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A view of DNA short sequences rich in guanines like potassium biosensor(*)

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Summary. — Pu23 and Pu27, single strand DNA sequences rich in guanines, can fold forming a G-quadruplex, in the presence of Na^+ and K^+. A G-quadruplex is a DNA secondary structure made up of at least two tetrads, an array made of four guanines, stacked on each other. Pu27, the 27-base–long wild-type sequence, is the c-myc transcription repressor. Pu23 is a 23-base–long mutant. In this work Pu27 and Pu23 are tested as watery K^+ biosensors, thanks to their K^+ affinity, even when Na^+ exceeds the K^+ concentration. The Pu27 and Pu23 biosensors are assayed in DMEM treated with a potassium solution. DMEM is a common medium where cancer cells can grow. This work shows that Pu27 and Pu23 can sense K^+ into treated DMEM folding, according to the G4 parallel conformation. The titration with Na^+ does not affect the Pu27 structural stability while, on the contrary, Pu23 displays a less stable folding. At fixed Na^+ concentration, Pu27 shows higher sensitivity and better resolution for K^+ detection with respect to Pu23; nevertheless, both sequences seem not to be adequate to measure a K^+ concentration difference of about 1 mM.

1. – Introduction

When scientists think about the DNA molecule, they usually think about the B form, relatively to what James Watson and Francis Crick published in 1953. In spite of their seminal paper, it is mandatory to look beyond the described structure because the living systems show to have DNA also in A and Z forms as well as with hairpin, triple helix (triplex) and G-quadruplex (G4) structures [1,2]. In 1910, Bang observed that the highly concentrated guanilic acid forms a gel and, 50 years later (1962), the G4 crystal structure

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was resolved and published, outlining that 4 guanines are bound to form a planar structure, called tetrad, with the bases placed at the vertexes [3, 4]. The G4 structure forms if at least two tetrads are piled up on each other [5, 6]. Hoogsteen H bonds among the four guanines (in which each is both donor and acceptor of H bonds), the hydrophobic interactions (π-π interactions) among the piled tetrads and the presence of monovalent or divalent cations are the required conditions for G4 stability [4, 5, 7]. With reference to H bonds, the pairing of the N1 of the first guanine with the O6 of the second one, along with the pairing of N2 on the first guanine with the N7 of the second guanine, results in eight H bonds per G-tetrad [2]. Besides H bonds and hydrophobic interactions, the cations fit the hydrophobic core of the G4 to stabilize the structure with a stability strength that follows this order: $K^+ > Na^+ > NH_4^+ > Li^+$ [2, 8]. Computational genome analysis of various organisms came up with putative G4 sequences in specific genome regions. In particular, they are located within regulative regions (*e.g.*, gene promoters) and telomeric sequences [9]. Among the available sequences, which fold according to G4 structures, Pu27 (5-TGGGGAGGGTGGGGAGGGTGGGGAAGG-3) and Pu23 (5-TGGGGAGGGTGGGGAGAGTGGGG-3) are the sequences herein studied. Pu27 is a highly conserved nuclease hypersensitive element NHEIII1 located between the 142nd and the 115th bases upstream promoter 1 (P1) and P2 and downstream P0. It was demonstrated that Pu27 controls about 80–90% of c-myc transcription, no matter whether the P1 or the P2 promoter is used. It is possible to consider Pu27 as a silencer because, once folded, c-myc expression will be repressed [10]. The other sequence herein investigated is Pu23, which differs from Pu27 because it is 4 nucleotides shorter, because of 5-AAGG-3 deletion, and the 17th guanine is replaced by an adenine. The point mutation, which was found in 30% of human colon-rectal cancer, folds into a G4 structure which is not as stable as the wild-type triggering c-myc promoter three times active compared the wild-type one [10]. It was demonstrated that the treatment of several tumor cell lines with G4 stabilizer molecules (*i.e.*, TMPyP4) drives to a reduction of the expression of c-myc mRNA and protein, due to the presence of G4 steadily folded [11, 12]. Since the stability of NHEIII1 was extensively studied with molecules and cations, including K^+ [11, 13], all these findings lead to considering Pu27 and Pu23 as possible K^+ biosensors of watery K^+.

The aim of this investigation is to measure the variations of K^+ in Dulbeccos Modified Eagle Medium (DMEM), a cancer cell medium widely used to grow cells. DMEM is a complex solution with an excess of Na^+ (156.3 mM) compared with K^+ (5.3 mM). DMEM was also studied when a solution, namely K:D–rib, is added. K:D–rib is an aqueous solution of D–ribose and $KHCO_3$ with in vitro proven antioxidant and anticancer properties [14, 15]. Its anti-cancer features may be connected to a K^+ uptake by cancer cells. The work purpose is to validate a biosensor able to detect an uptake of K^+ after cancer cell treatment with K:D–rib 5 mM. The comparison of the wild-type sequence with the mutated one will assess whether the shorter one might adopt a G4 structure that leads to a different biosensor resolution or sensitivity. The circular dichroism (CD) spectroscopy is a technique used for exploiting the Pu27 and Pu23 behavior as K^+ biosensors. CD spectroscopy measures the difference in absorption of left and right circularly polarized light by chiral compounds. It is a technique used to a large extent for studying the macromolecules with chiral centers, such as sugars, proteins and nucleic acids. The latter group embraces G4. Although G4 structures are highly polymorphic, the CD spectroscopy allows to distinguish among the main G4 structural conformations: parallel, antiparallel and hybrid. The parallel structure shows a positive peak at $\sim$264 nm and a negative one at $\sim$245 nm. The G4 antiparallel structure displays a positive peak at

∼295 nm, at ∼240 nm and also a negative peak at ∼260 nm. The hybrid-type structure has positive peak at ∼295 nm, ∼260 nm and a negative peak at ∼245 nm. The mentioned spectrum characteristics are generally accepted for the G4 structures [16, 17], although some exceptions are permitted, such as the shoulder at 260–270 nm related to the hybrid group [17].

2. – Materials and methods

2˙1. *Oligonucleotides.* – Pu27 (5-TGGGGAGGGTGGGGAGGGTGGGGAAGG-3) and Pu23 (5-TGGGGAGGGTGGGGAGAGTGGGG-3) sequences were purchased by Tema Ricerca Integrated DNA technologies, Italy. The dried oligonucleotides were straightaway reconstituted by adding TE buffer: 10 mM Tris (Sigma Aldrich) *p*H 7.5 and 0.1 mM EDTA (Sigma Aldrich), so as to obtain a stock solution of 100 μM and stored at −20 °C. The DNA was quantified by UV/VIS spectroscopy at room temperature (RT) according to Lambert-Beer's law, with molar extinction coefficient ($\epsilon_{254} = 311453.4\,M^{-1}cm^{-1}$). Before the quantification, the DNA solution was heated at 95 °C for 5 minutes and then cooled back at 0 °C (water and ice) for 10 minutes.

2˙2. *Folding experiments.* – Each sample was prepared adding 10 μM of oligonucleotide (Pu27 and Pu23) at the folding buffer composed by EDTA 0.1 mM, G4 folding solution (see sect. **2˙1**) and distilled water. The DNA and EDTA concentrations were in agreement with the protocol published by Paramasivan *et al.* [16]. The folding reactions, taking 24 h, occur at RT in the darkness. Before adding DNA to the sample, its stock solution was heated at 95 °C for 5 minutes then cooled back at 0 °C (water and ice) for 10 minutes.

2˙3. *G4 folding solutions.* – DMEM was provided with penicillin/streptomycin 1% (Gibco), L-glutamine 1% (Gibco) and fetal bovine serum (FBS) 10% (Gibco). The medium was without phenol red (*p*H indicator) because it might interfere with the optical activity of G4. Before adding FBS to DMEM, FBS was kept at 56 °C for 20 minutes, so as to denature the complement proteins. In this paper, DMEM means DMEM supplemented by all the previously listed elements. To define the exact concentration of Na^+ and K^+, one must consider also the contribution of FBS as a source of Na^+ and K^+. So, the Na^+ and the K^+ concentrations are, respectively, 170.8 and 6.45 mM. The basic solution assayed is DMEM (low glucose − Gibco), treated with K:D–rib 5 mM, and diluted 1:4 with respect to the sample volume. K:D–rib is composed of D–ribose (Sigma Aldrich) and $KHCO_3$ (BDH Prolabo). The molar ratio D–ribose: $KHCO_3$ is 1:3. A solution of K:D–rib 250 mM was prepared with 150 mg of D–ribose and 300 mg of $KHCO_3$ in 4 ml of distilled water [14, 15]. Next, the DMEM treated with K:D–rib 5 mM was prepared and the final solution K^+ concentration of 21.45 mM was obtained. Before assaying the samples, DMEM with K:Drib 5mM was diluted 1:4 with respect to the sample volume, bringing the Na^+ concentration to 42.7 mM and the K^+ one to 5.4 mM. All the solutions tested for K^+ concentration were kept at 37 °C in atmosphere humidified with CO_2 at 5% for 48 h, for simulating the maintenance and/or the cancer cell treatments.

2˙4. *Titration with NaCl and KCl solutions.* – According to the paper aims, to detail the sensitivity range of Pu27 and Pu23, regarding the K^+ sensing activity, and to better investigate the Na^+ and K^+ role in Pu27 and Pu23 folding, two experimental phases are necessary. The first one is the folding analysis of the oligonucleotides at the lowest studied ion concentrations, and the second one is the folding analysis increasing Na^+ and K^+ concentrations. In order to reduce the ion concentrations, Pu27 and Pu23 were

TABLE I. – *Pu23 and Pu27 sample assayed concentrations.*

Titration salt	Pu23		Pu27	
	$[K^+]$	$[Na^+]$	$[K^+]$	$[Na^+]$
	4.5	35.5	4.5	35.5
NaCl	4.5	47.5	4.5	47.6
NaCl	4.5	73.5	4.5	74.6
	4.5	35.5	4.5	35.5
KCl	5.8	35.5	5.8	35.5
KCl	9.4	35.5	9.4	35.5
NaCl	9.4	48.7	9.4	48.8

folded in the presence of DMEM treated with K:D–rib 5 mM and diluted by 1:4 with respect to the sample volume. During the preliminary folding experiments, DMEM treated with K:D–rib 5 mM and diluted 1:2 were extensively tested. The obtained Na^+ and K^+ concentrations were 85.4 mM and 10.7 mM, respectively. Therefore, to approach the Na^+ and K^+ concentrations of the DMEM treated with K:D–rib 5mM when it is diluted 1:2, several titrations, with NaCl 500 mM and KCl 50 mM, of the previous 1:4 diluted samples, were performed.

All the assayed concentrations are collected in table I. Because the titrations also affect the concentration of DNA, the samples were normalized to 10 μM, which is the DNA concentration requested by the folding protocol (see sect. **2**˙2). Furthermore, the spectra collected were normalized to the same final volume and the K^+ and Na^+ concentrations were calculated in agreement with normalization volume.

2˙5. *Circular dichroism*. – The CD spectra were collected by means of a J−715 spectropolarimeter (Jasco), equipped by an electronic Peltier to control the cell holder temperature. All the spectra were acquired in the UV range (235–320 nm wavelength) with the following parameters: scanning speed 50 nm/min, 0.2 nm data pitch, bandwidth 1 nm and a time response of 8 s. Samples were held in a quartz cell with an optical path of 2 mm. The chamber of the lamp was constantly flushed with a nitrogen stream so as to avoid ozone formation, which can damage the lamp. All spectra were collected at 20 °C and the final one was the average of three acquisitions. The reference of each sample is the spectrum with TE instead of DNA and the same folding buffer. Each reference spectrum is subtracted from the corresponding sample spectrum.

3. – Results

The paper goal is to define Pu27 and Pu23 as easy-to-use biosensors for K^+ in complex water solution. The achievement of the goal is held by the K^+ affinity compared to those manifested for Na^+, even if Na^+ concentration exceeds those of K^+. The contributions of both ions with respect to Pu27 and Pu23 folding, were exploited through titrations with NaCl and KCl. The folding was followed through circular dichroism (CD) spectroscopy,

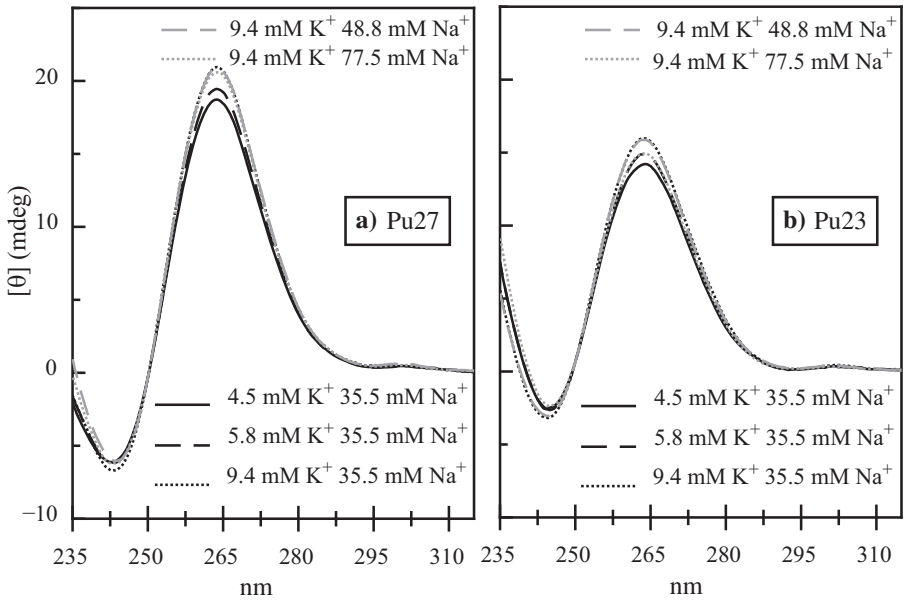

Fig. 1. – CD spectra (235 nm–325 nm) of Pu27 (a) and Pu23 (b) folded in the presence of DMEM treated with K:D–rib 5 mM and then diluted 1:4, with Na^+ and K^+ concentrations, respectively, of 35.5 mM and 4.5 mM (black solid line). Pu27 (a) and Pu23 (b), once folded, were firstly titrated with a KCl 50 mM solution so as to obtain the following concentrations: 5.8 (black dashed line) and 9.4 mM (black dotted line), keeping constant Na^+ at 35.5 mM (black solid line). Once Pu27 (a) and Pu23 (b) were folded at the biggest K^+ concentration, they were titrated with a solution of NaCl 500 mM in order to obtain the following Na^+ concentrations: 48.8 mM (gray dashed line) and 74.6 mM (gray dotted line). K^+ concentration was kept constant at 9.4 mM.

in order to detect if structural changes occurred with respect to the watery K^+ and Na^+ variations. All the folding reactions and titrations always came about in the presence of Na^+ excess. As reported in sect. **2**˙4, all the presented CD spectra were normalized to the same DNA concentration of 10 μM.

3˙1. *Pu27 titrated with KCl*. – In order to define the contribution of K^+ into Pu27 folding and for better understanding its K^+ sensing activity, the titration with KCl was carried out. Pu27 was folded in the presence of DMEM treated with K:D–rib 5 mM and the solution diluted 1:4. The concentrations of Na^+ and K^+ were respectively 42.7 mM and 5.4 mM. The titration brings the K^+ concentration towards those of DMEM treated with K:D–rib 5 mM, and diluted 1:2. The titration with KCl 50 mM takes two steps. As long as the folded Pu27 is titrated with KCl 50 mM the concentrations of K^+ and Na^+ are scaled by the dilution factor (sample volume/largest sample volume). For the sake of brevity herein are listed only the Na^+ and K^+ concentrations revised. The first step leads the K^+ concentration at 5.8 mM, and the second one up K^+ at 9.4 mM. The Na^+ concentration is 35.5 mM. All the spectra are shown in fig. 1(a). The folding of Pu27 according to the G4 structure is confirmed for all Na^+ and K^+ assayed concentrations. All spectra have a positive peak at ∼265 nm and a negative at ∼ 245 nm, confirming parallel topology [16, 17]. The more the K^+ concentration increases the more the ellipticity value

at ∼264 nm arises. The negative peak at ∼245 nm does not show significant modifications throughout the titration.

3˙2. *Pu27 folded at K^+ concentration of 9.4 mM titrated with NaCl.* – After defining the contribution of K^+ in the range 4.2 mM–9.4 mM, the Na^+ titration was performed in order to investigate its role in Pu27 folding. The final concentration of Na^+ will seek to reach those of DMEM treated with K:D–rib 5 mM and the solution is diluted 1:2, as detailed in sect. **2**. Titration takes two steps and it starts with K^+ and Na^+, respectively, at 9.4 mM and 35.5 mM. Firstly, the Na^+ concentration is brought to 48.8 mM and then it is increased till 77.5 mM. The above concentrations are those revised by the dilution factor. The CD spectra of Pu27 titrated samples are shown in fig. 1(a). The spectrum of Pu27 folded in the presence of Na^+ 35.5 mM and K^+ 9.4 mM has been detailed previously. The spectra corresponding to the Na^+ concentrations of 47.6 mM and 74.6 mM share the positive peak at about 265 nm and the negative one at about 245 nm; these are typical CD spectra features of a G4 parallel structure, in agreement with the literature [16, 17]. At the highest Na^+ concentration the CD spectra show a trivial ellipticity decrease (see fig. 1(a)).

3˙3. *Pu27 folded at K^+ concentration of 4.5 mM titrated with NaCl.* – To define the Na^+ contribution in the Pu27 folding, another Na^+ titration was performed keeping the K^+ concentration constant at 4.5 mM. Pu27 was folded in the presence of DMEM treated with K:D–rib 5 mM and the solution was then diluted 1:4. The concentrations of K^+ and Na^+ were 9.4 mM and 35.5 mM. As happened for other titrations, the CD

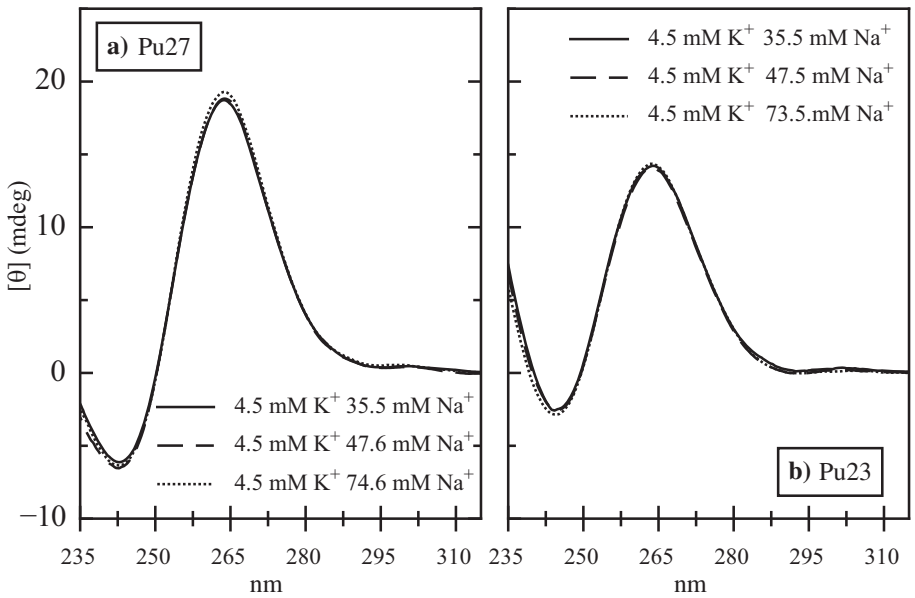

Fig. 2. – CD spectra (235 nm–325 nm) of Pu27 (a) and Pu23 (b) folded in the presence of DMEM treated with K:D–rib 5 mM and the solution diluted 1:4, with Na^+ and K^+ concentrations of 35.5 mM and 4.5 mM (black solid line) respectively. Pu27 (a) and Pu23 (b), once folded, were titrated with a solution of NaCl 500 mM, so as to obtain the following Na^+ concentrations: 48.8 mM (black dashed line) and 77.5 mM (black dotted line). K^+ concentration was kept constant at 4.5 mM.

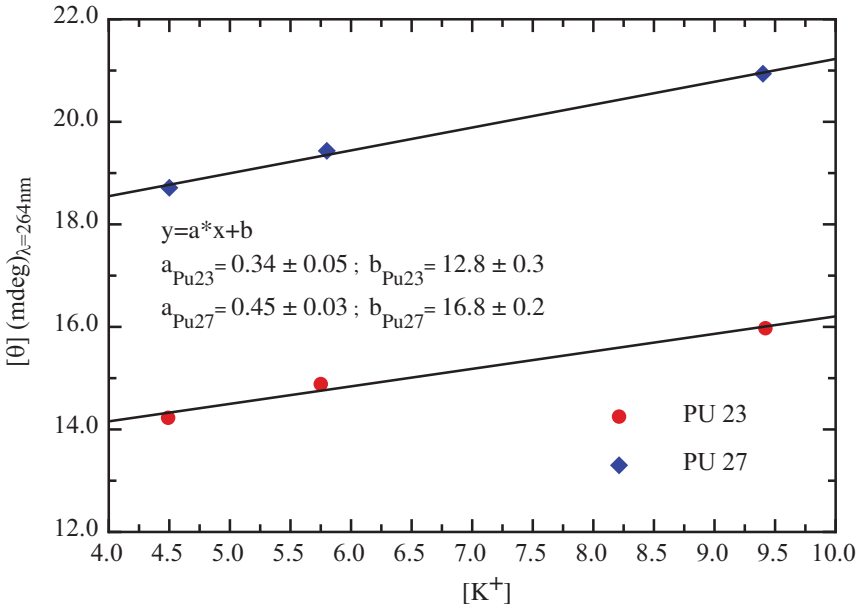

Fig. 3. – CD absorption values at 264 nm as a function of the overall K^+ concentrations in case of Pu27 (diamond) and Pu23 (circle) sequences. The lines are the fitted linear regressions. For each sequence the linear regression parameters are reported. The error bars are included in the dimension of marker symbols.

spectra must be corrected by the dilution factor, so as to take into account the dilution caused by titration. The Na^+ concentrations obtained were 48.8 mM and 77.5 mM. The highest Na^+ concentration approaches that of DMEM treated with K:D–rib 5 mM and diluted 1:2, with respect to the initial concentration. The CD spectra of NaCl titrated Pu27 samples are shown in fig. 2(a). The spectra (see fig. 2(a)) show the typical folding according to a parallel structure, in agreement with the literature data [16, 17], with the positive peak at ∼265 nm and the negative one at ∼245 nm.

3˙4. *Calibration curve of Pu27*. – The ellipticity at ∼264 nm, plotted as a function of $[K^+]$ exhibits a linear dependence, whose linear fit can be considered to be the biosensor calibration curve (see fig. 3). In the reported potassium range the biosensor has a resolution of ∼2.19 mM and sensitivity of $0.44 \pm 0.02\,\mathrm{mdeg} * \mathrm{mM}^{-1}$.

3˙5. *Pu23*. – Pu23 is the mutated form of Pu27 with a deletion of the final sequence, 5-AAGG-3, and a point mutation of the 17th guanine replaced with adenine. In particular, the second one was discovered in some cancer patients (*e.g.*, colon cancer patients) [11, 18]. The aim is to look throughout the Pu23 capability to sense watery K^+ in the presence of an enhanced Na^+ concentration. On the other hand, it is possible to determine the role of Na^+ and K^+ on the stability structure, considering Pu23 titrations. The aforementioned experimental approach followed for Pu27 was engaged for Pu23.

3˙6. *Pu23 folded and treated with KCl*. – Pu23 has been folded in the presence of DMEM treated with K:D–rib 5 mM and the solution was diluted 1:4, in the presence

of Na^+ and K^+, respectively, of 42.7 mM and 5.4 mM (see table I). The KCl titration was performed adding KCl 50 mM so as to approach K^+ concentration of DMEM treated with K:D–rib 5 mM and then diluted 1:2. As detailed above, K^+ and Na^+ concentrations need to be adjusted by the dilution factor. Thus the initial concentrations become, respectively, 4.5 mM and 35.5 mM. In the same way followed for the Pu27 samples, two titrations were done, raising K^+ concentration to 5.8 mM and 9.4 mM, as reported in the table I. The CD spectra of Pu23 folded and titrated are shown in fig. 1(b). According to the CD spectra, Pu23 folds as a G4 structure, with parallel topology signatures [16, 17]. The three spectra have the positive peak at ∼264 nm and the negative ones at ∼245 nm. The ellipticity value at ∼264 nm, typical of the parallel conformation, increases with increasing K^+.

3˙7. *Pu23 folded at 9.4 mM of K^+ and titrated with NaCl.* – After defining the K^+ contribution on Pu23 folding, it is noteworthy to investigate Na^+ contribution with NaCl 500 mM titration, as was done in the case of Pu27. The final Na^+ concentration is planned to be close to that of DMEM treated with K:D–rib 5 mM and diluted by 1:2. The titration started with the last KCl titrated sample (K^+ 9.4 mM and Na^+ 35.5 mM). The first supplement of NaCl leads the Na^+ concentration to 48.7 mM, while the second one to 77.5 mM. Concentrations include corrections because of the dilution due to titration. The CD spectra are collected in fig. 1(b). They indicate that the parallel structure is steadily maintained, since they have a positive peak at ∼264 nm and the negative one at ∼245 nm, according to the literature [16, 17]. While the first NaCl supplement increases the structural response of Pu23, the last NaCl amount causes a trivial ellipticity drop off.

3˙8. *Pu23 folded at 4.5 mM of K^+ and titrated with NaCl.* – To define more how Na^+ is involved on Pu23 folding, one more titration with NaCl 500 mM was performed. Pu23 was previously folded in the presence of K^+ and Na^+, respectively, of 4.5 mM and 35.5 mM (Na^+ and K^+ concentrations of DMEM treated with K:D–rib 5mM and the solution was diluted 1:4). The aforementioned titration protocol was followed. Two supplements of NaCl 500 mM led Na^+ to 48.5 mM and to 77.5 mM. The K^+ concentration was maintained at 4.5 mM. The outlined concentrations are those corrected by the dilution factor. All the spectra are collected in fig. 2(b) confirming that the parallel folding of Pu23 is maintained, in spite of NaCl titration [16, 17]. The first NaCl supplement increases the structural response of Pu23 while the last NaCl addition causes a marginal ellipticity drop off.

3˙9. *Calibration curve of Pu23.* – The ellipticity values of the positive peak at ∼264 nm are plotted with respect to the K^+ concentrations reached during KCl titration (see fig. 3). The graph evidences a linear response between the ellipticity and K^+ concentration. A linear interpolation fits the data. The slope of the line indicates the sensitivity of the device; in our case 0.341 mdeg $*$ mM^{-1}. The biosensor calculated resolution is 4.5 mM.

4. – Discussion

The main purpose of this article is the investigation of Pu27 and Pu23, single strand oligonucleotides which fold according to G4 structures, as watery K^+ biosensors. Beside the K^+ sensing activity, it was necessary to define some other biosensor characteristics, such as the K^+ concentration range resolution and sensitivity. Pu27, also called

NHEIII1 (nuclease hypersensitivity element), is located between −115 and −142 upstream the beginning site of the c-myc transcription [13]. Pu23 is the mutated sequence with the 17th guanine replaced by an adenine [18] and the deletion of the last four bases (5-AAGG-3). The rationale of this investigation is related to the study of K:D–rib [14,15], the effects of which might be connected to the K^+ uptake by cancer cells. So, the biosensor should measure if the cell supernatant has a variation in the K^+ concentration, with respect to the DMEM treated with the same K:D–rib concentration (5 mM). The first constrain is that the biosensor must work in DMEM, a widely known cancer cell medium with remarkable complexity, within a Na^+ concentration strongly exceeding that of K^+. The CD spectra of Pu27 and Pu23 demonstrate that DMEM, treated with K:D–rib 5 mM and then diluted 1:4, promotes their folding according to the G4 structure with parallel topology. The CD spectra show a positive peak at about 264 nm and a negative one at about 245 nm, confirming K^+ affinity, in spite of the Na^+ excess [16, 17]. The ellipticity difference at about 264 nm between Pu27 and Pu23 may be due to the point mutation (17th guanine replaced with an adenine) or to the deleted portion (5-AAGG-3). However, this last hypothesis does not seem to be critical for G4 folding [10, 19]. It is even possible to assert that solution complexity does not impair G4 folding. Considering that Pu27 and Pu23 must work within a K^+ concentration range, the K^+ titration with KCl 50 mM was done in order to increase the concentration of K^+ only. Beyond scaling up the K^+ concentration, the titration permits to analyse the increase of K^+ effects on the folding of the oligonucleotides. As expected, Pu23 and Pu27 give a positive structural feedback within the inspected range of K^+ (4.5 mM–9.4 mM). The CD spectra show a sharply ellipticity increase at 264 nm confirming the stability of the G4 parallel structure. As regards the increase, that of Pu27 is bigger than that of Pu23, corroborating that the diverse structural performances might be due to point mutation. Both oligonucleotides display a linear increase in ellipticity with respect to the increase in the K^+ concentration and data are interpolated by a linear regression curve, whose slope indicates the biosensor sensitivity. The comparison between the calibration curves shows that Pu27 is more sensitive than Pu23, with a K^+ resolution of 2.19 mM *versus* 4.5 mM. Despite the small difference, the oligonucleotides reflect the diverse structural stabilities and capabilities to sense K^+, which could be related to the point mutation of Pu23 (17th G → A). As far as the Na^+ contribution on the folding is concerned, two titrations with NaCl 500 mM were performed so as to increase the Na^+ concentration. Pu27 and Pu23 folded in the presence of the highest K^+ concentration (the final step of K^+ titration) are supplemented of NaCl 500 mM twice. As concerns Na^+ titration, Pu23 confirmed to be less stable than Pu27. The structural instability is explained with the small ellipticity drop connected to the lost of parallel structure, which is evident at the third NaCl supplement (48.7 mM → 77.5 mM). The difference can be explained, once more, by the point mutation which affects the G4 stability bringing to a rather small but detectable ellipticity fall, in particular when the folding is perturbed by adding NaCl. The second NaCl titration, performed at the minimum K^+ concentration (4.5 mM), does not show any significant differences between Pu27 and Pu23. It was confirmed that G17 is important for G4 folding because it is part of a triplet run and because all the three guanines are required for folding a G4 thermodynamically stable [11, 20]. In any case, Pu23 CD spectra demonstrate that it is possible to fold Pu23 according to the G4 structure even though the point mutation G17 → A17 and the deletion of 5-AAGG-3.

5. – Conclusions

In conclusion, it is possible to claim that Pu27 and Pu23, in the presence of DMEM treated with K:D–rib 5 mM, and then diluted 1:4, fold according to G4 structures with parallel topology. Pu27 shows a higher folding performance than Pu23, in terms of obtaining a parallel structure. Both oligonucleotides detect K^+ differences into the range considered (4.5 mM–9.4 mM) in the presence of 35.5 mM of Na^+. Pu27 manifests both higher sensitivity and higher resolution than Pu23 and the effects of Na^+ (35.5 mM–77.5 mM) on Pu27 folding can be neglected. On the contrary, the Na^+ effects of Pu23 folding are demonstrated by an ellipticity decrease with lost of parallel structure (Na^+ titration in the presence of 9.4 mM of K^+). The differences in terms of folding stability, sensitivity and performances to sense K^+ are mainly due to the point mutation (G17 → A17) and perhaps to 5′-AAGG-3′ deletion. The biosensor performances, in terms of sensitivity and resolution, are not as good as some of those published so far [21-23]. The Pu23 biosensor resolution value (4.5mM) excludes Pu23 sequence to be a K^+ biosensor in this context. Pu27 is the wild-type coming from the living world while Pu23 is a mutant, and they hold a noteworthy thermodynamic stability, compared to other synthetic sequences, such as PS2.M. The stability is also explained by the biological role of Pu27. As a repressor of the gene expression, once it is folded it must be strongly stable. This process is under control when the system is in a physiological state. It is possible to infer that a connection may exist between the different structure stability, manifested by Pu27 in the presence of Na^+ concentration increase (perturbing element) and the different effectiveness as c-myc gene expression regulator (repressor) displayed by the Pu27 sequence with respect to the other mutants.

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