Study of Native DNA Thermal Stability in the Presence of TiO_2 Nanoparticles

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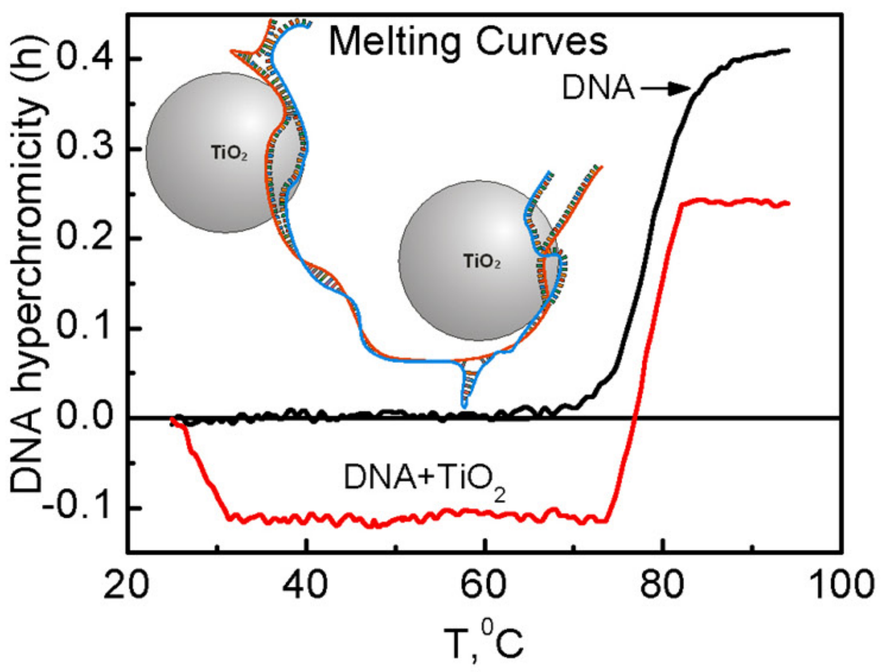

The intensive development of new nanomaterials during the last decades stimulates the detail study of the toxicological effect on the human health and the environment. The physico-chemical properties of the nanomaterials can differ significantly from intrinsic peculiarities to the bulk materials due to the geometric limitation of the propagation of quantum excitations. TiO_2 nanoparticles (NPs) occupy an important place in our life. They are applied for pharmaceuticals, paints, food colorants, cosmetics as well as antibacterial agents, etc. They are chemically inert, inexpensive, besides they have high thermal stability and high photocatalytic activity that can be revealed even under sunlight. The present work is devoted to the spectroscopic studies of the effect of the TiO_2 NPs ($d \le 100$ nm) on the DNA structural stability by UV spectroscopy and thermal denaturation. The sequential injection of the NPs portion into the DNA buffer solution (0.1 M Na^+, pH5) induced the partial DNA untwisting of the biopolymer structure. The appearance of the single-stranded DNA fragments can be caused by the damage effect produced by the reactive oxygen species generated by the NPs upon the light exposure. It's well known that this effect can be enhanced by the exposure of UV focused light. The study of the thermal stability of DNA in the presence of TiO_2 NPs revealed the stabilization of duplex structure with increasing the temperature from 25 to 44^{0}C. This stable structure keeps duplex one up to 77.5^{0}C. It is assumed that the observed biopolymer transformation is a consequence of the interaction of TiO_2 NPs with DNA, leading to the partial unzipping of duplex and then its ordering upon heating before duplex melting. The increase in the NPs concentration up to $1.75 \cdot 10^{-4}$M has induced the appearance of the "large" scattering centers. The performed estimation of their diameter size gives the value equaled to about 130 nm that is about 30 % more than the maximal size of NPs used in the present experiments. It can be explained by the convergence and interaction of NPs that are closely arranged on DNA. The most possible mechanisms of direct binding of TiO_2 NPs to DNA are discussed. The obtained results can be used in the development of self-cleaning antibacterial surfaces, as well as in the medical field.

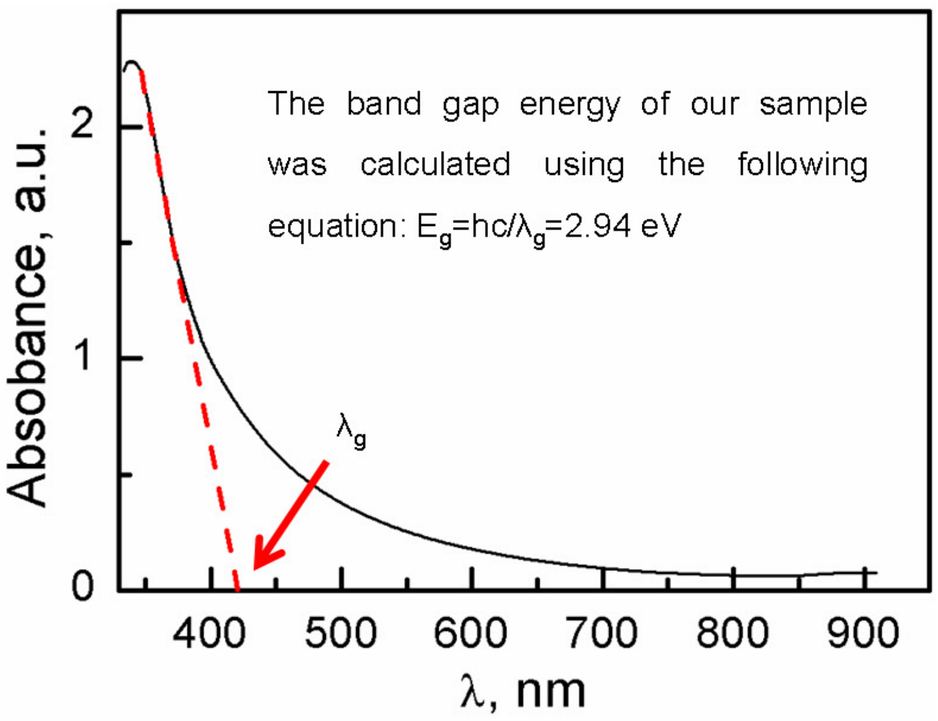

Fig.1. UV-Visible absorption spectrum of TiO_2 NPs dissolved in aqueous solution (for nanoparticles with d<100 nm).

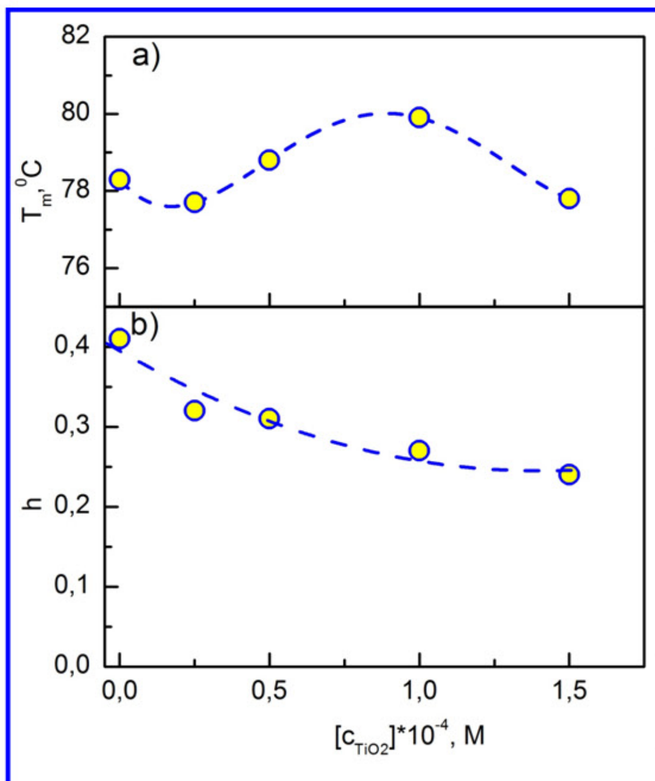

Fig. 3. **(a)** The DNA melting temperature dependence (T_m) vs $[c_{TiO2}]$. **(b)** The DNA hyperchromic coefficient dependence (h) vs $[c_{TiO2}]$. The blue dashed lines are B-spline interpolations. The size of error bars for T_m and h is smaller than that of the symbols.

The T_m dependence shows nonlinear behavior on $[c_{TiO2}]$ that may be caused by the complicated processes of the complexation of the biopolymer with NPs. The addition of $[c_{TiO2}]=2.5\times10^{-5}$ M to the DNA solution practically does not change the T_m value. The reason for this may be that the $T_m([c_{TiO2}])$ dependence is determined by the compensation effects inducing either increasing or decreasing the DNA thermal stability. They are caused by the realization of all possible types of binding of NPs to DNA.

The addition of $[c_{TiO2}]=2.5*10^{-5}$ M to DNA solution leads to the decrease in the h value by 18%. Further addition of TiO_2 to the solution practically does not change the h value.

The values of T_m and h extracted from the melting curves of DNA with $[c_{TiO2}]=1.75\cdot10^{-4}$ M and $[c_{TiO2}]=2\cdot10^{-4}$ M are not presented due to a light scattering distorting its shape.

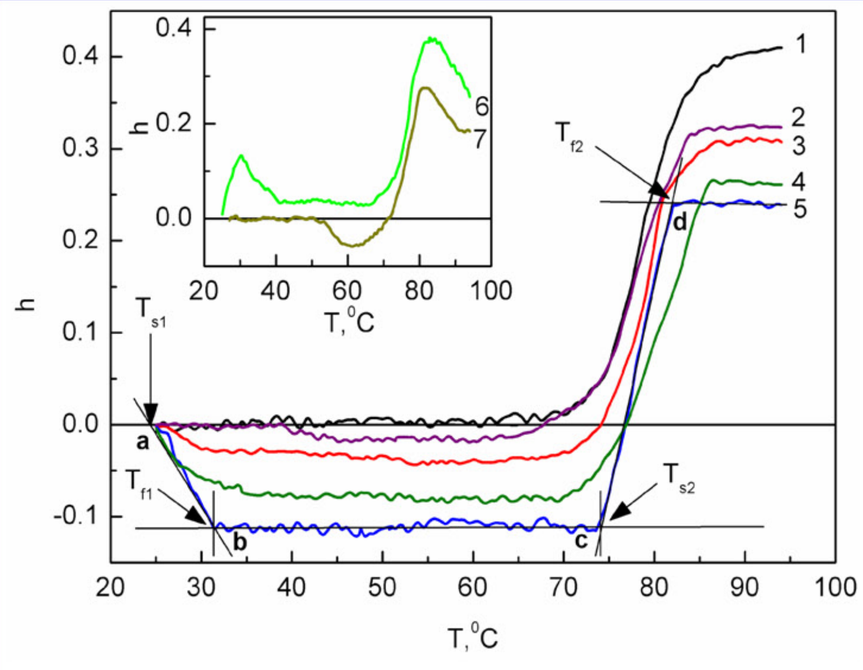

Fig.2 Temperature dependence of hyperchromic coefficient of DNA without (curve 1) and with (curves 2-7) TiO_2 NPs: «1» - $[c_{TiO2}]=0$; «2» - $[c_{TiO2}]=2.5\cdot10^{-5}$ M; «3» - $[c_{TiO2}]=5\cdot10^{-5}$ M; «4» - $[c_{TiO2}]=10^{-4}$ M; «5» - $[c_{TiO2}]=1.5\cdot10^{-4}$ M. The inset shows the temperature dependence of hyperchromic coefficient of DNA taken at $[c_{TiO2}]=1.75\cdot10^{-4}$ M (curve 6) and $[c_{TiO2}]=2\cdot10^{-4}$ M (curve 7).

The melting curves of DNA with TiO_2 in the nanoassemblies are characterized by the appearance of the trough that is increased with $[c_{TiO2}]$. As an example of the DNA melting curve in the complex with TiO_2 $[c_{TiO2}]=1.5\cdot10$-4 M, we'll show the its decomposition. In the segment of a-b, with an increase in temperature, the absorption hypochromism is observed, the value of which increases with the TiO_2 concentration. We believe that this effect is caused by the appearance of the additional stabilization of DNA in the presence of TiO_2 NPs. In the segment of b-c, the formed DNA structure remains stable (the value of h does not change). The defragmentation of the stable formed biopolymer structure occurs in the segment of c-d and ends with the plateau. With a further increase in $[c_{TiO2}]$, the biopolymer melting curves undergo strong changes. In particular, the absorption hypochromism is replaced by hyperchromism at the concentration NPs range of $1.75*10^{-4}$M-$2*10^{-4}$M, that is related to the formation of large DNA:TiO_2 NPs assemblies scattering light at $[c_{TiO2}]=1.75*10^{-4}$M and the appearance of the sediment at $[c_{TiO2}]=2*10^{-4}$M (curves 6 and 7 in inset). The estimation of the nanoassemblies size was carried out using Shifrin theory of light scattering (I.Y. Slonim, Opt. Spectrosk. **8,** 98 (1960)). The average radius of the formed DNA:TiO_2 NPs assemblies with $[c_{TiO2}]=1.75*10^{-4}$M is about R=680 Å, which is almost 30% larger than the declared maximum diameter of TiO_2 NPs used in the present studies.

CONCLUSION

The effect of TiO_2 NPs on the structural stability of DNA was studied using optical absorption spectroscopy with DNA thermal denaturation in the range of 20-94^{0}C. It was shown that upon DNA heating from about 25 to 44^{0}C the stabilization of DNA duplex occurs in the presence of TiO_2 NPs. This additional DNA stabilization indicates that partial DNA unwinding appears as a result of the direct binding of the biopolymer to NPs. DNA binding with TiO_2 NPs is manifested in the change of the DNA T_m and decreasing the hyperchromic coefficient that decreases with increasing NPs concentration. These changes of the parameters of DNA stability indicate the partial biopolymer duplex unwinding. The light scattering was observed in the TiO_2 NPs concentration range of $(1.75\div2)*10^{-4}$M. The size of the light scattering complexes of DNA with TiO_2 NPs was determined.

The data obtained in this work allow us to make the following conclusion that the adding of TiO_2 NPs in the buffer solution with DNA leads to partial DNA duplex unwinding. The consequence of this TiO_2 NPs effect on the living organism can manifest itself in a disruption of the normal functioning of the genetic apparatus of the cell, as well as mutagenesis and carcinogenesis. The obtained results can be also used in the creation of self-cleaning antibacterial surfaces, as well as in medicine.