Effect of sodium hypochlorite on nucleic acids of different primary and secondary structures

D N Osinnikova¹, E B Moroshkina¹, E S Mokronosova¹

¹St. Petersburg State University, 7/9 Universitetskaya Emb., St Petersburg 199034, Russia
E-mail: osinnikovadasha@yandex.ru

Abstract. The effect of sodium hypochlorite (NaClO) on nucleic acids (NAs) was investigated. The effect of biomolecular structure on resistance to hypochlorite was analysed: plasmid bacterial DNA, calf thymus DNA, synthetic polyadenylic-uridic acid samples were studied, as well as individual nucleotides (adenosine-5'-tetraphosphoric acid and guanosin-2',3'-cyclophosphoric acid). The effect of sodium hypochlorite on DNA was investigated depending on the concentrations of the components. We have also performed detailed analysis of the kinetics of the reaction between the NAs and NaClO. It was found that both the destruction of the secondary structure of DNA (denaturation) and the chemical modification of nitrogenous bases, presumably chlorination, occur. Presence of a stable double-stranded structure of DNA slows down the chemical reaction of sodium hypochlorite with nitrogenous bases of DNA.

1. Introduction

Currently, there is a huge amount of antimicrobial drugs, differing both in the type of action (destruction of the pathogen, stopping the reproduction of the pathogen), and the mechanism of action (for example, violation of the synthesis of nucleic acids (NAs)). Among them are compounds whose targets are nucleic acids and proteins, that ensures the destruction of bacteria with minimal risk of developing resistance.

Sodium hypochlorite (NaClO) is in the “top-100” list of chemical compounds, which are most relevant to practice [1]. It is widely used in medicine, food industry and agriculture as a bactericidal and sterilizing agent.

In living cell, sodium hypochlorite reacts with various biological molecules, including DNA, proteins and lipids [2-4]. It is known that the antimicrobial activity of the sodium hypochlorite is due to the presence of hypochlorite ion and its ability to oxidize and hydrolyze proteins [5]. High concentrations of the compound can also destroy the cell membrane of bacteria. However, the effect of NaClO on DNA remains underinvestigated.

It has been shown that the interaction of the hypochlorite with DNA in vitro resulted in DNA denaturation [5, 6] and various modifications of nitrogenous bases [7-9]. The reaction of the hypochlorite with individual nitrogenous bases at the first stage leads to their chlorination with the formation of various chloramines and radicals, leading to the destruction of the bases [10, 11].

In this work, a comparative study of the interaction of sodium hypochlorite with polynucleotides with and without a secondary structure (plasmid, calf thymus DNA, polyadenylic-uridic acid), as well as with individual nucleotides (adenosine-5'-tetraphosphoric acid and guanosine-2',3'-cyclophosphoric acid) was performed using spectroscopic approach.
2. Materials and methods

High molecular weight DNA (Sigma) from calf thymus (ctDNA) with molecular weight $M=10$ MDa was used. The concentration of sodium hypochlorite (assay quality, Merck) in aqueous solution was determined spectrophotometrically using the extinction coefficient $E_{292} = 350$ $M^{-1}cm^{-1}$ [12,13]. Plasmid DNA (pDNA) (pUC19 variant, 6386 b.p.) was extracted from E.coli using GeneJET Plasmid Kit (Thermo Scientific). Polyadenyl-uridylic acid (polyAU), adenosine-5′-tetraphosphoric acid sodium salt (ATPP), guanosin-2′,3′-cyclophosphoric acid sodium salt (cGMP) were purchased from Reanal.

Complexes of NAs with NaClO were prepared by mixing solutions of the individual components with appropriate concentrations. Concentration of DNA was determined spectrophotometrically using the absorption of hydrolyzed DNA at λ = 290 and 270 nm [14]. Concentrations of the other components were determined spectrophotometrically using the following extinction coefficients: $E_{290}$(polyAU) = 9100 $M^{-1}cm^{-1}$ [15], $E_{260}$(ATPP) =15400 $M^{-1}cm^{-1}$ [16]. $E_{260}$(cGMP) = 11500 $M^{-1}cm^{-1}$ [16]. Absorption spectra were recorded using Shimadzu UV-1800 spectrometer.

3. Results and discussion

3.1. Interaction of sodium hypochlorite with DNA

To analyze the effect of the secondary structure on interaction of NaClO with NAs we used ctDNA and pDNA samples. The absorption spectra (figure 1) of the ctDNA ($C=0.019$ $mM_{bp}$) and pDNA ($C=0.017$ $mM_{bp}$) in presence of sodium hypochlorite ($C_{NaClO}=0.15$ mM) were recorded at different time intervals (1 minute, 10 minutes, 30 minutes and 24 hours after preparation of the complexes). To estimate the concentration effects similar experiments in presence of higher hypochlorite concentrations ($C_{NaClO}=0.35$ mM, $C_{NaClO}=0.8$ mM) were also carried out (spectra are not shown, see fig.4a)

![Figure 1](image-url)

**Figure 1.** Absorption spectra of ctDNA (a) and pDNA (b) in presence of NaClO at different time intervals: 1 – 1 min., 2 – 10 min., 3 – 30 min., 4 – 24 hours

From the obtained spectra, it can be seen that, the absorption of the solution is close to the sum of the DNA and hypochlorite spectra. Noticeable changes in the shape of the spectrum and the absorption value at 260 nm are observed after 10–15 minutes. After 24 h, the DNA absorption band ($\lambda_{\text{max}} = 260$ nm) almost disappears, which most likely is a consequence of the damage of the structure of the nitrogenous bases of DNA.

3.2. Interaction of sodium hypochlorite with polyAU

As it was established above, the interaction of the hypochlorite with DNA leads to a complete loss of the secondary structure and the destruction of the heterocycles of the nitrogenous bases. To test the interaction of NaClO with a single-stranded NA, we carried out similar measurements with polyAU.
Figure 2 illustrates changes in the absorption spectra of polyAU (C=0.021 mM) in presence of the NaClO (C_{NaClO}=0.15 mM) with time. To estimate the concentration effects the measurements in presence of higher hypochlorite concentrations (C_{NaClO}=0.35 mM, C_{NaClO}=0.8 mM) were also carried out (spectra are not shown, see fig.4a).

![Figure 2](image)

**Figure 2.** Absorption spectra of polyAU in presence of NaClO at different time intervals: 1 – 1 min., 2 – 15 min., 3 – 24 hours

Unlike DNA, polyAU spectra demonstrate considerable changes within the 1st minute after mixing with NaClO. This indicates that the process of interaction is more efficient in the case of single-stranded NAs. At the same time, qualitatively the changes in the spectra with time are similar to those described above for DNA (figure 1).

3.3. **Interaction of sodium hypochlorite with mononucleotides**

To investigate the effect of hypochlorite on individual nucleotides the absorption spectra of ATPP (Figure 3a) and cGMP (Figure 3b) in presence of NaClO were analyzed. The concentrations of and the NaClO was 0.15 mM (Figure 3), 0.35 mM and 0.8 mM (spectra are not shown, see fig.4b).

![Figure 3](image)

**Figure 3.** Absorption spectra of ATPP (a) and cGMP (b) in presence of NaClO at different time intervals: 1 – 1 min., 2 – 15 min., 3 – 24 hours

In contrast to the polynucleotides, the spectral changes of the mononucleotides depend on the concentration of the sodium hypochlorite. At low NaClO concentrations, spectra contain an isobestic point (fig.3), indicating changes in the structure of nitrogenous bases, resulted from a chemical reaction with hypochlorite. At high concentrations of the hypochlorite, changes in the spectra with time lead to the disappearance of the absorption band of nitrogenous bases, indicating the destruction
of their cyclic structure. At intermediate concentrations of the hypochlorite, both processes take place: the first one (chlorination of nitrogenous bases) immediately after mixing, the second (the destruction of the cyclic structure of nitrogenous bases), the slower one, - within the 24 hours [8].

3.4. Concentration and time dependences

Next series of measurements was aimed to determine the effect of the hypochlorite concentration on the rate of DNA degradation. Figure 4 shows the dependence of the relative change in the absorption of nitrogenous bases in DNA composition on the concentration of sodium hypochlorite for polynucleotides (DNA and polyAU).

![Figure 4. The dependence of the relative absorption of $D_{NA}/D_{complex}$ at 260 nm on the concentration of NaClO for polynucleotides (a) and monomucleotides (b) at different time intervals (red – 1 min, black – 15 min, blue – 24 hours). (a): ■ – pDNA, ● – ctdNA, ▲ – polyAU; (b) – ATPP, ● – cGMP](image)

As it can be seen (figure 4a), the main changes of the absorption already occur at the minimal concentration of hypochlorite ($C_{NaClO}$=0.15 mM). A slight increase in the absorption at the hypochlorite concentration of 0.8 mM is due to the spectral contribution of the hypochlorite itself, since at the given concentration the excess of the hypochlorite remains in the solution after the reaction. Therefore, it can be assumed that the maximum effect on the samples under study is exerted by hypochlorite at a concentration of $C_{NaClO}$=0.35 mM.

Within 1 minute after the preparation, an increase in absorption of NAs is observed in the case of plasmid and thymus DNA. In the case of polyAU, the absorption significantly drops within the 1st minute. This suggests that the reaction rate is considerably higher in this case. It can be assumed that it is the secondary structure (B-form of DNA in our case) that slows down the reaction, and denaturation of DNA is required for the chemical reaction of the nitrogenous bases with hypochlorite, which we observed as an increase in absorption (hyperchromic effect).

The dependence of the relative absorption of the nucleotides at the wavelength $\lambda = 260$ nm (DNA absorption band) on the concentration of sodium hypochlorite is shown in figure 4b. It can be seen the intensity of the absorption band of mononucleotides slightly depends on the hypochlorite concentration and only during the first minutes of the reaction.

Further increase in the concentration of NaClO leads to an additional drop in absorption, which indicates the destruction of nitrogenous bases. For cGMP this process begins at lower concentrations of hypochlorite, than for ATPP.

The decrease in the relative absorption of mononucleotides is much greater than that of polynucleotides.

Figure 5a shows the dependence of the relative absorption at $\lambda=260$ nm on the interaction time for polynucleotides at $C_{NaClO}$=0.15 mM.
Figure 5. The dependence of the relative absorption of $D_{DNA}/D_{complex}$ at the $\lambda=260$ nm on the interaction time for polynucleotides (a) and mononucleotides (b). (a): ■ –pDNA, ● –ctDNA, ▲ – polyAU; (b): ● –cGMP, ● –ATPP

As it can be seen from the figure 5, the time dependencies are similar. The initial point of polyAU is lower than that of DNA, since the interaction manifests itself immediately after mixing.

Based on the time dependencies, we can estimate the time when half of the initial reagent transforms into the final product (half-decay time): $t_{1/2} \approx 10$ min.

Figure 5b shows the dependence of the relative absorption at $\lambda=260$ nm on the time of interaction with hypochlorite for mononucleotides. The kinetics of the reaction and the final values depend on the hypochlorite concentration. When the hypochlorite concentration is $C_{NaClO}=0.15$ mM, the final values are $D_{ATP}/D_{complex}=0.58 \pm 0.02$ and $D_{cGTP}/D_{complex}=0.50 \pm 0.02$. If the concentration $C_{NaClO}=0.35$ mM, then $D_{ATP}/D_{complex}=0.25 \pm 0.02$ and $D_{cGTP}/D_{complex}=0.28 \pm 0.02$, which is significantly lower. The reason for such differences is the presence of two processes: the first – chlorination of nitrogenous bases (fast), the second – the destruction of the cyclic structure of nitrogenous bases (slow), which starts at high concentrations.

4. Conclusion

The analysis of the data obtained suggests that the chemical reaction between NaClO and NAs involves two stages.

Stage 1. The primary process that takes place at low concentrations of hypochlorite ($C_{NaClO}=0.15$ mM) is chlorination. As the result, nucleotide chloramines are formed [7, 8, 10].

Stage 2. The second process, which begins at higher concentration of hypochlorite ($C_{NaClO}=0.35$ mM) is the destruction of the cyclic structure of nitrogenous bases, resulting from the reaction of hypochlorite with the products of primary reaction (stage 1) [9, 10].

It can be assumed that at the above experimental conditions, polynucleotides are participate in both processes. This is evidenced by the final absorptions of the mono- and polynucleotides (polyAU and DNA) at $\lambda = 260$ nm.

The secondary structure of the NAs affects the process of the interaction between the polynucleotides and hypochlorite, preventing the immediate modification of nitrogenous bases after the preparation of the complex. To initiate the reaction between hypochlorite and nitrogenous bases, the destruction of hydrogen bonds (denaturation) is required.

Thus, the reaction of hypochlorite with DNA causes its denaturation and destruction of cycles of nitrogenous bases.

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