Molecular Crystals and Liquid Crystals

ISSN: 1542-1406 (Print) 1563-5287 (Online) Journal homepage: http://www.tandfonline.com/loi/gmcl20

Luminescence of telomeric fragments of DNA macromolecule

Valeriy M. Yashchuk, Vladislav Yu. Kudrya, Igor Ya. Dubey, Kateryna I. Kovalyuk, Olesya I. Batsmanova, Volodymyr I. Mel'nik & Georgiy V. Klishevich

To cite this article: Valeriy M. Yashchuk, Vladislav Yu. Kudrya, Igor Ya. Dubey, Kateryna I. Kovalyuk, Olesya I. Batsmanova, Volodymyr I. Mel'nik & Georgiy V. Klishevich (2016) Luminescence of telomeric fragments of DNA macromolecule, Molecular Crystals and Liquid Crystals, 639:1, 151-159, DOI: 10.1080/15421406.2016.1255068

To link to this article: http://dx.doi.org/10.1080/15421406.2016.1255068

Published online: 14 Dec 2016.

MOLECULAR CRYSTALS AND LIQUID CRYSTALS
2016, VOL. 639, 151–159
http://dx.doi.org/10.1080/15421406.2016.1255068

Taylor & Francis
Taylor & Francis Group

Luminescence of telomeric fragments of DNA macromolecule

Valeriy M. Yashchuk[a], Vladislav Yu. Kudrya[a], Igor Ya. Dubey[b], Kateryna I. Kovalyuk[a], Olesya I. Batsmanova[a], Volodymyr I. Mel'nik[c], and Georgiy V. Klishevich[c]

[a]Faculty of Physics, National Taras Shevchenko University of Kyiv, Kyiv, Ukraine; [b]Institute of Molecular Biology and Genetics of NAS of Ukraine, Kyiv, Ukraine; [c]Institute of Physics of NAS of Ukraine, Kyiv, Ukraine

ABSTRACT

Optical absorption, fluorescence and phosphorescence spectra of the telomere fragment d(AGGGTTAGGGTTAGGGTTAGGG) (Tel22) were studied and compared with those of the native DNA. Main centers of optical absorption in telomeres are A, T and G nucleic bases and G-quadruplexes. The fluorescence of telomeres is associated mainly with G-bases and other long-wave centers, possibly G-quadruplex structures, whereas their phosphorescence is associated with AT-sequences as it takes place for the native DNA. A significant increase of phosphorescence-to-fluorescence intensity ratio was observed for Tel22 as compared to DNA. Results obtained are promising for the detection of DNA macromolecules containing the extended telomeric sequences.

KEYWORDS

telomere; DNA; G-quadruplex; fluorescence; phosphorescence; electronic excitation energy transfer

1. Introduction. Structure and functionality

Telomeres are the structures found at the ends of chromosomes consisting of a large number of repeating short guanine-rich nucleotide sequences (in humans – TTAGGG) called telomeric repeats (fig. 1). It was shown [1, 2] that these terminal DNA sequences determine the number of possible cell divisions and therefore the life span of cells. Telomeres participate in many key biological processes, including the apoptosis, aging and tumor transformation of the cell. So the studies, including the spectroscopic investigations, of these specific molecular systems are of high importance for biology, medicine and anticancer drug design [3, 4].

Up to now the spectral studies of the telomeres were focused mainly on optical absorption. These studies led to the discovery of so called G-quadruplexes in telomeres. G-quadruplex (G4) structures are formed by the stacks of guanine quartets connected by specific non-canonical systems of hydrogen bonds [3, 5-9]. In this paper we have studied the autofluorescence and autophosphorescence of model molecular system, 22-mer oligonucleotide d(AGGGTTAGGGTTAGGGTTAGGG), a fragment of the human telomeric DNA containing three full and one incomplete telomeric repeats (further referred to as Tel22).

2. Experimental

The oligonucleotide d(AGGGTTAGGGTTAGGGTTAGGG) (Tel22) was purchased from Eurogentec (Belgium), the salmon sperm DNA was obtained from Roth (Germany), deoxynucleoside monophosphates (dAMP, dGMP and dTMP) and ribonucleotides (rAMP,

CONTACT Valeriy M. Yashchuk yashchukvaleriy@gmail.com

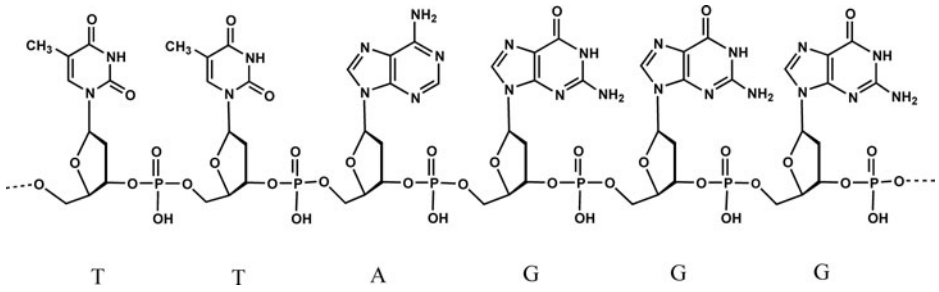

Figure 1. The structure of repeating fragment of telomere DNA (telomeric repeat).

rGMP and rCMP) were from Fluka (Switzerland). DNA concentration in water solution was determined spectrophotometrically using the extinction coefficient $\varepsilon_{260} = 6600\ M^{-1}cm^{-1}$ (per mole of nucleotide pairs) [10].

The steady state fluorescence and time-resolved phosphorescence measurements were performed with a Hitachi MPF-4 spectrofluorimeter. Fluorescence and phosphorescence were excited in the wavelength range 240–310 nm. Optical absorption spectra were recorded on a Specord UV-VIS spectrophotometer (Karl Zeiss Jena, Germany). The measurements were carried out in water at 4.2K, 77K and at ambient temperature, as previously described [11–15].

3. Results and discussion

3.1. The spectral properties of the DNA

As can be seen from the chemical structure of telomere (fig. 1), this molecular system contains T, A and G nucleic bases (fig. 2). So, for the understanding of properties of this system let us review the main photophysical features of DNA and nucleotides which are the elementary DNA fragments.

It is known [11–14] that DNA bases contribute mainly independently to total absorption spectrum of DNA macromolecule. This provides the ground to build the electronic energy diagram of DNA based on the excited singlet (S_1) and triplet (T_1) levels of model compounds dAMP, dGMP, dTMP and dCMP (note the last is absent in the human telomeric sequence) [11–13]. This diagram can be evaluated from their optical absorption, fluorescent and phosphorescent spectra. The slight difference between absorption spectral bands of the DNA and the sum of corresponding model nucleotides spectral bands according to [16] is connected with slight interaction between complementary π-electron systems of base pairs.

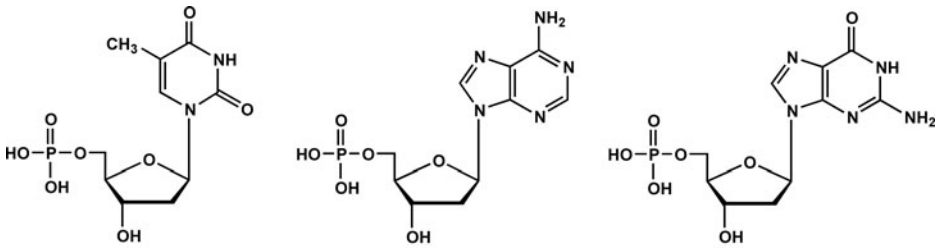

Figure 2. Deoxymononucleotides – the elementary fragments of the telomeric DNA.

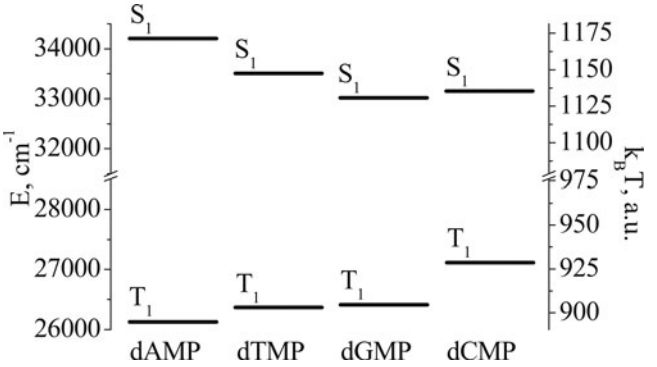

Figure 3. The energy diagram for the DNA.

Let us examine the DNA electronic energy diagram presented in our previous papers [11–13] (fig. 3). The similar tendency for the energy levels displacement was obtained for the RNA macromolecule too (the only difference is the thymidine is changed for uridine) [13]. Our scheme is more correct than one proposed in [17] (that is shown in [12]).

From this illustration it is easy to conclude that singlet excitations tend to localize on G bases (i.e. on the lowest S_1 level). As it follows from [11–13] the levels of G and C bases were very close (the difference between the C and G energy levels is less than value of kT at 77K). So only G and C bases contribute to the fluorescence spectrum of the DNA (and that of a model oligonucleotide d(CCCGGGTTTAAA) specially designed to study the nature of singlet and triplet excitation traps in the DNA) (fig. 4,a) [11–13]. The same situation was observed for the RNA (fig. 4,b) [13, 15].

As it follows from the energy diagram, the triplet excitations have to localize on adenine bases. We have shown that this tendency is indeed realized in the RNA [13, 15]. The electronic vibration structure that is intrinsic for adenine phosphorescence (fig. 5,c.4 – model compound rAMP, for comparison see fig. 5,c.5 – d(AAAAAAAAAAAA) homodeoxyoligonucleotide) is observed in the RNA phosphorescence spectrum, fig. 5,c.3).

In contrast to the RNA, in the DNA (fig. 5,c.1) [11–14] (as well as in d(CCCGGGTTTAAA) model oligonucleotide – see fig. 5,c.2) triplet traps associated with AT-sequence are formed. That's why the phosphorescence of DNA is associated with the emission of AT-complex.

3.2. Main features of electronic excitation energy transfer (EEET) in DNA

The electronic excitation energy transfer (EEET) was the goal of many researchers. Part of them was carried out at 77K in 1960–1970 [17–18]. Starting from 80-th the papers appeared where electronic processes in the DNA were studied at ambient temperatures [19–23]. Auto-luminescent investigations on the DNA at ambient temperatures became possible due to using synchrotron [24] and laser excitations [23, 25] as well as luminescence enhancement by nanoparticles using [26] (quantum yield of the DNA is $B \sim 10^{-5}$ at ambient temperatures). In the majority of these works authors prove only the existence of EEET in the DNA and the models of EEET [23]. Only in one paper [27] the attempt to evaluate the resulting spreading lengths of moving excitations was done. Our investigations were carried out not only at 77K but at 4,2K and 35K. Besides the proofs of EEET presence in the DNA and RNA the resulting spreading lengths of mobile singlet and triplet excitations were evaluated. The main arguments in favor of the existence of EEET are: (1) the quenching of DNA/RNA fluorescence or phosphorescence by little amount of aromatic guest molecules incorporated into a host

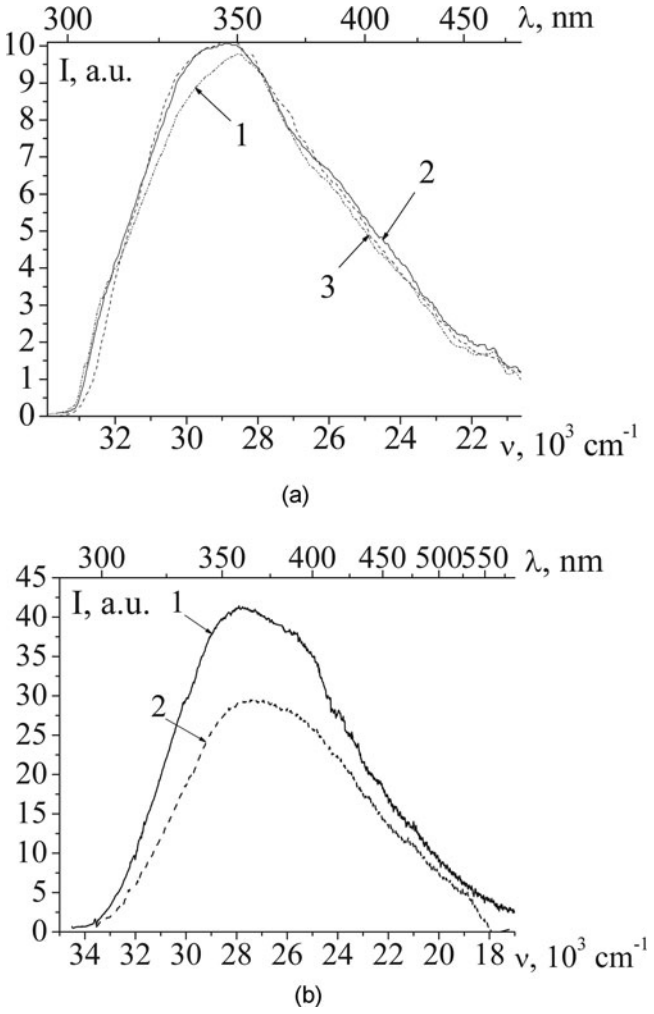

Figure 4. The fluorescence spectra of: (a) DNA (1), d(CCCGGGTTTAAA) (2) and linear combination of the spectra of deoxyribonucleotides dGMP and dCMP (3); (b) RNA (1) and linear combination of the spectra of ribonucleotides rGMP and rCMP (2). Excitation at 260 nm. Water solutions, $C = 10^{-4}$ M, T = 4,2K.

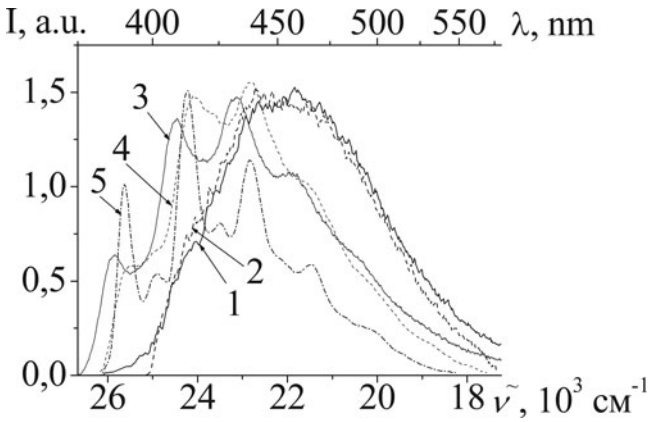

Figure 5. The phosphorescence spectra of DNA (1), d(CCCGGGTTTAAA) (2), RNA (3), rAMP (4) and d(AAAAAAAAAAAA) (5). Excitation at 300 nm. Water solutions, $C = 10^{-4}$ M, T = 4,2K.

macromolecule under excitation in DNA/RNA main band; (2) the depolarization of guest molecules fluorescence under excitation in the main band of a host macromolecule; (3) the appearance of delayed fluorescence of guest molecules under excitation in the main band of a host macromolecule [28–30].

From our data for the migrating singlet excitations in DNA the resulting (l_s) migration displacement length is about 10 base pairs [30], for the triplet excitations – l_t is about 20 base pairs [11–13, 28]. For the migrating S,T-excitations in RNA, $15<l_s<30$ base pairs, $l_t>30$ base pairs [13, 29].

3.3. Main peculiarities of the spectral properties of telomeres

3.3.1. Absorption, G-quadruplex structures

Optical absorption spectrum of the telomeric oligonucleotide Tel22 (fig. 6) is close to the DNA absorption spectrum. However, a weak shoulder in 295 nm region appears which is not observed in the DNA spectrum. According to a number of papers [6–9, 31], this additional absorbance is associated with G-quadruplex structures formed by the folding of specific guanine-rich nucleotide sequences, including that of Tel22. G-quadruplex assemblies are typically stabilized by K^+ and Na^+ cations [3, 5]. In our experiments performed in water these structures were not stabilized, so their content in the set of oligonucleotide conformations present at ambient temperature, most probably, was not high, and they were destroyed upon the heating of oligonucleotide sample up to 90°C.

The 295 nm band (that is associated with with G-quadruplex structures, see inset in fig. 6) was extracted from the absorption spectrum curve of the intact Tel22 oligonucleotide by the subtraction of the absorption spectrum curve of this oligonucleotide heated to 90°C (fig. 6).

So, an additional optical center appears in the telomere. The spectral manifestation of this center in absorption proves that it possesses the ground state, in contrast to AT trap [11–14] that is not observed in the absorption spectrum.

3.3.2. Fluorescence

At room temperature the telomere does not demonstrate any luminescence. On fig. 7 the fluorescence of investigated telomere fragment Tel22 at low temperature is presented. Under

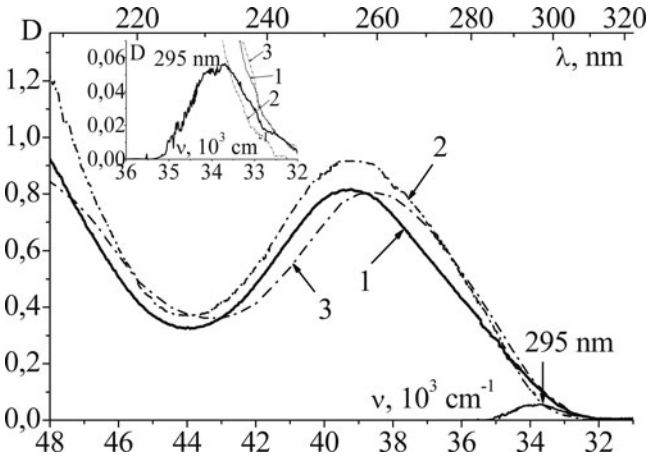

Figure 6. The optical absorption spectra of intact Tel22 at ambient temperature (1), Tel22 heated to 90°C (2), DNA (3) in water. Inset – a detailed view of the extracted 295 nm shoulder.

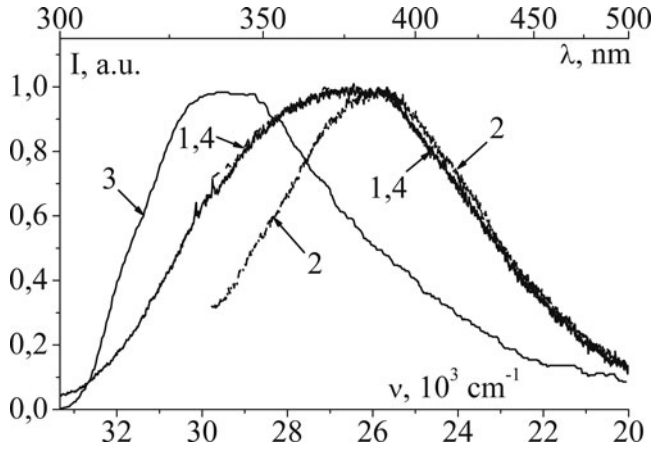

Figure 7. The fluorescence spectra of Tel22 oligonucleotide with $\lambda_{ex} = 260$ nm (1) and $\lambda_{ex} = 310$ nm (2), dGMP (3), the linear combination of the spectral curves of dGMP and the sample 2 (ratio 2:3) (4). Water solutions, $C = 10^{-4}$ M, T = 4,2K.

excitation by wavelength 260 nm the fluorescence spectrum (fig. 7,c.1) is complete and significantly differs from the DNA fluorescence spectrum (fig. 4a,c.1). Under excitation by wavelength 310 nm the fluorescence spectrum with single long-wave shifted band (maximum 380 nm) was observed (fig. 7,c.2). It is easy to see that the fluorescence spectrum of Tel22 excited at 260 nm consists of two bands. One of them (short-wave) could be associated with DNA fluorescence with dominant guanine contribution. Another (long-wave) one is not presented in typical fluorescence spectrum of DNA and coincides with the fluorescence band of Tel22 excited at 310 nm. This band is possibly associated with G-quadruplex optical center formed in Tel22. This is confirmed by the fact that this emission is dominant under long-wave excitation at 310 nm where any model compound – free nucleotide – cannot be excited.

The curve of linear combination of the fluorescence spectra of dGMP and Tel22 excited at 310 nm (taken in 2:3 ratio) (fig. 7,c.4) is close to the curve of the fluorescence spectrum of Tel22 excited at 260 nm (fig. 7,c.1). That is the additional confirmation of the presence of two fluorescent centers in the investigated telomere fragment.

3.3.3. Phosphorescence

The phosphorescence spectrum of investigated fragment of telomere (Tel22) does not depend on excitation wavelength in the range 240–310 nm and practically coincides with the phosphorescence spectra of the DNA and a number of poly- and oligonucleotides which, according to [11–14], are based on the emission of AT-complex (fig. 8).

3.3.4. The phosphorescence/fluorescence intensities ratio for telomeres

A significant increase of the ratio of phosphorescence-to-fluorescence intensity was observed for Tel22 oligonucleotide. This ratio was ~2,4 times higher for Tel22 in comparison with the DNA (fig. 9). The nature of this interesting effect and possible role of G-quadruplex structures in this phenomenon has to be studied yet. This future study should include the experiments with stable, specially prepared individual G-quadruplexes stabilized with Na^+ or K^+ cations. Nevertheless, this effect can be potentially used for the detection of the DNA sequences containing long telomeric tail.

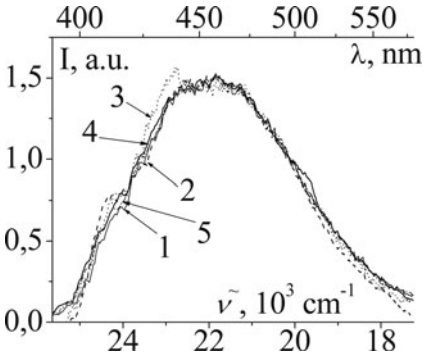

Figure 8. The phosphorescence spectra of DNA (1), d(CCCGGGTTTAAA) (2), poly(dAdT)$_2$ (3) under excitation $\lambda_{ex} = 300$ nm; Tel22 under excitation $\lambda_{ex} = 260$ nm (4) and $\lambda_{ex} = 300$ nm (5) at T = 4,2K.

3.3.5. EEET in telomeres

The fluorescence spectra of telomeric fragment Tel22 obtained in this work are not just simple combinations of the spectra of nucleic bases present in this oligonucleotide. As it was mentioned above, these spectra can be associated with the contribution of two emitting centers that have the lowest S_1 energy levels (fig. 10): guanine bases and some long-wave singlet excitations traps (possibly, G-quadruplexes).

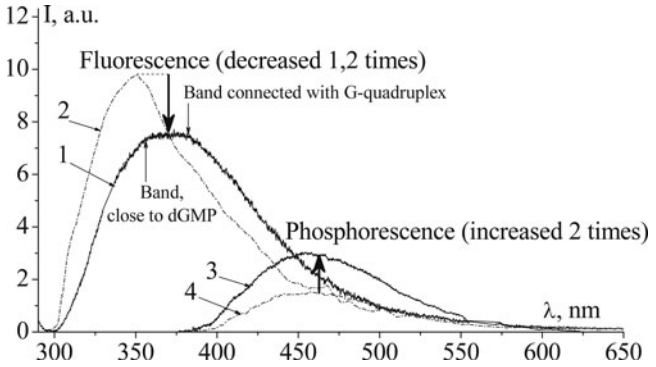

Figure 9. The fluorescence (1,2) and phosphorescence (3,4) spectra of Tel22 (1,3) and DNA (2,4). T = 4,2K, $\lambda_{ex} = 280$ nm.

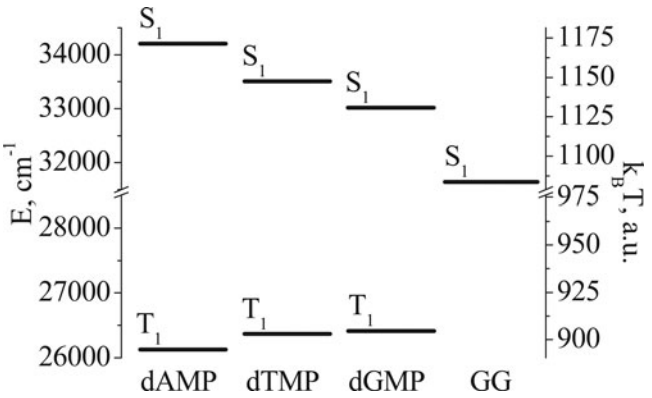

Figure 10. The energy diagram for the telomere (GG is G-quadruplex).

The resulting distance of the spreading of mobile singlet excitations in the DNA [11, 12, 30] is about 10 bases sequence. So, short-wave G-traps fluorescence must dominate. Nevertheless, some contribution of long-wave traps to the telomere fluorescence was observed. This fact demonstrates that part of singlet excitation energy transfers to these traps (possibly, G-quadruplexes).

Triplet EEET in the telomeres leads to the domination of AT-traps in their phosphorescence.

4. Conclusions

Main conclusions of the present spectroscopic study on the telomere fragment – oligonucleotide d(AGGGTTAGGGTTAGGGTTAGGG) (Tel22) – are summarized as follows.

1. Main centers of optical absorption in the telomeres are A, T and G bases and G-quadruplexes.
2. Fluorescence of the telomere is associated mainly with G-bases and other unknown long-wave emitting centers, possibly G-quadruplexes.
3. Phosphorescence of the telomere is associated with AT-sequences. The 2.4-fold increase of the phosphorescence-to-fluorescence intensity ratio for telomeric oligonucleotide over DNA is observed. The nature of this phenomenon has to be further investigated.
4. EEET in the telomeres is the main reason of domination of AT-traps in their phosphorescence. The singlet traps act rather weakly in the telomere fragment.
5. Possible application of the observed effects is the detection of DNA macromolecules containing long telomeric sequences, mainly using the autofluorescence of long-wave telomeric optical centers and the anomalous phosphorescence/fluorescence intensity ratio.

References

[1] Armanios, M., & Blackburn, E. H. (2012). *Nature Rev. Genet.*, *13*, 693.
[2] Rudolph, K. L. (2010). *Telomeres and Telomerase in Aging, Disease, and Cancer*, Springer: Berlin-Heidelberg, Germany.
[3] Xu, Y. (2011). *Chem. Soc. Rev.*, *40*, 2719.
[4] Ruden. M., & Puri, N. (2013). *Cancer Treat. Rev.*, *39*, 444.
[5] Burge, S., Parkinson, G. N., Hazel, P., Todd, A. K., & Neidle, S. (2006). *Nucleic Acids Res.*, *34*, 5402.
[6] Phan, A. T., & Mergny, J. -L. (2002). *Nucleic Acids Res.*, *30*, 4618.
[7] Gray, R. D., & Chaires, J. B. (2008). *Nucleic Acids Res.*, *36*, 4191.
[8] Vummidi, B. R., Alzeer, J., & Luedtke, N. W. (2013). *ChemBioChem.*, *14*, 540.
[9] Changenet-Barret, P., Hua, Y., & Markovitsi, D. (2015). *Topics Curr. Chem.*, *356*, 183.
[10] Reichmann, M. E., Rice, S. A., Thomas, C. A., & Doty, P. (1954). *J. Am. Chem. Soc.*, *76*, 3047.
[11] Yashchuk, V., Kudrya, V., Losytskyy, M., Suga, H., & Ohul'chanskyy, T. (2006). *J. Mol. Liq.*, *127*, 79.
[12] Yashchuk, V. M., Kudrya, V. Yu., Losytskyy, M. Yu., Dubey, I. Ya., & Suga, H. (2007). *Mol. Cryst. Liq. Cryst.*, *467*, 311.
[13] Kudrya, V. Yu., & Yashchuk, V. M. (2012). *Ukr. Phys. J.*, *57*, 187.
[14] Yashchuk, V., Kudrya, V., Gryn, D., Yatsenko, L., Volchenskova, I., Naumenko, A., & Yevtushenko, N. (2010). *J. Mol. Liq.*, *153*, 159.
[15] Kudrya, V. Yu., Yashchuk, V. M., Levchenko, S. M., Mel'nik, V. I., Zaika, L. A., & Govorun, D. M. (2008). *Mol. Cryst. Liq. Cryst.*, *497*, 93.
[16] Emanuele, E., Markovitsi, D., Milli, Ph., & Zakrzewska, K. (2005). *ChemPhysChem.*, *6*, 1387.
[17] Gueron, M., Eisinger, J., & Shulman, R. G. (1967). *J. Chem. Phys.*, *47*(10), 4077.

[18] Helene, C., & Longworth, J. W. (1972). *J. Chem. Phys.*, *37*(1), 399.
[19] Nordlund, T. M., Xu, D., & Evans, K. O. (1993). *Biochemistry*, *32*, 12090.
[20] Kawai, M., Lee, M. J., Evans, K. O., & Nordlund, T. M. (2001). *J. Fluorescence*, *11*, 23.
[21] Georghiou, S., Zhu, S., Weidner, R., Huang, C. -R., & Ce, G. (1990). *J. Biomolecular Structure and Dynamics*, *8*(3), 657.
[22] Markovitsi, D., Onidas, D., Gustavsson, T., Talbot, F., & Lazzarotto, E. J. (2005). *J. Am. Chem. Soc.*, *127*, 17130.
[23] Vaya, I., Gustavsson, T., Douki, T., Berlin, Y., & Markovitsi, D. (2012). *J. Am. Chem. Soc.*, *134*, 11366.
[24] Ballini, J. P., Vigny, P., & Daniels, M. (1983). *Biophys. Chem.*, *18*, 61.
[25] Anders, A. (1981). *Chem. Phys. Lett.*, *81*(2), 270.
[26] Lakowicz, J. R., Sen, B., Gryczynski, Z., D'Auria, S., & Gryczynski, I. (2001). *Biochem and Biophys. Ressearch Comm.*, *286*, 875.
[27] Bersohn, A., & Isenberg, I. (1964). *J. Chem. Phys.*, *40*, 3175.
[28] Tokar, V. P., Losytskyy, M.Yu., & Yashchuk, V. M. (2008). *Ukr. Phys. J.*, *53*, 1149.
[29] Levchenko, S. M., Tokar, V. P., & Yashchuk, V. M. (2011). *Functional Materials*, *18*(3), 1.
[30] Losytskyy, M.Yu., Yashchuk, V. M., & Yarmoluk, S. M. (2000). *Functional Materials*, *7*(4), 1.
[31] Mergny, J. -L., Phan, A. -T., & Laxcroix L. (1998). *FEBS Lett.*, *435*, 74.