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Photosensitized damage to telomere overhang and telomerase RNA by riboflavin

Yuxia Liu, Fuqiang Du, Weizhen Lin *, Tiecheng Tu, Wenxin Li, Nianyun Lin

Key Laboratory of Nuclear Analysis Techniques, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China

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Abstract

By ESR spin elimination and photocleavage assay, the mechanisms of one-electron oxidation damage of oligonucleotides by excited triplet state of riboflavin (Rb) have been elucidated. The results demonstrate that Rb, an endogenous photosensitizer, is capable of cleaving single-stranded telomeric overhang and the template region of telomerase RNA under UVA irradiation, resulting in blocking of reverse transcription of telomeric DNA which leads to the apoptosis of cancer cells ultimately.

Keywords: Telomere; Telomerase; Riboflavin; ESR spin elimination; Excited triplet state

1. Introduction

Telomeres are special DNA–protein structures found at the ends of all vertebrates linear chromosomes [1]. Telomeric DNA in vertebrate consists of tandem repeats of guanine rich sequences (TTAGGG)*n*. Telomerase is a ribonucleoprotein enzyme that maintains telomere length by adding telomeric sequences onto chromosome ends. The RNA component of telomerase contains a template sequence responsible for synthesis of telomeric DNA and complete replication of chromosome ends [2,3]. The primary function of telomeres is to protect the ends of linear chromosomes from recombination, fusion and degradation [4]. Each replication of telomeric DNA results in 3′-end bases loose of telomeres in normal somatic cells as a consequence of that the ends of telomeric DNA cannot be completely replicated by DNA polymerase. This process eventually leads to critical shortening of telomeric DNA, which results in cell apoptosis or cell death. This is known as "end replication problem" and telomeres are called "molecular clock" thereby. In contrast to somatic cells, in 90% tumor cell telomerase becomes active and stabilizes the length of telomeres [5], leading to the immortality of cancer cells. Nowadays, telomeres and telomerase are of great research interest due to the special relationship between activity of telomerase and cancer cells.

Riboflavin (Rb) or vitamin B_2 widely exists in human organs and a variety of fruits in free and conjugated forms, where it plays significant roles in most important biological functions. Rb-induced one-electron oxidation of biomolecules can readily occur via Type I photosensitized oxidation [6] because of a high quantum yield of the excited triplet state of Rb ($^3Rb^*$) and a higher reduction potential [7], which further lead to the damage of biological target molecules. The results from *in vitro* and *in vivo* studies demonstrate that Rb is capable of inducing damage of cellular DNA, protein and other biomolecules via photosensitized oxidation process under the irradiation of UVA or visible light, resulting in cell death eventually.

Previous ESR spin elimination and laser photolysis studies revealed that $^3Rb^*$ can react with all nucleic acid bases via electron abstraction reaction at deaerated condition with a variety of reaction rates [6]. Therefore, Rb can

* Corresponding author. Tel.: +86 21 59554603; fax: +86 21 59553021.
E-mail address: linweizhen@sinap.ac.cn (W. Lin).

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be recognized as a strong endogenous photosensitizer capable of photocleaving the single-stranded telomeric overhang and template region of telomerase RNA, inducing them to serious breakages of functional sequences which results in the death of cancer cells ultimately. Thus, we are aiming at developing a novel cancer phototherapeutic approach which is different from traditional photodynamic therapy (PDT) [8] of cancers.

2. Materials and methods

2.1. Detection of the reactivity of $^3Rb^*$ with a series of single-stranded telomeric sequence repeats by ESR spin elimination method

Rb-induced photosentized oxidation of single-stranded telomeric sequence repeats were detected on a Varian E-112 spectrometer (X band, 100 kHz field modulation, microwave power of 5 mW and modulation amplitude of 1.0 G) combined with spin-trapping methods [6].

2.2. Rb-induced photocleavage of ^{32}P-5′-end-labeled single-stranded telomeric sequence and the "DNA model" of template region of telomerase RNA

The oligonucleotides were ^{32}P-5′-end-labeled and purified according to the reported methods [9]. The samples to be irradiated contained ^{32}P-5′-$(TAGGGT)_4$ or ^{32}P-5′-CGTC TAACCCTAACTGAGAAGGC [3] (Takara, China), 60 μM Rb (Sigma) and sonicated calf thymus DNA in a total volume of 14 μl. The samples were irradiated at 365-nm wavelength at room temperature for 30 min. After irradiation, all reaction mixtures were ethanol-precipitated and dried, then redissolved in 1 M piperidine. After being heated at 90 °C for 30 min and quickly chilled on ice, all samples including the A + G and G-sequencing markers [10] were resuspended in denaturing gel-loading buffer and run on a 20% denaturing polyacrylamide gel and electrophoresed at 10 W for approximately 2 h. The sites of cleavaged bands were analyzed by autoradiography.

2.3. Rb-induced photocleavage of ^{32}P-5′-end-labeled template region of telomerase RNA

The methods for irradiation and treatment of samples were almost the same as the above-mentioned except that the telomerase RNA to be irradiated contained 200 μM Rb. After irradiation, the samples were treated with 1 M aniline acetate (Sigma), heated at 60 °C for 20 min (kept away from light) and quickly chilled on ice. The samples were ready for the next procedure.

2.4. Digestion of template region of telomerase RNA with RNase T1

The reaction mixture in a microtube contained ^{32}P-5′-RNA (Takara, China), 10 mg/ml tRNA, 1 U/μl RNase T1 (Sigma), 0.0012 g urea, 10 mM Na–citrate (pH 5.0) in a total volume of 8 μl. This reaction mixture was dissolved in the DEPC-treated deionized water, heated at 50 °C for 12 min, then additional 0.0022 g urea was added into it after the reaction mixture was chilled on ice.

2.5. Digestion of template region of telomerase RNA with alkaline hydrolysis

The reaction mixture contained ^{32}P-5′-RNA, 50 mM $Na_2CO_3/NaHCO_3$ (pH 9.0), 1 mM EDTA (pH 8.0), 0.25 mg/ml tRNA in a total volume of 30 μl. This reaction mixture was put in a 90 °C water bath for 15 min after being kept at 0 °C for 10 min, then quickly chilled on ice for the next procedure.

3. Results and discussion

3.1. Reactivities of $^3Rb^*$ with single-stranded telomeric sequence repeats

A stable free radical, 4-oxo-TEMPO, can capture $Rb^{-\cdot}$ produced from reactions of $^3Rb^*$ with nucleotides or DNA at room temperature. Thus, we can measure the yield of $Rb^{-\cdot}$ according to the percentage of diminution of 4-oxo-TEMPO and thereby determine the reactivities of $^3Rb^*$ with a series of single-stranded telomeric DNA [6,11–14], which exhibited that reactivities increase with the decrease of $D_{0.4}$ value. Fig. 1 and Table 1 demonstrate that the reactivities of the single-stranded telomeric DNA $(TAGGGT)n$ ($n = 1$–5) increase with the increment of repeats (n). It is supposed that the ionization potential (IP) of single-stranded telomeric DNA decreases with the increment of repeats (n) due to stacked purine repeats $(AGGG)n$, which indicates a tendency of degressive IP similar to that of $(–G_n–)$ [15]. Evidently, it seems that there exists certain structural similarity between single-helical telomeric DNA and double-helical DNA. But the previous

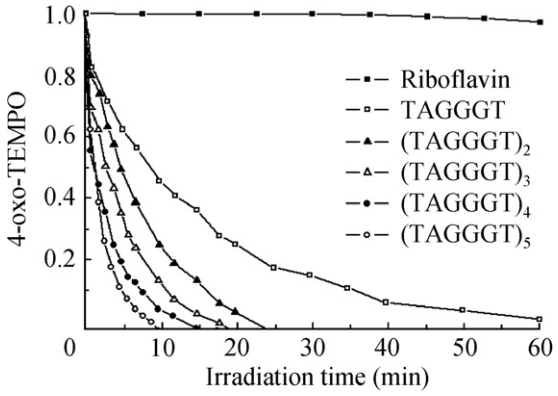

Fig. 1. Decrease of the ESR signal of 4-oxo-TEMPO aqueous solution during the irradiation. The samples contained 5 μM 4-oxo-TEMPO and 0.1 mM Rb, 1 mM $(TAGGGT)n$ ($n = 1$–5). The samples were buffered with 10 mM sodium phosphate at pH 7.0, deaerated by high purity nitrogen and *in situ* irradiated by 365 nm light.