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Short communication

Pushing the detection limits: The evanescent field in surface plasmon resonance and analyte-induced folding observation of long human telomeric repeats

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ABSTRACT

Conventional analysis of molecular interactions by surface plasmon resonance is achieved by the observation of optical density changes due to analyte binding to the ligand on the surface. Low molecular weight interaction partners are normally not detected. However, if a macromolecule such as DNA can extend beyond the evanescent field and analyte interaction results in a large-scale contraction, then the refractive index changes due to the increasing amount of macromolecules close to the surface. In our proof-of-principle experiment we could observe the direct folding of long, human telomeric repeats induced by the small analyte potassium using surface plasmon resonance spectroscopy. This work demonstrates the feasibility of new evanescent field-based biosensors that can specifically observe small molecule interactions.

1. Introduction

The impact of surface plasmon resonance for the analysis of molecular interactions has been tremendous since its introduction in 1983 (Liedberg et al., 1983). This becomes obvious by the amount of publications growing almost exponentially each year (Knoll et al., 2008). Some of the most straightforward SPR experiments involve nucleic acid interactions, such as hybridization and protein binding, typical for nucleic acid biochemistry. However, in addition to encoding the genetic information, nucleic acids are known to form complex secondary structures different from double helices, such as tRNAs that can fold in a certain structure important for its biological function. A well known three-dimensional structure of nucleic acids is the G-quadruplex which is formed *in vitro* by DNA and RNA sequences occurring in telomeres, promoter regions, recombination sites, RNA packaging sites and RNA dimerization domains (Shafer and Smirnov, 2000; Arthanari and Bolton, 2001; Simonsson, 2001), or artificially generated aptamers (Griffin et al., 1993; Gatto et al., 2009). In 2009 Blackburn, Greider and Szostak were awarded the Nobel Prize in Medicine for the discovery of the mechanism of telomeric protection of chromosomes and the enzyme telomerase (Szostak and Blackburn, 1982; Greider and Blackburn, 1985, 1989). Telomeres, i.e. the termini of the chromosomes, consist of a $d(GGGTTA)_n$ repeat sequence, which is the same in all vertebrate genomes. Human telomeres measure between 5 and 15 kilobases (Samassekou et al., 2010), of which the 3′-end of the G-rich strand forms an overhang, measuring between 35 and 600 nucleotides (Makarov et al., 1997; McElligott and Wellinger, 1997; Stewart et al., 2003). Besides protecting the end of chromosomes, it is shown that the overhang of telomeres can fold into G-quadruplexes in vivo (Blackburn, 1991). In these G-rich sequences guanine bases interact via Hoogsteen base pairing to form a three-dimensional G-quadruplex structure. The folding is supported by binding to monovalent cations. The binding is mainly dependent on the ion radius with a strong preference for potassium over other cations, especially under physiologically relevant concentrations (Hardin et al., 1991; Renciuk et al., 2009).

The structural change can be measured directly by e.g. fluorescence resonance energy transfer (FRET) with the disadvantage of using labels (Simonsson and Sjöback, 1999) or direct probing by atomic force microscopy (Basnar et al., 2006).

Using the principle of surface plasmon (SPR) resonance, a direct and on-line measurement without the need of labels can be

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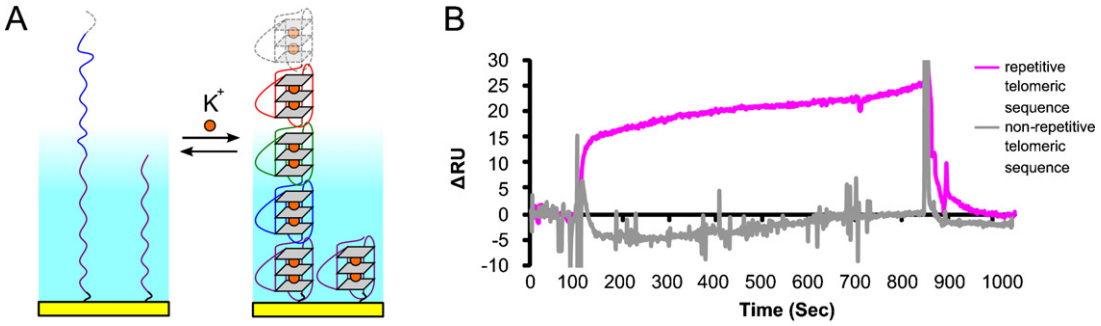

Fig. 1. (A) Schematic diagram showing the folding of concatemeric and single non-repetitive telomeres in response to potassium; evanescent field shown as a fading background. (B) SPR signal of a repetitive and non-repetitive telomeric sequence in 150 mM potassium chloride; injection at 100 s, washing at 850 s, a blank streptavidin coated surface was used as a reference for normalization.

performed. With SPR it is possible to detect changes in the refractive index as a result of binding events or changes of the structure of biomolecules, such as RNA (Lisdat et al., 2001; Willander and Al-Hilli, 2009). However, structural changes of one G-quadruplex forming sequence were not detectable with this method so far (Redman, 2007). Until now, only indirect measurements of the folding of a telomeric sequence have been applied (Zhao et al., 2004). Since long telomeric repeats forming G-quadruplexes occur naturally, these can be ideally synthesized *in vitro* by rolling circle amplification (RCA) (Pomerantz et al., 2008). In this process, a circular template is elongated by the highly processive φ29 DNA polymerase to form extremely long single-stranded concatemeric repeats mainly depending on the incubation time (Banér et al., 1998). Here, we present the proof-of-principle of combining the advantages of SPR with the potential of RCA for direct measurement of the structural changes of a repetitive human telomeric motif in the presence of potassium ions. We intend to capitalize on the limitations of SPR represented by the extent of the evanescent field allowing refractive index change measurements only close to the surface. Experiments conducted by Lavine et al. (2007) using the differential swelling behavior of molecularly imprinted polymers in SPR have shown that this can be done in principle. Therefore, very long single-stranded nucleic acids with the repetitive quadruplex-forming motif and oligonucleotides with a repetitive scrambled telomeric sequence are synthesized by RCA from circularized templates. The scrambled telomeric sequence is used as a control, for another comparison an oligonucleotide with only one telomeric motif is applied (Fig. 1A). More detailed information about materials and methods used is provided in the Supporting Information.

2. Results and discussion

To evaluate the advantage of a repetitive telomeric sequence over a sequence with one telomeric motif, we first observe the SPR signal in the presence of 150 mM potassium chloride for single and long telomeric repeats (Fig. 1B).

As expected, it is not possible to detect the G-quadruplex folding induced by potassium ions directly in case of single non-repetitive telomeric sequences. On the other hand, we find an increasing SPR signal for the repetitive telomeric sequence after contact with the potassium containing buffer. This shift in signal is possibly caused by the formation of G-quadruplexes in the presence of potassium ions. For further studies we use isomolar buffers of 150 mM monovalent ions with varying concentrations of potassium to lithium (Fig. 2). This setup is chosen to prevent large bulk index shifts that overlay the test signal. DNA strands with the scrambled telomeric sequence are used as a negative control, as they cannot form G-quadruplex structures (Pomerantz et al., 2008). As can be seen from Fig. 2B, no change in the refractive index can be detected, in contrast to the repetitive G-quadruplex forming DNA. Thus, the SPR signal change for the latter can be attributed to a conformational change of this DNA-layer.

Besides the occurrence of conformational changes, it is also possible to observe the kinetics of the folding process since the signal increases with injection time (Fig. 2A). The response curves for various potassium concentrations indicate a fast response followed by a slower process. Obviously, repetitive structures are not able to fold in a single step because folding of such large molecules is sterically challenging and is combined with contraction. Repetitive scrambled strands show no specific signal in the presence of various potassium concentrations. In contrast, repetitive telomeric strands reveal a signal, which is dependent on the potassium ion concentration (Fig. 2B). Changes in

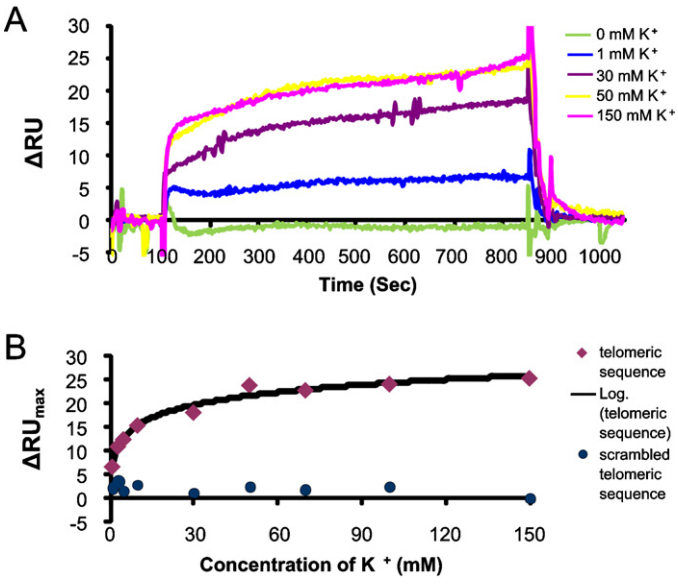

Fig. 2. Concentration dependence of the SPR signal on different potassium concentrations. (A) Representative sensorgrams of the repetitive telomeric sequence for potassium concentrations from 0 to 150 mM; injection at 100 s, washing at 850 s, a blank streptavidin surface was used as a reference for normalization. (B) Maximal association signal for the different concentrations in comparison of repetitive scrambled telomeric strand (blue dots) and repetitive telomeric strand (purple diamonds) showing a logarithmic dependence of the potassium concentration for the telomeric sequence.