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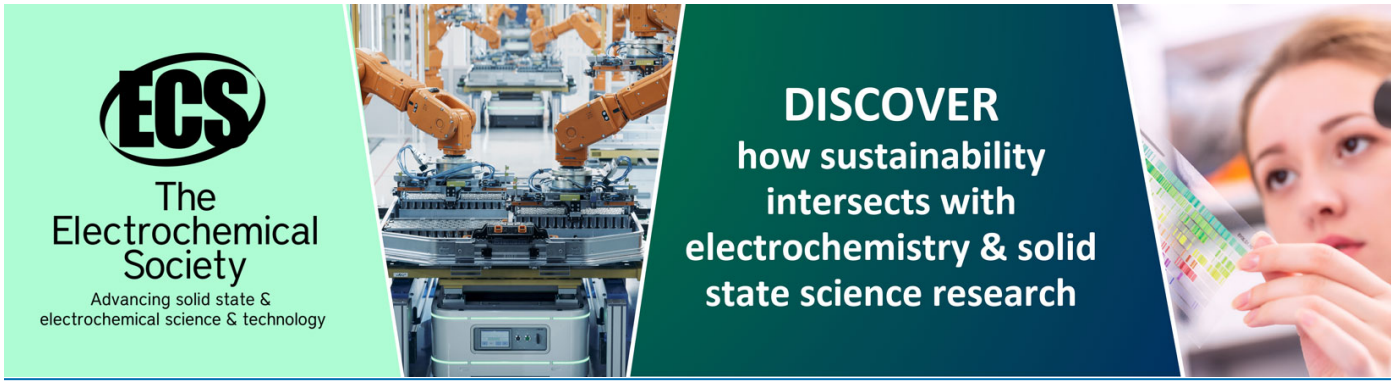

Effect of sodium hypochlorite on the structure of nucleic acids studied using infrared spectroscopy

D N Osinnikova[1], E B Moroshkina[1] and A M Polyanichko[1,2]

[1]Faculty of Physics, St Petersburg University, 7/9 Universitetskaya nab., St Petersburg, Russia, 199034
[2]Institute of Cytology, RAS, 4 Tikhoretsky ave., St Petersburg, Russia, 194064

E-mail: d.osinnikova@spbu.ru

Abstract. The effect of sodium hypochlorite (NaClO) on nucleic acids (NAs) was investigated depending on the concentration of the NaClO. We have performed detailed analysis of the FTIR and UV spectra of the NAs incubated with NaClO. It was found that both the destruction of the secondary structure of DNA (denaturation) and the chemical modification of nitrogenous bases occur.

1. Introduction

There are a lot of antiseptic agents targeted at nucleic acids and proteins to ensure the destruction of bacteria and viruses. Among them one of the most relevant to practice is sodium hypochlorite (NaClO) [1]. It is widely used in medicine, food industry and agriculture as a bactericidal and sterilizing agent.

In living cells, 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. 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 [10, 11]. Recently, it has been demonstrated that the secondary structure of the NAs affects the interaction of polynucleotides with hypochlorite, preventing the immediate modification of nitrogenous bases [12].

In this work, we analyze the interaction of sodium hypochlorite with calf thymus DNA using FTIR and UV spectroscopy.

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 $\mathcal{E}_{292}$ = 350 $M^{-1}cm^{-1}$ [13, 14].

Complexes of DNA with NaClO were prepared by direct mixing of the solutions of individual components with appropriate concentrations. Concentration of DNA was determined

spectrophotometrically using the absorption of hydrolyzed DNA at λ = 290 and 270 nm [15]. Absorption spectra were recorded using Shimadzu UV-1800 spectrometer.

Samples for IR spectroscopy were prepared as described earlier [16]. Prior to FTIR measurements all sample solutions were freeze dried. The obtained powder was further used to prepare KBr pellets of each sample. The samples were carefully blended with the KBr powder (approx. 1% mixture w/w), using agate mortar and pestle. 7 mm pellets were formed using 50 mg of obtained uniform powder and a hand press (PIKE Technologies, USA). IR spectra of the pellets were recorded using the Tensor 27 FTIR spectrometer (Bruker), purged with dry nitrogen and equipped with MCT detector. Each spectrum was recorded with 4 cm^{-1} resolution and averaged by 128 accumulations. Background subtraction and baseline correction were performed using OPUS software provided by the spectrometer manufacture based on the criteria described elsewhere [17].

3. Results and discussion

To analyze the effect of NaClO on ctDNA we obtained UV and IR absorption spectra of DNA-NaClO complexes at different [Cl]/[P] ratios R. The typical absorption spectra of the ctDNA (C=0.040 $mM_{p.}$) in presence of sodium hypochlorite are presented in Figure 1.

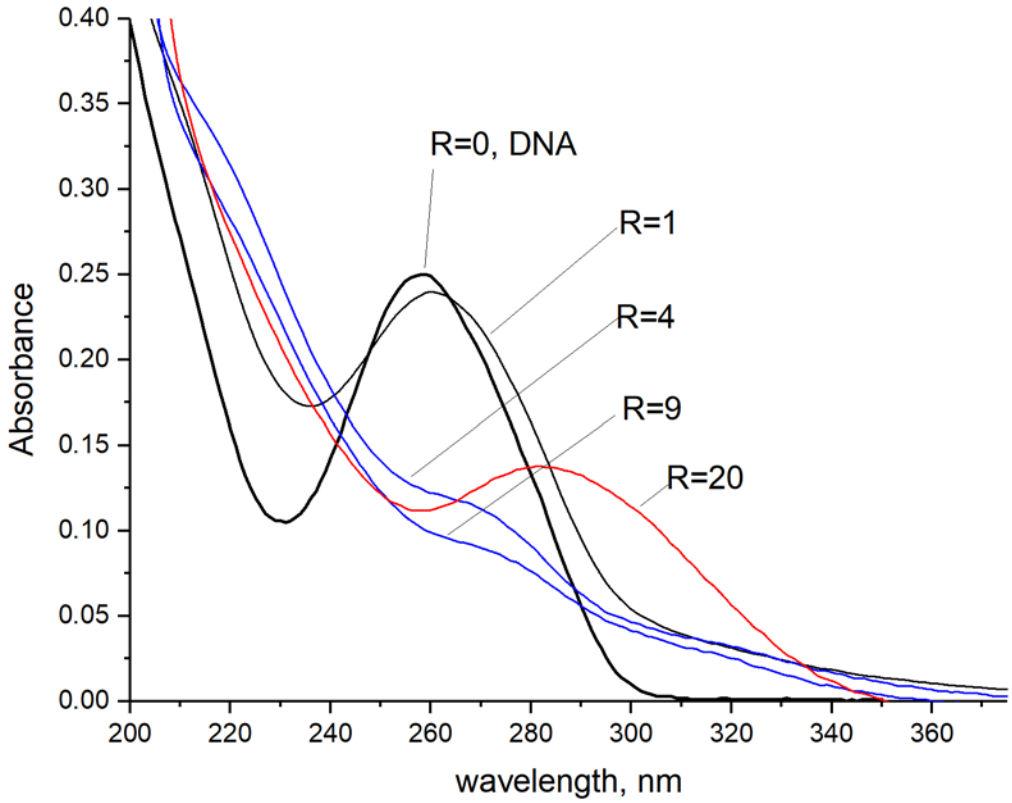

Figure 1. UV absorption spectra of ctDNA in presence of NaClO at different hypochlorite to phosphates molar ratios R.

From the obtained spectra, it can be seen that, the absorption of DNA gradually decreases and shifts towards longer wavelengths with increasing NaClO concentrations revealing considerable modification of the chromophore (i.e. nitrogenous bases) structure. At higher NaClO concentrations (R≥4) the DNA absorption band (λ_{max} = 260 nm) almost disappears, which most likely is a consequence of the damage of the structure of the nitrogenous bases of DNA [12], and by R=20 the

absorption of the sample is mostly due to the absorption of the excess of the hypochlorite in the solution.

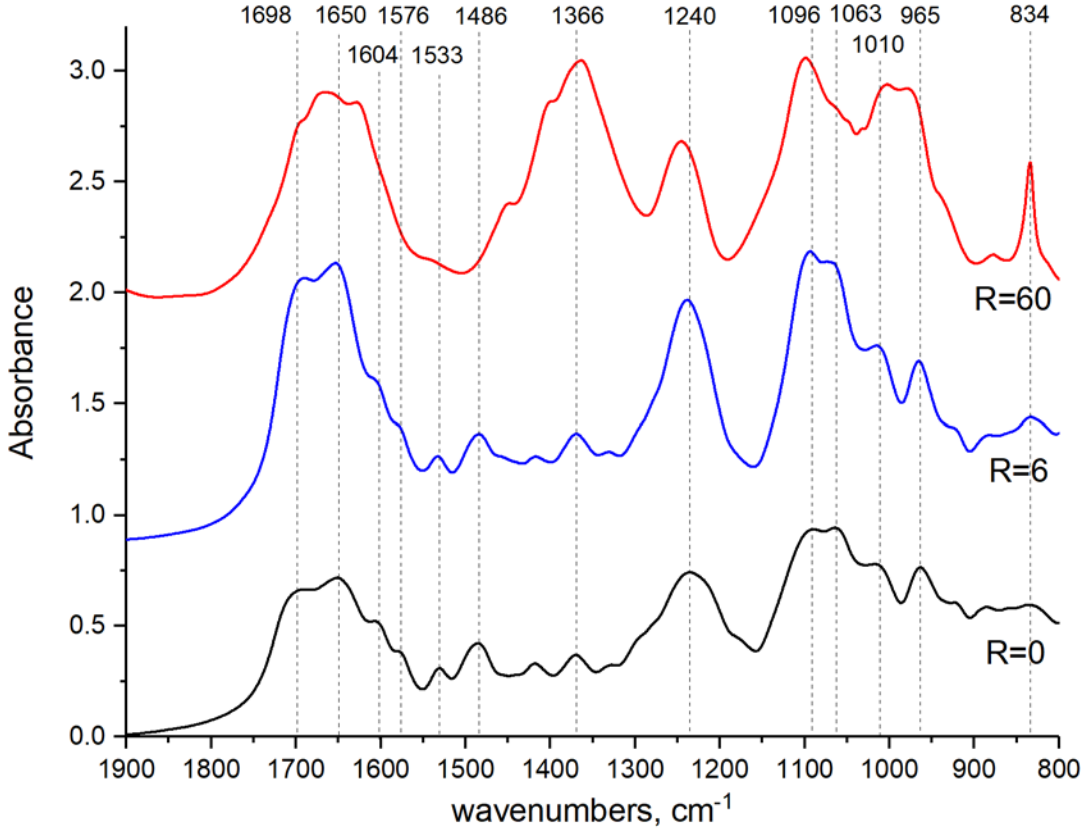

Figure 2. FTIR spectra of ctDNA in presence of NaClO at different hypochlorite to phosphates molar ratios R.

To investigate the effect of high concentrations of the hypochlorite on DNA structure in more details we have analyzed infrared spectra of the dried complexes at different [Cl]/[P] ratios R presented in Figure 2. The mid-IR spectra of DNA contain two major regions corresponding to the vibrations of the nitrogenous bases (1800 – ca. 1300 cm^{-1}) and to the vibrations of the sugar phosphate backbone (ca. 1300 - 800 cm^{-1}). The major assignments are based on the data published earlier regarding vibrational spectra of nucleic acids [16, 18, 19].

In presence of NaClO distinct changes are observed in the spectra of DNA in both regions, mentioned above, attributed to the vibrations of the nitrogenous bases and the sugar phosphate backbone. Comparison of the absorption spectra of pure DNA and the spectra of the complexes reveals the appreciable changes in vibrations at 1698 and 1576 cm^{-1}. The former might be due to the guanine (G) C=O stretching and NH_2 scissoring vibrations and interaction of the bases in the pairs, while the latter is typical for the interactions with N7 atom of guanine. Also the changes in vibrational modes of other bases, mostly those of cytosine, can be clearly seen at 1650, 1604, 1533 and 1486 cm^{-1}. Vibration at 1650 is attributed to the hydrogen bonded cytosine C4=O4, while the others at 1604, 1530 and 1490 cm^{-1} are involved in in-plane ring vibrations, NH and CH in-plane deformation modes of hydrogen bonded cytosine, adenine and thymine. It is also worth mentioning that (1) most of the above spectral features are characteristic for the base paring, indicating that the double helical structure of DNA is significantly distorted by R=6; and (2) the above characteristic vibrations of nitrogen containing groups within the rings of the bases are completely disappear at higher R (illustrated by

R=60), implying that the nitrogen atoms undergo chemical modifications and the ring structures are seriously damaged or even completely destroyed. The latter is also supported by the shifts of the carbonyl bands, and emerging vibrations at 1366 and 834 cm^{-1}, which are most likely due to the vibrations of the fragments of degraded rings. The major changes in vibrations of the sugar phosphate backbone at 1240, 1096, 1063, 1010, 965 cm^{-1} indicate that interaction of NaClO with DNA results in considerable changes of the backbone geometry by R=6 and degradation of the sugar rings by R=60.

4. Conclusions

The analysis of the data obtained confirmed that the chemical reaction between NaClO and NAs involves two stages. At low concentrations of hypochlorite the loss of the DNA secondary structure and formation of nucleotide chloramines occur [7, 8, 10, 12]. At higher concentrations of hypochlorite the destruction of the cyclic structure of nitrogenous bases is observed [9, 10, 12].

Earlier it was demonstrated, that the secondary structure of the NAs affects their interaction with hypochlorite, slowing the modification of nitrogenous bases [12], and the destruction of hydrogen bonds in the pairs was required to initiate the reaction between hypochlorite and nitrogenous bases.

Here, based on the analysis of FTIR spectra we confirmed that the reaction of hypochlorite with DNA causes its denaturation and destruction of cycles of nitrogenous bases.

Acknowledgements

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