

Catalytic Activity of DNA-Pt Complex

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Introduction

Enzyme promotes metabolic reaction as biocatalyst. In evolutionary RNA and protein function as a biocatalyst, but DNA has not been played this role. Although RNA carried out the information storage task of genetic information as well as catalytic roles in a very primitive self-replicating system, double helix structure of DNA is believed to be impossible to form intricate active enzymatic site. In addition, the chemical stability of DNA prevents self-modifying reactions¹.

However, recent development of DNA engineering enables to create artificial enzymatic ability of DNA (deoxyribozyme) such as RNA cleavage and DNA modification. DNA-based asymmetric catalysis such as metal-catalyzed reaction using chirality of DNA double helix has been also investigated.

However, there was no report to show that DNA bound with metallic complex like platinum complex reacts as activity of the enzymes. We found DNA-Pt complex has enzymatic activity that is similar to peroxidase in this study.

The number of active sites of DNA-Pt complex can be enormous in contrast to that of enzyme or protein, when substantial transition metal complexes bond to DNA. Furthermore, DNA-Pt complex is chemically stable than nature enzyme or proteins, because phosphodiester bonds of DNA are 100-fold more resistant to hydrolytic degradation than the peptide bonds of proteins.

We investigated optimal conditions for enzymatic activity of DNA-Pt complex, and compared it with that of horse radish peroxidase. We report here that base sequence of DNA, pH and temperature affect the enzymatic activity of DNA-Pt complex.

Experimental

DNA oligonucleotide (A20mer, G20mer, C20mer, T20mer and AG20mer) and single stranded DNA (from salmon testes, MW=468,000) was dissolved in ultrapure water and adjusted to be 1mg/mL.

Potassium tetrachloroplatinate(II) ($K_2[PtCl_4]$) was dissolved in ultrapure water and adjusted to be 83.3 mg/mL in dark place.

70 μ L of phosphate buffer (pH 7), 5 μ L of $K_2[PtCl_4]$ solution and 25 μ L of oligonucleotide solution were mixed in microtube in dark place at 25°C for 24, 48, 72, 96 and 120 h. 70 μ L of borate buffer (pH 9) and 70 μ L of disodium hydrogen phosphate-sodium hydrate buffer (pH 11) were used for pH 9 and pH 11 of DNA-Pt complex solution, respectively.

DNA-Pt complex solution was precipitated by 3M sodium acetate and ethanol by centrifugation (15,000rpm, 4°C, 15 minutes). Precipitate of DNA-Pt complex was dissolved in TE buffer, and the concentration was adjusted to be 50 nmol/L.

Enzymatic reaction was measured with TMB (3,3',5,5'-tetramethylbenzidine) Microwell Peroxidase Substrate System. The difference of the absorbance (450-595nm) was measured using microplate reader (BIO-RAT, Model 550) to determine the reaction of TMB solution catalyzed with DNA-Pt complex. Peroxidase (from Horseradish, 1,000 units/mg, HRP) was also used as the comparison of enzymatic reaction to DNA-Pt complex.

Results and discussion

Dependence of enzymatic activity of DNA-Pt complex prepared from 20mer DNA oligonucleotide [(AG)_n] was investigated at various pH for 24-120h. 50nmol/L of 20mer-DNA (AG) of DNA-Pt complex was used for the reaction with TMB. The reaction of TMB increased with the increase of reaction time of DNA and Pt during reaction time until 120h at pH 7, pH 9 and pH 11. It is also found that the enzymatic activity of DNA-Pt complex was found to be higher when DNA-Pt complex was prepared with 20mer DNA at higher pH.

Effect of DNA sequence on enzymatic activity of various 20mer DNA (MW=5721.8-6522.2)-Pt complex was investigated where DNA-Pt complex was prepared at pH 7, 9 and 11.

DNA-Pt complex prepared from AG 20mer DNA and A 20mer DNA showed higher reactivity with TMB than the other DNA-Pt complex prepared from G, T or C 20mer DNA at pH 7. This is explained as, cis-configuration platinum likely to bound N7 of guanine or adenine. DNA-Pt complex prepared from AG 20mer DNA showed the highest enzymatic reactivity for TMB at any pH in this study. When pH of reaction solution is higher than pH 9, pyrimidine nucleotide (thymine and cytosine) also bound with Pt and showed relatively high reactivity with TMB. The sequence of DNA was found to affect the reactivity with TMB. This should be originated from the amount of Pt bound on DNA.

Fig. 1 shows enzymatic activity of HRP and DNA (single stranded salmon testes DNA as well as 20mer DNA)-Pt complex for TMB. Single stranded salmon testes DNA-Pt complex reacted at pH 11 has similar enzymatic activity of HRP.

Fig. 2 shows comparison of enzymatic activity of HRP and DNA-Pt after treated by heat for 30 minutes from 20°C to 80°C. HRP is denatured over 60°C, while DNA-Pt complex still remains its enzymatic activity at 50-80°C. Therefore, DNA-Pt complex is more stable biocatalyst than HRP at high temperature. Furthermore, the reaction of TMB by DNA-Pt complex was not be influenced by plasma component such as platelet poor plasma, while HRP increased the enzymatic activity with TMB in the presence of platelet poor plasma.

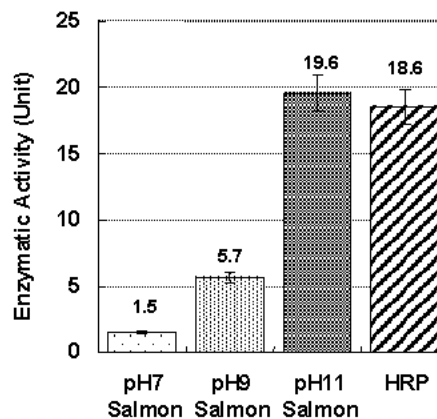


Fig. 1 Effect of reaction condition of pH on enzymatic activity of S.S. Salmon Testes DNA-Pt complex compared with HRP

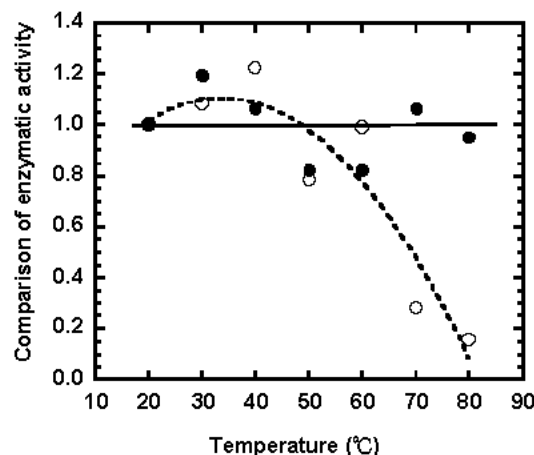


Fig. 2 Comparison of enzymatic activity after heat treatment of HRP and DNA-Pt complex

References

- [1] Ronald R. Breaker, Making Catalytic DNAs, Science 290, 2000