Effect of TiO$_2$ nanoparticles on the thermal stability of native DNA under UV irradiation

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Abstract

TiO$_2$ nanoparticles (NPs) are widely used in the environmental engineering, medicine, chemical and food industries due to their unique photocatalytic and biocidal properties. NPs may generate reactive oxygen species and, hence, have the toxic effect on the living cells via oxidative stress. An external UV irradiation may magnify the photocatalytic properties of TiO$_2$ NPs. In this regard, we have analyzed the influence of TiO$_2$ NPs on the conformation and thermal stability of native DNA in a buffer suspension without and under UV irradiation exploiting absorption spectroscopy and thermal denaturation in the range of 20–94 °C. The TiO$_2$ NPs size distribution and polydispersity index were examined by dynamic light scattering (DLS) and confirmed by TEM. The DNA:TiO$_2$ NPs assemblies were revealed and characterized by DLS and TEM. Upon heating the DNA suspension with TiO$_2$ NPs from about 25 to 44 °C we have observed decreasing the hyperchromicity coefficient ($h$) on the DNA melting curves. That is explained by the intensive formation of the DNA:TiO$_2$ NPs assemblies. We have revealed, that partial DNA disordering appears at initial contacts with NPs. DNA binding to TiO$_2$ NPs is manifested in the change of the DNA melting temperature ($T_m$). We showed that the performed UV treatment of DNA during 3 h leads to partial unwinding of the biopolymer structure. The NPs injection to the biopolymer suspension induced the additional effect on the DNA thermal stability under UV irradiation. The performed analysis of the experimental data suggests that the nature of the impact of NPs on the biopolymer is complex.

Keywords TiO$_2$ nanoparticles · DNA · Differential UV spectroscopy · Thermal denaturation

1 Introduction

Recently, in nanoscience, considerable interest has been paid to the study of the problems of the effect of nanomaterials on biological systems. First of all, this is caused by a breakthrough in the complex multistep synthesis of new nanoscale materials which are found its wide application in the various spheres of human life.

In the nanomaterials, due to the confined geometry of the propagation of quantum excitations, new physical properties are generated which are not characteristic to their bulk material equivalents. Among such materials, TiO$_2$ nanoparticles (NPs) occupy the one of the important places in the nanotechnology. They are chemically inert, inexpensive, besides they have high thermal stability and high photocatalytic activity that can be revealed even under natural sunlight exposure. The latter factor can be enhanced by absorbing the focused UV irradiation. Under ultraviolet exposure, electrons in the valence band of TiO$_2$ NPs absorb the light with energy equal or more than the energy gap between the valence and conduction bands. The light absorption induces generating the electron–hole pairs: the excited electron from the valence band jumps to the conduction band, leaving the hole in the valence band. Two photoexcited electrons can participate in the reduction of oxygen and the holes can oxidize the water molecules adsorbed on the photocatalytic surface of NPs. The photoexcited electron can react with...
oxygen generating the reactive oxygen species (ROS), such as hydroxyl (OH) and peroxy (HO2) radicals, superoxide anions (O2−), and hydrogen peroxide (H2O2) which take part, for example, in the degradation of the organic pollutants [1]. It makes TiO2 nanoparticles attractive for antibacterial and self-cleaning surface coatings [2]. In addition, TiO2 NPs might be used as the promising carriers for drug delivery applications and the sensitive nanosensor elements for O2, H2O2, monitoring relative humidity, etc. [3–6]. Moreover, by the tuning of the size and shape of TiO2 NPs, it is possible to increase their biological activity and enhance the penetration ability into the body [7, 8]. Despite the large scale of production and extensive use of TiO2 NPs in various areas of human activity, their toxic properties have not been sufficiently studied, although at the moment extensive studies are being carried out to evaluate their safety and potential risks [3, 9–13]. Penetrating into the cell, nanoparticles are able to bind to the biological macromolecules such as DNA, lipids, causing their damage [14]. The stability of the DNA molecular structure plays a main role in the functioning of the cell. Similarly, drugs, temperature, and light are among the external environmental factors that can inhibit DNA replication, thereby influencing the way of the development and functioning of living organisms. All of that, ultimately, can affect the normal life processes of all organisms ranging from prokaryotes to mammals [15]. At the moment, there are already a number of works devoted to the study of the physicochemical properties of NPs (see, e.g., [16, 17]), as well as the interaction of DNA with TiO2 NPs (see, e.g., [18, 19]). In particular, in [20], the effect of TiO2 NPs on DNA was studied by spectroscopic methods. The published data indicate the presence of electrostatic interaction along with the formation of the chemical bonds between TiO2 NPs and DNA [20]. This binding induces the structural changes in the biopolymer itself.

The molecular mechanisms of DNA damage into HepG2 cells induced by TiO2 NPs were recently studied in Ref. [21]. There was shown that TiO2 NPs are recently studied in Ref. [21]. There was shown that TiO2 NPs are able to influence the gene expression in DNA. Also, authors confirmed an activation of the elevated oxidative stress, including the generation of ROS, with increased hydrogen peroxide levels, decreased glutathione peroxidase, and reduced glutathione and activated caspase-3 levels in cells exposed to TiO2 NPs. It was observed that TiO2 NPs induce the expression of seventeen DNA damage marker genes [21].

An experimental study including both chromosome aberration and comet assays at low dose exposure TiO2 NPs was reported in Ref. [14]. They performed the study of the binding affinity of NPs with DNA using fluorescence titration method. This work also reported that NPs have a high affinity for DNA and they can directly bind to the biopolymer. In addition, it has been shown that NPs are able to strongly inhibit DNA replication and change the polynucleotide conformation that can lead to genotoxicity [14]. The binding constant of TiO2 NPs with DNA was estimated to be ~ 4.2 × 106 M−1 [14]. Such a rather high value of the binding constant indicates a strong interaction between TiO2 NPs and DNA.

As mentioned above, other exogenous factors also have the destructive effect on DNA. One of them is UV radiation. It is known that focused long-term UV exposure can lead to damage to DNA and important photosynthetic structures, a cellular damage or even cell death. For example, in work [15], devoted to the study of the effect of UV radiation on DNA, it is reported that UV radiation is one of the powerful agents that can cause various mutagenic and cytotoxic DNA damage as well as disrupting the integrity of the genome. At the moment, there is no data in the literature on the study of the temperature effect on the stability of DNA conformation in the presence of TiO2 NPs as well as combined influence of the UV irradiation and TiO2 NPs on the biopolymer thermal stability.

The aim of this study is obtaining the information on the thermal stability of DNA upon the addition of TiO2 NPs in the wide temperature of 20–94 °C and NPs concentration range, as well as to study the interaction between DNA and TiO2 NPs under and without an exposure to intensive UV radiation. The TiO2 NPs size distribution and polydispersity index were examined by dynamic light scattering (DLS) and confirmed by transmission electron microscopy (TEM). The DNA:TiO2 NPs assemblies were revealed and characterized by DLS and TEM. We have found from TEM observations that the nanoassemblies formed in a buffer suspension remain stable after a precipitation on the film surface. Upon heating the hydrogen bonds between complementary bases in double strand DNA are broken creating unwound single strands. One of the stages of DNA replication in the living cell is unwinding the two strands by breaking the hydrogen bonds between the paired bases of the double helix [22]. This process is carried out by a special enzyme called helicase. Therefore, the thermal denaturation helps to estimate the stability DNA in the different surrounding and it is an analogue of the operation of helicase. Thus, the thermal studies can qualitatively characterize the efficiency of the binding of DNA and TiO2 NPs in nanoassemblies. We have revealed the addition of TiO2 NPs to the buffer suspension of DNA leads to the appearance of the unwound segments in its secondary structure. Heating of the DNA:TiO2 NPs suspension in the temperature range of about 25–44 °C leads to a decrease in the hyperchromicity coefficient (h). This effect may be due to the intensification forming the nanoassemblies as a result of the partial unwinding of DNA and the binding of DNA to TiO2 NPs. It was also found that UV irradiation of the DNA:TiO2 NPs suspension for 3 h causes partial denaturation of the biopolymer structure, and the intensification of the formation of the DNA:TiO2 NPs
assemblies occurs at higher temperatures in the range from 38 to 66 °C. The analysis of the obtained experimental data suggests that the interaction of TiO$_2$ NPs with a biopolymer has complex nature and could be explained by both the direct and indirect effects of TiO$_2$ NPs on DNA.

2 Materials

The TiO$_2$ NPs powder purchased from Sigma-Aldrich (particle diameter ($d$) < 100 nm (BET), ≥ 97%, contains 1% Mn as dopant, Product code: 677,469) was used in the present work. TiO$_2$ NPs were dispersed in distilled water with pH of 6.65 which is close to the isoelectric point. The NPs suspension was ultrasonicated with $\nu$ = 22 kHz for 40 min at room temperature. The complexes of TiO$_2$ NPs with DNA were prepared as follows: 1) the salmon sperm DNA ($M_w = (4–6) \times 10^6$ Da) purchased from Serva (Germany) was added to a buffer solution with $10^{-3}$ M sodium cacodylate (CH$_3$)$_2$AsO$_2$Na•3H$_2$O from Serva (Germany), 0.099 M NaCl at pH 5; 2) the required amount of the TiO$_2$ NPs was added to the DNA buffer suspension. The DNA phosphorous concentration [P] was $(7.14 \pm 1) \times 10^{-5}$ M that had been determined by the molar extinction coefficient at $\nu_m = 38,500$ cm$^{-1}$ [23]. To study the effect of UV radiation on the structural features of the biopolymer, the assemblies of TiO$_2$ NPs with DNA were irradiated with an ultraviolet light-emitting diode ($\lambda = 360–365$ nm) for 3 h (the power density of the incident light at the cuvette surface was $P = 19$ mW cm$^{-2}$). In the present work, DLS study of the DNA:TiO$_2$ NPs nanoassemblies was performed in the cacodylate buffer suspension (0.1 M Na$^+$, pH 5) at $[c_{TiO2}] = 1.5 \times 10^{-4}$ M. The concentration of polynucleotide phosphates [P] equaled to $8 \times 10^{-5}$ M.

3 Methods

3.1 Transmission electron microscopy (TEM)

Electron microscopic studies were performed by transmission electron microscope SELMI EM-125. For in situ electron diffraction studies, samples were sequentially condensed into fresh cleavages of KCl single crystals with an amorphous carbon sublayer deposited on them. A 3-μL sample drop was deposited and adsorbed for 1 min, and then, the excess of the suspension was removed with a piece of a filter paper.

3.2 Dynamic light scattering

The hydrodynamic size distribution of the suspended DNA:TiO$_2$ NPs nanoassemblies was measured using the dynamic light scattering (DLS) method. The hydrodynamic size ($d_{H}$) of nanoparticles consists of the real diameter plus the diameter of the electrostatic ionic potential around them. Thus the hydrodynamic size exceeds the real size of the nanoparticles. The particle size distributions were determined via DLS using Zetasizer Nano ZS (Red badge) ZEN 3600 Malvern Instruments apparatus. To measure the diffusion speed, the speckle pattern produced by illuminating the particles with a 4 mW 632.8 nm He–Ne gas laser and 175° detection optics allowing analyze the scattering information at close to 180°. The laser power was automatically attenuated so that the count rate from the sample, especially high scattering samples, was within acceptable limits.

3.3 UV-spectroscopy

The absorption spectra are caused by absorption of the light with the frequency $\nu$ by the molecule. The light absorption induces the transitions of the molecule from the ground to excited electronic states [24]. The light absorption within the ultraviolet range (UV) of 30,000–50,000 cm$^{-1}$ is caused by $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ electronic transitions in the nucleobases of the biopolymer. In the DNA double helix, the nitrogenous bases stack one above the other. The change in the conformation and structural stability of nucleic acids occurs under the influence of various factors (for example, temperature, ionic conditions, UV exposure, etc.) and causes significant changes in the UV absorption spectrum [25]. The extinction coefficient of a double helix depends on mutual orientation of the intrinsic dipole moments of stacking nitrogenous bases. If the moments of two stacked bases are collinear, the light absorption will decrease (hypochromism). But at the same time, the disordering of the stacked biopolymer structure induces the delocalization of the moments and the increase in the light absorption (hyperchromism). In this work, the dependence of the optical density of suspensions (A) of DNA with TiO$_2$ NPs on the wavenumber at room temperature ($T = T_0 = 25$ °C) was recorded using UV–visible spectrophotometer (Specord M40, Carl Zeiss Jena, Germany). The light power density of the radiation source was quite low that did not lead to a noticeable degradation of the samples. The taken optical spectra measured in this paper correspond to an equilibrium situation, because their shape and intensity remained unchanged for 20–30 min. The deuterium electrical lamp was used as UV radiation source and for and tungsten filament one was employed as a source of UV–visible optical ranges.

3.4 Thermal denaturation

The thermal measurements on DNA are important for study of the possible structural biopolymer conformations. The analysis of the thermal measurements allows to determine
some thermodynamic binding parameters of ligand to DNA, such as the melting temperature, the temperatures of the beginning and end of the helix-coil transition, the transition interval. In the present work, thermal denaturation was used to studying the structural stability of DNA in the presence of TiO₂ NPs. Upon increasing the temperature, the ordered structure of DNA becomes disturbed. As a result, the biopolymer forms loops which appear because of breaking some H-bonds between the nitrogen paired bases. When the temperature reaches a value close to the melting temperature (Tₘ), the number of the helical regions becomes equal to the number of the unwound regions. In other words, Tₘ is the temperature at which 50% of a DNA sequence is in the helix conformation, and the other 50% is present as single strands. A further increase in the temperature leads to a strong shift of equilibrium toward the increase in the fraction of the single strands. This process is accompanied by an increase in the UV absorption intensity. The dynamics of this increase stops when the double helix of the biopolymer is completely unwound. The melting curve describes the dependence of the UV absorption intensity on temperature. The melting curves of DNA with different TiO₂ concentrations were recorded using UV-spectrophotometer at fixed wavenumber of νₘ = 38,500 cm⁻¹ that corresponds to the maximum absorption of DNA. We have used the laboratory software allowing to perform the registration of the melting curves as the temperature dependence of the hyperchromicity coefficient: h(T)=[ΔA(T)/Aₜ₀]ᵥ, where ΔA(T) is a change in the optical density of the DNA suspension upon heating and Aₜ₀ is the optical density at T = T₀. Thus, h(T) is the quantitative characteristic of hyperchromism. The registration of the absorption intensity was carried out using the double-cuvette scheme: identical suspension of DNA or their complexes with TiO₂ were placed in both channels of the spectrophotometer. The reference cuvette was thermostated at T = T₀ ± 0.5 °C, while the sample cuvette was slowly heated at a rate of 0.25 °C/min from 20 to 94 °C.

## 4 Results

### 4.1 Transmittance electron microscopy

TEM was used to examine the TiO₂ NPs size distribution. TEM image of TiO₂ sample is shown in Fig. 1a. It is clearly seen that the TiO₂ material consists of mostly spherical nanoparticles. The histogram of particle size distribution is depicted in the inset in Fig. 1a. From the TEM images, we determined the mean size of TiO₂ NPs equals about 74 nm. Figure 1b shows TEM image of the DNA:TiO₂ NPs nanoassemblies.

![Fig. 1](https://example.com/fig1.png)

**Fig. 1** TEM images of a TiO₂ NPs material precipitated from aqueous suspension. The inset in a is the size histogram TiO₂ NPs material determined from TEM images. b–d DNA:TiO₂ NPs nanoassemblies

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nanoassemblies precipitated from the buffer suspensions. We can distinguish the individual TiO₂ NPs combined into nanoassemblies by the DNA strands. The agglomeration of individual DNA:TiO₂ NPs nanoassemblies into nanoaggregates makes it difficult to estimate the size of both structures from TEM. The closer look at the nanoaggregates shows the presence of the individual nanoassemblies bound by the biopolymer strands (Fig. 1c). It is obvious that the DNA:TiO₂ NPs nanoassemblies formed in the aqueous suspension remain stable and after precipitation on the film surface. Along with the observed bound nanoassemblies there were revealed the separately standing nanoassemblies. The detailed analysis of the individual nanoassembly presented in the center of Fig. 1d shows that the biopolymer (the semitransparent covering) may by wrapped around of the nanoparticle (the dark opaque spot) forming DNA corona. It can be formed by the polymer wrapping in several layers around the nanoparticle that was shown by the example of the biopolymer multilayer covering of single-walled carbon nanotubes [26].

4.2 Dynamic light scattering

In order to obtain more information about the size and morphology of the nanoassemblies, we turned to DLS analysis for colloidal suspensions. First of all we have analyzed the TiO₂ NPs ([c₇O₂] = 1.5 × 10⁻⁴ M) colloidal suspension at pH 6.65 and T = 25 °C. The size distribution of the TiO₂ NPs is shown in Fig. 2. The distribution by intensity, by volume, and by number is slightly different. The polydispersity index (PdI) equaled about 0.17 ± 0.04, suggesting that NPs have a spherical shape. This observation agrees well with TEM figures (Fig. 1a). Now we turn to the estimation of the NPs diameter. At first, it should be noted that the European Commission defined that nanomaterials should be characterized as nanoscale objects in terms of the number size distribution of their constituent particles [27–29]. So, it seems reasonable that we used the DLS size distribution by number. Moreover, by TEM methodology we obtained the distribution of nanoparticles size by number of these particles [29, 30]. So, we can compare only distributions by number from DLS and TEM results. The number-based average diameter of studied TiO₂ NPs estimated by DLS was 75 ± 5 nm. That is in a good accordance with TEM data (the inset in Fig. 1a). The zeta potential of the TiO₂ NPs suspension was found to be of −51 ± 5 mV.

It should be noted that DLS measurements were carried out in the colloidal suspension environment but in the case of TEM studies the nanoparticles were precipitated from the suspensions. Comparing the TEM and DLS data of nanoparticle size distribution we can conclude that the distribution by number is better correlated with results taken by TEM. Thus, further to study the stability of the DNA:TiO₂ NPs nanoassemblies in aqueous suspension we will use the size distribution by number.

DLS measurements of DNA:TiO₂ NPs nanoassemblies were performed at 25 °C (0.1 M Na⁺, pH 5) (Fig. 3). There is one cone nanoassembly size distribution with maximum at about 90 nm. It’s well known that hydrodynamic size of a nanoassembly can be composed of roughly the nanoparticle diameter plus the diameter of the diffuse polymer electrostatic shell. The diameter of the double strand DNA is about 2 nm that is too small value to compensate a difference between average size of NPs and nanoassemblies. In keeping with TEM image presented in Fig. 1c we suggest that the difference between sizes of NPs and nanoassemblies could be explained by the biopolymer multilayer covering of individual NPs. It should be noted that a sufficiently high ionic force (0.099 M NaCl) of buffer suspension facilitates multilayer covering as cations weaken the repulsion between the phosphate groups of DNA. We also do not exclude that the biopolymeric shell could bind two or more nanoassemblies into nanoaggregates. The zeta potential of the DNA: TiO₂ NPs suspension was measured to be −49.2 ± 0.8 mV.
4.3 Absorption spectroscopy of TiO2 NPs

Figure 4 shows the absorption spectrum of aqueous suspension of TiO2 NPs used in the present work. We have focused on the low-energy part of UV spectra of TiO2 particles with maxima at about 340 nm that can be mainly attributed to the ligand-to-metal charge transfer $O_2^- \rightarrow Ti^{4+}$ ($O_{2p} \rightarrow Ti_{3d}$). The band gap energy (eV) of our sample was calculated using the following equation:

$$E_g = \frac{hc}{\lambda_g}$$

where $\lambda_g$ is the absorption onset wavelength (nm) of the exciting light, $c$ is velocity of light and $h$ is Planck’s constant. The band gap energy value calculated using the presented equation was evaluated as 2.94 eV. The recorded absorption spectrum of TiO2 NPs corresponds to the calculated data presented in Ref. [31] for nanoparticles with a primary $d \leq 100$ nm. That correlates with our TEM and DLS data presented in Figs. 1a and 2, respectively.

4.4 Study of DNA conformation in the presence of TiO2 NPs

The typical temperature dependence of hyperchromic coefficient ($h$) of DNA has the usual S-like shape: heating induces the helix-coil structural transition, which is reflected in the appearance of the hyperchromism ($h > 0$) (Fig. 5, curve 1). The analysis of the DNA melting curve without TiO2 NPs allows us to determine the thermodynamic parameters of the biopolymer: $T_m = 78.5 \, ^\circ C$ and $h_{m0} = 0.41$ (Fig. 5) (where $h_{m0}$—the value of the hyperchromic coefficient in the absence of TiO2 NPs). However, the addition of TiO2 NPs to the DNA buffer suspension causes the dramatic changes in the shape of the biopolymer melting curve. Upon heating the trough appears in the temperature range of about 30–75 °C. The depth of the trough increases with the TiO2 concentration ($[c_{TiO2}]$). As $[c_{TiO2}]$ increases, several segments can be clearly distinguished in the melting curve. Figure 5 shows the example of the decomposition of the DNA melting curve in the presence of TiO2 with $[c_{TiO2}] = 1.5 \times 10^{-4} \, M$. The characteristic points: $T_{s1}$ and $T_{s2}$ as well as $T_{f1}$ and $T_{f2}$ were calculated using an extrapolation of the linear segments of the melting curves. They are connected to the beginning ($T_{s1}$, $T_{s2}$) and finish ($T_{f1}$, $T_{f2}$) of the definite ongoing processes discussed below. In the segment of $a-b$, with an increase in temperature, absorption hypochromism is observed, the value of which increases with $[c_{TiO2}]$. We believe that this effect may be due to the intensification of the formation of the nanoassemblies of DNA with TiO2 NPs that is induced by a heating and the unwound single-stranded DNA ends. As it was mentioned above, one of the mechanisms of TiO2 binding to DNA is the electrostatic interaction of positively charged TiO2 NPs with negatively charged phosphate groups of DNA [20]. It is known that heating leads to the formation of the unwound regions at the ends as well as in the helix segments located far from the biopolymer ends. The unwound regions can effectively interact with NPs. In the segment of $b-c$, the formation of the nanoassemblies is ongoing. The helix-coil transitions in the DNA structure occurs in the segment of $c-d$ and it ends with the plateau (Fig. 5).

4.5 UV–irradiation effect on DNA conformation with TiO2 NPs

As mentioned above, one of the known exogenous factors having the destructive effect on DNA structure is UV radiation that is able to induce the appearance of the loops as well as single-stranded intermediates in double-stranded DNA. Figure 6 shows the temperature dependence of the hyperchromic coefficient of DNA obtained without and in the presence of TiO2 NPs under UV irradiation that was carried out during 3 h. The melting curves for non-irradiated and irradiated DNA suspensions demonstrate the different dynamics. The melting temperature of DNA decreases by...
more than 6 degrees and the \( h_{m0} \) is equal to 0.35 under UV irradiation for three hours. This effect may be caused by the partial denaturation of DNA and the appearance of single-stranded fragments under the UV irradiation [15, 32]. The formation of single-stranded fragments in the biopolymer in the complexes with TiO\(_2\) NPs under the UV irradiation is confirmed by the presence of non-cooperative "tails" in DNA melting curves (see, the \( a'–a \) segment in Fig. 6). The appearance of less cooperative melting segments on irradiated DNA:TiO\(_2\) NPs melting curves (Fig. 6) (in comparison with the melting curves of the non-irradiated biopolymer assemblies presented in Fig. 5) is due to the fact that the melting of single-stranded DNA fragments formed as a result of UV irradiation of the native biopolymer contributes to the total dependence of the hyperchromic coefficient of double-stranded DNA. With increasing \([c_{\text{TiO}_2}]\), a trough appears in the melting curves in the temperature range of 60–75 °C (Fig. 6). It should be noted that the temperatures of the beginning \( T_{s1} \) and end \( T_{f1} \) intensive formation of the DNA:TiO\(_2\) NPs nanoassemblies shift to the high-temperature region relative to the melting curve of non-irradiated DNA suspension (Figs. 5, 6). So, for example, at \([c_{\text{TiO}_2}]=1.5\times10^{-4}\) M, \( T_{s1} =25.1\) °C and \( T_{f1} =31.4\) °C in the absence of irradiation, and \( T_{s1} = 58.1\) °C and \( T_{f1} = 66.7\) °C after UV irradiation (Figs. 5, 6).

### 4.6 Comparative analysis of \( T_m \) and \( h \) dependences for the UV-irradiated and non-irradiated DNA: TiO\(_2\) assemblies

We compare the \( T_m \) and \( h \) dependences extracted from the irradiated and non-irradiated DNA melting curves in absence and presence of TiO\(_2\) NPs as the function of \([c_{\text{TiO}_2}]\) in Fig. 7a–b. The \( T_m \) dependence shows nonlinear behavior on \([c_{\text{TiO}_2}]\) that may be caused by the complicated processes of the complexation of the biopolymer with NPs. In the absence of UV irradiation, the addition of \([c_{\text{TiO}_2}]=2.5\times10^{-5}\) M to the DNA suspension practically does not change the \( T_m \) value (Fig. 7a). On the contrary, under UV irradiation, the addition of even \([c_{\text{TiO}_2}]=5\times10^{-6}\) M to the DNA suspension leads to a sharp increase in the melting temperature by more than 10 °C. A further increase in \([c_{\text{TiO}_2}]\) leads to weak changes in the value of this parameter (the changes are within 3 °C) for both the irradiated and non-irradiated DNA: TiO\(_2\) NPs assemblies. The reason for this may be that the \( T_m([c_{\text{TiO}_2}]) \) 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 DNA to TiO\(_2\) NPs [20]. The \( h([c_{\text{TiO}_2}]) \) dependences extracted from the melting curves for the non-irradiated and irradiated DNA:TiO\(_2\) NPs samples are presented in Fig. 7b. In general, both curves demonstrate the similar evolution in the studied concentration range of NPs. In the absence of TiO\(_2\) NPs, the UV irradiation of the DNA suspension for 3 h leads to a 15% decrease in the \( h \) value as compared to non-irradiated DNA. The addition of TiO\(_2\) NPs to the DNA suspension induces decreasing in the \( h \) value, in the absence of UV irradiation. This character of the dependence persists even after a three-hour UV irradiation of the DNA:TiO\(_2\) NPs suspension.

In the absence of any other conformational transitions, the maximum value of \( h(T) \) achieved with complete separation of double-stranded polymer \( (h_m) \) can be considered as a measure of its degree of helicity \( (\Theta) \) at \( T=T_{cP} \). Analysis of the
(\(h_m\)) evolution shows that in the absence of UV irradiation \((h_{m0}) = 0.41\), and after three hours of irradiation \((h_{m_0}) = 0.35\), that corresponds to the degree of helicity \(\Theta_{T_0} = 1\). Hence, the degree of DNA helicity in the presence of TiO2 NPs can be determined by the formula: \(\Theta = \frac{(h_T + h_m)}{(h_{m0})}\). Our performed estimates show that the degree of helicity of non-irradiated DNA with TiO2 at \([c_{TiO2}] = 1.5 \times 10^{-4} \text{ M}\) is \(\Theta = (0.12 + 0.24)/0.41 = 0.88\), and after three hours of UV irradiation \(\Theta = (0.05 + 0.2)/0.35 = 0.71\). Thus, we can conclude that the addition of TiO2 NPs to the DNA suspension leads to a decrease in its degree of the helicity, and UV irradiation is an additional factor that enhances the appearance of the single-stranded unwound regions in DNA.

5 Discussion

Recently [33, 34], it was shown that one of the main factors determining biological (and toxic) properties of the NPs is their charge. It is known [35], that TiO2 NPs are positively charged at low pH (pH < 6.5) and become negative at high pH (pH > 6.5). NPs are electrically neutral at pH ~ 6.5. More-
found that the TiO$_2$ NPs injection to the DNA buffer suspension leads to partial disordering of double-stranded DNA, and UV irradiation enhances the toxic effect of these NPs. The consequence of such an effect of TiO$_2$ NPs on a 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 also be used in the creation of self-cleaning antibacterial surfaces, as well as in medicine.

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Author contributions All authors discussed the results and commented on the manuscript. VV and EU carried out the spectroscopic measurements, thermal denaturation and analyzed data. AS and AL performed and analyzed the DLS data. SP performed TEM characterization. AG and VK planned and coordinated the project. EU, AG, and VK wrote the paper.

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Declarations

Conflict of interest The authors do not have any commercial or associative interest that represents any conflict of interest in connection with the work submitted.

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