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Hypochlorous acid induced structural and conformational modifications in human DNA: A multi-spectroscopic study

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ABSTRACT

Hypochlorous acid (HOCl) is generated by activated phagocytes at the site of inflammation. Exposure of DNA to HOCl results in base and nucleotide modifications causing DNA damage, which is one of the leading causes of various pathological conditions including carcinogenesis. In the present work, various biophysical techniques were used to study HOCl induced structural and conformational changes in human placental DNA. The HOCl modified DNA showed hyperchromicity, reduced fluorescence and decrease in melting temperature. Circular dichorism (CD) and Fourier transform infra-red (FT-IR) studies exhibited conformational changes and shift in band positions of DNA, respectively, suggesting structural changes. Agarose gel electrophoresis and scanning electron microscopy showed strand breakage and decreased aggregation. These results suggest that HOCl causes conformational and structural perturbations in mammalian DNA, which may consequentially lead to DNA mutations resulting in perturbation of epigenetic signals leading to cancer and autoimmune diseases.

1. Introduction

HOCl is generated by myeloperoxidase (MPO) in activated neutrophils using chloride ions and hydrogen peroxide (H_2O_2) produced by NADPH oxidase, as substrates [1,2]. Although HOCl is meant for host defence owing to its bacterial killing ability but sometimes excessive or sustained production may result in tissue damage and this may lead to progression of various diseases like chronic inflammation, atherosclerosis and some types of cancers like lung and gastric cancer [1,2]. Under pathological conditions, $HOCl/OCl^-$ is found at concentrations up to 200 μM [3]. However, at the site of inflammation it can be 3–8 mM while concentrations of 20–50 mM have also been reported [4,5].

HOCl is a highly reactive compound that reacts readily with biomolecules, including proteins, lipids, and DNA [1,6,7]. Proteins are major targets for HOCl, owing to their abundance and high reactivity with HOCl [8]. Protein-derived *N*-chloramines are formed readily on reaction of HOCl with lysine and histidine side-chains [6]. These species are reactive and can mediate further protein damage [9] and induce the oxidation of other biological molecules, including lipids [10] and DNA [11].

The heterocyclic (ring) NH groups of guanosine and thymidine derivatives are more reactive towards HOCl than the exocyclic NH_2 groups of guanosine, adenosine, and cytidine derivatives resulting in both structural changes and chemical modification [12]. Semistable chloramines (RNHCl and RR′NCl species) are formed upon reaction of HOCl with these groups [13]. The formation of these species can lead to the dissociation of double stranded DNA due to the disruption of hydrogen-bonding [14] and formation of nitrogen-centered radicals [15]. The heterocyclic chloramines formed with guanosine and thymidine react more rapidly with thiols and other primary amines (via chlorine atom transfer) than the exocyclic NH_2-derived chloramines formed on guanosine, adenosine, and cytidine [12,14,16].

HOCl reacts with DNA repair enzymes [17] and can potentiate H_2O_2-mediated DNA strand breaks in human mononuclear leukocytes [18]. Exposure of isolated human placental DNA to HOCl generates thymine glycol and 5-OH-uracil, two modified pyrimidine base products, as major products [19]. HOCl can

Abbreviations: HOCl, hypochlorous acid; CD, circular dichorism; FT-IR, Fourier transform infra-red; SEM, scanning electron microscopy; NaOCl, sodium hypochlorite; NaPi, sodium phosphate buffer; MPO, myeloperoxidase; NADPH, reduced nicotinamide adenine dinucleotide phosphate; DLS, differential light scattering; ITC, isothermal calorimetry; EtBr, ethidium bromide; EDTA, ethylenediaminetetraacetic acid; HATR, horizontal attenuated total internal reflection.

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chlorinate/oxidize pyrimidines and purines. Henderson et al. [20] reported that the HOCl-derived Chlorine can chlorinate 2′-deoxycytidine (dC) to form 5-chloro-2′-deoxycytidine (5-CldC) as the major product. These alterations could lead to miscoding, base mis-incorporation, and mutation if unrepaired prior to DNA replication [21,22]. Disruptions of epigenetic patterns are reported in numerous human tumors and are considered critical for understanding cancer etiology [23,24]. Effects of HOCl on the structural changes of DNA have been examined and each study has added some information to the previous knowledge [12,15,19]. However, a detailed study on the HOCl- induced conformational changes in DNA has not been done so far.

The aim of this study was to investigate the HOCl concentration dependent changes in human placental DNA. These changes were studied by physicochemical techniques like UV–vis, florescence spectroscopy, circular dichorism (CD), Fourier transform infrared red (FTIR) spectroscopy, thermal denaturation studies, agarose gel, dynamic light scattering (DLS), isothermal calorimetry (ITC), scanning electron microscopy (SEM). Thus HOCl can cause oxidative modification and conformational changes in double stranded DNA.

2. Materials and methods

2.1. Chemicals

Human placental DNA, sodium hypochlorite (NaOCl), nuclease S1, ethidium bromide (EtBr), agarose and dialysis tubing were purchased from Sigma–Aldrich, USA. Chloroform, ethanol, sodium hydroxide, isoamyl alcohol, sodium chloride, sodium acetate and ethylenediaminetetraacetic acid (EDTA) were from Qualigens, India. All other reagents/chemicals were of the highest analytical grade available.

2.2. Modification of human placental DNA by HOCl

Sodium hypochlorite (NaOCl) was directly used as a source of HOCl [25]. HOCl concentration was determined at 292 nm (pH 12, $\varepsilon = 350\ M^{-1}\ cm^{-1}$) [26]. Human placental DNA was used in these studies. It was purified free of proteins, RNA and single stranded regions as described earlier [27] and the purity of DNA was confirmed by A_{260}/A_{280} ratio. Human placental DNA (25 μg/ml) was incubated with 0.025, 0.05, 0.075, 0.1 and 0.125 mM HOCl at 37 °C for 15 min in 0.05 M sodium phosphate (NaPi) buffer, pH 7.4. The reaction mixture was quenched by addition of five times methionine to HOCl present in the samples and then dialysed against 0.05 M NaPi buffer, pH 7.4 for 24 h.

2.3. UV–visible spectrophotometry

The absorbance spectra of native (unmodified) and HOCl modified DNA samples were recorded in the wavelength range of 200–400 nm using quartz cuvettes of 1 cm path length on a Shimadzu UV-1700 spectrophotometer. Hyperchromicity at 260 nm was calculated by the following equation:

$$\%\ \text{hyperchromicity at 260nm} = \frac{OD_{\text{HOCl modified DNA}} - OD_{\text{native DNA}}}{OD_{\text{native DNA}}} \times 100$$

2.4. Fluorescence studies

The fluorescence emission spectra of native and modified DNA samples (25 μg/ml) were recorded from 300 to 700 nm using quartz cuvettes on Shimadzu RF-5301 spectrofluorometer using an excitation wavelength of 325 nm [28]. Ethidium bromide (2.5 μg/ml) was used as an external chromophore. Fluorescence emission spectral analysis of native and modified DNA was calculated from the following equation.

$$\%\ \text{decrease in FI} = \frac{FI_{\text{HOCl modified DNA}} - FI_{\text{native DNA}}}{FI_{\text{native DNA}}} \times 100$$

Where FI is florescence intensity.

2.5. Circular dichroism

CD spectroscopic measurements of native and HOCl modified DNA were recorded with 1 mm path length quartz cuvette on Jasco-J720 CD spectrophotometer [29]. Measurements were done in the wavelength range of 220–400 nm in the nitrogen atmosphere. Sample spectra were collected at room temperature with a scan speed of 100 nm/min and the base line was adjusted with NaPi buffer, pH 7.4. Molar ellipticities [θ] were calculated in terms of base pair concentration according to the following equation:

$$\{\theta\} = \theta/10\,cd$$

Where θ (theta) is the measured ellipticity (mdeg), c is the molar concentration of DNA (in terms base pair), and d is the path length (in cm).

2.6. Thermal denaturation analysis

Native and HOCl modified DNA samples were subjected to heat denaturation on Perkin Elmer UV/Vis Lambda-25 spectrophotometer coupled with a temperature programmer PTP–1 + 1 Peltier system and Julabo 5A controller assembly [30]. Samples were heated from 30 °C to 95 °C at a rate of 1 °C/min. Percent denaturation was calculated using the formula:

$$\%\ \text{denaturation} = \frac{A_T - A_{30}}{A_{Max} - A_{30}} \times 100$$

Where, A_T = absorbance at temperature T°C, A_{max} = maximum absorbance on completion of denaturation (95 °C), A_{30} = initial absorbance at 30 °C.

2.7. Agarose gel electrophoresis (alkaline and non-alkaline conditions)

Native and HOCl modified DNA samples were subjected to 0.8% agarose gel electrophoresis under alkaline and non-alkaline conditions and migration pattern was observed. Alkaline gel electrophoresis was done as described by McDonnell et al. [31] with slight modifications. Gel was prepared in alkaline electrophoresis buffer (50 mM NaOH and 1 mM EDTA, pH 8.0). Samples were precipitated with ethanol and dissolved in alkaline loading buffer (50 mM NaOH, 1 mM EDTA, 5–10% glycerol, 0.025% bromophenol blue), loaded in wells of gel and electrophoresed for 8 h at 2 V/cm. Under non-alkaline condition native and modified DNA were separated on 0.8% agarose gel, which was electrophoresed at 30 mA for 2 h in TAE buffer (40 mM Tris–acetate, 2 mM EDTA, pH 8.0). DNA bands were visualized under UV light after staining with ethidium bromide (0.5 mg/ml) [32]. The gels were then photographed by Gel Doc instrument.

2.8. FT-IR spectroscopy

FT-IR spectra of native and HOCl-modified DNA were recorded on Perkin-Elmer Spectrum Two FT-IR spectrometer keeping the wave number range from 1800 to 700 cm^{-1} in order to cover the major absorption bands of DNA [33]. Ten μl of native and HOCl treated DNA samples were placed on the horizontal attenuated total internal reflection (HATR) crystal and interferograms were

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