of telomerase offer one possible mechanism for interfering with telomere synthesis.

ACKNOWLEDGMENTS

We thank Bing Mao and Dr. Nick Geacintov for their help with fluorescence measurements.

REFERENCES

Blackburn, E. H. (1991) *Nature 350*, 569–573.
Blackburn, E. H., & Szostak, J. W. (1984) *Annu. Rev. Biochem. 53*, 163–194.
Bresloff, J. L., & Crothers, D. M. (1975) *J. Mol. Biol. 95*, 103–123.
Caruthers, M. H. (1982) in *Chemical and Enzymatic Synthesis of Gene Fragments* (Gassen, H. G., & Lang, A., Eds.) pp 71–79, Verlag Chemie, Weinheim.
Crothers, D. M. (19710 *Biopolymers 10*, 2147–2160.
Guo, Q., Seeman, N. C., & Kallenbach, N. R. (1989) *Biochemistry 28*, 2355–2359.
Hardin, C. C., Henderson, E., Watson, T., & Prosser, J. K. (1991) *Biochemistry 30*, 4460–4472.
Henderson, E. R., Hardin, C. C., Walk, S. K., Tinoco, I., & Blackburn, E. H. (1988) *Cell 51*, 899–908.
Henderson, E. R., Moore, M., & Malcolm, B. A. (1990) *Biochemistry 29*, 732–737.
Hertzberg, R. P., & Dervan, P. B. (1982) *J. Am. Chem. Soc. 104*, 313–315.
Jin, R., Breslauer, K. J., Jones, R. A., & Gaffney, B. L. (1990) *Science 250*, 543–546.
LePecq, J. B., & Paoletti, C. (1967) *J. Mol. Biol. 27*, 87–106.
Lipps, H. J. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 4104–4108.
Maxam, A. M., & Gilbert, W. (1980) *Methods Enzymol. 65*, 499–560.
Mergny, J. L., Collier, D., Rougee, M., Montenay-Garestier, T., & Helene, C. (1991) *Nucleic Acids Res. 19*, 1521–1526.
Oka, Y., & Thomas, C. A. (1987) *Nucleic Acids Res. 15*, 8877–8898.
Scaria, P. V., & Shafer, R. H. (1991) *J. Biol. Chem. 266*, 5417–5423.
Sen, D., & Gilbert, W. (1988) *Nature 334*, 364–366.
Sen, D., & Gilbert, W. (1990) *Nature 344*, 410–414.
Sundquist, W. I., & Klug, A. (1989) *Nature 342*, 825–829.
van Dyke, M. W., & Dervan, P. B. (1983) *Nucleic Acids Res. 11*, 5555–5567.
Williamson, J. R., Raghuraman, M. K., & Cech, T. R. (1989) *Cell 59*, 871–880.
Wiseman, T., Williston, S., Brandts, J. F., & Lin, L. N. (1989) *Anal. Biochem. 179*, 131–137.
Zahler, A. M., Williamson, J. R., Cech, T. R., & Prescott, D. M. (1991) *Nature 350*, 718–720.
Zakian, V. A. (1989) *Annu. Rev. Genet. 23*, 579–604.

Structure and Stability of Sodium and Potassium Complexes of dT_4G_4 and dT_4G_4T†

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Received November 26, 1991; Revised Manuscript Received January 7, 1992

ABSTRACT: The ends of eukaryotic chromosomes contain specialized structures that include DNA with multiple tandem repeats of simple sequences containing clusters of G on one strand, together with proteins which synthesize and bind to these sequences. The unit repeat in the protozoan *Oxytricha* with the cluster dT_4G_4 can form structures containing tetrads of guanine residues, referred to G4 DNA, in the presence of metal ions such as Na^+ or K^+. We show here that, in the presence of Na^+, dT_4G_4 forms a tetramer with parallel strands by means of a UV cross-linking assay. In the presence of K^+, two further interactions are observed: at low temperature, higher order complexes are formed, provided the 3′ end of the strand is G; a single 3′T inhibits this association in dT_4G_4T. At high temperature, these complexes dissociate, leading to a tetramer with a different ordered structure that melts only at very high temperatures. These results suggest that the cohesive properties of DNA containing G clusters might depend on associative interactions driven by a free 3′G terminus in the presence of K^+, as well as by connecting antiparallel G hairpins as has been postulated.

One role of telomeres is to stabilize the ends of chromosomes against breakage (Blackburn & Szostak, 1984; Zakian, 1989; Blackburn, 1991) by inhibiting degradation of DNA or fusion processes. DNA containing the sequences repeated in telomeres can show unusual cohesive behavior in solution (Lipps, 1980; Oka & Thomas, 1987), suggesting that short overhanging G clusters might be capable of connecting duplexes, thereby maintaining integrity of chromosome ends (Blackburn, 1991). This has been confirmed by studies of short oligomeric model sequences containing G clusters (Henderson et al., 1987, 1990; Sen & Gilbert, 1988, 1990, 1992; Sundquist & Klug, 1989; Williamson et al., 1989; Hardin et al., 1991). Oligonucleotides corresponding to two copies of dT_2G_4 form an intramolecular, double-stranded hairpin loop structure under low ionic strength. Upon addition of Na^+ or K^+ ions, the two hairpins dimerize to assume a compacted, antiparallel fold-

† This work was supported by Grant CA-24101 from the National Institutes of Health and by the generous support from the W. M. Keck Foundation.
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0006-2960/92/0431-2455$03.00/0

back structure (Sundquist & Klug, 1989; Williamson et al., 1989). Sen and Gilbert (1988) proposed that single-stranded G-rich DNA can self-associate to form a parallel four-stranded structure in Na^+, in which layers of four guanines arranged in planar tetrads are stabilized by Hoogsteen hydrogen bonding. On the other hand, an intramolecular, four-stranded fold-back structure has been deduced for repeats such as $d(T_4G_4)_4$ and $d(T_2G_4)_4$ in the presence of Na^+ or K^+ (Williamson et al., 1989). DNA oligomers with a terminal guanine can generate, in addition to tetramers, higher order products in the presence of K^+ (Sen & Gilbert, 1992).

In this work, the structural properties and thermal stabilities of two short oligomers, dT_4G_4 and dT_4G_4T, are compared in Na^+ and K^+. Differences in the behavior of these two models suggest that free guanine at the 3′ end of chromosomal telomeres might play a crucial role in the formation of higher order products. We also describe a UV cross-linking assay that shows the tetrameric structure of both these oligomers has parallel strand arrangement in Na^+ and K^+.

Materials and Methods

Synthesis and Purification of Oligonucleotides. Oligonucleotides used in this study were synthesized on an ABI 391 PCR MATE DNA synthesizer and deprotected by routine phosphoramidite procedures (Caruthers, 1982). Strands were purified by preparative HPLC on a Du Pont Zorbax Bio Series oligonucleotide column, following the manufacturer's recommended elution protocol. Oligonucleotides were labeled at their 5′ termini using T4 polynucleotide kinase (Boehringer); the labeled strands were purified by polyacrylamide gel electrophoresis.

Solution Preparation. The concentration of DNA strands was determined spectrophotometrically at 260 nm and 80 °C (Cantor et al., 1970). DNA solutions were prepared in a 10 mM Tris·HCl buffer containing 0.1 mM EDTA and 200 mM NaCl or KCl, adjusted to pH 7. The DNA strands of dT_4G_4 and dT_4G_4T were evaporated to dryness at 50 °C over 10 h and then dissolved in 10 mM Tris·HCl buffer (pH 7) and 0.1 mM EDTA (TE buffer). The DNA samples were incubated at 4 °C for 48 h.

DMS Protection Assay. Methylation protection experiments were performed as described by Maxam and Gilbert (1980). A total of 1 μL of a fresh 1:100 dilution of DMS in water was added to 10 μL of DNA samples (100 μM) in TE buffer without or with indicated salts for 30 min at room temperature. The reactions were terminated by two sequential rapid ethanol precipitations and then lyophilized. The DNAs were cleaved at the methylation sites by treatment with 100 μL of 1 M piperidine at 90 °C for 30 min and lyophilized extensively.

Circular Dichroism (CD) Spectroscopy and CD Melting Curves. CD spectra were recorded using an AVIV Model 60DS CD spectropolarimeter equipped with a programmable, thermoelectrically controlled cell holder (Aviv Associates, Lakewood, NJ). Each spectrum corresponds to an average of three scans from which the buffer background was subtracted. CD melting curves were recorded at 265 nm. Ellipticities at 5 °C were normalized to 1.0. Samples were heated at a rate of 0.5 °C/min. These melting curves allow us to measure the transition temperatures, T_m, which are the midpoints of the order–disorder transition of these DNA molecules.

UV Cross-Linking. Irradiations were performed with a Sylvania G8T5 lamp (8 W, Germicidal) at 254 nm in microtiter plates with conical wells at a distance of 9 cm from the lamp at room temperature, in a volume of 5 μL. DNA samples were dissolved in loading buffer and run on a denaturing 14% polyacrylamide gel. In order to identify sites of cross-linking, bands corresponding to monomer and cross-linked species were cut out and soaked overnight at 37 °C in 0.4 mL of buffer (0.5 M ammonium acetate, 1 mM EDTA). The DNA strands were precipitated twice with ethanol and lyophilized. The DNA cross-linking sites were then treated with 100 μL of 1 M piperidine at 90 °C for 30 min and extensively lyophilized (Williamson et al., 1989). Ratios of counts in the dimer, trimer, and tetramer bands were determined by cutting out the bands and scintillation counting. Gel slices were placed in vials with 12 mL of scintillator solution containing 4 g of PPO and 0.05 g of POPOP (Amersham) in toluene (Aldrich) and counted on a liquid scintillation spectrometer (Intertechnique SL 30). Gel slices that did not contain radioactivity were counted as the background.

Gel Electrophoresis. Native polyacrylamide gels were run on 6% native gels (19:1 monomer/bis ratio) at specific temperatures for 20 h at 100 V (ca. 8 V/cm). The electrophoresis plates were jacketed and cooled or heated with circulating water to provide a specific running temperature ± 1 °C in the gel throughout the electrophoresis. The buffer system contained 53.4 mM Tris–borate–EDTA buffer, pH 8.3 (0.6× TBE), with added 50 mM KCl. No tracking dyes were added to samples in these runs. The gels were exposed to X-ray film for 1 h without an intensifying screen. For denaturing polyacrylamide gels, the samples for electrophoresis were taken up in formamide loading buffer, heated briefly to 90 °C, cooled, and then run on a 20% denaturing polyacrylamide gel for 3 h at 2000 V (ca. 50 V/cm) and 40 °C. The gels were dried immediately on a vacuum-drying apparatus (Hoefer) and exposed at room temperature to X-ray film.

Results

The Tetrameric Structure in dT_4G_4 and dT_4G_4T Differs in Structure and Stability. Since the single-stranded overhangs of G-rich naturally occurring telomeres have free guanine at their 3′ ends, it is of interest to determine how an additional T at the 3′ end affects the structure and stability of model complexes. Accordingly, we synthesized the two oligomers, dT_4G_4 and dT_4G_4T, both of which form stable tetramers in the presence of Na^+ or K^+. By use of the electrophoretic assay developed by Sen and Gilbert (1990), we verified that five bands result in a nondenaturing gel when either dT_4G_4 or dT_4G_4T is mixed with a marker strand of distinct mobility, $dT_{10}G_4$. We have also performed the DMS protection experiment that has been useful in probing the formation of G4 DNA structures (Sen & Gilbert, 1988; Williamson et al., 1988). Figure 1 shows that each G N7 within the G clusters of dT_4G_4 and dT_4G_4T is fully protected from methylation in Na^+ or K^+, suggesting formation of tetraplex structure (Zimmerman et al., 1975). We and others (Jin et al., 1990; Hardin et al., 1991; Guo et al., 1992) have shown that tetraplex G structures have a characteristic CD spectrum. CD spectra of dT_4G_4 and dT_4G_4T in K^+ are shown in Figure 2. The spectrum of dT_4G_4 or dT_4G_4T is characterized by a peak at 265 nm with a trough at 242 nm in both Na^+ (data not shown) and K^+ (Figure 2). Differences in behavior of these two oligomers can be seen in their CD melting profiles in Na^+ or K^+ (Figure 3). The additional 3′T residue in the oligomer dT_4G_4T stabilizes the tetrameric structure in this chain by nearly 20 °C over that in dT_4G_4 in the presence of 200 mM Na^+ (Figure 3). In the presence of K^+, however, the situation is quite different. The oligomer dT_4G_4 forms a new ordered structure on heating, with different molar ellipticity at 265 nm, while the dT_4G_4T unfolds to a new state that also retains considerable order at 100 °C. K^+ thus induces extremely

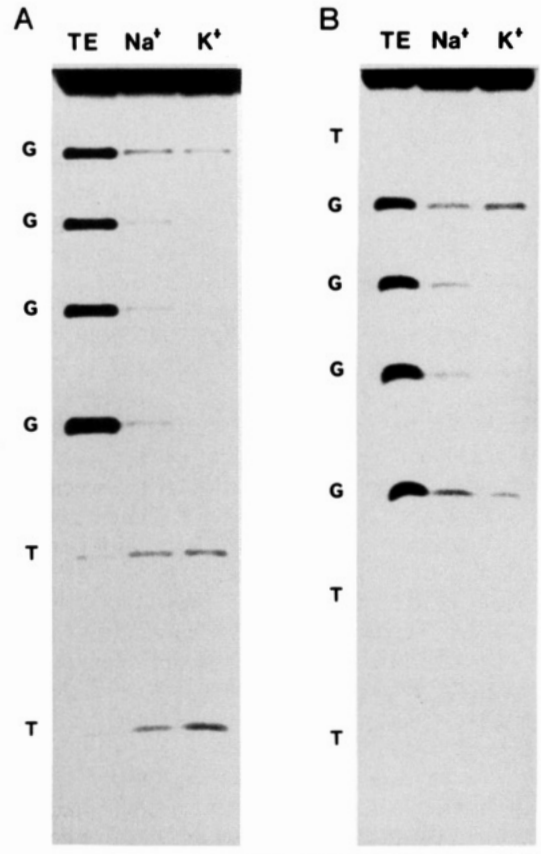

FIGURE 1: Methylation protection of dT_4G_4 (A) and dT_4G_4T (B). An autoradiogram of the 20% denaturing polyacrylamide gel is shown of methylation protection. The DNA strand 5′-labeled with ^{32}P was methylated by DMS in TE buffer, or TE buffer with 200 mM NaCl or KCl added, and then cleaved with piperidine.

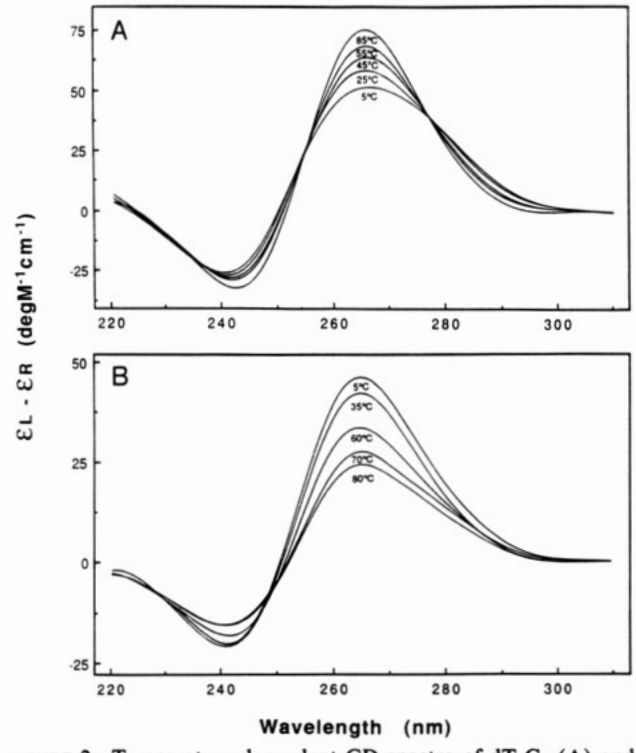

FIGURE 2: Temperature-dependent CD spectra of dT_4G_4 (A) and dT_4G_4T (B) in 10 mM potassium phosphate buffer (pH 7), 0.1 mM EDTA, and 200 mM KCl. The total DNA strand concentration is 100 μM. Samples were preequilibrated at 5 °C for 1 h.

stable structures in these short chains, which might have a biological role provided they occur also under less severe

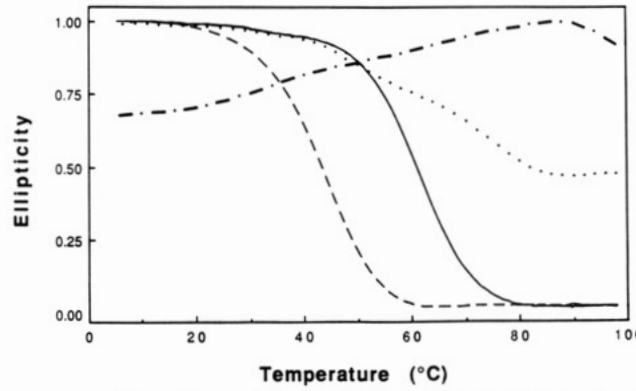

FIGURE 3: CD melting profiles for dT_4G_4 in 200 mM Na^+ (– –) and K^+ (–·–) and for dT_4G_4T in 200 mM Na^+ (—) and K^+ (···) in 10 mM phosphate buffer (pH 7) and 0.1 mM EDTA. The total DNA strand concentration is 100 μM. Samples were preequilibrated at 5 °C for 1 h.

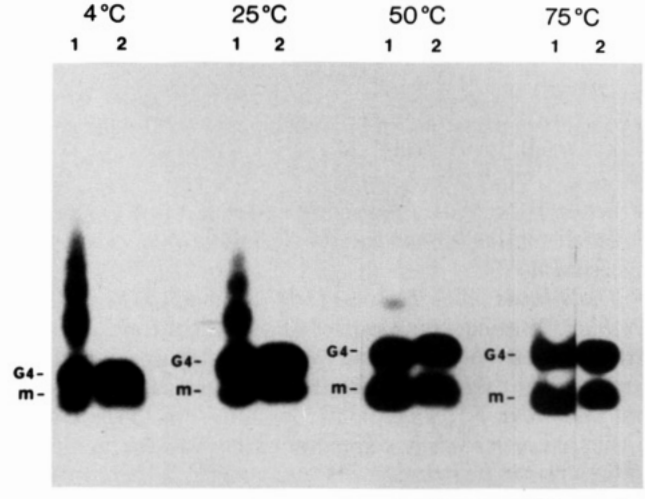

FIGURE 4: Electrophoresis of dT_4G_4 and dT_4G_4T in K^+ in nondenaturing gels. Autoradiograms of the native gels are shown of the monomer (m), tetramer (G4), and other high-order products of dT_4G_4 (lane 1) and dT_4G_4T (lane 2). The DNA samples were incubated at specific temperatures as indicated for 1 h and then run on 6% native gels at the same temperature as incubated.

conditions. It is interesting to note that the structure in these oligomers is different from one to another, as well as from that in tandem repeats of the same sequence, as can be seen from their characteristic CD spectra (Guo et al., 1992).

The Associative Interaction in the Tetramer $(dT_4G_4)_4$ Is Driven by a Free 3′G Terminus in the Presence of K^+. It has been suggested by Sen and Gilbert (1992) that G-rich oligomers having a terminal guanine form, in addition to G4 DNA, superstructures with K^+, or Rb^+, but not with Na^+. The CD melting profiles (Figure 3) also indicate that the behavior of G clusters is more complicated in the presence of K^+. One obvious possibility is that the unusual melting behavior of the oligomer dT_4G_4 in K^+ is due to formation of high-order polymeric complexes. In order to test this hypothesis, we performed an electrophoretic experiment with these two oligomers on nondenaturing gels, following incubation at different temperatures. As shown in Figure 4, at low temperatures, a series of higher molecular weight complexes with K^+ migrating more slowly than the $(dT_4G_4)_4$ tetramer can be resolved. Incubation at higher temperatures shows that these structures dissociate with increasing temperature. Interestingly, the presence of a single 3′T residue inhibits this association; dT_4G_4T does not show this effect (Figure 4), consistent with the results reported by Sen and Gilbert (1992). By contrast, both dT_4G_4 and dT_4G_4T form a unique, defined G4 DNA

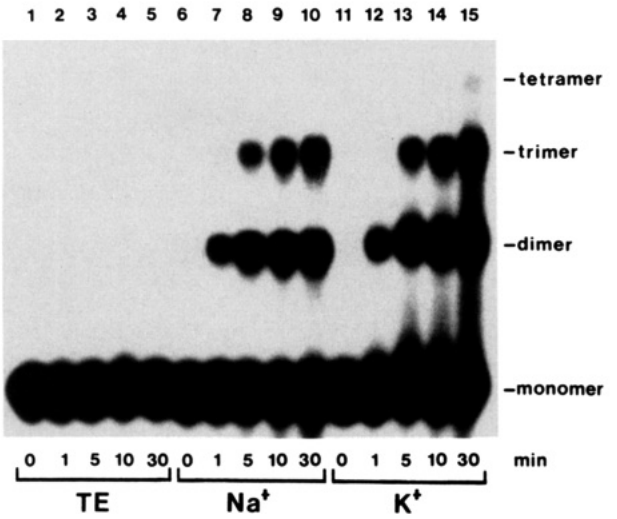

FIGURE 5: UV cross-linking of dT_4G_4. An autoradiogram of the 14% denaturing polyacrylamide gel is shown of the cross-linking products of dT_4G_4 upon irradiation at 254 nm at room temperature for 1, 5, 10, and 30 min. The total DNA strand concentration is 100 μM. The DNA samples were prepared in 10 mM Tris·HCl (pH 7), 0.1 mM EDTA (TE) (lanes 1–5), or TE with 200 mM NaCl (lanes 6–10) or KCl (lanes 11–15) added.

structure in Na^+ (data not shown). Hence a free 3′G seems essential for the K^+-mediated association observed in this experiment.

The Strands in the Tetramers $(dT_4G_4)_4$ and $(dT_4G_4T)_4$ Are Parallel. Why does the oligomer dT_4G_4 generate higher order products in K^+ but not in Na^+? We might suspect that the strands in the tetrameric structure could be antiparallel in Na^+, but parallel in K^+, which might facilitate the formation of higher molecular weight complexes. Thus we were prompted to ask whether the strands in the tetramer $(dT_4G_4)_4$ are parallel or antiparallel in Na^+ or K^+. Although Sen and Gilbert (1988) measured complete DMS protection of G N7 in several G-cluster DNA sequences that they studied, concluding that this required a parallel arrangement, direct evidence to support this notion is lacking. Therefore, we have used a UV cross-linking assay to establish unequivocally whether the strands are parallel or antiparallel. The reasoning is as follows. If dT_4G_4 forms a parallel G4 structure in the tetramer form, it should be possible to cross-link the T tails by means of thymine dimer formation in the presence of UV light so as to generate products that include dimers, trimers, and tetramers. On the other hand, if the tetramer is antiparallel, formation of dimers should represent the maximum end product. Figure 5 shows the result of this experiment. Trimers and tetramers can be detected in Na^+ or K^+ complexes of dT_4G_4 (Figure 5) and dT_4G_4T (data not shown). Thus both species assume a parallel strand G4 structure in Na^+ or K^+. For this test to work, we need to establish that cross-linking of these strands involves the T's, not the G's. To ascertain this, we mapped sites of the UV-induced cross-linking of dT_4G_4 in Na^+ by treating the irradiated species with piperidine, which has been found to cleave UV cross-linking sites (Williamson et al., 1989). As shown in Figure 6, the major cross-linking site is at T_3, with minor activity at T_1, T_2, and T_4 in both the dimer and trimer cross-linked species. There is no indication of cross-linking at the G positions in this experiment with comparable intensity. In addition, the pattern of nonuniform activity at each T site suggests that the thymine tail in the G4 structure may not be completely flexible. This argument is also supported by the relative intensities of the dimer, trimer, and tetramer bands (57:19:1).

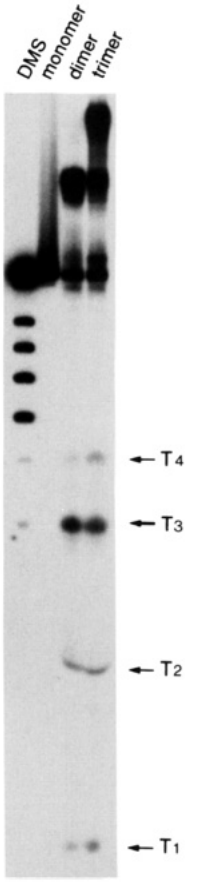

FIGURE 6: Sites of UV cross-linking of dT_4G_4 in Na^+. An autoradiograph of the 20% denaturing polyacrylamide gel is shown of the piperidine cleavage products in the cross-linked dimer and trimer of dT_4G_4 in Na^+. Lane DMS is a ladder of unirradiated dT_4G_4 with DMS–piperidine treatment as a marker. Lane monomer is an irradiated but un-cross-linked species. Lanes dimer and trimer contain the cleavage products of cross-linked dimer and trimer species, respectively. The arrows indicate the cross-linking positions.

Discussion

The presence in telomeric DNA of multiple tandem repeats of sequences with G clusters on one strand, such as dT_4G_4, endows these molecules with unique cohesive properties that could protect the chromosome ends from dissociation by breaks. The most likely structure that forms in these chains is an antiparallel fold-back structure that has been detected in solution (Henderson et al., 1988, 1991; Williamson et al., 1989; Sundquist & Klug, 1989; Hardin et al., 1991). This is a different structure from that formed in tetrameric complexes with a single dT_4G_4 sequence. Our results here show that, in principle, a single overhanging dT_4G_4 sequence has the potential to complex similar single-stranded sequences to form parallel tetramers that can associate further in the presence of K^+. This kind of complex could result in damaging the ends and suggests that there might be more than one kind of cohesive interaction present in telomeres. This complex is stabilized in the presence of sodium ions by a 3′T. Potassium ions stabilize the structure in dT_4G_4 enormously (see Figure 3). If the 3′G terminus is free, a second parallel structure forms at high temperature in the presence of K^+. The stability of this complex suggests that K^+ might be able to promote and maintain large-scale structures in a cell relying on a minimal set of overhanging G clusters. Depending on the context, this offers an alternate mechanism for stabilizing DNA against dissociation by breakage or a pathway for genetic interactions in sequences bearing G clusters. Liberation of individual dT_4G_4 overhangs can lead to strong intermolecular association

driven by K^+ ions. However, the presence of a single 3′T appears to inhibit this process, although a residual high-temperature structure still forms in dT_4G_4T (Figure 3).

Structurally, we can say little at the moment about the remarkably stable high-temperature structure in either of these complexes in K^+. Since the CD spectrum changes in magnitude but not in shape, it seems likely that no large-scale change in geometry is involved. One possibility is that a change in hydration or ion binding occurs on heating the tetramers, but this is by no means established. Structural and thermodynamic analysis of this form of the complex is presently under way to attempt to distinguish these from other possibilities.

Registry No. dT_4G_4, 108050-57-3; dT_4G_4T, 138693-69-3; Na, 7440-23-5; K, 7440-09-7.

References

Blackburn, E. H. (1991) *Nature 350*, 569–573.

Blackburn, E. H., & Szostak, J. W. (1984) *Annu. Rev. Biochem. 53*, 163–194.

Cantor, C., Warshaw, M. W., & Shapiro, H. (1970) *Biopolymers 9*, 1059–1077.

Caruthers, M. H. (1982) in *Chemical and Enzymatic Synthesis of Gene Fragments* (Gassen, H. G., & Lang, A., Eds.) pp 71–79, Verlag Chemie, Weinheim.

Guo, Q., Lu, M., Marky, L. A., & Kallenbach, N. R. (1992) *Biochemistry* (preceding paper in this issue).

Hardin, C. C., Henderson, E., Watson, T., & Prosser, J. K. (1991) *Biochemistry 30*, 4460–4472.

Henderson, E. R., Hardin, C. C., Walk, S. K., Tinoco, I., & Blackburn, E. H. (1987) *Cell 51*, 899–908.

Henderson, E. R., Moore, M., & Malcolm, B. A. (1990) *Biochemistry 29*, 732–737.

Jin, R., Breslauer, K. J., Jones, R. A., & Gaffney, B. L. (1990) *Science 250*, 543–546.

Lipps, H. J. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 4104–4108.

Maxam, A., & Gilbert, W. (1980) *Methods Enzymol. 65*, 499–560.

Oka, Y., & Thomas, C. A. (1987) *Nucleic Acids Res. 15*, 8877–8898.

Sen, D., & Gilbert, W. (1988) *Nature 334*, 364–366.

Sen, D., & Gilbert, W. (1990) *Nature 344*, 410–414.

Sen, D., & Gilbert, W. (1992) *Biochemistry 31*, 65–70.

Sundquist, W. I., & Klug, A. (1989) *Nature 342*, 825–829.

Williamson, J. R., Raghuraman, M. K., & Cech, T. R. (1989) *Cell 59*, 871–880.

Zakian, V. A. (1989) *Annu. Rev. Genet. 23*, 579–604.

Zimmerman, S. B., Cohen, G. H., & Davies, D. R. (1975) *J. Mol. Biol. 92*, 181–192.

Average Density and Size of Microclusters of Epidermal Growth Factor Receptors on A431 Cells†

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Received October 28, 1991; Revised Manuscript Received January 14, 1992

ABSTRACT: For some hormone receptors, the early events of signal transduction depend on their molecular arrangement and interactions at the cell surface. An understanding of the mechanism of signal transduction in general needs a careful analysis of the receptor distribution. Here, we present the first quantitative measurement of epidermal growth factor receptor distribution on A431 cells obtained by scanning fluorescence correlation spectroscopy. Prior to epidermal growth factor binding, the A431 cell membrane presents an average surface density of 7.7–8.4 microclusters/μm^2, each containing an average of 130 receptors.

There is strong evidence that the molecular organization and the lateral distribution of receptors in cell membranes are important for their function (Lichtner & Schirrmacher, 1990). In many cases, it is clear that the distribution of receptors is neither random nor homogeneous, but rather the receptors are partly aggregated (Van Belzen et al., 1988). The organization and distribution of receptors at the cell surface frequently change as a consequence of their interactions with specific hormones or ligands (Van Belzen et al., 1988; Haigler et al., 1978). Yet, some important questions about receptor distribution remain unanswered: Are receptors distributed uniformly, randomly, or among domains on the cell surface prior to the binding of hormone? If clusters exist, can their density and size be determined? What is the proportion of clustered and nonclustered receptors? What is the nature of the receptor–receptor interactions and what constraints arise from them? Answers to these and related questions will influence the way we understand the dependence of the cellular response on receptor mobility, receptor–receptor interactions, and receptor–ligand interactions. An important starting point is to measure the receptor distribution on cell surfaces *prior* to hormone binding.

The polypeptide hormone epidermal growth factor (EGF) acts through its cell surface receptor to induce cell proliferation (Schlessinger, 1986; Defize et al., 1989). Its mode of action appears to involve receptor microclustering (Schreiber et al., 1983; Yarden & Schlessinger, 1986), a mechanism thought to be common to many receptor systems. The A431 epidermoid carcinoma cell line has been used extensively in the study of the mechanism of action of the EGF receptors and is very well characterized biochemically and biophysically (Kawamoto et al., 1983; Moolenaar et al., 1987; Wiley, 1988; Roy et al., 1989; Bellot et al., 1990). Detailed analysis of the EGF-receptor organization on A431 cells has been attempted using ferritin-labeled EGF (Kawamoto et al., 1983; Mckanna et al., 1979) and monoclonal antibodies directed against the EGF receptors which are then labeled with gold-conjugated protein

† This work was supported by the Natural Sciences and Engineering Research Council of Canada.

0006-2960/92/0431-2459$03.00/0
