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Hypochlorous Acid

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Abbreviations

CYA, Cyanuric acid

MPO, Myeloperoxidase

DMPO, 5,5-dimethyl-1-pyrroline-*N*-oxide

NaOCl, Sodium hypochlorite

EPR, Electron paramagnetic resonance

OCl⁻, Hypochlorite ion

HOCl, Hypochlorous acid

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Abstract

When chlorine is added to the water it produces hypochlorous acid (HOCl) and HOCl is used widely to control bacteria and algae. It is a weak acid and strong oxidant so it reacts with a wide variety of reductants. HOCl is a highly reactive species that participates in both oxidation and chlorination reactions. Inside cells, HOCl is generated by the enzyme myeloperoxidase in which H_2O_2 and Cl^- ions serve as substrates. Thiols and thioethers are particularly reactive biologic targets of HOCl and the main biological chlorination reactions are with amine groups to give chloramines. Some specific reaction products are taken as biomarkers to monitor the activity of HOCl through various methods including electron paramagnetic resonance (EPR).

Introduction

Most of people use bleach everyday and some of them know that hypochlorous acid (HOCl) is the main active component of bleach. However, few of people learn that we produce “bleach” by ourselves inside our body. As a weak acid and strong nonradical oxidant, HOCl both controls bacteria and algae and oxidizes organics.

Chemistry of Hypochlorous Acid

When chlorine is added to the water it produces hypochlorous acid (HOCl).

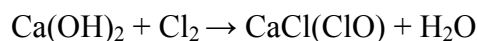


Hypochlorous acid further dissociates to hypochlorite ion (OCl^- , the weaker form of free chlorine) and free hydrogen (H^+):

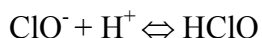


This dissociation is reversible, and pH driven. At pH 7.53, concentrations of HOCl and OCl^- are equal. At pH = 10, the predominant form is hypochlorite ion (OCl^-). HOCl is the “active ingredient” while the OCl^- is a bank, or reservoir of less active chlorine. So chlorine is most efficient in bacteria control at a pH of approximately 7.4 - 7.6. At low pH chlorine tends to use itself up too quickly. At a high pH chlorine doesn't produce very much disinfectant or HOCl-it is mostly in the form H^+ and OCl^- . As HOCl is used to kill algae, or as it evaporates, OCl^- shifts back to HOCl to maintain the pH-mandated equilibrium [2].

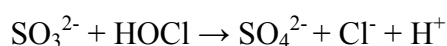
When passing chlorine over calcium hydroxide, bleaching powder is obtained.



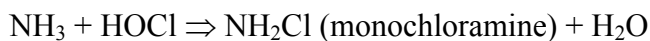
Dissolved in water, HOCl is produced.



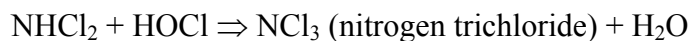
HOCl is an extremely active oxidizing chemical that will react with many reducing substances such as hydrogen sulfide, ferrous iron, manganese or sulfurous anion. It also reacts with organic matter and ammonia.



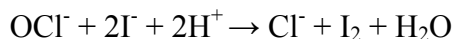
The reaction of HOCl with ammonia produces a series of chlorine-like odorous, irritating compounds called chloramines or combined chlorine. These chloramines irritate eyes and mucous membranes and are often confused with chlorine. Combined chlorine has little ability to kill bacteria. Formation of chloramines is based on the following equation:



Further reaction with HOCl forms nitrogen trichloride

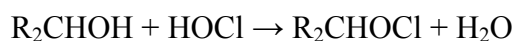


In acidic solution, iodide is oxidized by hypochlorite to iodine:



The iodine then reacts with the starch in the test paper to produce the characteristic purple colour of the starch-iodine complex.

Aqueous sodium hypochlorite (NaOCl) can be used to oxidize secondary alcohols to ketones. The reaction occurs more rapidly under acidic conditions, so it is thought that the actual oxidizing agent is HOCl, generated by the acid base reaction between sodium hypochlorite and acetic acid. In alcohol oxidations, the reaction probably proceeds *via* E2 elimination of the alkyl hypochlorite produced by reaction of the initial alcohol with HOCl.



Though HOCl is used as disinfecting agent widely, the precise mechanism of the disinfecting action of chlorine is not fully known. Chlorine is capable of undergoing a wide variety of reactions and probably reacts with the microbial cell at several levels. At high concentrations, massive oxidation takes place and membranes and all organic components are affected. At lower concentrations chlorine probably affects vital protein systems as well as membranes.

Biochemistry of HOCl

1. Formation of HOCl

The 'respiratory burst' of phagocytes such as neutrophils generates superoxide which forms H_2O_2 by dismutation. H_2O_2 and Cl^- ions serve as substrates for the enzyme myeloperoxidase to generate HOCl [3].

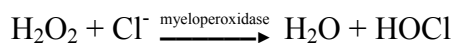
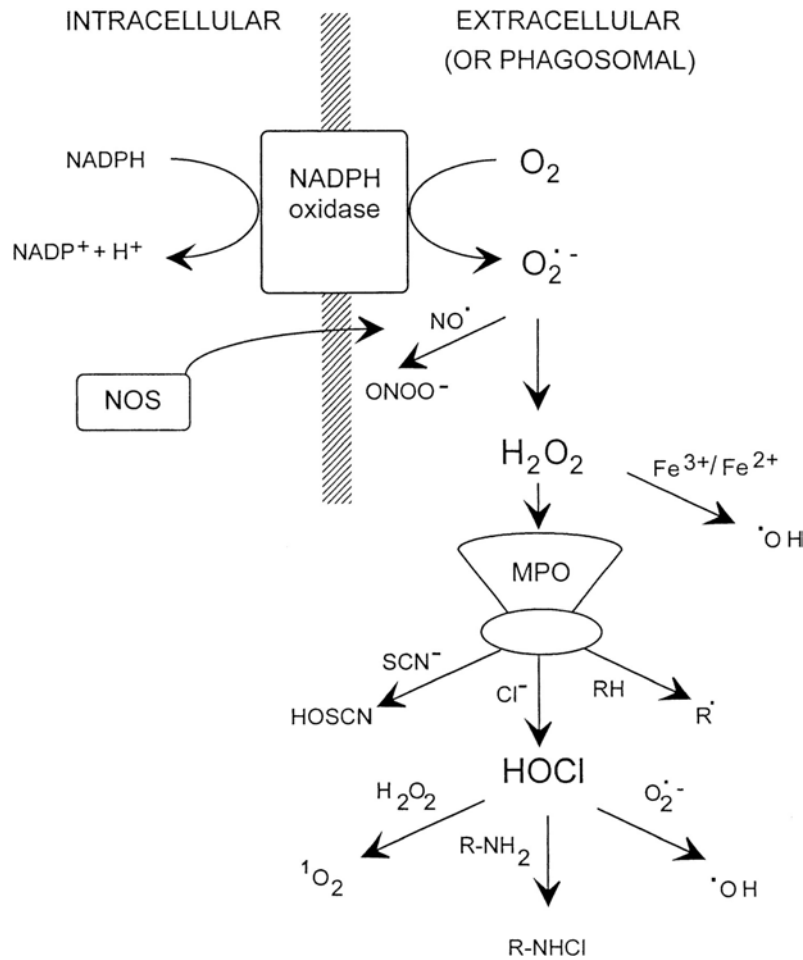


Figure 1 shows a possible pathway of formation of HOCl in cells [4].



2. Reaction of HOCl

(1) Oxidation

Hypochlorous acid is a strong nonradical oxidant of a wide range of biological compounds, but more selective than hydroxyl radical. The preferred substrates of HOCl are thiols and thioethers. Cysteine and methionine residues react rapidly with HOCl to give oxyacids and cystine (from Cys) and sulphoxides (from Met)[5]. The latter type of reaction is believed to be responsible for the inactivation of α_1 -proteinase inhibitor. Other compounds, including ascorbate, urate, pyridine nucleotides, and tryptophan are oxidized by HOCl, although not as rapidly. The treatment of isolated proteins with HOCl is known to result in the alteration of amino acid side chains, protein

fragmentation [6] and cross-linking/aggregation [7]. In some cases these reactions can render the protein more susceptible to degradation by intracellular protease enzymes. It has been shown, for example, that HOCl-treated fibronectin is more susceptible to degradation by elastase due to changes to both the primary and tertiary structures of the protein [7].

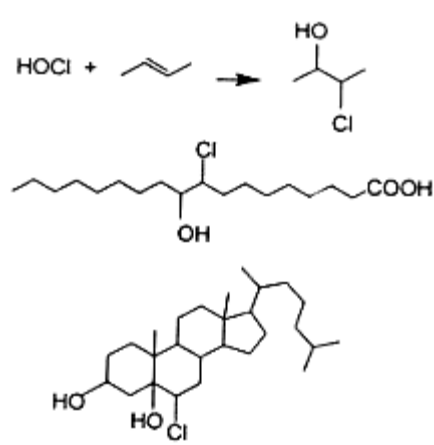


Figure 2. Route to chlorohydrin formation and isomeric examples of oleic acid and cholesterol chlorohydrin [8].

(2) Chlorination

Apart from oxidation reactions, HOCl also reacts with biomolecules through chlorination. The main biological chlorination reactions are with amine groups to give chloramines. The free amino groups of lysine residues are known to form chloramine (RNHCl) intermediates readily on reaction with HOCl [9]. This is believed to be of particular importance for two reasons. First, lysine residues are often present in proteins in higher amounts than other reactive residues. Secondly, the long lifetime of chloramines compared with those of HOCl or H₂O₂ means that it is likely that these materials, which retain the oxidizing equivalents of HOCl, diffuse a considerable distance and hence might cause damage at remote sites. HOCl also reacts with tyrosine residues to give ring chlorinated products, including 3-chlorotyrosine and 3,5-dichlorotyrosine [Figure 3], the former has been employed as a marker of HOCl-induced damage. [8] Besides the chlorination of amino acids, HOCl reacts with unsaturated lipids and

cholesterol to give chlorohydrins[Figure 2] and with nucleic acids to give ring chlorination of cytosine residues.

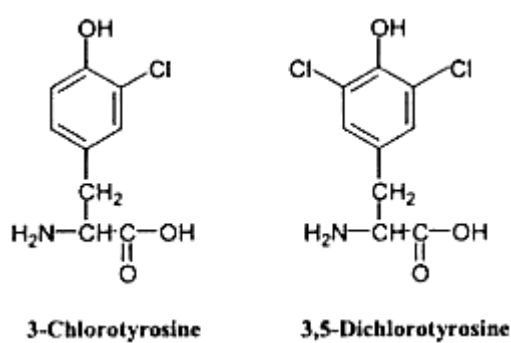


Figure 3. Chlorinated tyrosines formed from the reaction of HOCl with tyrosine residues in proteins [7].

Detection of HOCl and its products

1. The ultraviolet absorption spectra were used to determine the concentrations of HOCl and OCl⁻ in electrolyzed oxidizing water and the chemical equilibrium of these species with change in pH and amperage. Figure 4 (Left) shows an example of high resolution spectra of deuterated hypochlorous acid.

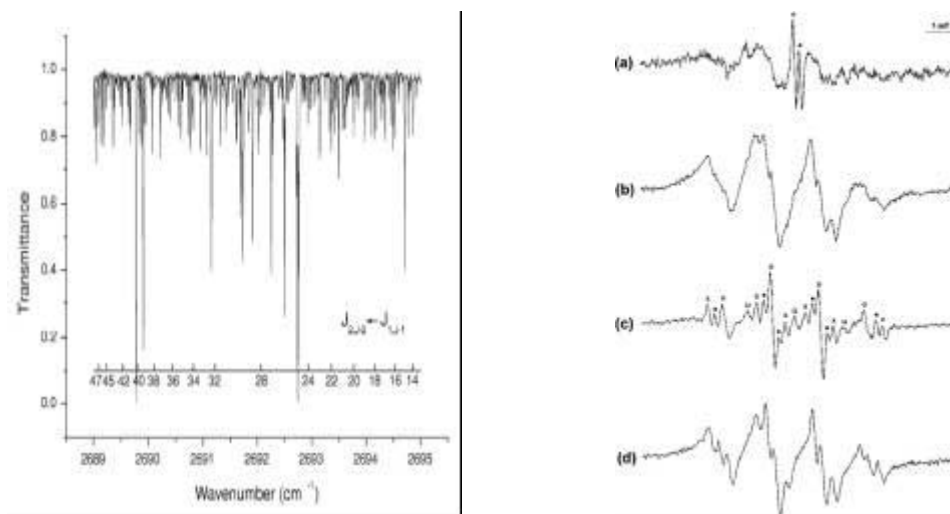


Figure 4 (Left) A portion of the $2\nu_2$ spectrum of DOCl[10]; (Right)EPR spectra observed on reaction of plasma with HOCl in the presence of the spin trap DMPO [11].

2. Treatment of plasma and isolated plasma proteins with a wide range of HOCl concentrations gives rise to nitrogen-centered protein-derived radicals [12]. The exact mechanism of formation of these protein-derived nitrogen-centered radicals from chloramines remains to be established. There are two hypotheses. One of them is homolysis of the N–Cl bond to give RNH^\bullet and Cl^\bullet and another is one-electron reduction by trace metal ions to give RNH^\bullet and Cl^- [12]. Figure 4(right) shows one example of EPR spectra observed on reaction of plasma with HOCl in the presence of the spin trap DMPO.

Summary

As a strong oxidant, HOCl reacts with a wide variety of reductants including biomolecules. So HOCl is used widely to control bacteria and algae. HOCl is also generated by the enzyme myeloperoxidase in which H_2O_2 and Cl^- ions serve as substrates inside cells. Through oxidation and chlorination reactions, HOCl reacts with proteins, lipids and nucleic acids. The reactions of HOCl in cells can be measured by taking some specific reaction products as biomarkers through various methods including electron paramagnetic resonance (EPR).

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