



Short communication

# The binding efficiency of RPA to telomeric G-strands folded into contiguous G-quadruplexes is independent of the number of G4 units

Astrid Lancrey<sup>a</sup>, Loyal Safa<sup>a</sup>, Jean Chatain<sup>a</sup>, Emmanuelle Delagoutte<sup>a</sup>,  
Jean-François Riou<sup>a</sup>, Patrizia Alberti<sup>a, \*\*</sup>, Carole Saintomé<sup>a, b, \*</sup>

<sup>a</sup> Structure et Instabilité des Génomes, Sorbonne Universités, Muséum National d'Histoire Naturelle, CNRS UMR 7196, INSERM U1154, 43 rue Cuvier, F-75005, Paris, France

<sup>b</sup> Sorbonne Universités, UPMC Univ Paris 06, UFR927, F-75005, Paris, France

## ARTICLE INFO

### Article history:

Received 9 October 2017

Accepted 24 November 2017

### Keywords:

G-quadruplex

Telomere

Replication protein A

Fluorescence resonance energy transfer (FRET)

Gel electrophoresis

## ABSTRACT

Replication protein A (RPA) is a single-stranded DNA binding protein involved in replication and in telomere maintenance. During telomere replication, G-quadruplexes (G4) can accumulate on the lagging strand template and need to be resolved. It has been shown that human RPA is able to unfold a single G4. Nevertheless, the G-strand of human telomeres is prone to fold into higher-order structures formed by contiguous G-quadruplexes. To understand how RPA deals with these structures, we studied its interaction with telomeric G-strands folding into an increasing number of contiguous G4s. The aim of this study was to determine whether the efficiency of binding/unfolding of hRPA to telomeric G-strands depends on the number of G4 units. Our data show that the number  $n$  of contiguous G4 units ( $n \geq 2$ ) does not affect the efficiency of hRPA to coat transiently exposed single-stranded telomeric G-strands. This feature may be essential in preventing instability due to G4 structures during telomere replication.

© 2017 Elsevier B.V. and Société Française de Biochimie et Biologie Moléculaire (SFBBM). All rights reserved.

## 1. Introduction

The G-rich telomeric sequence can adopt non-canonical DNA conformations known as G-quadruplexes (G4s). These four-stranded structures are based on guanine quartets stabilized by Hoogsteen hydrogen bonds and coordinated by central cations. Evidence is accumulating that G4s can form at telomeres during lagging strand DNA replication and at the 3' G-overhang [1–4]. Several helicases (such as the Werner helicase, WRN) able to unwind G4s are essential for telomere stability and replication [5–7]. In addition, several non-helicase single-stranded DNA binding proteins, involved in telomere maintenance (replication and capping), have been shown to unfold G4s. These proteins are the POT1-TPP1 complex [8,9] (two Shelterin components [10]), RPA [11–14] (a key player in DNA replication, recombination and repair,

that is highly conserved in eukaryotes [15]), and the CST complex [16] (a RPA-like complex [17]). The recruitment of these proteins is tightly coordinated through the cell cycle, ensuring telomere replication and telomere protection [18,19]. Here, we focus on human Replication Protein A (hRPA). In human cells, RPA associates with telomeres [20]; it has been proposed that it binds telomeric single-stranded DNA at replication forks (or telomere ends) during early to middle S-phase [18]. A recent study shows that yeast RPA ensures correct replication of the telomeric lagging strand, and suggests a role of RPA in preventing DNA secondary structures on the telomeric lagging strand template [21]. So far, studies concerning the interaction of hRPA with G4s have focused on a single quadruplex formed by four repetitions of the human telomeric motif (GGGTTA) [11,22–24]. Results obtained with a single G4 may not necessarily reflect the binding properties of hRPA to long telomeric G-strands, which are prone to fold into contiguous (or multimeric) G4s, like beads-on-a-string [25–29]. In the present work, we address, for the first time, the question of how hRPA deals with contiguous (or multimeric) telomeric G4s, with the aim of determining whether its efficiency of binding/unfolding long telomeric G-strands folded into contiguous G4 depends on the number of G4 units.

\* Corresponding author. Structure et Instabilité des Génomes, Sorbonne Universités, Muséum National d'Histoire Naturelle, CNRS UMR 7196, INSERM U1154, 43 rue Cuvier, F-75005, Paris, France.

\*\* Corresponding author.

E-mail addresses: [alberti@mnhn.fr](mailto:alberti@mnhn.fr) (P. Alberti), [carole.saintome@mnhn.fr](mailto:carole.saintome@mnhn.fr) (C. Saintomé).

## 2. Materials and methods

### 2.1. Materials

Recombinant hRPA was expressed in the *Escherichia coli* BL21 (DE3) strain transformed with the plasmid pET<sub>11a</sub>hRPA that permits the co-expression of RPA1, RPA2 and RPA3. hRPA was purified as previously described [21]. Oligonucleotides were purchased from Eurogentec.

### 2.2. Electrophoretic mobility shift assays

Oligonucleotides were labeled with  $\gamma$ [<sup>32</sup>P]ATP using T4 polynucleotide kinase. For all EMSA experiments, hRPA was diluted and pre-incubated (20 min at 4 °C) in a buffer containing 50 mM Tris-HCl pH 7.5, 100 mM KCl, 1 mM DTT, 10% glycerol, 0.2 mg/mL BSA and 0.1 mM EDTA. In a standard reaction, radiolabeled oligonucleotide (2 nM) (slowly annealed from high to low temperature) was incubated with various amounts of protein in 10  $\mu$ L reaction buffer [50 mM HEPES pH 7.9, 0.1 mg/mL BSA, 100 mM KCl or NaCl and 2% glycerol] for 20 min at 20 °C. Samples were then loaded on a native 1% agarose in 0.5 $\times$  TBE buffer. After electrophoresis, the gel was dried and exposed on a phosphorimager screen, scanned with a Typhoon instrument (Molecular Dynamics). Quantification was made with ImageQuant version 5.1. For each hRPA concentration, the fraction of radiolabelled oligonucleotide bound to hRPA was calculated as follow:  $I_{DNA \text{ bound to hRPA}} / (I_{free \text{ DNA}} + I_{DNA \text{ bound to hRPA}})$ .

### 2.3. Fluorescence titrations

Fluorescence titrations were carried out on a SPEX Fluorolog spectrofluorimeter (Jobin Yvon) equipped with a circulating water bath to regulate the temperature of the cell holder. Fluorescence spectra of 100 nM H69-Q1,2,3 oligonucleotides (doubly-labeled with a FAM and a TAMRA) were recorded at 20 °C in a buffer containing 50 mM KCl, 2 mM MgCl<sub>2</sub>, and 5 mM lithium cacodylate (pH 7.2). H69-Q1,2,3 were pre-incubated 10 min at 20 °C. The protein (from 100 to 1000 nM) was directly added to the solutions containing the oligonucleotides. The spectra were collected after 2 min of incubation while exciting at 470 nm. The fluorescence intensity of the donor FAM ( $I_D$ ) was measured at 518 nm, the fluorescence intensity of the acceptor TAMRA ( $I_A$ ) was measured at 586 nm; the percentage of opened labeled G4 units at 20 °C was calculated as:

$$(P - P_f) \times 100 / (P_u - P_f),$$

where:

$$\begin{aligned} P &= I_D / (I_D + I_A) \text{ at a given hRPA concentration at } 20^\circ\text{C}, \\ P_f &= I_D / (I_D + I_A) \text{ in the absence of hRPA at } 20^\circ\text{C}, \\ P_u &= I_D / (I_D + I_A) \text{ in the absence of hRPA at } 80^\circ\text{C}. \end{aligned}$$

We assumed that, in the absence of hRPA, at 20 °C all the labeled G4 units are folded, while at 80 °C all the labeled G4 units are unfolded. Under these assumptions,  $P_f$  is the  $P$  value corresponding to 0% of unfolded labeled G4 units, while  $P_u$  is the  $P$  value corresponding to 100% of unfolded labeled G4 units. The expression  $(P - P_f) \times 100 / (P_u - P_f)$  provides, hence, the percentage of unfolded labeled G4 units for a given hRPA concentration.

## 3. Results and discussion

We carried out our study in the presence of the biological relevant potassium cation. We first investigated if hRPA is able to

unfold contiguous G4s. To this purpose, we carried out fluorescent resonance energy transfer (FRET) titration experiments using the telomeric sequence H69 ((G<sub>3</sub>T<sub>2</sub>A)<sub>3</sub>G<sub>3</sub>), which folds into three contiguous G4 units. Three modified H69 oligonucleotides, doubly labeled with a 6-carboxyfluorescein (FAM) and a tetramethylrhodamine (TAMRA) at different positions, were used. In H69-Q1, the fluorophores are placed at both sides of the first G4 unit; in H69-Q2, they are placed at both sides of the second G4 unit; in H69-Q3, they are placed at both sides of the third G4 unit (Supplementary Information, Table S1). The doubly labeled G4 units of these three constructs have temperatures of half-denaturation in the range of 50–60 °C, as previously assessed by melting experiments followed by fluorescence [26]. For all H69-Q1,2,3, fluorimetric titration with hRPA revealed an increase of FAM fluorescence and a decrease in TAMRA fluorescence upon hRPA addition, indicating that hRPA is able to open each of the three G4 units, independently of its position (Fig. 1). These results demonstrate that hRPA is able to unfold all the G4s present in a multimeric structure.

Having shown that hRPA unfolds multimeric G4s, we next investigated whether its binding efficiency depended on the number of G4 units. To this purpose, we carried out electrophoretic mobility shift assays (EMSA) using four oligonucleotides: H27 (T<sub>2</sub>A(G<sub>3</sub>T<sub>2</sub>A)<sub>3</sub>G<sub>3</sub>T<sub>2</sub>A), H51 (T<sub>2</sub>A(G<sub>3</sub>T<sub>2</sub>A)<sub>7</sub>G<sub>3</sub>T<sub>2</sub>A), H75 (T<sub>2</sub>A(G<sub>3</sub>T<sub>2</sub>A)<sub>11</sub>G<sub>3</sub>T<sub>2</sub>A) and H99 (T<sub>2</sub>A(G<sub>3</sub>T<sub>2</sub>A)<sub>15</sub>G<sub>3</sub>T<sub>2</sub>A). We previously characterized the structures formed by these oligonucleotides: we showed that H51, H75 et H99 fold into two, three and four contiguous G4 units, respectively, which are non-interacting and have identical stabilities; while the single G4 formed by the shortest sequence H27 has a higher stability [26] (Supplementary Information, Table S1). We point out that the binding constant of hRPA to dT<sub>50</sub>, dT<sub>60</sub> and dT<sub>70</sub> oligonucleotides (longer than the size of the 30-nt RPA extended-binding mode and able in principle to bind more than one RPA, up to three RPA for dT<sub>70</sub>) does not depend on their length [30]. Hence, any significant difference in the binding of RPA to H51, H75 and H99 should not be due to oligonucleotide length, but solely on the number of G4 units. H27, H51, H75, H99 oligonucleotides in the presence of increasing hRPA concentrations showed retarded bands on agarose gel (Fig. 2A). The number of bands increased with the length of the oligonucleotide, revealing hRPA:DNA complexes of increasing stoichiometry, where one, two, three, four (or more) hRPA are bound. These results show that hRPA is able to bind telomeric strands structured in multimeric G4s and to form complexes where several proteins are present along the DNA strand. For each oligonucleotide, we quantified the percentage of DNA bound to hRPA as a function of hRPA concentration and determined the hRPA concentration to obtain 50% of bound oligonucleotide (Fig. 2B and C). The binding curves showed two main features: (i) hRPA bound more efficiently the multimeric G4 sequences (H51, H75 and H99) than the monomeric G4 sequence (H27); (ii) the efficiency of hRPA binding to the multimeric G4 sequences (H51, H75 and H99) was independent of the number of formed G4 units (two, three or four). In addition, the non-sigmoidal shape of the binding curves, the coexistence of complexes of different stoichiometries (CI, CII, CIII and CIII+) up to 25–50 nM hRPA concentrations (quantification of the percentage of each complex from Fig. 1 is shown in Supplementary Information, Fig. S1) and data fitting according to a model of binding to a one-dimensional homogeneous lattice [31] (Supplementary Information, Fig. S2) further suggest a poorly cooperative binding mode of hRPA to H51, H75 and H99, as previously observed with long non-structured single-stranded oligonucleotides [30,32]. The higher hRPA binding efficiency ([hRPA]<sub>50</sub> around 5 nM) toward multimeric G4s compared to the single G4 ([hRPA]<sub>50</sub> around 20 nM) can be explained by the lower stability of these higher-order structures

Download English Version:

<https://daneshyari.com/en/article/8304238>

Download Persian Version:

<https://daneshyari.com/article/8304238>

[Daneshyari.com](https://daneshyari.com)