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Coordination complex between haemin and parallel-quadruplexed d(TTAGGG)†

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Received (in Cambridge, UK) 2nd April 2003, Accepted 22nd May 2003
First published as an Advance Article on the web 13th June 2003

Haemin, iron(III)–protoporphyrin IX complex, and parallel-quadruplexed d(TTAGGG) have been shown to form a stable coordination complex which exhibits spectroscopic properties remarkably similar to those of haemoproteins.

Interactions of porphyrin derivatives with quadruplexed DNA have been extensively studied to develop antitumor drugs possessing telomerase inhibition activity[1–3] as well as to explore catalytic DNAs that mimic haemoprotein functions.[4–6] The size, planarity and hydrophobicity of the porphyrin ring are well-suited for intercalating into or stacking with the G-quartets. Among porphyrin derivatives, haemin, iron(III)–protoporphyrin IX complex, is unquestionably the most studied prosthetic group in biological macromolecules, because of its biological relevance, ubiquitous occurrence in the active sites of various b-type haemoproteins and characteristic physicochemical properties. We report herein the formation of a coordination complex between haemin and quadruplexed DNA composed of d(TTAGGG).

We first examined interaction of haemin with the DNA using absorption spectra. The Soret absorption of haemin exhibited both hyperchromic and bathochromic effects, together with isosbestic points at 382 and 419 nm, with increasing DNA concentration (Fig. 1A). Since d(TTAGGG) is known to be assembled into a stable parallel-quadruplex in solution, which exists as a back-to-back dimer through intermolecular π–π stacking interaction between the two G-quartets,[7,8] these spectral changes clearly indicated that haemin interacts with the quadruplexed DNA. Scatchard plots of the absorbance at 404 nm observed upon the titration of haemin with the DNA, illustrated in Fig. 1B, were represented as a straight line and yielded the binding constant of 4.2×10^7 M^{-1} with the stoichiometric ratio of about 1 : 2 between haemin and the quadruplexed DNA. The high binding affinity of haemin to the quadruplexed DNA was manifested in the large binding constant. Additionally, as shown in Fig. 1C, the absorption spectrum of the haemin–DNA complex highly resembled that of sperm whale aquomet myoglobin (Mb),[9] in which haem iron is coordinated to a histidyl imidazole and a water molecule as axial ligands. The spectral similarity strongly suggested that the haemin environments in the haemin–DNA complex and aquomet Mb are highly alike. The circular dichroism spectrum of the haemin–DNA complex exhibited a positive maximum at ~ 260 nm, which is characteristic of a parallel-quadruplexed DNA,[10] and an induced negative intensity at ~ 400 nm, which is indicative of π–π stacking interaction between the porphyrin ring of haemin and the base pair of DNA[11] (results not shown). These results further confirmed the complexation of haemin with the parallel-quadruplex of d(TTAGGG).

Similar titrations have been carried out with different DNA sequences such as d(TTAGGGT) and d(TTTGGG). The formation of a back-to-back dimer from the DNA quadruplex has been shown to be hampered in d(TTAGGGT) due to the presence of an extra T at the 3′-terminus.[7] We found that the haemin binding affinity to quadruplexed d(TTAGGGT) was considerably lower than that to quadruplexed d(TTAGGG), while d(TTTGGG) was essentially identical to d(TTAGGG) in terms of the binding affinity. These results, together with the observed stoichiometric ratio of about 1 : 2 between haemin and quadruplexed DNA in the complex, dictated that haemin is sandwiched between the G-quartets at the 3′-termini of two quadruplexed DNAs.

We next examined the pH dependence of the Soret absorption of the haemin–DNA complex to infer the coordination structure of haem iron in the complex. The Soret absorption of the complex exhibited hypochromic and bathochromic effects with increasing pH and the pH dependence of the absorbance at 404 nm exhibited a titration curve with the midpoint at pH 8.6 (Fig. 2B). Aquomet Mb exhibits similar pH-dependent absorption changes due to the deprotonation of Fe-bound H_2O at higher pH with pK value of 8.5–9.0.[12] The similarity in the pH-dependent properties of the absorption spectra between the two systems not only demonstrated the coordination of H_2O to haem iron in the haemin–DNA complex, but also demonstrated that chemical environments around Fe-bound H_2O in the complex and aquomet Mb are similar. This conclusion was further supported by NMR and EPR spectra.

The 600 MHz ^{1}H NMR spectrum of the complex exhibited well-resolved paramagnetically shifted signals arising from

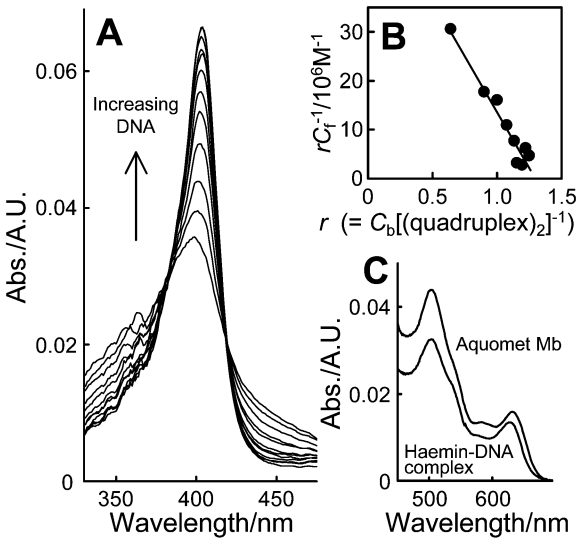

Fig. 1 (A) Soret absorption of 0.5 μM haemin in 300 mM KCl and 50 mM phosphate butter at pH 7.0, in the presence of various d(TTAGGG) concentrations of 0–15 μM, at room temperature. 0.17 w/v% Triton X-100 and 0.5 v/v% DMSO were added to the solution mixture to prevent haemin aggregation. (B) Scatchard plots for the absorbance at 404 nm, which yielded the binding constant of 4.2×10^7 M^{-1} and the stoichiometric ratio of about 1 : 2 between haemin and the quadruplexed DNA. (C) The portions of the absorption spectra of the haemin–DNA complex (bottom) and sperm whale aquomet Mb (top) in 300 mM KCl and 50 mM phosphate buffer at pH 7.0 and room temperature.

† Electronic supplementary information (ESI) available: CD and NMR spectra. See http://www.rsc.org/suppdata/cc/b3/b303643j/

DOI: 10.1039/b303643j

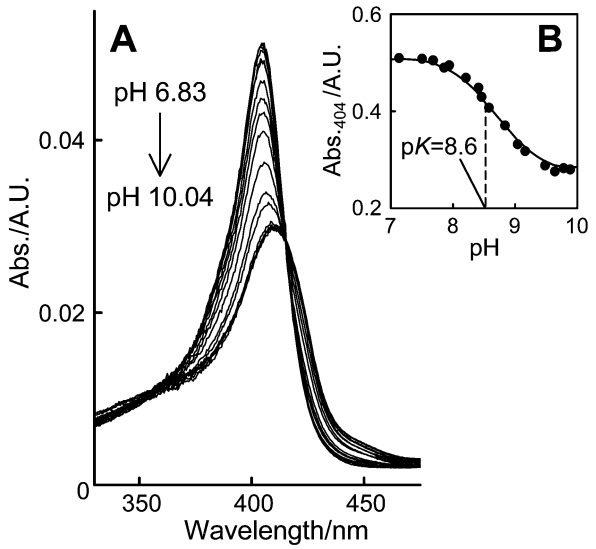

Fig. 2 (A) pH dependence of Soret absorption of 5 μM haemin–DNA complex in 300 mM KCl at room temperature. pH was varied from 6.83 to 10.04. (B) Plot of the absorbance at 404 nm against pH. The midpoint was observed at pH 8.6.

haem peripheral side-chain protons due to unpaired electrons at haem iron (traces A–C in Fig. 3). A specific complexation between haemin and DNA was manifested in the appearance of a single set of paramagnetically shifted signals, which reflected a unique haem electronic structure in the complex. The Curie plots, shifts *vs*. reciprocal of absolute temperature, for the resolved signals over the temperature range examined, 278–338 K, gave a straight line, indicating that they obey the Curie law. This result is consistent with the high binding affinity of haemin to the DNA quadruplex revealed by the absorption studies. Four relatively large peaks at 60–80 ppm in trace A could be attributed to haem methyl protons, and the shift pattern of the haem methyl proton signals of the complex resembled that of aquomet Mb in trace A′, indicating that structural features around haem iron in the two systems are alike. Furthermore, the low-temperature EPR spectrum of the complex exhibited signals at $g = \sim 2$ and ~ 6 (trace D), which are characteristic of a ferric high-spin haemin complex,[12] and was similar to that of aquomet Mb at neutral pH. These results also supported the

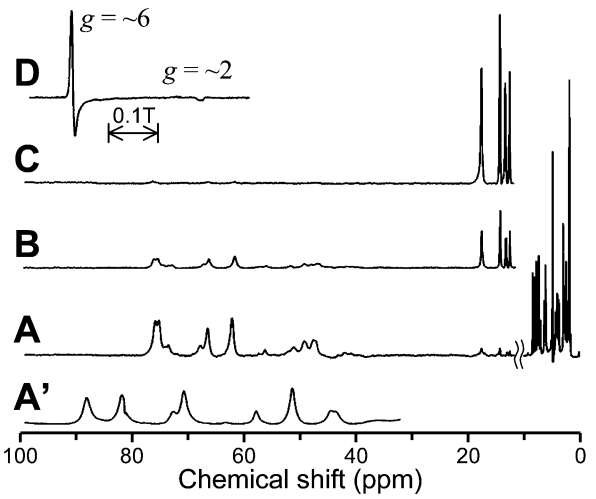

Fig. 3 NMR and EPR spectra of the haemin–DNA complex. The 600 MHz ^{1}H NMR spectra of the haemin–DNA complex in 300 mM KCl in 90% H_2O–10% D_2O at 298 K and pH 7.04 (A), pH 7.81 (B), and pH 8.58 (C), and sperm whale aquomet Mb at 298 K and pH 7.00 (A′) and low temperature (15 K) EPR spectrum of the haemin–DNA complex in 300 mM KCl and 50 mM phosphate buffer at pH 7.0 (D). In trace A, the y-gain for the spectral region of 0–10 ppm was reduced by 1/200 relative to that below 10 ppm.

proposal that haem iron in the complex is coordinated to H_2O. Recently, a DNA aptamer which recognises haemin has been reported and the axial ligands to haem iron in such an aptamer complex have been proposed to be a guanine residue and H_2O.[6] Therefore, similarly to the coordination structure in the aptamer complex, haem iron in the present haemin–DNA complex may be coordinated to a guanine residue at the 3′-terminus of quadruplexed d(TTAGGG), although further structural characterisation is required to identify the axial ligand to haem iron in the complex.

A new set of NMR signals appeared in the chemical shift range of 10–20 ppm at the expense of the signals below 40 ppm, with increasing pH (traces A-C). The pH dependence of the NMR spectra of the complex could be attributed to the deprotonation of Fe-bound H_2O at higher pH, as manifested in the pH dependence of its Soret absorption. Consequently, signals in trace C could be assigned to haem side-chain protons, possibly methyl protons, of the haemin–DNA complex with Fe-bound OH^-. The chemical shift separation of > 40 ppm between the haem methyl proton signals of the complexes with Fe-bound H_2O and OH^- dictated that the ligand exchange rate is $<< 2 \times 10^4$ s^{-1}. The slow ligand exchange could be attributed to hydrophobic environments around haemin in the complex, demonstrating a significant contribution of hydrophobic interaction between haemin and G-quartets of the DNA quadruplex to form the complex.

The results presented in this study demonstrated that haemin forms a stable coordination complex with two-equivalent quadruplexed d(TTAGGG) and that haem environments in the complex are highly similar to those of haemoproteins. Physicochemical properties of the haemin–DNA complex could be controlled by chemical modification of haem peripheral side-chains and the substitution of the centre metal ion as well as by designing the three-dimensional structure in the assembly of nucleic acids through the primary sequence. Studies of the haemin–DNA complex would provide detailed information about intermolecular interactions operative upon the complexation, which could facilitate a molecular basis for constructing "haem–nucleic acid".

This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (2), "Dynamic Control of Strongly Correlated Soft Materials" (No. 413/14045203), from the Ministry of Education, Science, Sports, Culture, and Technology. The NMR spectra were recorded on a Bruker AVANCE-600 spectrometer at the Chemical Analysis Centre, University of Tsukuba.

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