

Is Free Halogen Necessary for Disinfection?

D. E. WILLIAMS,^{1,2} E. D. ELDER,³ AND S. D. WORLEY^{1*}

Department of Chemistry, College of Sciences and Mathematics,¹ and Department of Pathobiology, College of Veterinary Medicine,² Auburn University, Auburn, Alabama 36849, and Department of Biology, Georgia Southwestern College, Americus, Georgia 31709³

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The principle of Le Chatelier was used in demonstrating that 3-chloro-4,4-dimethyl-2-oxazolidinone (compound 1) itself kills *Staphylococcus aureus* rather than the very small amount of free chlorine in hydrolysis equilibrium with compound 1. On the other hand, when the *N*-bromo analog of compound 1 (compound 1B) was used as the disinfectant, the mixture of combined compound 1B and free bromine formed in the hydrolysis equilibrium provided disinfection. When the hydrolysis equilibrium for 1B was suppressed to the level at which a negligible amount of free bromine remained in solution, combined compound 1B was much more efficacious than combined compound 1 at killing *S. aureus*.

Work in these laboratories in recent years has focused on the development of new disinfectants, particularly those which are very stable in aqueous solution and in the solid state. Such stable disinfectants are needed for a variety of applications, such as to prevent biofouling in cooling towers and closed-cycle cooling-water systems, to disinfect swimming pools and hot tubs, and to provide a disinfecting residual for potable water in remote locations. Several *N*-halamine compounds have been investigated in these laboratories which indeed possess the necessary stability requirement; these include 3-chloro-4,4-dimethyl-2-oxazolidinone (compound 1), 1,3-dichloro-4,4,5,5-tetramethyl-2-imidazolidinone (compound A), and the bromochloro derivative of A (compound ABC) (1, 2, 12, 14). The bromo derivative of compound 1 (compound 1B) and the dibromo derivative of A (compound AB) are less stable than the *N*-chloramine analogs because of the inherently weak N-Br bond, but they are more stable than other *N*-bromamines developed to date (14). The structural feature responsible for the stability of these new *N*-halamines is the presence of the two methyl groups bonded to the rings adjacent to each nitrogen-halogen bond. The structural formulas for these compounds are shown in reference 10.

The efficacies of disinfection for these compounds have been determined for a variety of microorganisms in water at variable pH, temperature, and water quality (9-11, 13). In general the *N*-chloramine compounds 1 and A tend to require significantly longer contact times at a given total chlorine concentration than does free chlorine to provide sterile water. The *N*-bromamines 1B and AB provide more rapid disinfection but are less stable than the *N*-chloramine analogs. A possible reason for this is that the former liberate some free bromine in aqueous solution, while the latter liberate very little free chlorine. Compound ABC provides rapid initial disinfection due to the N-Br moiety and slower long-term disinfection as a result of the N-Cl. It has been suggested to us that the mode of disinfection for all of these new compounds is through the small amount of free halogen liberated in hydrolysis equilibria as illustrated in Fig. 1.

This hypothesis obviously cannot be the case for microorganisms which are more susceptible to disinfection by the combined halamines than by free halogen, but it could

conceivably be true for microorganisms which are much more resistant to the *N*-halamines than to free halogen. The purpose of this work was to test this hypothesis.

Our procedure was to use the principle of Le Chatelier (4), which states that when a system at equilibrium is perturbed, the equilibrium will be displaced in such a manner as to oppose the perturbation. We added increasing amounts of product amine so as to displace the equilibria (equations 1 and 2, Fig. 1) to the reactant side, thus reducing the amount of free halogen (HOCl or HOBr) in each case. We tested each solution for disinfection efficacy against *Staphylococcus aureus*; this bacterium was chosen because it is particularly resistant to our *N*-halamine compounds, much more so than to free halogen. If the rates of disinfection were not affected by the reduction in free halogen, our assumption would be that the disinfectant action was not provided by the small amount of free halogen in the equilibrated system. A reduction of disinfection rate caused by the decrease in free halogen concentration should demonstrate the converse.

The equilibrium constants at pH 7.0 and 25°C for the reactions represented by equations 1 and 2 were measured to be 2.3×10^{-9} and 3.1×10^{-5} , respectively. The concentration of free chlorine in equilibrium reaction 1 was too small to be measured with standard titrimetric procedures; it was measured with a model 2124 multirange HOCl selective electrode (Delta Scientific). The equilibrium constant for reaction 2 was determined by use of *N,N*-diethyl-*p*-phenylenediamine (DPD) and a modification of the procedures outlined by Palin (5, 6) and Sollo et al. (8). Potassium permanganate was used as a standard for calibration of the DPD response curve. Spectroscopic data were collected with a Guilford model 250 spectrophotometer.

In the disinfection experiments, the amine product 4, 4-dimethyl-2-oxazolidinone of both reactions was added at three concentrations and was allowed to equilibrate with compound 1 or 1B for 30 min at pH 7.0 and 25°C in demand-free water before addition of bacteria. *S. aureus* ATCC 25923 was then added to a final cell density of 10^6 CFU/ml, and the rates of disinfection (10^6) were measured by using procedures outlined previously (10, 11). The results were compared with the disinfection rates for compounds 1 and 1B with no amine product added. Testing also included controls with demand-free buffer and with the appropriate product amine concentrations in demand-free buffer.

Neither control showed any reduction in viable organisms

* Corresponding author.

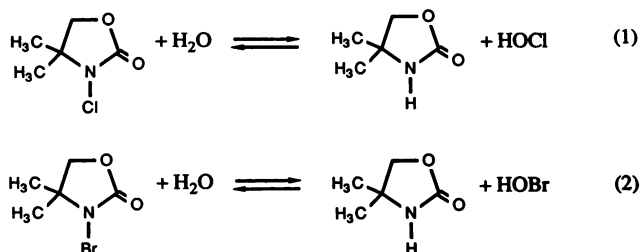


FIG. 1. Equations showing liberation of small amounts of free halogen in hydrolysis equilibrium for compounds 1 (equation 1) and 1B (equation 2).

during the maximum contact time (240 min) used in these experiments. Thus, all disinfection in this study can be attributed to the *N*-halamine compounds or free halogen. Predicted times necessary for 10^6 -fold inactivation of *S. aureus* were calculated by using the General Linear Models procedure of the SAS Institute, Inc. (3, 7, 10). The treatment variable of molar ratio of amine to *N*-halamine was tested for its effect on calculated disinfection rates by testing for the heterogeneity of the slopes of the generated regression equations. The significance level chosen for these comparisons was $P < 0.05$.

Table 1 shows the measured molar concentrations for compounds 1, 1B, and amine product added for the various experiments. It also gives the calculated concentrations of free halogen as dictated by the equilibrium constants for reactions 1 and 2 and Le Chatelier's principle. It can be seen that 10 mg of total chlorine per liter from compound 1 provided only 0.028 mg of free chlorine per liter at equilibrium; 1 mg of total chlorine per liter from compound 1 gave only 0.009 mg of free chlorine per liter. By addition of an equimolar concentration of amine product to the solutions, the amount of free chlorine was reduced to 0 and 0.00008 mg/liter, respectively. When the amine product concentration was increased by another order of magnitude or more, there was negligible free chlorine concentration from compound 1. On the other hand, the free bromine concentration for the compound 1B hydrolysis equilibrium was more difficult to suppress, requiring almost a 500-fold excess of amine product to eliminate the measurable free bromine product.

The data presented in Table 2 show that compound 1 disinfection times were completely independent of added product amine at both compound 1 concentrations studied. It is thus clear that the small amount of free chlorine present at equilibrium (0.028 or 0.009 mg/liter) is not responsible for the disinfection action of compound 1 against *S. aureus*. On the other hand, the data in Table 2 show that the free

TABLE 2. Predicted inactivation times for compounds 1 and 1B with and without the presence of excess amine

Total halogen concn (μM)	Amine added (μM)	Predicted inactivation time (min) ^a	SE ^b (min)	R ²
Compound 1				
28.21	0	254	36	0.84
	28.21	251	33	0.87
282.1	282.1	252	33	0.85
	2,821	254	37	0.83
	0	58.4	3.7	0.95
	282.1	57.5	3.4	0.95
2,821	58.2	6.4	0.93	
Compound 1B				
28.16	0	0.99	0.13	0.93
	281.6	2.25	0.17	0.77
	2,816	4.47	0.45	0.66
	14,080	4.70	0.40	0.80

^a Contact time predicted for a 10^6 -fold reduction in CFU of *S. aureus* per milliliter (see text).

^b Standard error of contact time estimate.

bromine in equilibrium with compound 1B was responsible in part for the disinfection action of that compound, as addition of increasing amounts of amine product caused a decline in disinfection efficacy. After addition of 1.408×10^{-2} M amine product, the amount of free bromine declined to zero; the 4.70-min contact time necessary for a 10^6 -fold kill of *S. aureus* under those conditions represented the efficacy of the combined *N*-bromamine itself. Combined 1B required 54-fold less contact time than did combined 1 at approximately the same molar concentration.

It can be concluded from this work that the mode of action of compound 1 against *S. aureus* involves the whole *N*-chloramine molecule rather than the limited amount of free chlorine produced in hydrolysis equilibrium. Furthermore, the *N*-bromamine 1B required considerably less contact time than did the *N*-chloramine compound 1 for a comparable disinfection performance, even when the free bromine had been suppressed by utilization of Le Chatelier's principle. However, compound 1 was considerably more stable in solution than 1B (14), so the *N*-halamine disinfectant of choice is dependent on the intended application.

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TABLE 1. Free halogen concentrations for compounds 1 and 1B with and without excess amine added

Compound	Total halogen concn (M) ^a	Amine added (M)	Free halogen (M) ^a
1	2.821×10^{-5} (1 mg of Cl per liter)	0	2.535×10^{-7} (0.009 mg of Cl per liter)
	2.821×10^{-5}	2.821×10^{-5}	2.300×10^{-9} (0.00008 mg of Cl per liter)
	2.821×10^{-5}	2.821×10^{-4}	0
	2.821×10^{-5}	2.821×10^{-3}	0
	2.821×10^{-4} (10 mg of Cl per liter)	0	8.044×10^{-7} (0.028 mg of Cl per liter)
	2.821×10^{-4}	2.821×10^{-4}	0
1B	2.816×10^{-5} (2.25 mg of Br per liter)	2.821×10^{-3}	0
	2.816×10^{-5}	0	1.782×10^{-5} (1.424 mg of Br per liter)
	2.816×10^{-5}	2.816×10^{-4}	2.744×10^{-6} (0.22 mg of Br per liter)
	2.816×10^{-5}	2.816×10^{-3}	3.036×10^{-7} (0.024 mg of Br per liter)
	2.816×10^{-5}	1.408×10^{-2}	0

^a Data in parenthesis are the masses of halogen represented by the indicated molar concentrations.

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