EFFECT OF TiO_2 NANOPARTICLES ON THE THERMAL STABILITY OF NATIVE DNA UNDER CONDITIONS CLOSE TO PHYSIOLOGICAL ONES

E. L. Usenko[1], A. Yu. Glamazda[1,2], V. A. Valeev[1], V. A. Karachevtsev[1]

[1]B.Verkin Institute for Low Temperature Physics and Engineering of NAS of Ukraine, 47 Nauky Ave., Kharkiv, 61103, Ukraine

[2]V. N. Karazin Kharkiv National University, 4 Svobody sq., Kharkiv, 61022, Ukraine

e-mail: usenko@ilt.kharkov.ua

At the present, TiO_2 nanoparticles (NPs) are quite intensively used in the environmental engineering, cosmetology, pharmaceuticals, and medicine [1]. But, despite the growing demand for these NPs in production and medicine, numerous works are currently appearing, indicating their possible negative impact on human health, as well as their genotoxicity [2].

The present work is devoted to the study of the thermostability of native DNA upon its binding to TiO_2 NPs depending on their concentration at near physiological ionic conditions (0.1 M Na^+, pH 7) using thermal denaturation method.

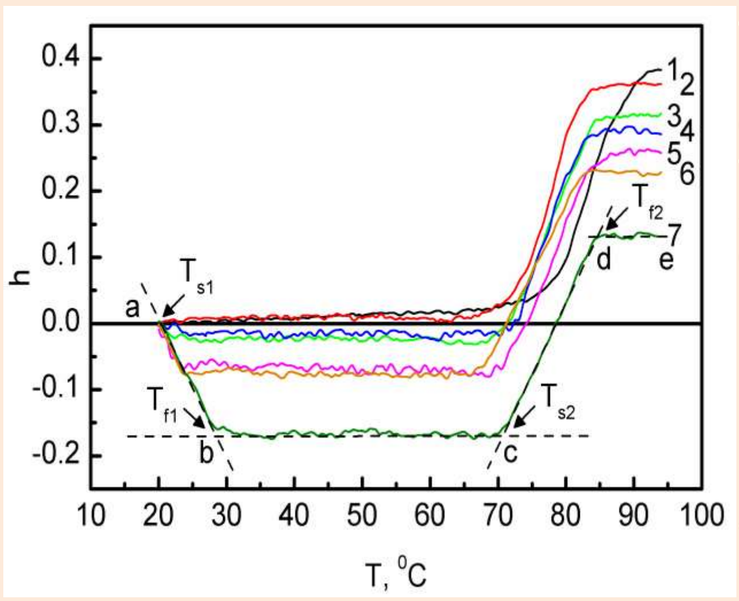

Fig. 1. Temperature dependence of hyperchromic coefficient (h) of DNA without (curve 1) and with (curves 2-7) TiO_2 NPs (0,1 M Na^+, pH 7): «1» - $[c_{TiO2}] = 0$; «2» - $[c_{TiO2}] = 2.5 \times 10^{-5}$ M; «3» - $[c_{TiO2}] = 5 \times 10^{-5}$ M; «4» - $[c_{TiO2}] = 10^{-4}$ M; «5» - $[c_{TiO2}] = 1.5 \times 10^{-4}$ M, «6» - $[c_{TiO2}] = 1.75 \times 10^{-4}$ M; «7» - $[c_{TiO2}] = 2 \times 10^{-4}$ M.

The melting curve of DNA exhibits the usual S-like curve upon heating caused by only one spiral-coil transition (Fig. 1). Upon the TiO_2 NPs injection into the DNA suspension, there are no noticeable changes in the shape of the melting curve up to $[c_{TiO2}] = 2.5 \times 10^{-5}$ M. However, there is a trough appears in the shape of the melting curve upon injection of $[c_{TiO2}] = 5 \times 10^{-5}$ M. According to Ref. [3], the absorption hypochromism observed in the segment of a-b with increasing temperature is caused by the formation of a more ordered structure of the biopolymer bound to TiO_2 NPs. In the segment of b-c, the formed DNA:TiO_2 NP nanoassemblies remain stable. This is somewhat different from the studies performed at pH 5 [4], where the agglomeration of DNA:TiO_2 NP nanoassemblies was observed over the segment of b-c. The observed hyperchromism on the melting curve at T > 70 °C (the segment of c-d) is due to the helix-coil transition.

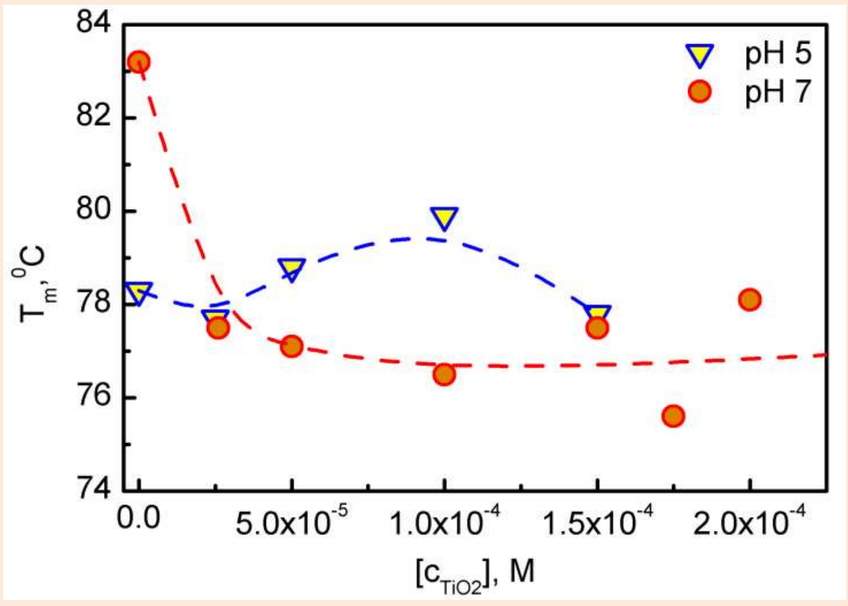

Fig. 2. The DNA melting temperature dependences on $[c_{TiO2}]$ at pH 5 [3] and pH 7. The dashed lines are for the eye guideline.

From this figure, it is seen that the addition of $[c_{TiO2}] = 2.5 \times 10^{-5}$ M to the DNA solution leads to a noticeable decrease in the DNA melting temperature by ~ 6 °C. The further increase in $[c_{TiO2}]$ stimulates slight changes in T_m (changes are within 1.9 °C). The decrease in T_m observed at $[c_{TiO2}] \leq 2.5 \times 10^{-5}$ M is because that the main contribution to the concentration dependence of the melting temperature is caused by the interaction of TiO_2 NPs with nitrogenous bases of DNA. T_m describes a very weak dependence on $[c_{TiO2}]$ exceeding 2.5×10^{-5} M that is apparently due to compensation for the effects causing an increase and decrease in the thermal stability of DNA due to the implementation of all possible types of binding of DNA to TiO_2 NPs. It should be noted that in our previous studies performed at pH 5, slight changes in the melting temperature were observed in the entire studied range of $[c_{TiO2}]$ [3].

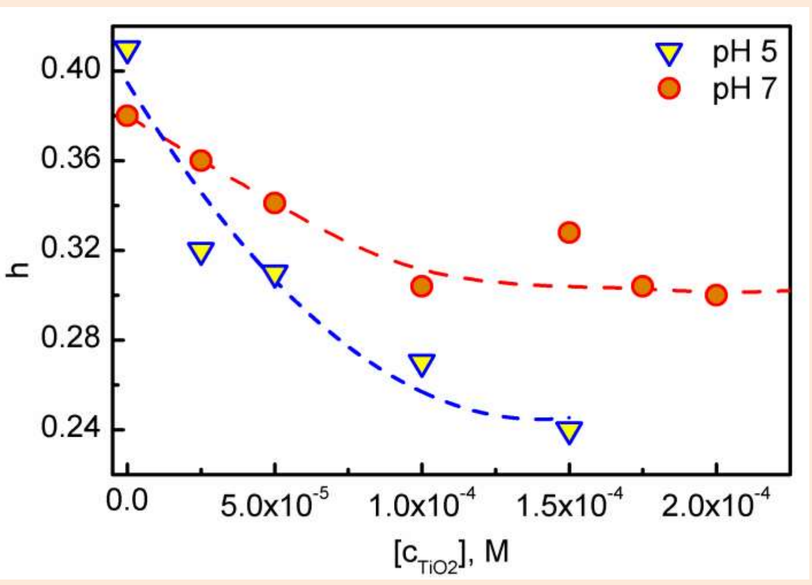

Fig. 3. The dependence of the DNA hyperchromic coefficient (h) in the presence of TiO_2 NPs on $[c_{TiO2}]$ at pH 5 [3] and pH 7. The dashed lines are for the eye guideline.

It can be seen from this figure that the addition of $[c_{TiO2}] = 1.0 \times 10^{-4}$ M to the DNA suspension leads to a decrease in the value of h($[c_{TiO2}]$) by ~ 21 % at pH 7 and by ~ 34 % at pH 5. Further addition of TiO_2 NPs to the DNA solution practically does not change the value of this parameter. As noted above, the decrease in the melting temperature is associated with the interaction of nitrogenous bases of DNA (in particular, the N7 atoms of DNA guanine [3]) with a surface of TiO_2 NPs. It leads to the appearance of unwound regions in the structure and, accordingly, to a decrease in the degree of helicity of the polynucleotide. Thus, the observed decrease in h is precisely caused by this effect. In addition, as noted above, at pH 7, TiO_2 NPs are negatively charged (at pH 5, NPs are positively charged). Despite the fact that the high ionic strength of the suspension partially compensates for the negative charge on the NP surface, nevertheless, the remaining electrostatic repulsion between TiO_2 NPs and DNA phosphate groups weakens the interaction between NPs and DNA. Such an effect is reflected in a more smooth change in the value of h at pH 7 compared with pH 5.

Conclusions

The study of the effect of temperature and TiO_2 NPs concentration on the stability and conformation of DNA under conditions close to physiological (0.1 M Na^+, pH 7) was performed by the thermal denaturation. The analysis of the DNA melting curves in the presence of TiO_2 NPs revealed a temperature range in which the light absorption of DNA decreases. We believe that the observed effect is explained by the unwound DNA regions can bind to NPs and form more ordered structure on the NP surface than double-stranded DNA at initial moment. The injection of $[c_{TiO2}] = 2.5 \times 10^{-5}$ M into DNA suspension leads to a decrease in the DNA melting temperature by ~ 6°C at pH 7. It is assumed that this effect is due to the predominant interaction of the nitrogenous bases of DNA with these NPs.

The results obtained in this study can be used to create various hygiene products, as well as medicine and pharmacology.

References

[1] S. Shabbir, M. Fakhar-e-Alam Kulyar, Z. A. Bhutta, P. Boruah, and M. Asif, BioNanoScience. 11, 621 (2021). https://doi.org/10.1007/s12668-021-00836-3.

[2] S. Patel, P. Patel, and S.R. Bakshi, Cytotechnology. 69, 245 (2017). https//doi.org/10.1007/s10616-016-0054-3.

[3] E. Usenko, A. Glamazda, V. Valeev, A. Svidzerska, A. Laguta, S. Petrushenko, and V. Karachevtsev, Appl. Phys.A. 128, Article number: 900 (2022). https://doi.org/10.1007/s00339-022-06043-5.

[4] E. Usenko, A. Glamazda, A. Svidzerska, V. Valeev, A. Svidzerska, A. Laguta, S. Petrushenko, and V. Karachevtsev, J. Nanopart. Res. 25,113 (2023). https//doi.org/10.1007/s11051-023-05770-x.