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Influence of the structural organization of nucleic acids on the interaction with hypochlorite: ATPP, PolyA and DNA

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Received December 01, 2022
Revised January 13, 2023
Accepted January 18, 2023

The action of hypochlorite on various biological molecules in a living cell has been actively studied for years. However, the influence of the structural organization of nucleic acids on their interaction with hypochlorite remains underinvestigated. In this work, using ultraviolet and infrared spectroscopy, we analyzed the effect of the structure of nucleic acids on the reaction with hypochlorite using the example of the three most common and biologically significant types of nucleic acids (NA): double-stranded DNA in the B-form, single-stranded RNA, and nucleotide phosphates. It was found that the rate of the initial stage of the reaction of hypochlorite with endocyclic nitrogen atoms depends on the presence/absence of base pairing in the NA structure. At the same time, the polymeric structure of NC significantly accelerates and increases the efficiency of the subsequent stages of the reaction associated with the chlorination of exocyclic nitrogen atoms and the destruction of the ring structure of nitrogenous bases.

Keywords: UV absorption, IR spectroscopy, hypochlorite, nucleic acids.

DOI: 10.61011/EOS.2023.06.56671.113-23

Introduction

The interaction of reactive chlorine-containing compounds with various bioorganic molecules, such as amines, amino acids, proteins, lipids, carbohydrates, and nucleic acids (NA), is the focus of constant attention not only of chemists, but also of molecular biologists and physicians [1,2]. The study of chemical mechanisms of the formation of various chlorine derivatives under physiological conditions is of particular practical importance. One type of these compounds are hypochlorites, i.e. salts of hypochlorous acid. Being a strong oxidizing agent, hypochlorous acid acts as a natural antiseptic [3–5]. Its dissociation constant is $pKa = 7.59$ [6], therefore at physiological pH values hypochlorous acid is present in solution both as HOCl and as ClO^- hypochlorite. With the development of inflammatory reactions, the production of hypochlorite in the body can lead to tissue damage and provoke the development of malignant neoplasms. [7,8].

The effect of hypochlorite on various biological molecules in a living cell has been actively studied for a number of years [1]. In particular, it was found that in the course of reactions of hypochlorite with NA, chlorination of nitrogenous bases occurs with the formation of chloramines of the RNHCl form (for exocyclic nitrogen) and RR'NCl form (for endocyclic nitrogen) [9]. At the same time, RNHCl-derivatives were found during the interaction of hypochlorite with all nucleosides containing exocyclic nitrogen (adenosine, guanosine and cytidine), and endocyclic chloramines were experimentally observed only after reactions with guanosine and thymidine [10].

At the same time, the effect of the structural organization of NAs on their interaction with hypochlorite remains much less studied. At present, there are only a few studies where the effect of hypochlorite on the spatial structure of DNA has been indirectly analyzed by electrophoresis, viscometry methods, and also there are some studies on the efficiency of staining with ethidium bromide [9–11].

In this study, UV and IR spectroscopy methods are used to analyze the effect of the NA structure on the course of the reaction with hypochlorite using the example of the three most common and biologically significant types of NA: double-stranded DNA in the B-form, single-stranded RNA, and nucleotide phosphates.

Materials and methods

High-molecular calf thymus DNA (Sigma), polyadenylic acid (polyA), and adenosine-5'-tetraphosphoric acid (ATPP) (Reanal) were used in the study. The concentration of NA solutions was determined spectrophotometrically using the method of hydrolysis in the presence of perchloric acid [12] for DNA; molar extinction coefficients ε_{258} equal to 15400 and 9800 $M^{-1}cm^{-1}$were used for ATPP and polyA solutions, respectively. The concentration of NA in all complexes was $7.6 \cdot 10^{-5}$ M.

The solutions of sodium hypochlorite (NaOCl) used in the study were produced at the FBIS Pasteur Institute of Epidemiology and Microbiology by electrolysis of 3% sodium chlorite solution with a current of 3 A for 30 min. The content of chlorine in the initial solutions was 801 and 1566 mg/L. The concentration of sodium hypochlorite in

an aqueous solution was determined spectrophotometrically using the extinction coefficient of $\varepsilon_{292} = 350\,\mathrm{M}^{-1}\mathrm{cm}^{-1}$ [13]. The concentration of sodium hypochlorite in all complexes was $7.3 \cdot 10^{-3}$ M.

Complexes of NA and sodium hypochlorite were prepared in 1 mM NaCl solutions by direct mixing of equal volumes of solutions of the corresponding components in the required concentrations. The molar ratio of hypochlorite to nitrogenous bases in all studied complexes was 5 : 1.

The UV spectroscopy

Immediately after preparation, all studied solutions were placed in a spectrophotometer. The measurements were carried out in quartz cells with an optical path length of 1 cm. Absorption spectra were recorded using a Shimadzu UV-1800 scanning spectrophotometer (Japan). When recording time dependences, the reaction was carried out directly in the cell. The spectra were recorded at times from 1 to 3 min with an interval of 0.5 min, from 3 to 10 min with an interval of 1 min, from 10 to 120 min with an interval of 10 min, and additionally 24 h after the start of the reaction.

The IR spectroscopy

To record the IR absorption spectra, the complexes obtained 120 min after the start of the reaction were freeze-dried, after which they were triturated with KBr powder in the ratio of 0.5 mg sample per 100 mg KBr, and pellets were formed using a hand press. The spectra were recorded on a Tensor 27 IR Fourier spectrometer (Bruker, Germany) equipped with a low-noise MCT (HgCdTe) detector. The optical paths were purged with dry nitrogen. Infrared spectra were recorded in the region of $4000-800\,\mathrm{cm}^{-1}$ with a resolution of $2\,\mathrm{cm}^{-1}$, each spectrum was averaged over 128 accumulations. The obtained spectra were processed using the software supplied with the instrument.

Results and discussion thereof

According to currently available data [1], the main target in the interaction of nucleic acids with hypochlorite are nitrogen atoms within the nitrogenous bases, however the effect of the spatial organization of NA on the efficiency of this reaction remains unclear. To analyze the effect of the structure of NAs on their interaction with hypochlorite, three samples different in their structural organization were used in the study: double-stranded DNA in the B-form (high-molecular calf thymus DNA), single-stranded RNA molecule (polyadenylic acid, polyA), and nucleotide phosphates (adenosine tetraphosphate, ATPP). In this study, reactions running under conditions of a slight excess of hypochlorite with respect to the number of potential targets (nitrogen atoms) in the composition of NAs were analyzed. The number of nitrogen atoms per base cannot exceed five

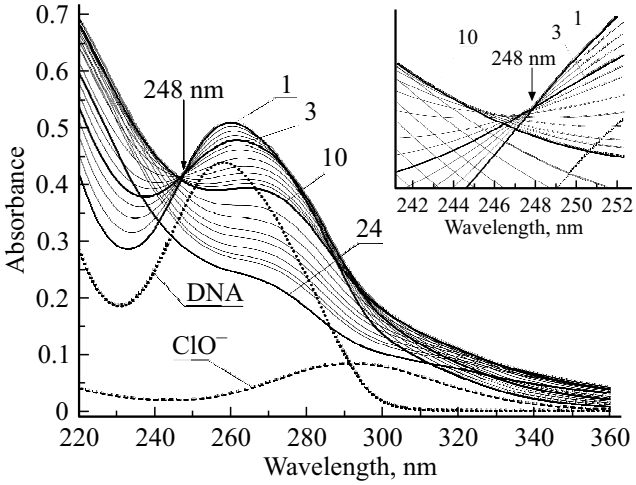

Figure 1. UV absorption spectra of DNA complexes with sodium hypochlorite. The arrow in the figure shows position of the isosbestic point at 248 nm; this area is shown in more detail in the insert. The spectra marked with callouts correspond to 1, 3, 10 min, and 24 h after the start of the reaction. Dashed lines indicate the spectra of DNA and hypochlorite at concentrations corresponding to the initial time of the reaction.

(for purines), of which no more than four are available for interaction. Thus, to create a minimum guaranteed excess of hypochlorite in the system, it is sufficient to use the molar ratio of hypochlorite to nitrogenous bases of $R = 5$.

Study of the kinetics of the reaction of hypochlorite with NA

At the first stage, a comparative analysis of the kinetics of reactions between hypochlorite and selected NA samples was carried out. The interaction of hypochlorite with nitrogenous bases leads to changes in their spectral characteristics, which makes it possible to follow the course of the reaction by the UV absorption spectra of NAs. To monitor changes in the spectra of NaOCl/NA complexes, the reaction mixture was placed in a spectrophotometer, and the absorption spectra of the solution were recorded at different time points in the range from 1 min to 24 h after the start of the reaction.

Kinetics of the reaction with double-stranded DNA

The characteristic UV absorption spectra of the complexes of DNA and hypochlorite, as well as the dynamics of changes in the spectra over time, are shown in Fig. 1. The same figure shows spectra of DNA and NaOCl solutions before the start of the reaction. In the DNA spectrum an absorption band is observed with a peak at 258 nm, the spectrum of hypochlorite is characterized by the presence of an absorption band with a peak at 292 nm, which corresponds to the ClO^- ionized form [14]. The first spectrum of the DNA-sodium hypochlorite complex

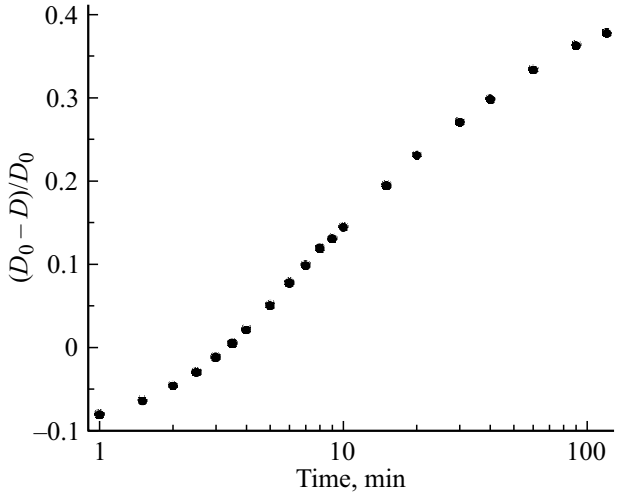

Figure 2. Time-dependence of the relative change in the absorption of the DNA-NaOCl complex at a wavelength of 260 nm. D_0 is taken equal to the sum of absorptions of DNA and hypochlorite at the initial moment of time.

was recorded 1 min after the start of the reaction. For this spectrum, there is an increase in the absorption at the maximum of the band by 16% relative to the sum of the absorption of the initial solutions of DNA and sodium hypochlorite at the same wavelength and accompanied by a bathochromic shift of the peak of the DNA absorption band by 2 nm. In all subsequent spectra of the complexes (from 1.5 min to a day), the absorption in this region of the spectrum decreases monotonically. The spectra corresponding to the interval from 1 to 3–3.5 min are characterized by the presence of an isosbestic point at 248 nm, which indicates the only type of interaction that manifests itself in the spectra in this time interval. The spectra corresponding to the first 10 min of the reaction demonstrate a bathochromic shift of the DNA absorption band peak, which shifts to 266 nm by the tenth minute. Further transformations in the system are accompanied by a decrease in the DNA absorption band up to its almost complete disappearance 2 h after the start of the reaction. In parallel, a weak absorption band is formed in the spectrum in the region of 325 nm, which can probably be attributed to inorganic chlorine-containing reaction products [15]. In the subsequent 22 h no significant changes in the absorption spectra are observed in the system.

The dynamics of changes in the concentration of chromophores responsible for DNA absorption is illustrated by the dependence of the relative change in absorption at 260 nm over time (Fig. 2). The main stages of the reaction are more clearly demonstrated by the time-dependence of the reaction rate, which corresponds to the graph of the first derivative of concentration over time. In our case, the time derivative of the dependence of the relative change in absorption can be considered (Fig. 3). Three stages are clearly distinguished on this dependence, corresponding to the occurrence of various reactions in the system in the following time intervals: from the beginning of the reaction to the third minute after the start of the reaction, from the third minute to the ninth minute, from the tenth minute onwards.

In order to obtain more detailed information about the changes in the composition of the chemical groups of DNA upon interaction with sodium hypochlorite, we recorded IR spectra of the corresponding systems. Fig. 4 shows the

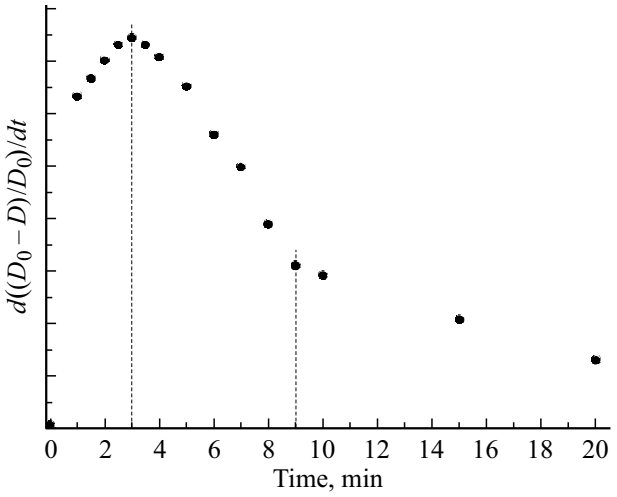

Figure 3. Time-dependence of the first derivative of the relative change in the absorption peak of the DNA-NaOCl complex.

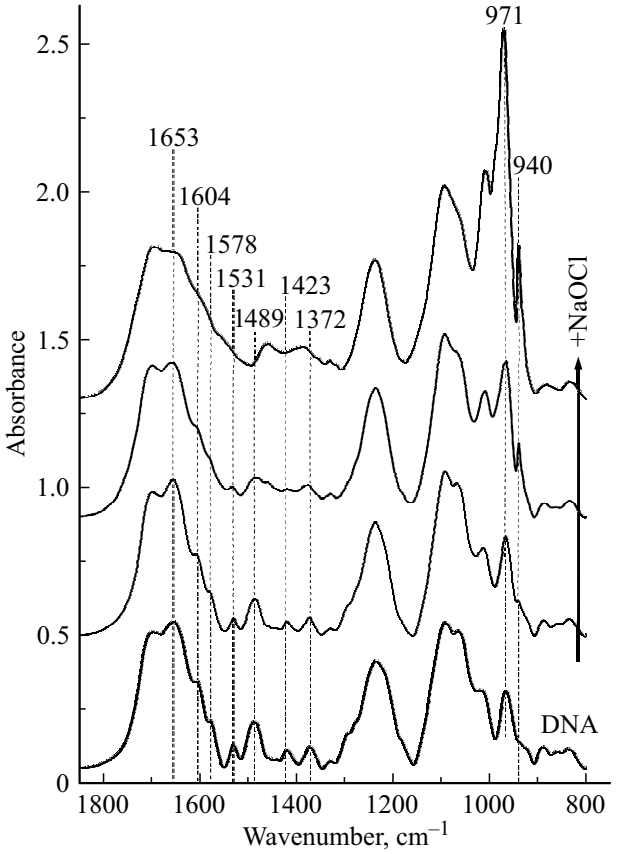

Figure 4. IR absorption spectra of DNA and sodium hypochlorite complexes.

IR spectra of the original DNA sample and DNA after interaction with hypochlorite under conditions of excess and deficiency of hypochlorite. The main changes in the DNA spectrum are observed in the region of 1800−1550 cm^{-1}, which corresponds mainly to the vibrations of carbonyl and amino groups within the nitrogenous bases, as well as in the region of 1300−1550 cm^{-1}, where vibrations of the rings are observed, as well as vibrations of bonds with involvement of heteroatoms in the rings of DNA bases [16–22]. In the course of the reaction with hypochlorite, first a gradual disappearance of the bands at 1653, 1604, and 1578 cm^{-1}, corresponding to vibrations of amino groups predominantly in the composition of adenine, is observed in the spectrum. The disappearance of the bands may be indicative of either a chemical modification of the amino groups accompanied by a shift in the vibrational frequencies beyond the studied range, or their destruction. Bands at 1531, 1489, 1423, 1372 cm^{-1}, corresponding to vibrations of rings and C−N bonds in them, demonstrate similar behavior, but with some delay. At the same time, bands at 940 and 971 cm^{-1}, related to the stretching vibrations of the O−Cl and N−Cl bonds of hypochlorite derivatives, begin to appear in the spectrum [23–25].

Thus, based on the analysis of the data obtained, it can be concluded that the reaction of hypochlorite with DNA bases under the experimental conditions consists of at least three stages. The first and the fastest stage completes by the third minute. At the same time, based upon the stepwise increase in absorption and the shift of the band peak in the first recorded spectrum of the complex (one minute after the start of the reaction), further changes in the spectra observed in the interval of 1−3 min correspond to the end of the first stage of the reaction, which was running in the first tens of seconds (Fig. 1 and 3). According to the previously obtained data [1,9–11], the fastest stage in the interaction of DNA with hypochlorite corresponds to the formation of RR, NCl unstable endocyclic chloramines. Chlorination of nitrogenous bases can affect the properties of chromophores, which could explain the observed effect. At the same time, chlorination of endocyclic nitrogen atoms involved in the formation of complementary pairs between nitrogenous bases is possible only after the corresponding hydrogen bonds are broken. The latter circumstance will inevitably lead to at least partial DNA denaturation, which is characterized by both hyperchromism (up to 40%) and a bathochromic effect, leading to a shift in the DNA absorption band peak from 258 to 259 nm [26]. However, the changes in the spectrum observed at the first stage correspond to a shift in the absorption peak to 261 nm and a gradual decrease in absorption after the initial jump, which cannot be explained by the denaturation effect only. This behavior of the spectra most likely corresponds to the simultaneous occurrence of several processes at once. In the first seconds, the process of hydrogen bonds breaking in the double helix dominates, which leads to a rapid increase in absorption and a slight shift of the peak. Then the reaction with the formation of endocyclic chloramines becomes

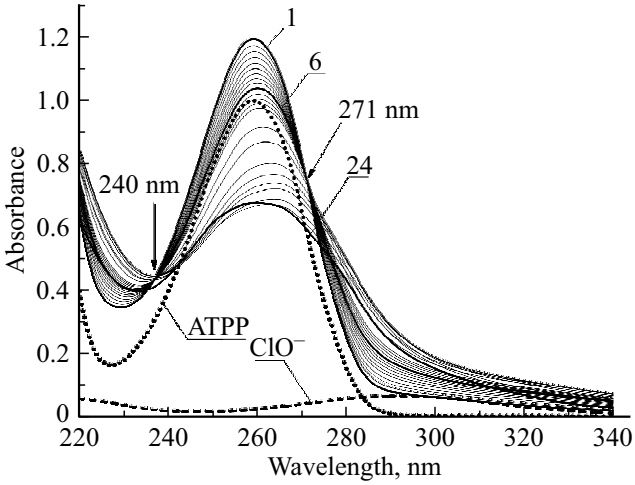

Figure 5. UV absorption spectra of ATPP complexes with sodium hypochlorite. Arrows in the figure show positions of isobestic points at 240 and 271 nm. The spectra marked with callouts correspond to 1, 6 min and 24 h after the start of the reaction. Dashed lines indicate the spectra of ATPP and hypochlorite at concentrations corresponding to the initial time of the reaction.

dominant, which is accompanied first of all by a drop in the absorption of nitrogenous bases down to a level close to the absorption of DNA before entering into a reaction with hypochlorite. The observed slight shift of the absorption peak at this stage may also be due to the formation of endocyclic chloramines. However, it cannot be ruled out that the bathochromic shift reflects the beginning of the next stage of the reaction, during which (in the interval of 3−9 min) a shift by another 5 nm is observed. At the second stage of the reaction of DNA with hypochlorite, chlorine is transferred to exocyclic nitrogen atoms with the formation of more stable derivatives of the RNHCl form [1,9–11]. This reaction is mostly completed by the tenth minute (Fig. 3), when the third stage of the reaction begins. At the final stage, a gradual degradation of RNHCl occurs, first of all due to the interaction with neighboring nitrogenous bases, which is also accompanied by the destruction of the latter. At this stage, the spectra no longer exhibit a bathochromic shift, and a decrease in the number of nitrogenous bases is accompanied by a gradual disappearance of the DNA absorption band in the spectrum.

Thus, it can be assumed that an important factor affecting the course of the second and the third stages of the reaction in DNA is the presence of neighboring nitrogenous bases in the immediate environment of endocyclic chloramines. Availability for the reaction of similar „close neighbors“ is ensured first of all by the polymeric nature of DNA.

Kinetics of the reaction with ATPP

Other conditions for the reaction can be created in a solution of monomers without the polymer fraction. In this study, the reaction of hypochlorite with adenosine

tetraphosphate (ATPP) was investigated. For this purpose, the UV absorption spectra of the ATPP/ClO^- complexes were recorded in the time interval from 1 min to 24 h after the start of the reaction (Fig. 5). In the spectrum of free ATPP, there is a single absorption band with a peak at 259 nm. The first spectrum of the ATPP/ClO^- complex was recorded 1 min after the start of the reaction. For this spectrum, there is an increase in the absorption at the maximum of the band by 20% relative to the sum of the absorption of the initial solutions of ATPP and sodium hypochlorite at the same wavelength. In contrast to the reaction with DNA considered above, the bathochromic shift of the absorption band peak for the ATPP/ClO^- complex is about 0.5 nm. In the subsequent spectra of the complexes (from 1.5 to 120 min), the absorption in this region of the spectrum decreases monotonically approximately to the absorption level of ATPP before the start of the reaction. The spectra corresponding to the interval from 1 to 6 min are characterized by the presence of two isosbestic points at 240 and 271 nm. The spectra corresponding to the first six minutes of the reaction demonstrate a small bathochromic shift of the ATPP absorption band peak, which shifts to 260 nm by the sixth minute. Further transformations in the system are accompanied by a decrease in the ATPP absorption band and a continuing bathochromic shift, which ends 50 min after the start of the reaction at 264 nm. It is interesting to note that two hours after the start of the reaction, a distinct ATPP absorption band is observed in the spectrum, which persists even after a day. Moreover, after 24 h the peak of this band returns to ~ 260 nm. Also, in contrast to DNA, the ATPP/ClO^- spectra do not contain a band in the vicinity of 325 nm, which we attributed to inorganic chlorine-containing reaction products.

The main stages of the reaction are more clearly demonstrated by the dependence of the reaction rate on time (Fig. 6). Only two sections are clearly observed on it, corresponding to various reactions running in the system in the following time intervals: from the second minute to the sixth minute and from the seventh minute onwards.

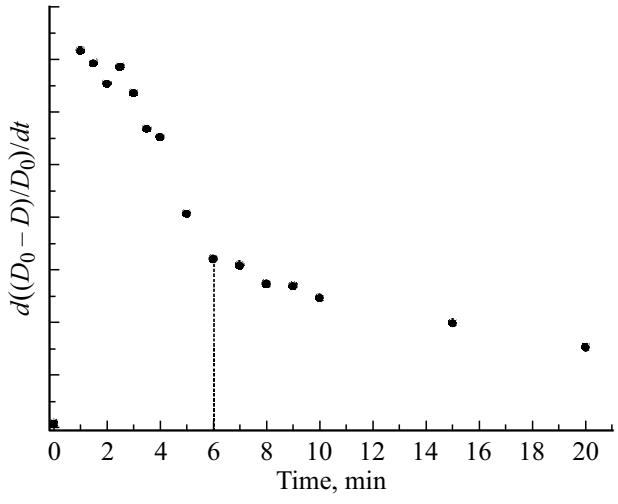

Figure 6. Time-dependence of the first derivative of the relative change in the absorption peak of the ATPP-NaOCl complex.

Based on the above dependences, a conclusion can be made that for the reaction of hypochlorite with ATPP under conditions of the experiment we have succeeded to reliably observe two stages. In all appearance, the first most rapid stage of the formation of endocyclic chloramines in the case of monomeric ATPP is completed after 1.5–2 min, and under the conditions of this experiment it is not reliably observed. Perhaps this is a consequence of the fact that nitrogenous bases do not form hydrogen bonds in this system, and nitrogen atoms N1 and N6 are available for hypochlorite, which facilitates the initial stages of the interaction. Then, interpreting the time-dependence of the reaction rate (Fig. 6) in the same way as in the case of the DNA/ClO^- complex described above in detail, it can be said that the reaction observed in the interval of 1.5–6 min corresponds to the second stage, where chloramines of the RNHCl form are formed. Then, a slow degradation of external chloramines takes place, however, in this case, it does not lead to the complete destruction of nitrogenous bases in the solution due to the absence of direct contacts between them. In all appearance, this circumstance allows some of the nitrogenous bases to retain their structure, which explains the presence of the corresponding absorption band in the spectrum even after a day. At the same time, during this period the degradation of external RNHCl chloramines is completed, which leads to a hypsochromic shift of the absorption band peak and its return to the 260 nm position corresponding to the state of the system before the start of chlorine transfer to exocyclic nitrogen.

Thus, a conclusion can be made that the absence of a structure of complementary base pairs ensured the availability of nitrogen atoms in the first and sixth positions for the reaction with hypochlorite and considerably accelerated the first stage of the reaction. At the same time, the absence of polymer organization in ATPP led to the preservation of the structure of heterocycles in a significant part of nitrogenous bases.

Kinetics of the reaction with single-stranded RNA

Polyadenylic acid is a single-stranded polynucleotide where nitrogenous bases do not form hydrogen bonds with each other, but are located on a common sugar-phosphate backbone. To study the reaction of hypochlorite with single-stranded NAs, the UV absorption spectra of polyA/ClO^- (Fig. 7) were analyzed. The spectrum of polyadenylic acid contains one absorption band in the region under study with a peak at 255 nm.

The first spectrum of the PolyA/ClO^- complex was also recorded 1 min after the start of the reaction. In contrast to the systems previously described in the study, the spectrum of this complex one minute after the start of the reaction demonstrates all signs of the completion of the second stage of interaction with hypochlorite. The absorption of this complex turned out to be lower than the initial

absorption of the free NA. The position of the absorption peak in the spectrum is shifted by 3 nm to the long wavelength region, to 258 nm. In the subsequent spectra of the complexes (from 1.5 to 120 min), the absorption in this region of the spectrum rapidly decreases, which is accompanied by a bathochromic shift of the peak to 266 nm as early as by the sixth minute after the start of the reaction. By this time, the shape of the spectrum completely changes, which is indicative of the beginning of the destruction of heterocycles of nitrogenous bases. Further transformations in the system lead to almost complete disappearance of the adenine absorption band in the spectrum as early as in 20–30 min after the start of the reaction. Also, starting from approximately the tenth minute, a band in the region of 325 nm begins to form in the spectra of PolyA/ClO^-. The time-dependence of the reaction rate (Fig. 8) demonstrates two sections corresponding to the running of various reactions in the system at times before and after 6 min.

Based on the above dependences, a conclusion can be made that for the reaction of hypochlorite with polyadenylic acid under conditions of the experiment we have succeeded to observe the final stage of the formation of exocyclic chloramines and their subsequent degradation accompanied by rapid and complete destruction of the structure of heterocyclic rings of nitrogenous bases. This behavior of the reaction fully confirms the above assumption about the effect of the structure of polynucleotides on the course of the reaction with hypochlorite. Moreover, the absence of a structure of complementary pairs in the presence of a polymeric organization of bases significantly accelerated not only the stage of initial chlorination of heterocycles, but also increased the efficiency of subsequent stages of the reaction. This circumstance probably is indicative of the possibility of a reaction between chlorinated heterocycles no later than

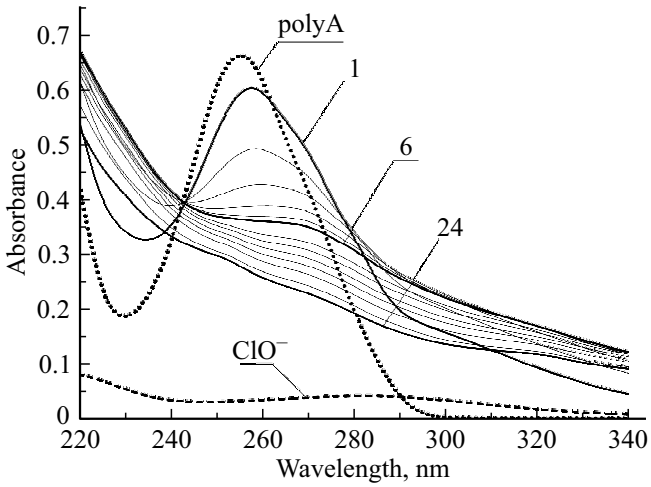

Figure 7. UV absorption spectra of polyA complexes with sodium hypochlorite. The spectra marked with callouts correspond to 1.6 min and 24 h after the start of the reaction. Dashed lines indicate the spectra of polyA and hypochlorite at concentrations corresponding to the initial time of the reaction.

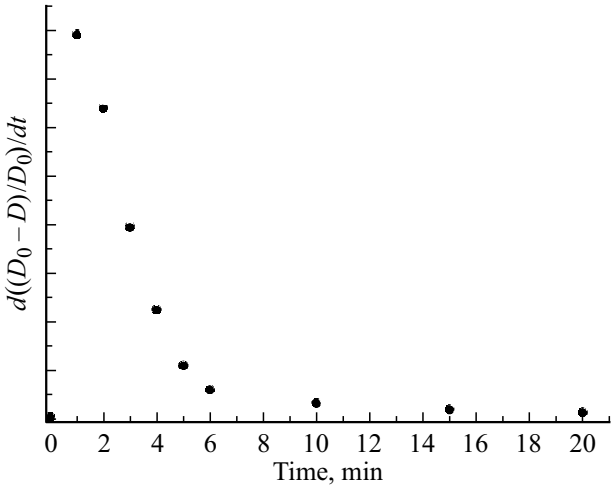

Figure 8. Time-dependence of the first derivative of the relative change in the absorption peak of the polyA-NaOCl complex.

the second stage of the reaction. It is also possible that the increased efficiency of the destruction of heterocycles was affected by the formation at the first stages of other reaction products, in addition to the chloramines themselves.

Thus, a conclusion can be made that the structural organization of NAs has a significant effect on their interaction with hypochlorite. The presence of complementary base pairs stabilized by hydrogen bonds seems to be the limiting factor in the onset of the reaction of hypochlorite with endocyclic nitrogen atoms. At the same time, the polymeric structure of NA significantly accelerates and increases the efficiency of the subsequent stages of the reaction related to the chlorination of exocyclic nitrogen atoms and the destruction of the ring structure of nitrogenous bases.

Acknowledgments

The authors are grateful to Anna Gennadievna Afinogenova, PhD in biology, for kindly provided hypochlorite solutions. The work was carried out using the equipment of the Research Park of St. Petersburg State University (Optical and laser methods of substance investigations; Center for diagnostics of functional materials for medicine, pharmacology and nanoelectronics, Cryogenic department).

Conflict of interest

The authors declare that they have no conflict of interest.

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Translated by Y.Alekseev