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NO OR LITTLE PRODUCTION OF SINGLET MOLECULAR OXYGEN IN HOCl OR HOCl/H_2O_2

A MODEL SYSTEM FOR MYELOPEROXIDASE/H_2O_2/Cl^-

Yoshio Ushijima
Clinical Laboratory, School of Medicine, Gunma University
Maebashi, Gunma, Japan

Minoru Nakano*
College of Medical Care and Technology, Gunma University
Maebashi, Gunma, Japan

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Summary: The HOCl in chlorine-water oxidizes DPF to *cis*-DBE in parallel to the HOCl concentration. The addition of H_2O_2 produces singlet molecular oxygen, and a bimol collision above pH 6.0, but not in the pH region 3.0 to 4.0. The DPF conversion to *cis*-DBE is initiated by a 1,2-position attack of OH^- and Cl^+, followed by the HCl elimination. The oxidation potency of HOCl is much greater than the singlet molecular oxygen generated in chlorine-water/H_2O_2 solution, on the pH range 6.0 to 8.0 where both HOCl and OCl^- are present.

Human polymorphonuclear leuckocytes ingest organisms and emit weak light in the visible region during phagocytosis (1,2). Among the microbicidal systems, myeloperoxidase, H_2O_2, and a halide cofactor (3), could generate HOCl near pH 4.5 (4).

Rosen and Klebanoff (5) demonstrated that the system, such as myeloperoxidase with H_2O_2/Cl^-, HOCl or HOCl/H_2O_2, convert DPF to *cis*-DBE, $^1O_2$ quenchers and traps are inhibitory, thus emphasizing the $^1O_2$ production. Harrison *et al.*(6) have implicated Cl_2 in the DPF conversion to *cis*-DBE by myeloperoxidase/H_2O_2/Cl^-. We know of no specific $^1O_2$ traps and quenchers

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* To whom correspondence should be addressed.

Abbreviations: $^1O_2$, singlet molecular oxygen; DPF, 2,5-diphenylfuran; cis-DBE, cis-1,2-dibenzoylethylene.

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usable in aqueous system. Since DPF is oxidized to *cis*-DBE either by $^1O_2$ or ·OH and by other oxidants (7), we ask whether the oxidant in chlorine-water solution might be $^1O_2$.

The present work reports: 1) A HOCl concentration dependence of the DPF conversion to *cis*-DBE in chlorine-water solution, and inhibition by the added H_2O_2, 2) the chlorine-water solutions do not emit weak light on the pH range 2.5 to 10.8, 3) in DPF/chlorine-water/H_2O_2 solution chemiluminescence is detected in the visible region above pH 6.0 and is enhanced significantly near pH 10, at which the DPF conversion to *cis*-DBE is not observed, 4) the DPF conversion to *cis*-DBE by HOCl does not require O_2.

MATERIALS AND METHODS

NaOCl solution; This was obtained from Nakarai Chem. Co. The concentration of NaOCl in the solution was determined as follows. Pipette 3 ml of 4-6% NaOCl solution into 100 ml flask containing 50 ml of water. Add 2g of potassium iodide and 2 ml of 30% acetic acid and titrate with 0.1N sodium thiosulfate. Add indicator near the end point and continue the titration until the disappearance of blue color. One ml of 0.1N $Na_2S_2O_3$ corresponds to 3.72mg NaOCl.

The DPF conversion to *cis*-DBE; The reaction mixture contained 50 mM buffer, 0.2mM DPF, 0.4mM NaOCl solution, 3% acetone, 0.8mM H_2O_2 or none, in a total volume of 2 ml. All components, except for the NaOCl solution, was preincubated at 37°C for 3 min. The reaction was initiated by the addition of NaOCl solution and the incubation was carried out 37°C for 30 min. in a water bath with continuous shaking, in the dark. The reaction was stopped by addition of 2 ml of ether. The ether extraction was repeated three times. The combined ether extracts were then dried over anhydrous sodium sulfate and evaporated to dryness in vacuo. The residue was dissolved in ether and applied onto Silica gel H thin layer plates. After developing in hexane-dioxane (3:1 v/v), the spots corresponding to an authentic sample of *cis*-DBE were scrapped and extracted with 2.0 ml of 95% ethanol. After centrifugation, *cis*-DBE in ethanol was determined by the method described by Rosen and Klebanoff (5).

Luminescence measurement; Luminescence was measured in a Beckmann Model LS-200B liquid scintillation counter with coincidence circuit turned off at 22°C. The standard reaction mixture is the same as that for the conversion of DPF to *cis*-DBE. The reaction was initiated by the addition of NaOCl and luminescence was measured for 12 sec. (each light intensity) first three times during 2 min. at room temperature. Each light intensity in log scale was plotted against the time measured. Maximal light intensity was then calculated by the extrapolation.

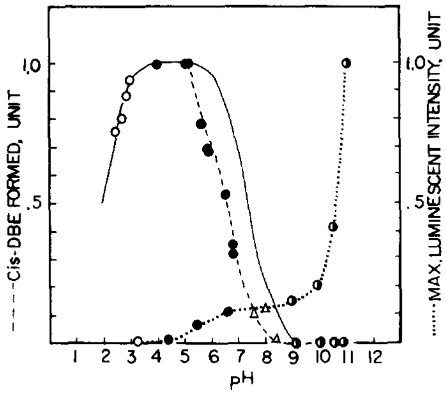

Fig. 1. Effect of pH on the conversion of DPF to cis-DBE in chlorine-water solution or on the luminescent intensity in chlorine-water/H_2O_2. The incubation conditions and assays were the same as those listed in text, save that the various buffer solution were used; 0.05M citrate-HCl (o), 0.05M acetate (●), 0.05M sodium phosphate (△) and borate-HCl or NaOH (◑). A curve showing theoretical concentrations of HOCl in chlorine-water solution (-) was obtained from the data descrived by Swartz (8). The results are shown relative to the values at pH 4.0 for the conversion of DPF to cis-DBE and at pH 10.8 for the maximal light intensity.

RESULTS AND DISCUSSION

It is known that in chlorine-water solution at low pH elementary chlorine (Cl•) predominates, at pH 2-3 chlorine (Cl_2) and hypochlorous acid (HOCl), around the neutral pH hypochlorous acid and hypochlorite (OCl^-), while at higher pH OCl^- alone exists (8). On the other hand, HOCl and Cl_2 possess higher oxidation potentials (+1.50 and 1.35 V, respectively) than OCl^- (0.95 V) (9). Thus oxidation activity of chlorine-water solution should be greater at low pH than at high pH because of the greater portions of molecular chlorine and hypochlorous acid which are present. If an oxidizable compound is added to the chlorine-water solution, it should be easily oxidized at low pH rather than at high pH.

When DPF was added to chlorine-water solution and incubated for 30 min. at 37°C, it was converted to *cis*-DBE on the final pH range 2.5 to 7.6, but not to *cis*-DBE at higher pH. As shown in Fig. 1, the DPF conversion to *cis*-DBE is parallel to the theoretical concentration of HOCl in chlorine-water solution (reported by Swartz (*8*)) in the pH region 2.5 to 5.0. However, such a good correlation between the oxidizability of DPF and the HOCl concentration in media was slightly deviated on the pH range 5.5 to 9.0. In the pH region 4.0 to 5.0, approximately 85% of DPF added was converted to *cis*-DBE. With chlorine-water solution a detectable chemiluminescence was not observed on the final pH range 3.0 t 11.0. As shown in the same figure, chlorine-water solution, when H_2O_2 was present, emits light significantly near pH 10.5, but not on the pH range 3.0 to 4.0.

Under our experimental conditions chemiluminescence appeared promptly by the rapid addition of NaOCl solution and lasted for less than 2 min. Therefore, the light intensity measured and calculated by our procedure should be erratic, compared with the data reported by Kajiwara and Kearns (10). However, our data indicate that chemiluminescence, which originates from bimol collision of $^1\Delta_g$ type singlet molecular oxygen (11, 12), is indeed weaker in acid solution in agreement to the report of Kajiwara and Kearns (10).

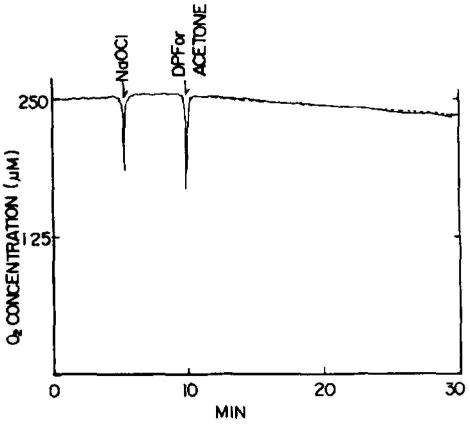

Fig. 2. Oxygen consumption of DPF in chlorine-water solution. Oxygen consumption was measured by the Yanako oxygenometer(Model PO 100A). Two components, 1.94 ml of water and 0.75 ml of 0.2 M acetate buffer were transfered to a cell, and 0.3 ml of 4 mM NaOCl and 0.01 ml of 60 mM DPF in acetone (–) or 0.01 ml of acetone alone (--) were added successively. Final pH was 4.0.

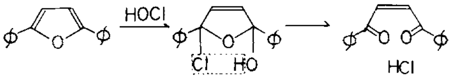

Fig. 3

In contrast to the experimental system in which NaOCl was gradually added to a cold H_2O_2 solution containing DPF (pH 10-11.0), little or no oxidation of DPF was observed, if any, in the present system on the final pH range 9.0 to 11.0. Under the standard incubation conditions 8.0mM H_2O_2 caused 20% inhibition on the DPF conversion to *cis*-DBE by HOCl at pH 4.0.

These results may indicate the lack of the accumulation of $^1O_2$ to react with DPF in NaOCl/H_2O_2 and the decomposition of HOCl by H_2O_2. The latter is probably through the following reaction (13),

$$HOCl + H_2O_2 \longrightarrow O_2 + H^+ + Cl^- + H_2O \qquad (I)$$

Oxygen generated by reaction (I) in the acid solution would be in ground state, $^3\Sigma_g^-$. Since the chlorine-water system at pH 4.3 does not require O_2 for DPF oxidation (Fig. 2), the DPF conversion to *cis*-DBE may occur through the reaction shown in Fig 3, identical to the DPF oxidation in a mixture of nitric acid-acetic acid (14). From these results, it may be considered that the generation of $^1O_2$ in myeloperoxidase/H_2O_2/Cl^- system at its optimal pH (4-5) is negligible.

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