Reaction field for efficient porphyrin metallation catalysis produced by self-assembly of a short DNA oligonucleotide

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The G-wire structure, which is formed by dGTGGGTTGGGTGGGTTGG in the presence of 50 mM K^+, becomes a catalytic reaction field for porphyrin metallation, inserting Zn^{2+} into porphyrin efficiently.

Many protein enzymes provide a reaction field for their substrates. RNA or DNA can catalyze the cleavage or ligation reaction of nucleic acids, and then has the potential to provide a reaction field for its substrate.[1–4] The catalytic RNA– or DNA–substrate complexes consist of many unpaired regions such as internal loops, hairpin loops and bulges, and the catalysis reactions occur in these unpaired regions.[1–4] Thus, catalytic RNA or DNA produces the reaction fields. If these reaction fields can be made by designing specific sequences of catalytic RNA or DNA, it would be an important development for ribozyme engineering. Recently, a DNA enzyme that can catalyze insertion of Zn^{2+} into mesoporphyrin IX (MPIX) was isolated from a DNA library using *in vitro* selection.[5,6] This minimum DNA enzyme is a 24-mer oligonucleotide and consists of a G-rich sequence that is able to form a G-quartet structure.[6] Although this reaction mechanism was investigated,[7] little is known about the detailed active structure of this DNA enzyme except for the G-quartet that produces the reaction field to catalyze the metallation reaction. Here, in order to develop the chemical engineering of nucleic acids, we have investigated the active structure of the DNA enzyme with catalytic activity for the porphyrin metallation by using an 18-mer DNA sequence, dGTGGGTTGGGTGGGTTGG. This oligonucleotide was designed to contain the G-rich sequence that forms the G-quartet structure and to be shorter than the previous minimum DNA enzyme isolated by Li and Sen.[5,6]

The ability of this 18-mer DNA to catalyze the insertion of Zn^{2+} into MPIX was investigated by HPLC. The reaction yields of the porphyrin metallation were calculated by the ratio of the peak areas at 400 nm between the reactant and the product (ZnMPIX). The metallation reactions were performed in the presence of 50 mM K^+ or Na^+, because the formation of the G-quartet structure is easier in the presence of K^+ than Na^+.[8–12] At the single turnover condition,‡ the reaction yield in the presence of 50 mM K^+ was 91% after 8 h. On the other hand, the reaction yield in the presence of 50 mM Na^+ was 46%. The reaction yields in the absence of K^+ or Na^+ were 20 or 17%, respectively. Further, the k_{cat} values of the 18-mer DNA in the presence of 50 mM Na^+ and K^+ were 8.0×10^{-3} and 1.0 min^{-1}, respectively.§ This value in the presence of K^+ is of the same order as that for ferrochlatase.[13] Thus, the 18-mer DNA was able to catalyze efficiently the porphyrin metallation and played a role as an enzyme in the presence of K^+. The result also suggests that the active structure of this novel DNA enzyme consists of the G-quartets. In order to investigate the effect of DNA sequences other than the G-rich sequence, the activity of 16-mer DNA, dGTGGGTGGGTGGGTGG (which lacks the sixth and fifteenth T residues of the 18-mer DNA oligonucleotide), was also measured in the presence of K^+. Under the single turnover condition, the reaction yield was 55% after 8 h, which is much smaller than that for the 18-mer DNA. The k_{cat} value was 5.6×10^{-3} min^{-1}. Thus, the sequence that joins the G-rich sequence is also important for the formation of the active structure.

The CD spectra of the DNA oligonucleotides were measured to determine the active structure, since CD spectra of nucleic acids are very sensitive to their overall structure.[14] Fig. 1 shows the CD spectrum of the 18-mer DNA oligonucleotide in 50 mM Tris-OAc (pH 7.4) solution containing 50 mM K^+ and 1 mM spermidine at 25 °C. The spectrum has a positive peak around 265 nm and a relatively weak and negative peak around 240 nm. On the other hand, the spectra of the 18-mer DNA in the presence of Na^+ and the 16-mer DNA in the presence of K^+ have two positive peaks around 265 and 295 nm and a relatively weak and negative peak around 230 nm. The intensity at 265 nm of the 18-mer DNA in the presence of K^+ was about 15-fold higher than those of the 18-mer DNA in the presence of Na^+ and the 16-mer DNA in the presence of K^+. The difference in metallation activities between the DNA species corresponds to the difference in the intensity values at 265 nm. Thus, the structural component that produces the positive peak around 265 nm in the CD spectrum must be the active structure for the metallation catalysis. The G-rich DNA species form a parallel or antiparallel G-quartet structure.[8–12,15,16] Previous studies indicated that the CD spectrum of the antiparallel G-quartet structure had 295 nm positive and 265 nm negative bands, while a parallel G-quartet structure with four or more strands exhibits a strong positive band at 260 nm and negative band at 240 nm.[15,16] Since the CD spectrum of the active structure of the DNA enzyme showed a strong positive band at 260 nm, the structure with the catalytic activity for the metallation should have mainly a parallel G-quartet structure with four or more strands. The structures of these DNA species were also investigated by nondenaturing gel electrophoresis. The mobility of the 18-mer DNA in the presence of 50 mM K^+ at 25 °C was demonstrated by the smearing of the band. This suggests that

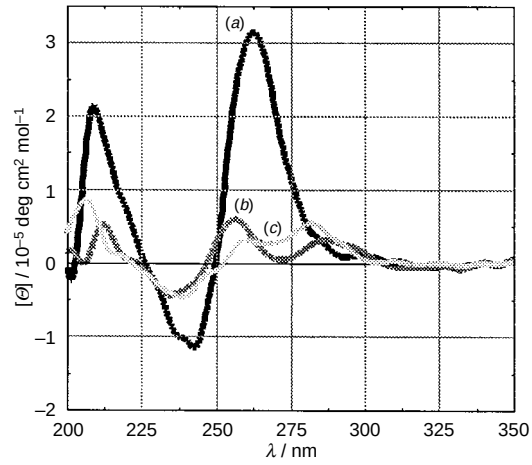

Fig. 1 CD spectra of dGTGGGTTGGGTGGGTTGG in the presence of (*a*) 50 mM K^+ and (*b*) 50 mM Na^+, and (*c*) dGTGGGTGGGTGGGTGG in the presence of 50 mM K^+ at 25 °C. Buffer contains 50 mM Tris-OAc (pH 7.4) and 1 mM spermidine. Concentration of each oligonucleotide was 20 μM.

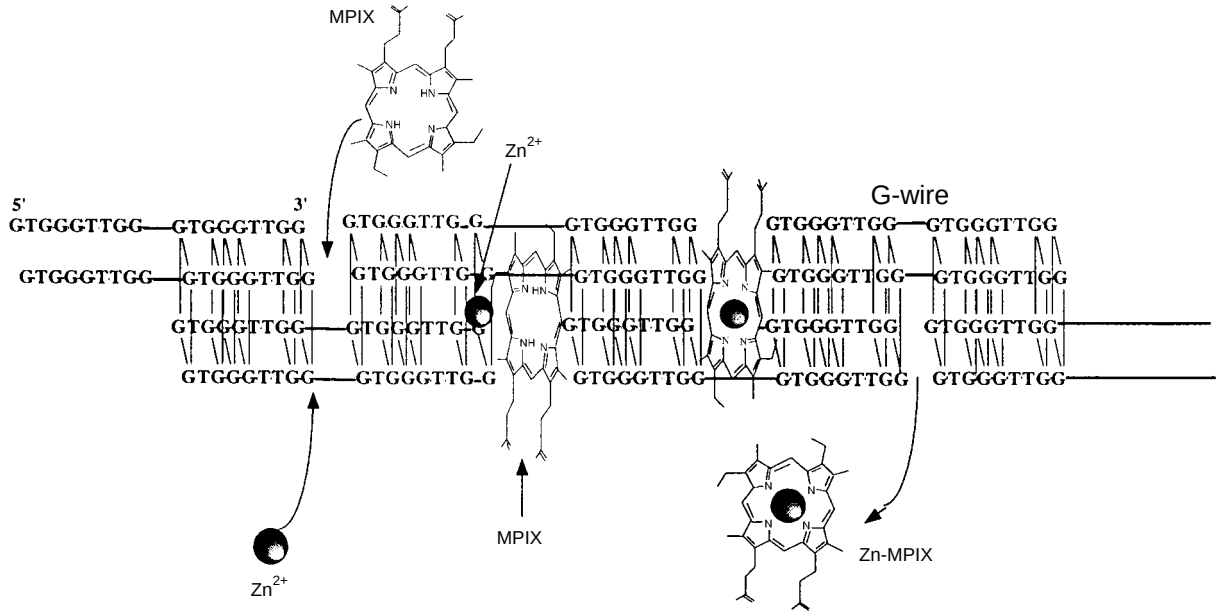

Fig. 2 Schematic illustration of the reaction mechanism between Zn^{2+} and MPIX in the G-wire of the 18-mer DNA species

the structure of the G-wire consists of a slipped tetraplex structure.[17,18] Thus, the results of the CD spectra and gel bands indicate that the active structure of the DNA enzyme is a G-wire structure stabilized by K^+.[17–19]

Fig. 2 shows our proposed reaction field for the porphyrin metallation. In the G-wire field, the porphyrin intercalates into the pocket formed by the junction between the G-quartet domains. Zn^{2+} also inserts into the cavity between two G-quartet planes near the pocket formed by the junction between the G-quartet domains. Following these intercalations, the porphyrin is close to Zn^{2+} so that the metallation occurs easily. The K_m value of the 18-mer DNA in the presence of K^+ was about two orders larger than the others. This value would suggest the possibility that G-wire DNA enzymes are easily able to release the complex between the porphyrin and Zn^{2+} as a product. Furthermore, the G-wire structure has many pockets into which the porphyrins may enter. Thus, this structure produces a good reaction field for the catalytic reaction. In this G-wire structure, the two T residues removed from the 18-mer DNA enzyme locate in the junction site between continuing G-quartet domains. If this region is shortened, the G-wire structure is destabilized by repulsion between the G-quartet domains. Thus, the difference in G-wire formation between the 18-mer and 16-mer DNA species is due to this repulsion between the junctions.

In summary, it was found that a novel 18-mer DNA enzyme, which was shorter than a previously reported DNA enzyme, catalyzed porphyrin metallation. This DNA enzyme formed a G-wire structure as the reaction field to catalyze the insertion of Zn^{2+} into mesoporphyrin IX. This G-wire structure shows promise as a reaction field for other catalytic reactions. Thus, our results in the present study indicate the possibility of DNA usage as materials and reaction fields in chemical nanotechnology.

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Notes and References

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‡ The metallation reaction was carried out in 50 mM Tris-OAc (pH 7.4) solution containing 5 μM DNA, 1 mM Zn^{2+}, 33 μM MPIX, 50 mM KOAc, 1 mM spermidine, 5% DMSO and 0.5% Triton X-100 at 25 °C for 8 h. Before the reaction was initiated, the DNA was reannealed by heating to 90 °C for 2 min, cooled to 25 °C, and incubated for 24 h in the reaction buffer without MPIX and Zn^{2+}. Then, the DNA was preincubated with MPIX for 10 min, and combined with appropriate volumes of the same solution containing $Zn(OAc)_2$. The reactions were stopped by the addition of a solution containing 200 mM Tris (pH 9.0) and 15 mM EDTA. The reactions were monitored by HPLC. The column used was a C18 column, and the mobile phase was 85% methanol and 15% 1 M ammonium acetate, pH 5.2.

§ The rates of metallation in the presence of the 18-mer or 16-mer DNA species were assayed for the concentration range of MPIX from 30 to 500 μM at a fixed concentration of 1 mM Zn^{2+} and 5 μM DNA. For initial rate measurements, good linear relationships between product formation and time were found in each case. The K_m and k_{cat} values were calculated form Lineweaver–Burk plots of $1/(V_{obs}-V_{background})$ *vs.* 1/[MPIX]. Each data point represents the average of at least two sets of independent measurement.

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