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Efficient DNA photocleavage promoted by a Tb(III) complex

Angélica Cavalett [a], Tiago Bortolotto [a], Patrícia Rabêlo Silva [a], Gilmar Conte [b], Hugo Gallardo [b], Hernán Terenzi [a,*]

[a] Centro de Biologia Molecular Estrutural, INBEB, Universidade Federal de Santa Catarina, Florianópolis, SC, 88040-900, Brazil
[b] Departamento de Química, INCT-Catálise, Universidade Federal de Santa Catarina, Florianópolis, SC, 88040-900, Brazil

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ABSTRACT

This work demonstrates the efficient photocleavage of DNA induced by a terbium complex. Mechanistic studies and DNA groove binding dependence were performed to better understand the photo-cleavage reaction, suggesting the presence of oxygen and carbon-centered radicals in the strand scission event. Furthermore, kinetic analyses were also achieved. The DNA photocleavage promoted by the title complex proceeds with a k_{obs} of ~10.2 h^{-1}, a reaction rate useful in molecular biology practices, with a $t_{1/2}$ of 4 min. This complex emerges as a new example of lanthanide compound with DNA cleavage ability by a non-hydrolytic mechanism.

Rare earth ions and their derived complexes are known by the high efficiency in cleaving DNA and hydrolyzing dinucleotides due to the lack of redox chemistry and the strong Lewis acidity [1,2], differing from classic DNA cleavage agents as $[Cu(phen)_2]$ described by Sigman and co-workers, which fragments DNA by an oxidative mechanism [3]. Large amounts of Ce(IV) ion successfully hydrolyze dideoxyribonucleotides as well as single-stranded DNA [4–8]. Curiously, Ce(III) ion can also promote DNA fragmentation by a Fenton-like free radical generation in the presence of hydrogen peroxide in contrast to the hydrolytic mechanism presented by Ce(IV) ion [9]. The efficient cleavage of double-stranded DNA by Ce(IV) based compounds was finally achieved with the binuclear Ce(IV) complex Ce_2(HXTA) (HXTA = 5-methyl-2-hydroxy-1,3-xylene-α,α-diamine-N,N,N′,N′-tetraacetic acid) with a rate constant of ~0.5 h^{-1}, which corresponds to a DNA half-life of ~1.4 h with only 10 μM of the complex [10].

Afterwards, many approaches using oligonucleotides attached to Ce(IV) ion as a conjugate were extensively reported to achieve specificity on DNA hydrolysis in terms of nucleotide sequence [11–18]. Other lanthanides complexes of Eu(III), La(III), Nd(III), Pr(III), Gd(III) are able to hydrolyze DNA [19–22]. However, new aspects concerning the cleavage of DNA by lanthanides were recently highlighted. New complexes of La(III) and Gd(III) [23] and Eu(III) containing 1,10-phenanthroline derived bases such dipyrido[3,2-*a*:2′,3′-*h*]quinoxoline (dpq) and dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz) show significant DNA cleavage when irradiated by UV-A light[24]. In both cases, free radical scavengers inhibited the complex activity suggesting the participation of Reactive Oxygen Species (ROS) on the DNA strand scission event such as hydroxyl radical (OH•) and singlet oxygen ($^1O_2$). These later evidences indicated an oxidative mechanism, in contrast to the several examples of preferential hydrolytic mechanism, toward DNA cleavage by lanthanide complexes.

Herein, we report a new example of oxidative cleavage of DNA by a rare earth complex with $[Tb(tdzp)(acac)_3]$ (**1**) (where tdzp = [1,2,5] thiadiazolo [3,4-f][1,10] phenanthroline and acac = acetylacetone), developed by us [25] (Fig. S1). The phenanthroline based ligand tdzp, previously described by Conte and co-workers [26], was already employed in the Fe(II) metal complex $[Fe(tdzp)_3]$ which presented high efficiency to photocleave DNA under UV-A light with no significant activity in dark conditions [27]. Chemical compounds with this behavior can be particularly useful in certain tumor treatment approaches such as photodynamic therapy (PDT) [28–31], since the lack of activity in the dark may avoid the potential side effects on healthy cells. Based on this evidence, this work will focus in the expected high activity of **1** toward DNA in the presence of UV-A light irradiation in comparison to that observed in dark conditions.

The DNA cleavage by **1** was evaluated following the conversion of pBSK II supercoiled DNA (F I) to the open circular (F II) and linear DNA (F III) using agarose gel electrophoresis to separate the cleavage products [32,33]. The cleavage reactions were carried out as earlier described [34]. Complex **1** shows extensive plasmid DNA cleavage in PIPES/NaCl buffer (25 mM, pH 7.0) after 5 min of light irradiation (UV-A, λ = 365 nm, 12 W) in a concentration dependent-manner (Fig. 1). The increase in complex concentration is followed by the increase of the plasmid DNA forms containing single (F I) and double-stranded breaks (F II) (Fig. 2A). Hussain and colleagues reported La(III) and Gd(III) complexes with DNA photocleavage activity under

* Corresponding author at: Centro de Biologia Molecular Estrutural, Universidade Federal de Santa Catarina, Florianópolis, SC, 88040-900, Brazil. Tel.: +55 48 3721 6426.
E-mail address: hterenzi@ccb.ufsc.br (H. Terenzi).

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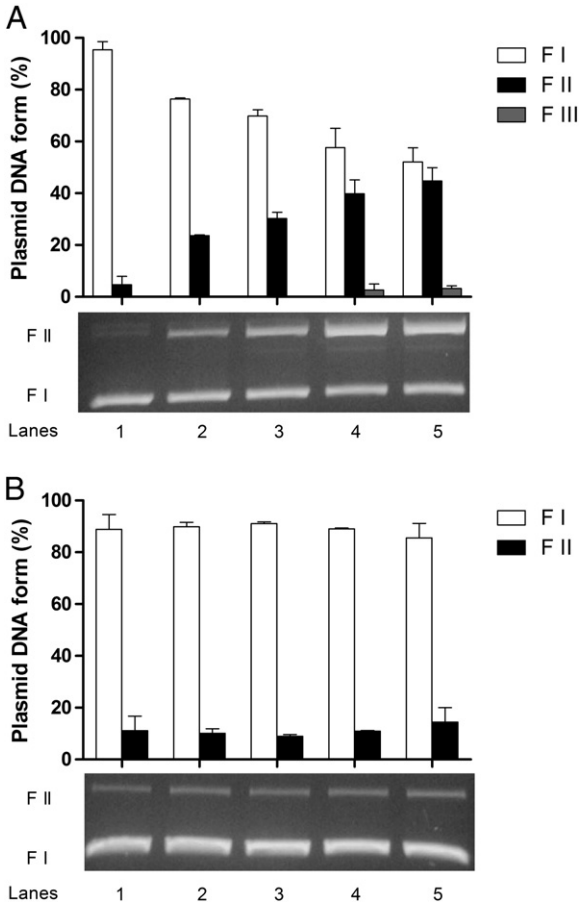

Fig. 1. DNA photocleavage performed by **1** in PIPES/NaCl buffer (25 mM, pH 7.0) incubated for 5 min at room temperature under UV-A light (λ = 365 nm, 6 W) (A) and for 24 h under dark conditions at 37 °C (B) with different complex concentrations. Lanes 1: DNA control (30 μM in bp); lanes 2: DNA + **1** (10 μM); lanes 3: DNA + **1** (25 μM); lanes 4: DNA + **1** (50 μM); lanes 5: DNA + **1** (100 μM). Representative data from three independent experiments.

UV-A irradiation (λ = 365 nm, 6 W) for 2 h [35]. In addition, similar results were recently reported for two Eu(III) complexes, $Eu(dpq)(acac)_3$ and $Eu(dppz)(acac)_3$ [36]. These complexes presents a much lower activity in comparison to **1**. In all the examples reported here [35,36], these lanthanide complexes can only photocleave DNA under UV-A light. To confirm this dependence, further studies with **1** were performed, and, in fact, no cleavage activity of the complex under a longwave red-light (λ = 635 nm, data not shown) was observed. Under dark conditions complex **1** did not show any activity after a 24 h incubation (Fig. 2B), demonstrating a strong and preferential dependence of UV-A photo-activation for plasmid DNA cleavage.

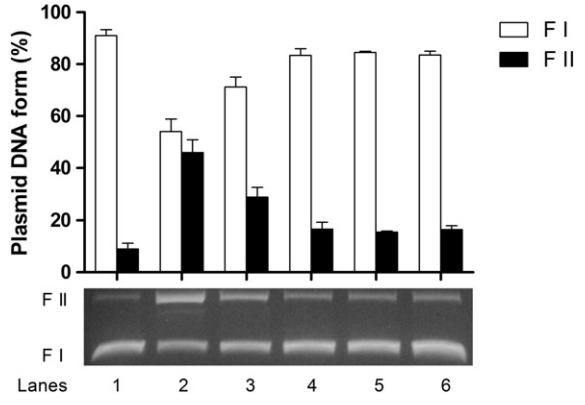

Fig. 2. DNA photocleavage performed by **1** in PIPES/NaCl buffer (25 mM, pH 7.0) incubated for 5 min at room temperature under UV-A light (λ = 365 nm, 6 W) in the presence of different radical scavengers. Lane 1: DNA control (30 μM in bp); lane 2: DNA + complex; lane 3: DNA + complex + DMSO; lane 4: DNA + complex + KI; lane 5: DNA + complex + NaN_3; lane 6: DNA + complex + TEMPO. Representative data from three independent experiments.

Since DNA is a negatively charged molecule, electrostatic interactions between **1** and DNA itself could contribute to the cleavage reaction. To test this hypothesis, increasing amounts of NaCl in the range of 50 to 300 mM were added to the DNA cleavage mixtures (Fig. S2). As the ionic strength increases in the reaction medium, the cleavage of DNA promoted by **1** decreases proportionally. This evidence suggests that electrostatic interactions probably contribute to DNA-binding and further strand cleavage process by **1**.

To elucidate whether the mechanism of DNA photocleavage performed by **1** could be radical-dependent, different inhibitors of reactive oxygen species (ROS) were added to the DNA reaction medium prior to the complexes (Fig. 2). The addition of DMSO (10%), KI (10 mM) or NaN_3 (10 mM) partially reduced complex activity, suggesting an oxidative mechanism in the strand scission event. In addition, further assays were conducted under anaerobic conditions (Fig. 3).

The lack of dissolved oxygen in the reaction medium did not completely abolished the photocleavage of DNA by **1**, suggesting that other radicals species instead of oxyradicals may be participating in the cleavage mechanism. This effect was somehow expected since the work of Souza and co-workers using another tdzp based complex, $Fe(tdzp)_3$, reports oxygen-independent DNA photo-cleavage trough the formation of ligand and metal-centered radicals [27]. Additional

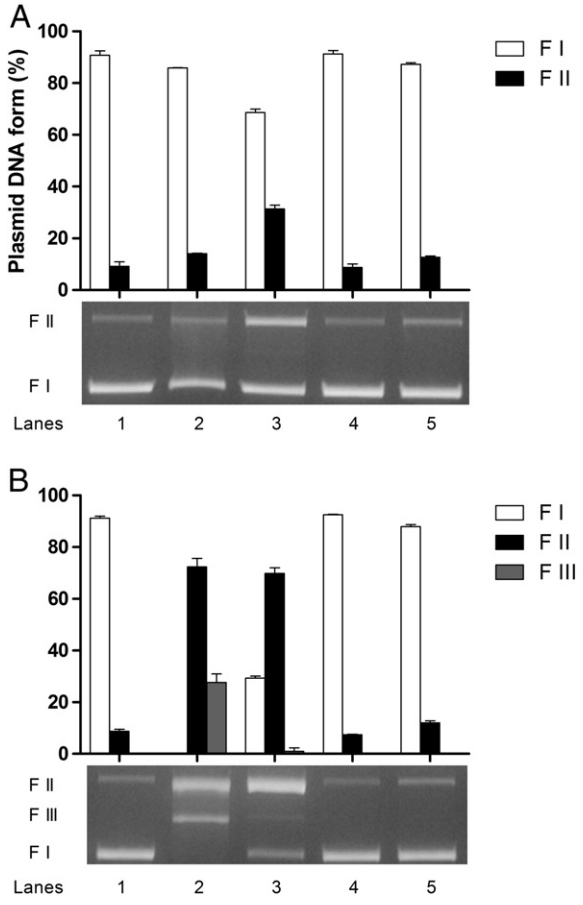

Fig. 3. DNA photocleavage performed by **1** in PIPES/NaCl buffer (25 mM, pH 7.0) incubated for 5 min at room temperature under UV-A light (λ = 365 nm, 6 W) in anaerobic conditions (A) and aerobic conditions (B). Lanes 1: DNA control (30 μM in pb); lanes 2: DNA + $CuCl_2$ + MPA; lanes 3: DNA + **1**; lane 4: DNA + TEMPO; lane 5: DNA + **1** + TEMPO. Representative data from two independent experiments.