Circular dichroism spectroscopic studies on structures formed by telomeric DNA sequences *in vitro*

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Abstract Telomere plays an important role in cellular processes, such as cell aging, death and carcinogenisis. Having special sequences, it can form quadruplex structure *in vitro*. Circular dichroism (CD) spectroscopic studies show that TTAGGG, $(TTAGGG)_2$ and $(TTAGGG)_4$ can all form quadruplex *in vitro* and exist mainly as parallel quadruplex without metal ions. Both K^+ and Na^+ can stabilize the tetrameric structure and facilitate the forming of anti-parallel conformation. Furthermore, the conformations of quadruplex can also be affected by sequence length, the nature and concentration of metal ions.

Keywords: human telomere, quadruplex, conformation, CD spectrum, K^+, Na^+, cation concentration.

Telomeres are special structures at the termini of linear eukaryotic chromosomes consisting of particular guanine-rich DNA sequences, and are involved in the chromosome stability, replication of the ends as well as chromosome organization within the nucleus[1]. Recently, someone proposed that the lengths of telomeres (molecular clock) regulate carcinogenisis and aging of cell[2]. In explaining how telomere, binding with telomerase, performs its special functions, the key point is to apprehend its structure but not the sequential specificity[3]. Consequently, to study the structure of telomeric DNA becomes the striking point to reveal the relationships between the telomere and those cellular processes, and thus has biological significance.

For over thirty years, it has been known that guanine-rich nucleotides and polyguanine can form four-stranded structures (G-DNA) containing guanine quartets (G-quartets, fig. 1(a))[4]. The stability of this kind of quadruplex structure exhibits a strong dependence on the monovalent metal ions (such as K^+ and Na^+)[5]. Recently, the telomeric DNA structure in certain eukaryotic species, $d(T_4G_4)$ for example, has been studied sufficiently and was proved to be quadruplex *in vitro* in the presence of monovalent cations[6].

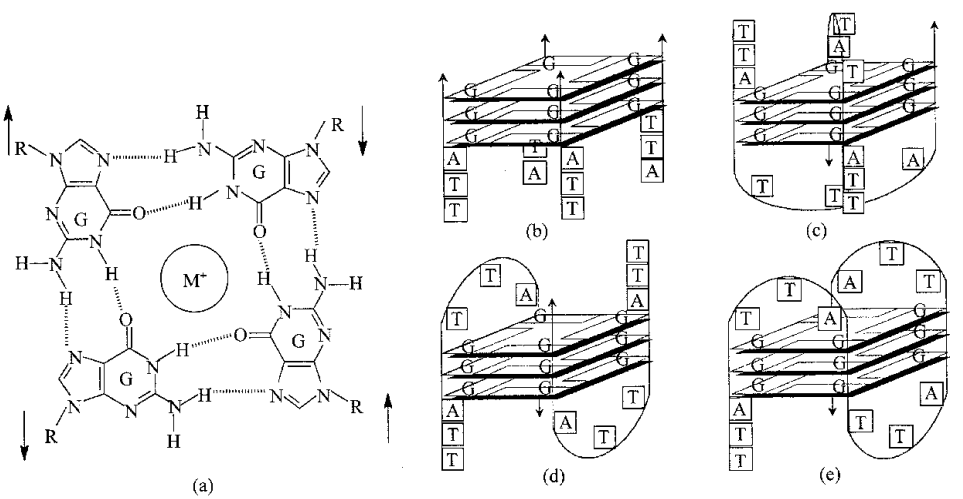

Fig. 1. Molecular configuration and sketch maps of quadruplex structures. (a) G-quartet; (b) parallel quadruplex (G-DNA); (c), (d) anti-parallel quadruplex (hairpin dimer quartets); (e) intermolecular anti-parallel quadruplex.

Because different oligonucleotide structures have different Cotton effects, the circular dichroism (CD) is very sensitive in detecting changes in structures of polynucleotides. In recent years, we have studied the structures of duplex, triplex and quadruplex DNA by applying the CD technology[7]. However, systematical CD spectral studies focused on human telomeric DNA sequence d(T_2AG_3) have not been reported until now. In this note, based on our previous work, we compared the effects of various factors on quadruplex structures formed by human telomeric DNA *in vitro* from two aspects——ions (K^+ and Na^+) and sequence lengths (repetitive frequency, *n*). We hope to provide some scientific evidence for the research on *in vivo* structure of human telomeric DNA and its dynamics, and thus help to further understand the relationships between telomere and cell aging, carcinogenisis and death.

1 Materials and methods

(ⅰ) Sample preparation. All the oligodeoxynucleotides were obtained from Life Technologies, Inc. (LTI, Hongkong). Purification and desalination were performed by reverse HPLC (C-18). The purity was determined to meet the experimental requirement by the UV spectrum. Oligodeoxynucleotides were solved in a 10 mmol/L Tris-HCl (pH=7.5) buffer containing a certain concentration of KCl and NaCl, respectively. Samples were heated at 80℃ for 5—10 min, then cooled slowly to room temperature, and then incubated at 4℃ for 5—6 h. The oligodeoxynucleotide sequences and cation concentrations in each sample are shown in table 1. Tris was obtained from the Sigma Chemical Company, other regents used were of A. R. grade produced in China.

Table 1 Oligodeoxynucleotide sequences and cation concentration *C*

Sample	A_0	A_{K1}	A_{K2}	A_{N1}	A_{N2}	B_0	B_{K1}	B_{K2}	B_{N1}	B_{N2}	C_0	C_{K1}	C_{K2}	C_{N1}	C_{N2}
Sequence		TTAGGG					$(TTAGGG)_2$					$(TTAGGG)_4$			
Repetitive frequency		*n*=1					*n*=2					*n*=3			
C/mmol · L^{-1}	–	40	80	40	80	–	40	80	40	80	–	40	80	40	80

Concentration of oligodeoxynucleotide in each sample is 50 μmol · L^{-1}. 0, samples without K^+ and Na^+; K, samples with K^+; N, samples with Na^+.

(ⅱ) CD spectrum analysis. Determination of CD spectrum was carried out on a JASCO-J700 spectropolarimeter. The instrument conditions were as follows: scan range, 320—200 nm; scan speed, 100 nm/min; quartz cell length, 1 mm; sensitivity, 5 mdeg; response, 4 s; resolution, 1 nm; accumulation times, 4; temperature, 20℃; reference sample, buffer solution containing the same cation condition.

2 Results and discussion

(ⅰ) Conformation and sequence length. Human telomeric DNA can form special structures due to its special sequences, and the conformations formed by sequences in various lengths are different. There is some relation between them. Fig. 2(a) shows that the CD spectra of samples A_0, B_0 and C_0 have a highly similarity: a negative band at 240 nm, and a positive band at around 260 nm, analogous to the CD spectrum of poly(dG) which is well known to form intermolecular parallel quadruplex structure[8]. In this conformation, each strand runs in the same direction and all G residues from different strands in the G-quartet are in anticonformation. Its characteristic CD spectrum exhibits a negative band at 240 nm and a positive band at 260 nm[9]. The results above suggested that samples A_0, B_0 and C_0 might exist as quadruplex DNA structure with a parallel formation (G-DNA) (fig. 1(b)) in the solution without monovalent metal ions. Further analysis shows that there are faint positive bands at around 290 nm in curves of B_0 and C_0 and the band is more intense for C_0. As is reported, G-rich sequences can fold back and form hairpin dimer structures with anti-parallel conformation[10]. In contrast to parallel quadruplex conformation, their guanine glycosidic torsion angles in the G-quartet appear an alternative syn- and anti-conformation and their CD spectra are characterized by a negative band at 260 nm and a positive band at 295 nm. Hence, it is most possible that when repetitive frequency *n* is 2 or 4, the oligonucleotide sequences can fold spontaneously into intramolecular or intermolecular

hairpin dimer quadruplexes in the solution respectively (fig. 1(c), (d), (e)). But the proportion is quite low and increases with the increase of repetitive frequency.

(ii) Conformation and metal cations. The aforementioned results have shown that CD spectra of sequences in different lengths are not the same, but the difference in spectra is minor. When 80 mmol/L Na^+ was added into solutions, the difference was enlarged greatly (fig. 2(b)). Compared respectively with curves A_0, B_0 and C_0 in fig. 2(a), fig. 2(b) shows that curve A_{N2} has not an obvious change; for curves B_{N2} and C_{N2}, 240 nm negative bands disappeared, 260 nm positive bands shifted to 250 nm. At the same time, a new negative band at 260 nm and a positive band at 290 nm were observed. The intensities of the two positive bands are almost equal for curve B_{N2}, while for curve C_{N2} there appeared a more visible blue shift and a comparable sharp drop between 260 nm and 290 nm bands, showing a dominant strong 290 nm band. It is obvious that the addition of Na^+ can change the structures of oligonucleotide notably and has different effects on sequences in different lengths, the longer the sequence is, the greater the change will be. As for the reasons, on one hand, the formation of quadruplex needs monovalent cations (such as K^+ and Na^+): the O-6 of guanine can form a coordination complex with either K^+ or Na^+ in G-quartets, and thus stabilize the structure; on the other hand, telomeric sequence can form different conformations of quadruplex structure in accordance with its

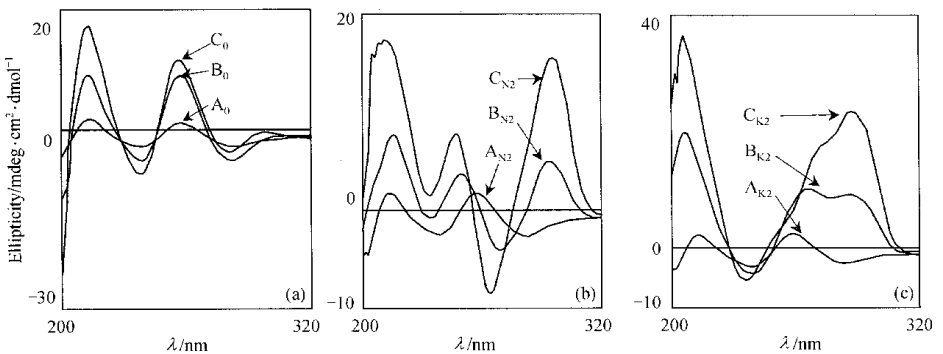

Fig. 2. CD spectra of TTAGGG, $(TTAGGG)_2$ and $(TTAGGG)_4$ in Tris-HCl buffer at 20℃, pH 7.5. (a) K^+ and Na^+ are absent; (b) 80 mmol/L Na^+ is in solution; (c) 80 mmol/L K^+ is in solution. Characters are sample names shown in table 1.

length[9], shorter sequences have less kinds of conformation than longer ones. As shown in fig. 1, when *n* is 1, the sequence can only adopt intermolecular quadruplex structure. When *n* is 2, it can also form intramolecular hairpin structure and then dimerize into anti-parallel conformation. While *n* increases to 4, it is even able to fold itself into monomolecular quadruplex structure; and moreover, its parallel conformation can exist as a right-handed helix[11]. Once metal ions were added into solutions, they interacted with oligonucleotide sequences and converted the sequences into more thermodynamically stable structures. Therefore, the CD spectra of sequence A did not change obviously, while CD spectra of sequences B and C changed greatly and showed a certain complexity. In a word, the longer a sequence is, the more complicated the case may be; the more complicated the case is, the greater effects the cations may have.

(iii) Comparison of the effects of K^+ and Na^+. As also is shown in fig. 2(b), the typical bands of parallel quadruplex decreased after the addition of Na^+ into the solution, while the typical bands of anti-parallel quadruplex appeared and increased gradually, indicating that Na^+ can promote sequences to form anti-parallel conformation. This is in accordance with the results reported in ref. [12]; that is to say, Na^+, while forming coordinate bond with O-6 of G residue, can make sequences form hairpin dimer quarduplex. Fig. 2(c) shows the CD spectra of the three sequences in the presence of 80 mmol/L K^+. The addition of K^+ also caused different changes of the CD spectra of sequences in different lengths. But contrary to Na^+, the addition of K^+ made these curves exhibit double positive bands between 260 and 290 nm. Unlike the two kinds of typical CD spectra described above, it is more like merging together of 260 nm band with the 290 nm band. According to refs. [11, 12], K^+ can stabilize G-

quartets both in parallel and anti-parallel structures, it is possible that the two conformations coexist in the same solution and give the special spectra. The results also show that K^+ and Na^+ have different effects on telomeric DNA structures, despite the fact that they are both monovalent cations and can stabilize G-DNA. Probably, the spaces inside the G-quartets of different conformations are different in size, and need cations of various diameters to stabilize them. So the differences in CD spectra are due to the differences of cations' diameters. However, it is also considered that the difference in effects of cations is the result of the difference in relative free energies of hydration of cations[4]. After all, there is no perfect explanation to this phenomenon yet; more research work needs to be done.

(iv) Effects of cation concentration. We have found in other experiments that there is a certain trend in changes of CD spectra for different concentrations of K^+ or Na^+. The positive band at 290 nm increases with the increase of cation concentration. Fig. 3(a), (b) and (c) show CD spectra of sequences A, B and C at two concentrations of K^+ and Na^+, respectively. There is still no appreciable change for CD spectra of sequence A (fig. 3(a)). In fig. 3(b), the positive band at 290 nm of sequence B increases with the increase of Na^+ concentration, suggesting that the proportion of anti-parallel quadruplex in the solution increases. This result is similar to the effects of sequence length, but the effects of cation concentrations are much weaker. Moreover, for curves B_{K1} and B_{K2}, the increase of the whole absorption was observed, the shoulder band around 290 nm also increased and took more proportion, indicating that the increase of cation concentration is helpful to the formation of anti-parallel quadruplex structure. Despite the lower change extent, results from fig. 3(c) are similar to those from fig. 3(b). It can be realized from the former analysis that sequence C can more easily form an anti-parallel quadruplex structure in the presence of cations. It is possible that sequence C can form completely anti-parallel conformation in a condition of comparably low concentration of K^+ or Na^+ and the concentration of 40 mmol/L is considerably higher to it, so the spectra of sequence C did not change much. Sequence B requests a higher cation concentration to form the anti-parallel conformation to full extent and 40 mmol/L of K^+ or Na^+ is still lower, so the CD spectra of sequence B changed greatly.

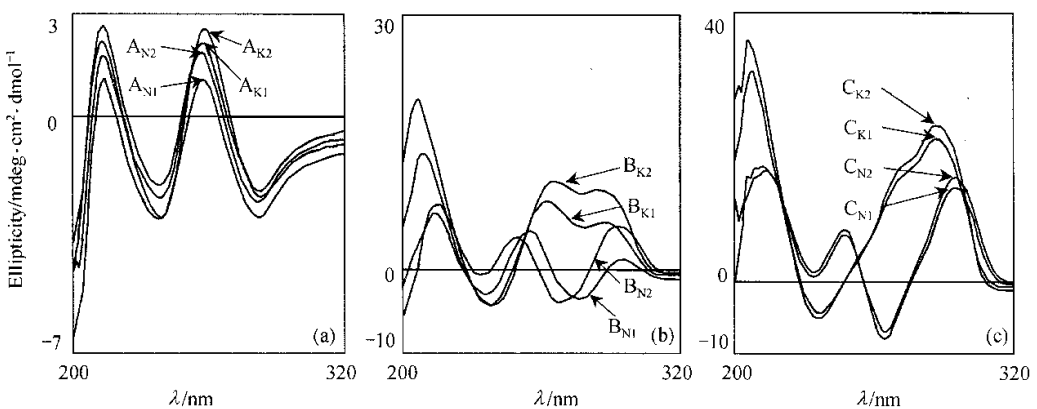

Fig. 3. CD spectra of sequences at different K^+ and Na^+ concentrations. (a) A, TTAGGG; (b) B, $(TTAGGG)_2$; (c) C, $(TTAGGG)_4$.

In conclusion, the note here suggests the following: i) Without metal ions, human telomeric DNA sequence can form quadruplex structures *in vitro* and exists mainly in parallel conformation. With the increase of sequence length (repetitive frequency), it tends to form anti-parallel quadruplex. ii) K^+ and Na^+ can stabilize the quadruplex structures and are of aid to the formation of anti-parallel conformation. iii) K^+ and Na^+ have different effects on the sequences: Na^+ can stabilize anti-parallel conformation while K^+ can do both conformations. iv) Monovalent metal ions benefit the formation of anti-parallel structure and their effects are intensified with their concentrations' going up.

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