\varepsilon = 71$), together with absorption maxima at 538 nm ($\varepsilon \approx 5$) and ca. 566 nm ($\varepsilon \approx 3$), and is quite similar to that of metMb(Im) (Supporting Information). The similarity in the spectral properties strongly suggested that the heme environment in heme–DNA(Im) is similar to that in metMb(Im), in which heme Fe^{3+} is coordinated to His and Im.

The ^1H NMR spectrum of the heme–DNA complex exhibit-

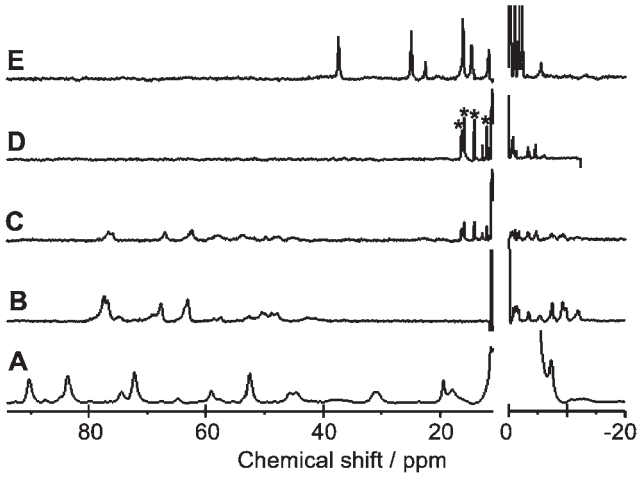

Figure 2. Downfiled-shifted portions of the 500 MHz ^{1}H NMR spectra of metMb (A), heme–DNA complex (B), a mixture of the heme–DNA complex and heme–DNA(Im) (C), heme–DNA(Im) (D), and metMb(Im) (E) in 90% H_2O/10% ^2H_2O, pH 7.0, at 25 °C.

ed well-resolved paramagnetically shifted signals arising from heme peripheral side-chain protons due to unpaired electrons at heme Fe^{3+} (Figure 2b). The specific complexation between the DNA and heme was manifested in the appearance of a single set of paramagnetically shifted signals, which reflected a unique heme electronic structure in the complex. Curie plots, i.e., shifts vs reciprocal of absolute temperature, for the resolved signals over the temperature range of 278–348 K, each gave a straight line, indicating that they obey Curie's law, this being consistent with the high binding affinity of heme to the DNA (Supporting Information). The four relatively large signals at 60–80 ppm could be attributed to heme methyl protons, and the shift pattern of the heme methyl proton signals of the heme–DNA complex resembled that of metMb (Figure 2a), indicating that electronic nature of heme, hence coordination environment around heme Fe^{3+}, in the two systems are similar. With the addition of Im, a new set of signals appeared in the chemical shift ranges of 10–20 ppm and −5–0 ppm at the expense of the signals for the heme–DNA complex (Figure 2c), and further addition of Im resulted in complete disappearance of the signals for the heme–DNA complex (Figure 2d). The newly emerging signals should arise from heme–DNA(Im) and, in particular, signals indicated by asterisks are assignable to heme methyl protons, judging from their intensities. The ca. 40 ppm separation between the signals of the heme–DNA complex and heme–DNA(Im) indicated that the exchange rate between the two states is $\ll 2 \times 10^4\ s^{-1}$. The paramagnetic shifts of heme methyl proton signals for heme–DNA(Im) are considerably smaller than those of metMb(Im) (Figure 2e). Fe^{3+} in metMb(Im) is known to exhibit a thermal spin equilibrium between the high spin ($S = 5/2$) and low spin ($S = 1/2$) states.[9] Fe^{3+} mostly exists in the low-spin state in the ambient temperature range and the fraction of the high-spin state gradually increases with increasing temperature. The thermal spin equilibrium in metMb(Im) is generally manifested in the non-linearity and anti-Curie behavior of heme methyl proton signals.[9b] On the other hand, the normal Curie behavior of heme methyl proton signals of heme–DNA(Im) (Supporting Information) suggested the negligible contribution of the high-spin state, if any. Consequently, the small paramagnetic shifts (10–20 ppm) of the heme methyl proton signals for heme–DNA(Im) could be attributed to the predominance of the low-spin state, as has been demonstrated in studies of various ferric porphyrin derivatives bearing Im as an axial ligand.[10] The X-ray data of metMb(Im) demonstrated that the heme active site structure is substantially altered by the coordination of bulky Im and that the Fe^{3+}–Im coordination structure in the protein is largely distorted compared to those in model compounds.[11] The distorted Fe^{3+}–Im coordination structure is possibly responsible for the thermal spin equilibrium of metMb(Im). Consequently, the predominance of the low-spin state in heme–DNA(Im) dictated that the Fe^{3+}–Im coordination structure is not significantly distorted.

The present study clearly demonstrated that the heme–DNA complex is remarkably similar to metMb in terms of heme electronic structure as well as Im binding affinity. These findings lead to the possibility of constructing a heme–DNA that exhibits a hemoprotein function. The properties of the heme–DNA complex could be controlled by chemical modification of heme peripheral side chains and substitution of the center metal ion as well as designing of the three-dimensional structure of a G-quadruplexed DNA through the sequence. Further structural and functional studies of a series of heme–DNA complexes are expected to unveil the principles underlying the structure–function relationship of the complexes.

This work was partly supported by a Grant-in-Aid (No. 17655072) from MEXT.

References and Notes

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Published on the web (Advance View) January 5, 2006; DOI 10.1246/cl.2006.126