

Chloroperoxidase Catalyzed Halogenation of Pyrimidine Bases

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Treatment of uracil or cytosine with H_2O_2 and potassium halides in the presence of chloroperoxidase in potassium phosphate buffer solution at pH 3.0 resulted in halogenation of the substrates, whereas no reaction occurred on thymine by a similar treatment.

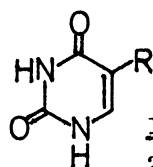
Enzymatic halogenation of biomolecules with H_2O_2 and halide ions is of interest in connection with the action of disinfection by myeloperoxidase which is present in neutrophil. Furthermore, peroxidase catalyzed halogenation of amino acids¹⁾ and important metabolites such as arachidonic acid²⁾ and prostaglandin E_1 ³⁾ with H_2O_2 and halide ions has been investigated. However, little attention has been paid to the reaction of nucleic acid bases, although oxidation of adenine and nucleic acids and incorporation of chlorine into RNA by myeloperoxidase in the presence of H_2O_2 and NaCl was reported.⁴⁾ On the other hand, chloroperoxidase catalyzed halogenation of β -keto adipic acid,^{5a)} monochlorodimedone,^{5b)} barbituric acids,^{5c)} cyclopropanes,^{5d)} alkynes,^{5d)} alkenes,^{5e)} trans-cinnamic acids,^{5f)} steroids,^{5g)} NADH,^{5h)} tyrosine,⁵ⁱ⁾ anisole,^{5j)} anilines,^{5k)} antipyrine,^{5l)} and thiazoles^{5m)} is known. These observations led us to investigate chloroperoxidase catalyzed halogenation of pyrimidine bases.

A solution of uracil (1) (1.5 mmol), chloroperoxidase⁶⁾ (1250 units), and KBr (3 mmol) in 0.25 mol dm^{-3} potassium phosphate buffer solution at pH 3.0 (120 ml) was stirred. The reaction was started by adding H_2O_2 and continued for 60 min at 25-28 °C. The solution was adjusted to pH 7.0 with KOH and catalase was added to remove any remaining H_2O_2 . The reaction mixture was evaporated to give a white solid mass. The residue was extracted with a large amount of MeOH. Droplet counter-current chromatography (Tokyo Rikakikai Co., DCC-300-G2) was used for preparative separation of the extract. The separation with $CHCl_3$ -MeOH- H_2O (5:5:3) by descending method resulted in the isolation of 5-bromouracil (2)⁷⁾ (41%) and 1 recovered (34%).

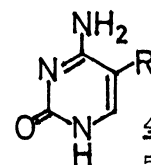
Under similar conditions, the reaction of cytosine (4) gave 5-bromocytosine (5)⁷⁾ in 41% yield. Furthermore, treatment of 1 (2 mmol) with KCl (4 mmol), H_2O_2 (4 mmol), and chloroperoxidase (1923 units) gave 5-chlorouracil (3)⁷⁾ (7%) and 1 recovered (70%). On the other hand, no reaction occurred by a similar treatment of thymine with the enzyme, although we reported the non-enzymatic chlorination of thymines in Clark and Lubs buffer solutions at pH 1.0-2.0.⁸⁾ In addition, 1, 4, and thymine were unreactive to a mixture of potassium halides and H_2O_2 in the absence of chloroperoxidase.

In this paper, we demonstrated the enzymatic halogenation of 1 and 4. On the other hand, antiviral activities of 5-halogenated uracils and cytosines and their nucleosides⁹⁾ and mutation caused by phagocytes¹⁰⁾ are known.

Therefore, the result suggests a possibility of damage of nucleic acids by peroxidase.



1 : R = H
2 : R = Br
3 : R = Cl



4 : R = H
5 : R = Br

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