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Human telomeric DNA sequences have a peroxidase apoenzyme activity[†]

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Abstract

Many G-quadruplex-forming, biological DNAs, such as telomeres and a c-Myc (an oncogene) promoter region, have been shown by an *in vitro* study to be peroxidase apoenzymes.

Telomeres are guanine-rich, repetitive DNA segments located at the ends of linear chromosomes of eukaryotic organisms.^{1,2} They protect the chromosome ends and maintain the integrity of eukaryotic genomes. Telomeres are involved in the aging process and have implications in a range of human diseases, including cancers.^{3–5} Fundamental studies related to the physical structures and biochemical properties of telomeres have recently received tremendous attention.⁶ However, the biological functions of telomeres have not been fully understood. Herein, we present experimental data to show an unrecognized chemical aspect of telomeres: telomeric DNAs possess peroxidase-like activities.

A short synthetic DNA oligomer, PS2.M, was derived from an *in vitro* selected DNA aptamer that could tightly bind to hemin (a porphyrin derivative).^{7,8} Such a hemin–DNA complex was later found to have a peroxidase-like activity.^{9,10} Hemin itself is known to be able to catalyze peroxidative reactions, but its activity is low.¹¹ When complexing with PS2.M, its catalytic efficiency significantly increases.⁹ Thus the DNA–hemin complex is a peroxidase holoenzyme that contains a DNA apoenzyme and a hemin co-factor. In nature, hemin-like molecules are active co-factors for many proteins that possess peroxidatic activities.¹² Likely, these protein apoenzymes provide environments that enhance the intrinsic peroxidatic properties of the hemin-like molecules. The DNA molecule PS2.M, as a peroxidase apoenzyme, might provide a similar environment. Even though its detailed catalysis mechanism is not clear, the DNA peroxidase has been explored for bioanalysis.^{13,14} PS2.M is proposed to fold into a G-quadruplex structure (Fig. 1, left) and the hemin interacts with the DNA aptamer by either intercalating into or stacking onto the G-quadruplex.^{7,8} In fact, it is well documented that porphyrin derivatives can readily interact with many G-quadruplex structures, including a number of biologically important DNAs, such as telomeres^{15,16} and c-Myc (an oncogene) promoters.¹⁷ It prompts our curiosity whether these biologically important DNAs can interact with hemin in a similar fashion as PS2.M does and function as peroxidase apoenzymes.

We first tested a short human telomeric DNA, d(TTAGGG)₄, that contains four tandem repeats of a hexanucleotide.¹⁹ This sequence, in the presence of K⁺, adopts a three-deck G-quadruplex structure with the linking trinucleotide loops (TTA) positioned on the exterior, as suggested by a crystallography study (Fig. 1, right).¹⁸ To analyze the enzymatic activity of this DNA, we

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followed a reported method¹⁴ and used hydrogen peroxide (H_2O_2) and 2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) as substrates for the catalytic reaction. ABTS is colorless, but the reaction product $\text{ABTS}^{•+}$ is blue-green colored (maximum absorption wavelength, $\lambda_{\text{max}} = 415 \text{ nm}$) and can provide a convenient, colorimetric signal. Fig. 2 (left) shows the absorbance change over the reaction time in the presence of the telomere-hemin complex. The fast increase in absorbance indicates an accelerated oxidation of ABTS. In a control experiment with hemin alone, the reaction is much slower than that catalyzed by the telomere-hemin complex. This result shows that human telomeric DNA, when complexing with hemin, can significantly catalyze peroxidatic reactions.

The catalyzed reaction rate is DNA concentration-dependent. The initial reaction rate linearly increases as the telomeric DNA concentration increases (Fig. 2, right). Besides the human telomeric DNA, we have used three other DNA molecules as controls: the previously reported DNA peroxidase (PS2.M), a non-related, random, single-stranded DNA (ssDNA), and a double-stranded DNA (dsDNA). Interestingly, the human telomeric DNA shows a much higher catalytic activity than PS2.M, the previously reported DNAzyme. It is not a surprise because PS2.M is not selected by its peroxidase activity but rather by its binding to porphyrin derivatives.⁷ Unrelated ssDNA and dsDNA do not accelerate the reactions when compared with the background, suggesting that the peroxidase activity belongs only to a specific group of DNAs.

$\text{d}(\text{TTAGGG})_4$ is the minimum unit for human telomeric DNA to form an intramolecular G-quadruplex. However, telomeres in cells are much longer and contain more repeating hexanucleotides. Therefore, telomeres contain multiple G-quadruplex domains, or multiple copies of DNA peroxidases, and will have higher peroxidase activities. This hypothesis has been tested by checking the activity of a longer telomeric DNA strand, $\text{d}(\text{TTAGGG})_{12}$. Fig. 3 shows the DNA length effect on the enzymatic activity of the human telomeric DNA. For both DNAs (4 and 12 repeats, respectively), there are linear correlations between the initial reaction rate and the DNA concentration. The activity of the long DNA is approximately 2.5 times more than that of the shorter DNA, despite its three-times as long sequence. It is reasonable since even though the long DNA can form three G-quadruplex domains, these domains are close to, and would likely affect, each other, either by distorting the quadruplex structures, or partially blocking the active sites, resulting in relatively lower enzymatic activity for each G-quadruplex domain compared to the individual G-quadruplex, $\text{d}(\text{TTAGGG})_4$.

DNA telomeres exist in all eukaryotes and are all G-rich sequences. They all have potential to form G-quadruplexes. We have surveyed the peroxidase activity for telomeric DNA sequences from different species and found that most of them exhibit significant peroxidase activities (Fig. 4). In general, the telomeric DNAs from higher organisms (*e.g.* higher plants, insects, and vertebrates) have high peroxidase activities, and the telomeric DNAs from immortal organisms (*e.g.* *Oxytricha*, *Tetrahymena*, and *Schizosaccharomyces pombe*) have low or no peroxidase activities.

Oxidative stress has been proposed to be a factor for telomere losses and cell senescence.²⁰ It has been demonstrated that mild oxidative stresses, such as chronic hyperoxia²¹ or hydrogen peroxide (H_2O_2),²² accelerate telomere shortening and decrease replicative lifespans. Since those conditions are similar to the experimental conditions used here, questions naturally arise: do telomere DNAs function as peroxidases in living cells? If so, how do such enzymatic activities influence the genome integrity and other biological processes, *e.g.* DNA damage, transcription, and tumor genesis? For example, the promoter region of an oncogene c-Myc contains a G-rich sequence, which has been proposed to be able to adopt G-quadruplex folding. Its conformation influences the c-Myc transcription.¹⁷ We have found that this DNA sequence also has a peroxidase activity (Fig. 4).

A variety of DNA enzymes have been developed from directed evolutions.^{23–26} However, no DNA enzymes have been found in cells with one arguable exception for a single-turnover, self-destructing DNA motif.²⁷ It is a general impression that DNA enzymes are unlikely to exist in cells because most biological DNAs are double stranded. Here, we provide evidence for a group of biological DNA enzymes that have multiple-turnovers. This study raises a question of whether other DNA enzymes exist in biology.

In conclusion, *in vitro* studies have shown that many biological G-quadruplex-forming DNAs, such as human telomeres, have peroxidase-like activity. At this point, it is not clear whether such enzyme activities exist in cells and, if they exist, what the biological implications are. Such questions are under investigation in our group. We are also asking what are the sequence and structural requirements of the DNA peroxidase and how to optimize the DNA peroxidase.

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Fig. 1. Structures of (left) PS2.M, a previously reported DNA peroxidase and (right) a reported structure of human telomeric DNA.¹⁸ The arrowed lines indicate the DNA polarity: from 5' → 3'. Note that the G-quadruplex motifs have different topologies in PS2.M and human telomeric DNA.

Fig. 2.

The human telomeric DNA–hemin complex has a peroxidase-like activity. (Left) Absorbance evolution at $\lambda = 405$ nm during the enzymatic reaction that converts colorless ABTS into blue–green $\text{ABTS}^{\bullet+}$. The DNA concentration is 100 nM. (Right) Dependence of initial reaction rates on the DNA concentrations. The initial concentrations of hemin, ABTS, and H_2O_2 are 500 nM, 1.17 mM, and 3.54 mM, respectively. Error bars are calculated from 5 repeating experiments.

Fig. 3. The peroxidase activity of human telomeric DNA is DNA-length dependent. The initial concentrations of hemin, ABTS, and H_2O_2 are 500 nM, 1.17 mM, and 3.54 mM, respectively. Error bars are calculated from 5 repeating experiments.

Fig. 4.

Survey of peroxidase activities of telomeric DNA sequences from different species. Each telomeric DNA contains four basic repeats. In general, higher organisms have higher peroxidase activities. Note that the last one is not a telomeric DNA; instead, it is a potential G-quadruplex-forming DNA segment found in the promoter region of c-Myc, an oncogene. The initial concentrations of DNA, hemin, ABTS, and H_2O_2 are 100 nM, 500 nM, 1.17 mM, and 3.54 mM, respectively. Error bars are calculated from 5 repeating experiments.