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Chemical Inactivation of Protein Toxins on Food Contact Surfaces

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S Supporting Information

ABSTRACT: We compared the kinetics and efficacies of sodium hypochlorite, peracetic acid, phosphoric acid-based detergent, chlorinated alkaline detergent, quaternary ammonium-based sanitizer, and peracetic acid-based sanitizer for inactivating the potential bioterrorism agents ricin and abrin in simple buffers, food slurries (infant formula, peanut butter, and pancake mix), and in dried food residues on stainless steel. The intrinsic fluorescence and cytotoxicity of purified ricin and abrin in buffers decreased rapidly in a pH- and temperature-dependent manner when treated with sodium hypochlorite but more slowly when treated with peracetic acid. Cytotoxicity assays showed rapid and complete inactivation of ricin and crude abrin in food slurries and dried food residues treated 0−5 min with sodium hypochlorite. Toxin epitopes recognized by ELISA decayed more gradually under these conditions. Higher concentrations of peracetic acid were required to achieve comparable results. Chlorinated alkaline detergent was the most effective industrial agent tested for inactivating ricin in dried food residues.

KEYWORDS: *ricin, abrin, bioterrorism, chemical inactivation*

■ INTRODUCTION

Regulatory agencies and the food industry recognize the importance of proper hygiene to minimize risks from food-borne contamination. Significant efforts are invested to reduce the possibility that pathogens and toxic agents enter the food supply inadvertently. Enhanced security measures, constant vigilance by governments and the food industry, effective medical counter-measures, and acceptable methods of remediation are a few critical elements of a food defense system that is prepared to thwart efforts to contaminate the food supply intentionally.

Ricin, a plant toxin extracted from the seeds of the castor bean plant, *Ricinus communis*, has a long history in toxicology of accidental poisonings and use in homicides, assassinations, and biological warfare.[1−4] Attempts by domestic and international extremists to obtain or use ricin have been reported in the popular press,[5−7] including an alleged plot to poison salad bars.[8] Ricin and abrin, a related toxin produced by rosary pea plant, *Abrus precatorius*, are potent ribosome-inactivating protein toxins that cause cell death by inhibiting protein synthesis. Ricin and abrin are heterodimeric proteins; each is composed of an A and B subunit linked by a single disulfide bond. The catalytic A-subunits are 28S rRNA-specific adenosine *N*-glycosidase enzymes that depurinate a critical adenine necessary for peptide chain elongation. The B subunits contain two galactose-binding domains that facilitate adhesion and uptake of the toxin via binding to exposed galactose residues of glycoproteins and glycolipids on target cells.[1]

Previous research performed in our laboratories showed that ricin and abrin are heat stable in a variety of food matrixes and that they retain their toxic activity during yogurt manufacture and storage.[9−11] The stability of these proteins suggests that significant toxicity could persist through the manufacturing process and distribution to reach consumers if toxic doses were introduced into foods during production.

Ricin and abrin are potentially fatal when ingested, but inhalation of airborne particles of dried food residues containing smaller amounts of the toxins may present a greater hazard. In the case of deliberate contamination of a food processing facility, remediation of food-contact surfaces must be done safely and effectively while minimizing worker exposure. Using respirators and soaking contaminated surfaces with chemical inactivation agents should reduce this hazard. Hilgren and co-workers[12] studied the effectiveness of different surface sanitizers for inactivating *Bacillus anthracis* spores in the presence of several model food matrixes (whole milk, egg yolk, flour). Although food matrixes had minimal influence on the sporicidal activity of peracetic acid and peroxide, the effectiveness of sodium hypochlorite was greatly reduced. MacKinnon and Alderton[13] found that incubation in 3 mM sodium hypochlorite (223 ppm) for 2−48 h at pH 7.0 led to fragmentation of ricin, inactivation of its cytotoxic activity, and decreased detectability with ELISA using rabbit polyclonal antibodies. Recently, Cole and co-workers[14] described chemical inactivation of ricin solutions buffered at pH 7.4 using dilute sodium hypochlorite (2.8−3.0 ppm; 38−40 μM) for 10−170 min.

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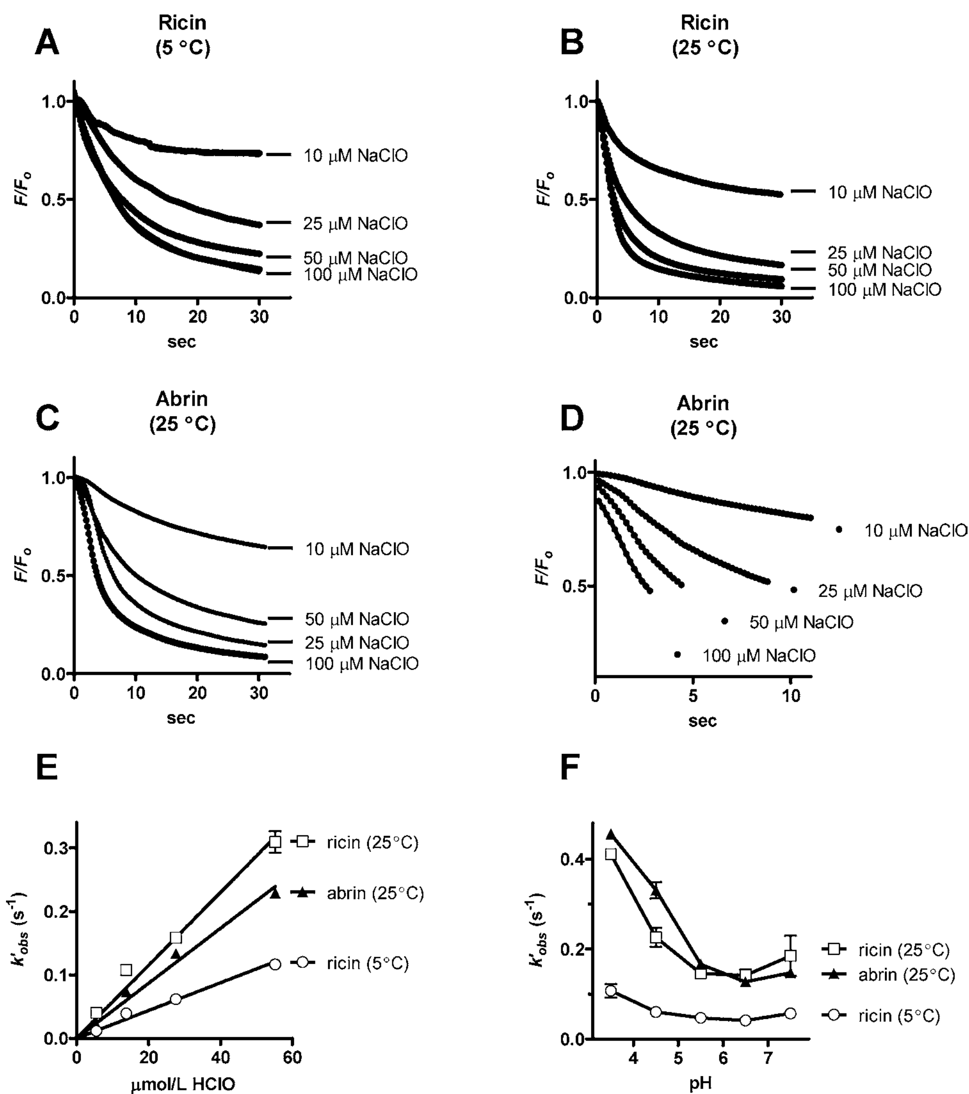

Figure 1. Reactions between sodium hypochlorite and toxins monitored with fluorescence. (A–C) Ricin or abrin was added to NaClO in malate/diethylamine buffer, pH 7.5, in a thermostatted cuvette, and the decay in fluorescence was monitored. (D) Initial reaction progress for abrin and NaClO. (E) Pseudo first-order rate constants plotted versus HClO concentration. (F) Effect of pH on pseudo first-order rate constants. Effects of NaClO concentration and pH on reaction rates are statistically significant ($p < 0.05$).

These investigators used decay in intrinsic protein fluorescence, cytotoxicity assays, gel electrophoresis, and size exclusion chromatography to evaluate the effects of chemical inactivation agents on the structure and activity of ricin and its two subunits. Those studies revealed the effectiveness of longer incubation for inactivating purified ricin using dilute sodium hypochlorite. Our present work compares the efficacies of sodium hypochlorite, peracetic acid, chlorinated alkaline detergent, phosphoric acid-based detergent, peracetic acid-based sanitizer, and quaternary ammonium-based sanitizer to inactivate ricin and abrin in food slurries and in dried food residues adsorbed to stainless steel.

MATERIALS AND METHODS

Materials. Purified ricin (*Ricinus communis* agglutinin II, 5 mg/mL in phosphate buffered saline) was purchased from Vector Laboratories (Burlingame, CA), and purified abrin was obtained from Toxin Technology (Sarasota, FL). The purity of commercial ricin and abrin stocks was verified using Laemmli gel electrophoresis. The concentrations of stock solutions were determined spectrophotometrically at 280 nm using published molar absorptivity values for ricin, 93 900 M^{-1} cm^{-1},[15] and abrin, 100 170 M^{-1} cm^{-1}.[16] An additional stock of crude abrin was extracted from *Abrus precatorius* seeds obtained from Toxin Technology. The seeds (110 g) were soaked overnight at 4 °C in 350 mL of phosphate-buffered saline (PBS, pH 7.3; Sigma-Aldrich, St. Louis, MO) containing 0.05% Tween 20 (Sigma-Aldrich), macerated using a food processor, and the homogenate was transferred to a large beaker. After the particulate residue was allowed to settle for 30 min, the supernatant layer was collected and then centrifuged 20 min at 10 000*g* at 4 °C. The clarified supernatant was passed through a Whatman #4 paper filter disk and stored in 2 mL aliquots at −20 °C. The abrin concentration in the filtered extract (1.3 mg/mL) was determined using a commercial ELISA kit (Tetracore, Gaithersburg, MD).

Sodium hypochlorite and peracetic acid stock solutions, catalase, butylated hydroxytoluene (BHT), and sodium thiosulfate were obtained from Sigma-Aldrich. Sodium hypochlorite concentrations in commercial stock solutions were determined by back-titration of starch/iodide reactions using sodium thiosulfate solutions standardized with potassium iodate solid.[17,18] Sodium hypochlorite concentrations in working solutions were confirmed spectrophotometrically (ε_{292} 360 M^{-1} cm^{-1})[19] immediately prior to use. Peracetic acid concentrations in stock solutions were confirmed by titration with potassium permanganate. Levels of active chlorine in sodium hypochlorite solutions and available oxygen in peracetic acid solutions were estimated

Table 1. Pseudo First-Order Rate Constants (k'_{obs}) Measured Using Fluorescence Quenching of Ricin and Abrin in the Absence of Food Residues[a]

toxin treatment	pH	k'_{obs} ($\times 10^{-3}$ s^{-1})	residual toxin at equilibrium (%)
490 nM ricin + 50 μM NaClO (5 °C)	3.5	110 ± 30	41 ± 6
	4.5	60 ± 7	35 ± 9
	5.5	47 ± 4	44 ± 3
	6.5	41 ± 1	22 ± 5
	7.5	57 ± 4	13 ± 9
184 nM ricin + 50 μM NaClO (25 °C)	3.5	410 ± 10	6 ± 2
	4.5	230 ± 20	15.8 ± 0.5
	5.5	150 ± 10	15.7 ± 0.9
	6.5	140 ± 10	6.8 ± 0.1
	7.5	190 ± 60	6 ± 4
99.3 nM abrin + 50 μM NaClO (25 °C)	3.5	460 ± 10	2.2 ± 0.1
	4.5	330 ± 30	5.7 ± 0.8
	5.5	170 ± 10	4.3 ± 0.1
	6.5	130 ± 10	0 ± 0
	7.5	150 ± 10	2.4 ± 0.4
200 nM ricin + 5.0 mM peracetic acid (25 °C)	3.5	1.54 ± 0.01	5.2 ± 0.9
	4.5	0.55 ± 0.04	8.4 ± 0.5
	5.5	0.022 ± 0.08	65 ± 11
	6.5	0.27 ± 0.02	64 ± 1
	7.5	0.156 ± 0.005	59 ± 1
100 nM abrin + 5.0 mM peracetic acid (25 °C)	3.5	3 ± 1	2.1 ± 0.1
	4.5	1.9 ± 0.3	1.1 ± 0.2
	5.5	0.300 ± 0.003	8 ± 3
	6.5	0.140 ± 0.005	45 ± 1
	7.5	0.089 ± 0.012	39 ± 1

[a]Ricin or abrin in buffer was added to 50 μM sodium hypochlorite or 5.0 mM peracetic acid, and the decay in fluorescence was monitored. Effects of temperature and pH on k'_{obs} were significant ($p < 0.05$).

using titration kits (Ecolab, St. Paul, MN) for reactions involving food residues.

Food materials used in these studies, peanut butter (smooth), pancake mix (powdered, incomplete), and infant formula (milk-based), were obtained at local supermarkets and used as described. Laboratory-grade water was used to prepare working solutions of chlorinated alkaline detergent (Exelerate CIP; Ecolab), phosphoric acid-based detergent (Bruspray Acid; JohnsonDiversey; Sharonville, OH), peracetic acid santizer (Vortexx; Ecolab), and quaternary ammonium compound-based sanitizer (Whisper V Sanitizer; Ecolab) at the concentrations specified in the text. All other reagents used were at least analytical grade.

Chemical Quenching of the Intrinsic Protein Fluorescence of Ricin and Abrin by Sodium Hypochlorite and Peracetic Acid. Sodium malate/diethylamine buffers (50 mM each) were adjusted to pH 3.5, 4.5, 5.5, 6.5, or 7.5 for fluorescence experiments. Both ricin and abrin exhibited fluorescence maxima at EX285/EM336 using a Perkin-Elmer model 55 spectrofluorimeter (Waltham, MA). Buffer containing sodium hypochlorite (0−100 μM final concentration in 2.00 mL) was placed in a thermostatted cuvette (1.0 × 1.0 cm) equipped with a micro stir bar and maintained at the desired reaction temperature (5 or 25 °C). Reactions were initiated by adding aliquots (5−20 μL) of concentrated ricin or abrin stocks at the zero time point, and the rapid decay in intrinsic protein fluorescence was monitored until the reactions were complete.

Reactions between peracetic acid and both toxins occurred too slowly to perform an adequate number of consecutive replicate trials with the technique described above. Instead, our fluorescence decay method was adapted for use in a 96-well quartz microplate with opaque walls (Hellma USA, Plainview, NY) using a Biotek Synergy 4 multimode plate reader (Winooski, VT) in fluorescence mode (EX285/EM336). Each well contained 5.0 mM peracetic acid and toxins (100 nM abrin or 200 nM ricin to yield similar initial fluorescence intensities) in 0.1 mL reactions buffered at pH 3.5, 4.5, 5.5, 6.5, or 7.5. The time-dependent decreases in intrinsic protein fluorescence were monitored for 120 min at 25 °C. The reaction order and rate constants were determined as described below.

Chemical Inactivation of Ricin Biological Activity Using Sodium Hypochlorite and Peracetic Acid. Ricin (200 nM) was diluted into buffer (pH 4.5 or 7.5) and treated with various concentrations of peracetic acid or sodium hypochlorite at room temperature. After incubation for 2 min, samples containing peracetic acid were neutralized with 0.1 mg/mL catalase and 0.1 mM BHT, and samples containing sodium hypochlorite were neutralized with 0.1 M sodium thiosulfate. The residual biological activity in treated samples was determined using cytotoxicity assays with RAW264.7 murine macrophage cells. Replicate sets of treated toxin samples exhibited negligible differences in apparent residual activity, whether prepared fresh and analyzed immediately or stored at −20 °C and then thawed for analysis (data not shown).

Determinations of Residual Ricin Cytotoxicity Using RAW264.7 Macrophage Cells. Triplicate samples of ricin or abrin treated with sodium hypochlorite or peracetic acid were neutralized and frozen as described. Zero-time point samples were thawed at 37 °C, vortexed 2 min, and 1.02 mL aliquots were taken from each tube. The three portions were mixed and diluted with 11.94 mL of cell culture media (MegaCell MEM supplemented with 10% FBS, 0.2 mM glutamine, 100 μg/mL streptomycin sulfate, and 100 U/mL penicillin G) to prepare a 20 nM ricin standard (1:4.90 dilution). Samples at all other time points were thawed and mixed in the same manner, and 0.306 mL aliquots were mixed with 1.19 mL of media to prepare ricin starting dilutions (1:4.90) corresponding to that of the pooled zero time point standard. In addition, starting dilutions of 2.0 mM cadmium chloride and 2.0 nM ricin in PBS positive controls were prepared. 2-fold serial dilutions of zero time point standards and samples were prepared in triplicate using media as the diluent; 2-fold serial dilutions of cadmium and ricin positive controls were prepared singly. Aliquots (0.1 mL) of serially diluted standards, samples, and controls were transferred to the corresponding wells of assay plates containing 0.1 mL/well overnight cultures of RAW264.7 macrophage cells (15 000 cells/well) and incubated for 48 h at 37 °C. Cell viability was determined using CellTiter Blue reagent (Promega, Madison, WI) fluorescence assays (EX550/EM590) according to the manufacturer's instructions. Fluorescence data were fitted to a four-point logarithmic function using Prism 5.0, which provided $\log(IC_{50})$ values for each treatment. Percent toxin activity was calculated from $\log(IC_{50})$ values according to eq 1, where α and β are $\log(IC_{50})$ values for the zero time point standard and individual time points, respectively.

$$\% \text{ toxin activity remaining} = 100\% \times 10^{(\alpha - \beta)} \quad (1)$$

Control experiments were performed in the presence and absence of ricin to confirm that neither food residues nor neutralization buffer exerted a detectable influence on the measured cytotoxicity at the level of dilution (1:730 and 1:5.4, respectively) used in this study (data not shown).

Chemical Inactivation of Protein Toxin Suspensions or Protein Toxins Dried onto Stainless Steel Surfaces in the Presence or Absence of Food Residues. Slurries were prepared from food items by blending thoroughly with distilled water (35 g of peanut butter + 100 g of water; 25 g of pancake mix + 100 g of water; 8.5 g of infant formula + 56 g of water). Concentrated ricin or abrin stock solutions were mixed with PBS or with food slurries to produce working stocks containing 1.0 mg/mL toxin. Identical 0.1 mL aliquots of toxin-containing working solutions were either placed into separate 15 mL tubes or dispensed carefully onto circular, type 304 stainless steel coupons (1.27 cm diameter; BioSurface Technologies Corp.; Bozeman, MT). The coupons were allowed to air-dry in a biosafety cabinet for 1 h and then placed carefully into separate 15 mL tubes. Chemical inactivation reagents were prepared by dilution of concentrated stocks to achieve the desired final concentrations (0.067−40 μM sodium hypochlorite; 6.6−130 mM peracetic acid; 0.5−5.0%

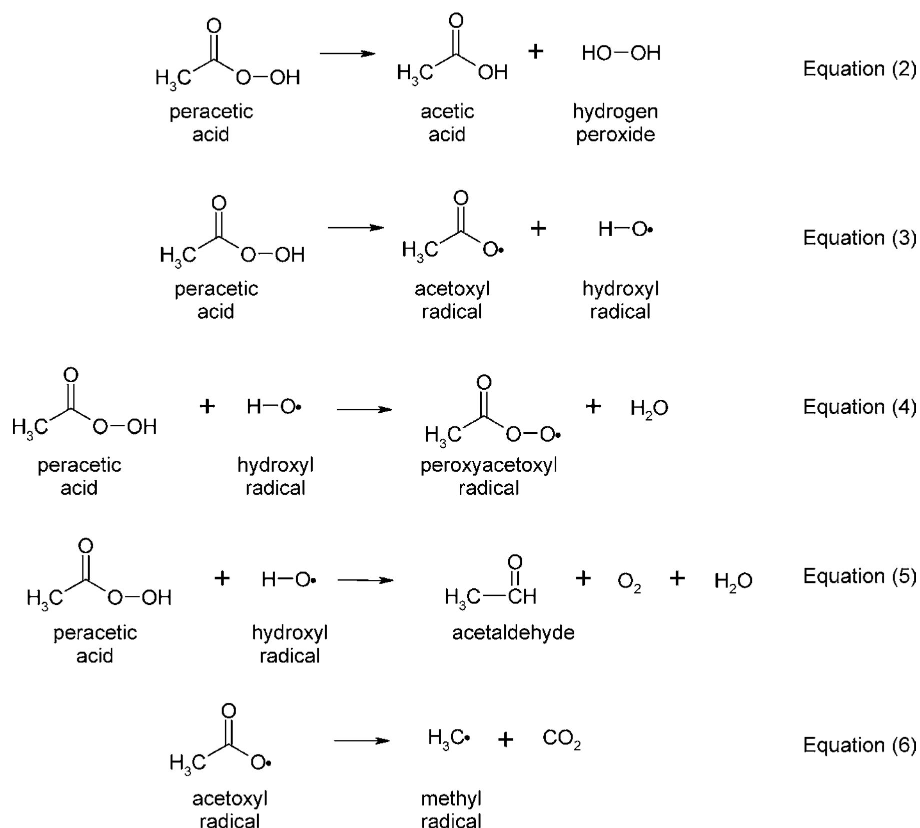

Figure 2. Reactive chemical species derived from peracetic acid. Under various conditions peracetic acid may form spontaneously: hydrogen peroxide, hydroxyl radical, acetoxyl radical, peroxyacetoxyl radical, acetaldehyde, and methyl radical.

chlorinated alkali detergent; 1.0−10.0% phosphoric acid-based detergent; 500−2000 ppm quaternary ammonium-based sanitizer; and 0.1−1.0% peracetic acid-based sanitizer). Reactions were initiated by adding each chemical inactivation agent contained in 1.4 mL to the tubes containing protein toxin suspensions or by adding each agent contained in 1.5 mL to the tubes containing protein toxins dried onto stainless steel coupons. Reactions were allowed to incubate for 0−5 min at room temperature and then quenched rapidly by adding 13.5 mL of neutralization buffer (BD Difco; Sparks, MD) supplemented with catalase. Ricin- and abrin-specific ELISA test kits (Tetracore) were used to measure remaining toxin in aliquots taken from neutralized reactions. ELISA was performed according to the manufacturer's instructions with the exception that seven-point calibration curves were used.[9,10,20] RAW264.7 macrophage cytotoxicity assays were used to assess the residual biological activity remaining in additional 2.0 mL portions taken from neutralized reactions.

Data Analysis. Three-dimensional structures for ricin (PDB ID: 2AAI)[21] and abrin (PDB ID: 1ABR)[22] were obtained from the RCSB PDB,[23] and their solvent-accessible features were identified using open-source Jmol software[24] (see the Supporting Information). Rate constants were determined by fitting the measured time-dependent decay in toxin concentration or biological activity to kinetic model equations using Prism 5.0 software (GraphPad, San Diego, CA). Significant differences between values derived from the fitted data were compared using Akaike's information criteria (AIC_c) with correction for small sample sizes.[25] The effects of reaction pH, temperature, and concentration on rate constants were evaluated using one-way analysis of variance (ANOVA). Combined effects of chemical treatments and drying onto stainless steel coupons or of chemical treatments and methods of detection were evaluated using two-way ANOVA, where appropriate. Otherwise, Kruskal−Wallis nonparametric ranked tests were used for multiple comparisons. Differences in mean values were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Effects of Temperature, pH, and Concentrations of Sodium Hypochlorite and Peracetic Acid on the Decay in Intrinsic Protein Fluorescence for Ricin and Abrin in the Absence of Food Residues. We treated ricin and abrin in simple buffers with sodium hypochlorite (20- to 100-fold molar excess) and monitored the rate of decay in the intrinsic fluorescence due to internal aromatic residues (Figure 1A−C). Fluorescence quenching data were fitted to various kinetic models to determine the reaction order and rate constants (Supporting Information). Having determined that a two-step, pseudo first-order model involving a fast and slow step described the data adequately, we focused on the earliest linear phase of the reaction to minimize complications due to the accumulation of uncharacterized secondary reaction products (Figure 1D).

Pseudo first-order rate constants (k'_{obs}) showed obvious effects of temperature and the concentration of hypochlorous acid, which was calculated from the hydrogen ion concentration at pH 7.5, bulk sodium hypochlorite concentration, and K_a for hypochlorous acid (Figure 1E). Hypochlorous acid accounts for >99%, 93%, and 55% of the total hypochlorite concentration as the pH increases from pH ≤5.5, 6.5, and 7.5, respectively. Apparent second-order rate constants obtained from the slopes of plots of k'_{obs} versus hypochlorous acid concentration at pH 7.5 were 2200 ± 100, 5700 ± 200, and 4300 ± 100 M^{-1} s^{-1} for ricin at 5 °C, ricin at 25 °C, and abrin at 25 °C, respectively. The magnitudes of these rate constants preclude slower reactions involving Tyr residues, peptide bonds, and Gln residues and implicate faster reactions with Met, Cys, His, amino termini of polypeptides, Trp, and possibly Lys.[26]

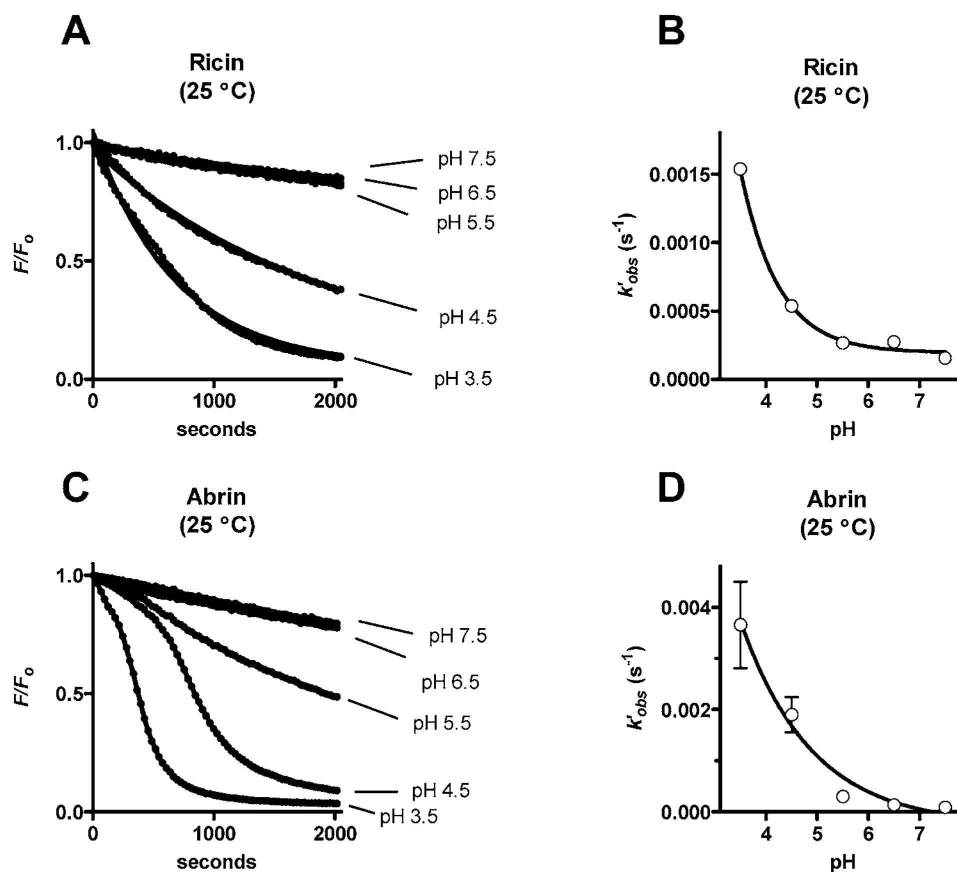

Figure 3. Reactions between peracetic acid and (A,B) ricin and (C,D) abrin exhibit pseudo first-order kinetics at room temperature. Ricin (200 nM) or abrin (100 nM) in sodium malate/diethylamine buffers (pH 3.5−7.5) was adjusted to 5 mM peracetic acid, and the decay in fluorescence intensity was monitored (A,C). Plots of pseudo first-order rate constants (k'_{obs}) versus pH reveal a logarithmic dependence on hydrogen ion concentration (B,D). Average values calculated from triplicate reactions are presented. Effects of pH on reaction rates are statistically significant ($p < 0.05$).

Additional reactions were performed with 50 μM sodium hypochlorite at 5 and 25 °C using ricin and abrin in buffers adjusted to pH 3.5, 4.5, 5.5, 6.5, and 7.5 (Figure 1F, Table 1). Pseudo first-order rate constants increased in a sodium hypochlorite concentration- and temperature-dependent manner. Pseudo first-order rate constants were greatest at pH 3.5 and lowest at pH 6.5. They decreased significantly as the pH dropped from pH 3.5 to pH 6.5 and increased slightly at pH 7.5. Interestingly, Pattison and Davies[26] reported that the reactivity with hypochlorous acid peaks at pH 7.5 for the His side chain analog 4-imidazole acetic acid.

Importantly, the calculated half-lives for ricin and abrin during the initial phase of the reaction ranged from 1.5 to 16.7 s, indicative of the speed of the reaction. Moreover, the residual toxin fluorescence was greater and pseudo first-order rate constants were lower for reactions that occurred at 5 °C, indicating that the structure of ricin is somewhat less reactive with sodium hypochlorite under refrigeration conditions, perhaps due to decreased local thermal motion that exposes additional functional groups to hypochlorous acid.

The physical organic chemistry of reactions between peracetic acid and proteins has been studied by others, but not as thoroughly as for reactions involving sodium hypochlorite.[33−42] Kerkaert and co-workers[27] reported that Trp and Met residues of caseins and whey proteins were oxidized more readily than Tyr, His, or Lys residues by peracetic acid. However, Finnegan et al.[28] found that the reactivity of free amino acids with peracetic acid followed the trend Gly > Lys > Cys, Met, His > Trp. The complexity of organic peracid reactivity may be due to the variety of reactive species in chemical equilibrium with the reagent (Figure 2), which may react with protein functional groups at different rates. Ionization of the acidic proton of peracetic acid is suppressed due to intramolecular hydrogen bonding with the carbonyl oxygen, rendering peracetic acid a much weaker acid (pK_a 8.2) than acetic acid (pK_a 4.8); it is only ≤2% ionized at pH ≤6.5 and 17% ionized at pH 7.5. Peracetic acid is in chemical equilibrium with acetic acid and hydrogen peroxide (K_{eq} 0.37 at room temperature; eq 2) and may undergo homolytic cleavage of the peroxy bond to produce hydroxyl radicals and acetoxyl radicals (eq 3). These radical species may react further to produce peroxyacetoxyl radicals (eq 4), acetaldehyde (eq 5), and methyl radicals (eq 6).[29,30] Relative concentrations of these various reactive species are influenced by additional factors, such as the concentration of peracetic acid, temperature, and the availability of metal ions (e.g., Fe^{2+} present in some foods may facilitate catalytic Fenton-like reactions to release hydroxyl radicals). Thus, the presence of multiple reactive species in varying concentrations complicates the predictability of reaction mechanisms involving peracids.

We monitored the effect of pH on the rates of reactions between excess peracetic acid and purified ricin and abrin using fluorescence quenching (25 000−50 000-fold molar excess). These reactions proceeded according to pseudo first-order kinetics (Figure 3A−C). Rate constants decreased logarithmically for reactions with ricin (10-fold decrease) and abrin (33-fold decrease) as the pH increased from pH 3.5 to pH 7.5

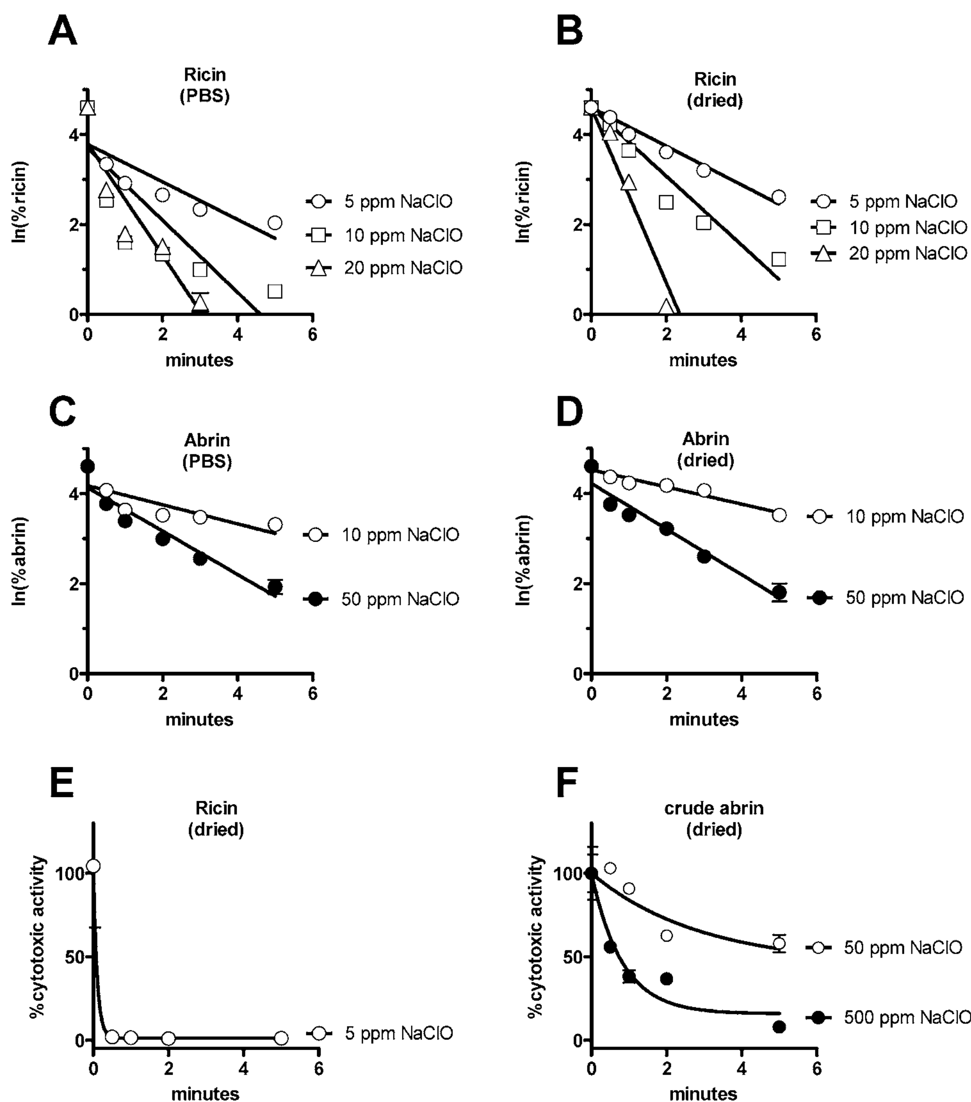

Figure 4. Inactivation of ricin and crude abrin in PBS (pH 7.3) or dried onto stainless steel coupons using 5−50 ppm sodium hypochlorite (67−670 μM) at room temperature. (A,C) Toxin solutions, (B,D−F) dried toxins. (A−D) Decay in ELISA detection; (E) decay in ricin cytotoxicity activity using 5 ppm NaClO; and (F) crude abrin cytotoxicity using 50 or 500 ppm NaClO (50.67−6.7 mM). Each point represents the average of three values. Error bars indicate one standard error of the mean. Effects of NaClO on reaction rates are statistically significant ($p < 0.05$).

(Table 1, Figure 3B,D). The greatest influence of pH on the rates of peracetic acid reactions with ricin and abrin occurred between pH 3.5 and 5.5, which correlates best with protonation of acidic amino acid side chains of the toxins.

Comparing the Inactivation of Ricin and Abrin in Solution or Adsorbed to Stainless Steel Using Sodium Hypochlorite and Peracetic Acid. ELISA was used to monitor the decay in ricin and abrin structural epitopes (Garber, E. FDA/CFSAN, personal communication) after 0.0, 0.5, 1.0, 2.0, and 5.0 min incubation in PBS (pH 7.3) at room temperature with chemical inactivation agents. The loss of toxin epitopes following treatment with excess sodium hypochlorite was consistent with pseudo first-order kinetics (Figure 4A−D). Pseudo first-order rate constants (k'_{obs}) for reactions between ricin or crude abrin and sodium hypochlorite are shown in Table 2. The rate of inactivation increased with the concentration of sodium hypochlorite, but drying the toxins only imparted minor differences.

In contrast to results obtained using ELISA, identical samples analyzed using cytotoxicity assays revealed rapid and complete inactivation of ricin biological activity following treatment at the lowest sodium hypochlorite concentration used (5 ppm; 67.4 μM), whether in solution or dried onto stainless steel coupons (Figure 4E, Table 2). The range of residual cytotoxicity activities detected in ricin samples after 30 s incubation, the shortest nonzero time point used, was 0.0−2.2% in reactions containing 5−20 ppm sodium hypochlorite (67.4−269 μM). The experimental error associated with low levels of residual toxin activity introduces significant uncertainty when using nonlinear regression, and we noted that 95% confidence intervals were wider for the rate constants for these reactions (data not shown). We determined empirically that slower reactions that exhibited rate constants ≤140 s^{-1} were associated with more reasonable confidence intervals. Therefore, the fastest reactions were reported with k'_{obs} > 140 s^{-1} when necessary. Treatment of crude abrin dried onto stainless steel with 50 ppm sodium hypochlorite (674 μM) resulted in rapid, but incomplete, inactivation (55 ± 5% residual toxin), but only 8 ± 2% activity remained after treatment with 500 ppm sodium hypochlorite (6.74 mM NaClO; Figure 4F, Table 2). The crude

Table 2. Pseudo First-Order Rate Constants (k'_{obs}) for Inactivation of Purified Ricin or Crude Abrin at Room Temperature in PBS (pH 7.3) Solution or Dried on the Surface of Stainless Steel Coupons Using Sodium Hypochlorite in the Absence of Food Residues[a]

toxin	method of analysis	matrix (solution or dried)	NaClO (ppm)	NaClO (μM)	k'_{obs} ($10^{-3} \times s^{-1}$)	residual toxin[b] (%)
ricin	ELISA	solution[c]	5	67	8.9 ± 0.8	7.8 ± 0.8
			10	130	18.1 ± 1.7	1.7 ± 0.1
			20	270	29 ± 2	0 ± 0
		dried[c]	5	67	7.7 ± 0.8	14 ± 2
			10	130	17.4 ± 1.7	3.4 ± 0.1
			20	270	26 ± 3	0 ± 0
	cytotox	solution	5	67	>140	0.8 ± 0.1
			10	130	>140	0 ± 0
			20	270	>140	1.2 ± 0.1
		dried	5	67	>140	1.3 ± 0.1
			10	130	>140	1.3 ± 0.1
			20	270	>140	0 ± 0
crude abrin	ELISA	solution[c]	10	130	9.8 ± 1.4	27.5 ± 0.1
			50	670	20.3 ± 1.9	7.0 ± 0.7
		dried[c]	10	130	3.7 ± 0.3	34 ± 2
			50	670	18.1 ± 1.9	6.3 ± 1.2
	cytotox	solution	50	670	80 ± 40	27 ± 5
			500	6700	>140	2.1 ± 0.3
		dried	50	670	9 ± 4	58 ± 5
			500	6700	19 ± 6	8 ± 2

[a]Values shown are the average ± standard error of the mean for three trials. Inequalities are given in cases where the uncertainty in k'_{obs} values exceeds the mean values. [b]Residual toxin detected after 5 min of treatment. [c]Significant effect of NaClO concentration on k'_{obs}.

Table 3. Pseudo First-Order Rate Constants (k'_{obs}) for Inactivation of Ricin in PBS Solution or Dried on the Surface of Stainless Steel Coupons Using Peracetic Acid (PAA) in the Absence of Food Residues[a]

toxin	method of analysis	matrix (solution or dried)	PAA (ppm)	PAA (mM)	k'_{obs} ($10^{-3} \times s^{-1}$)	residual toxin[b] (%)
ricin	ELISA	solution[c]	500	6.6	2.5 ± 1.5	40.2 ± 0.9
			1000	13	7.8 ± 1.9	0 ± 0
			2000	26	>140	0 ± 0
		dried[c]	500	6.6	14 ± 2	34.1 ± 0.4
			1000	13	6 ± 2	5.2 ± 0.1
			2000	26	>140	0 ± 0
	cytotox	solution[c]	1000	13	48 ± 13	0 ± 0
			2000	26	80 ± 30	6 ± 5
			5000	66	>140	0 ± 0
		dried	1000	13	60 ± 20	0 ± 0
			2000	26	75 ± 30	18 ± 2
			5000	66	>140	0 ± 0

[a]Values shown are the average ± standard error of the mean for three trials. Inequalities are given in cases where the uncertainty in k'_{obs} values exceeds the mean values. [b]Residual toxin detected after 5 min of treatment. [c]Significant effect of PAA concentration on k'_{obs}.

abrin extract likely contained abundant protein contaminants that acted as a sink to consume active hypochlorous acid.

The rapid inactivation of the cytotoxic activity of ricin and abrin by sodium hypochlorite is consistent with observations made by Cole et al.[14] who noted complete inactivation of toxin activity by the earliest time point tested, 10 min. Reaction rates measured using ELISA contrasted with rates measured using fluorescence quenching in this study. We feel that this difference in apparent kinetics is related to the differences in the measured species. We suggest that labile functional groups present in the structures of ricin and abrin react quickly with sodium hypochlorite, as indicated by fluorescence quenching. The damaged protein then undergoes a slower refolding reaction that destroys the structural epitopes recognized by the monoclonal antibodies used in our ELISA methods. We suggest that this rationalization is also applicable to a similar discrepancy between slower rates of ricin inactivation using peracetic acid (Tables 2 and 3) measured with ELISA as compared to cytotoxic activity.

The inactivation of ricin cytotoxic activity in the absence of food residues using 1000−5000 ppm peracetic acid (13−68 mM) was less rapid as compared to inactivation using 5−20 ppm sodium hypochlorite (68−270 μM; see Tables 2 and 3), with apparent pseudo first-order rate constants ranging from 48 to >140 s^{-1} in a dose-dependent manner. Cytotoxicity assays were used to compare the molar excesses of peracetic acid and sodium hypochlorite required to achieve 50% inactivation of ricin at pH 4.5 and at pH 7.5 after 2.0 min of incubation. The percentage of ricin activity remaining at 2.0 min plotted versus the concentrations of hypochlorous acid, after adjusting for the effect of pH on the concentration of the free acid, yielded IC_{50} values of 1.19 ± 0.06 and 0.99 ± 0.08 μM at pH 4.5 and 7.5, respectively (Figure 5A). Similar analysis performed for peracetic acid yielded IC_{50} values of 20 ± 6 and 45 ± 8 μM

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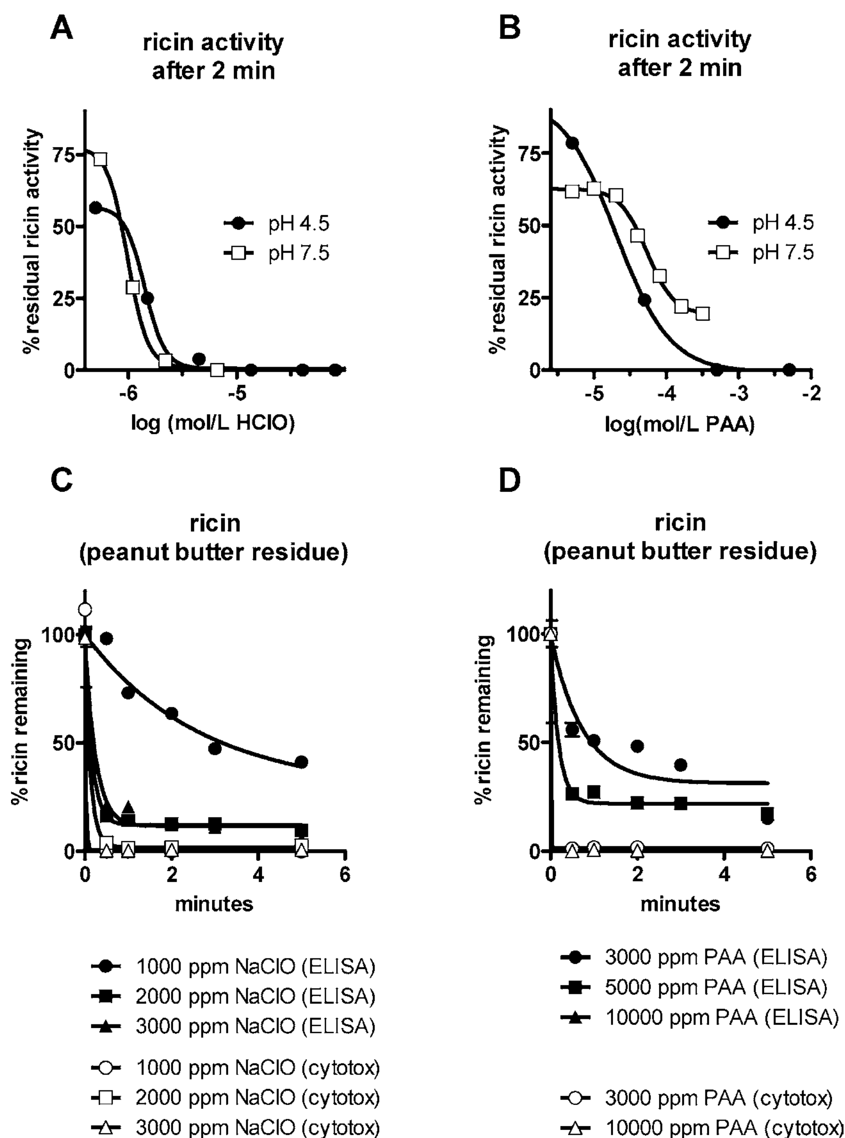

Figure 5. Residual ricin cytotoxic activity following exposure at room temperature to sodium hypochlorite or peracetic acid (PAA) at pH 4.5 and pH 7.5. (A) Residual cytotoxicity of ricin (200 nM) incubated with sodium hypochlorite (0.1−320 μM) for 2 min. Hypochlorous acid concentrations were calculated from pH, total hypochlorite concentration, and K_a. (B) Residual cytotoxicity of ricin (200 nM) incubated with peracetic acid (0.5−5000 μM) for 2 min. Reactions were performed in triplicate. Rates of inactivation for ricin in dried peanut butter residues using sodium hypochlorite (C) and peracetic acid (D) were compared using ELISA and cytotoxicity. Effects of HClO and pH on inactivation of ricin biological activity and effects of NaClO and PAA concentration on the rate of loss of ricin epitopes were statistically significant ($p < 0.05$).

at pH 4.5 and 7.5, respectively (Figure 5B). Division of IC_{50} values by the ricin concentration indicates that a 5−6 molar excess of hypochlorous acid or a 100−270 molar excess of peracetic acid for 2 min will inactivate 50% of ricin's biological activity.

Effects of Sodium Hypochlorite and Peracetic Acid on the Stability of Ricin in the Presence of Food Matrixes: Infant Formula, Peanut Butter, and Pancake Mix. The effects of sodium hypochlorite and peracetic acid on ricin inactivation in the presence of an infant formula matrix are given in Table 4. In general, pseudo first-order rate constants increased with higher concentrations of either sanitizer, whether measured using ELISA or cytotoxicity assays. Likewise, pseudo first-order rate constants for the inactivation of ricin in the presence of pancake mix (Table 5) increased with sodium hypochlorite concentration. Rates of inactivation of ricin in pancake mix using 500−1000 ppm peracetic acid (6.6−13 mM) measured using cytotoxicity assays were too rapid to demonstrate the effect of concentration. Rates of ricin inactivation in the presence of peanut butter are presented in Figure 5C,D and Table 6. Pseudo first-order rate constants increased as the concentration of sodium hypochlorite increased, and, again, reaction rates measured using cytotoxicity assays were too rapid to discern effects of concentration. A significant effect of peracetic acid concentration was only apparent in trials using ELISA detection for the inactivation of ricin in the presence of peanut butter. Rate constants measured using cytotoxicity assays were significantly greater than those determined using ELISA, regardless of the food matrix. Overall, the rates of ricin inactivation in the presence of foods were not significantly different whether suspended as slurries or dried onto stainless steel coupons, regardless of the type of food, method of detection, or which chemical agent was used. However, apparent rate constants were lower and percentages of residual toxin were

Table 4. Pseudo First-Order Rate Constants (k'_{obs}) for Sodium Hypochlorite- and Peracetic Acid (PAA)-Dependent Inactivation of Ricin in the Presence of Infant Formula Slurries or Food Residues Dried on the Surface of Stainless Steel Coupons[a]

toxin	method of analysis	matrix (solution or dried)	NaClO (ppm)	NaClO (mM)	k'_{obs} ($10^{-3} \times s^{-1}$)	residual toxin[b] (%)
ricin in infant formula	ELISA	slurry[c]	100	1.3	<1	98 ± 3
			500	6.7	12 ± 2	0 ± 0
			1000	13	10 ± 1	3.5 ± 0.3
		dried	100	1.3	22 ± 8	57 ± 4
			500	6.7	10 ± 2	3.5 ± 0.3
			1000	13	15 ± 2	0 ± 0
	cytotox	slurry[c]	100	1.3	52 ± 7	0.4 ± 0.4
			1000	13	>140	16 ± 11
		dried	100	1.3	60 ± 10	0.4 ± 0.4
			1000	13	90 ± 40	20 ± 2
toxin	method of analysis	matrix (solution or dried)	PAA (ppm)	PAA (mM)	k'_{obs} ($10^{-3} \times s^{-1}$)	residual toxin[b] (%)
ricin in infant formula	ELISA	slurry	500	6.6	<1	100 ± 3
			1000	13	<1	80 ± 8
			2000	26	2.1 ± 0.4	52.5 ± 1.0
		dried	500	6.6	<1	109 ± 3
			1000	13	4 ± 2	66 ± 5
			2000	26	2.3 ± 0.9	51.4 ± 0.1
	cytotox	slurry	500	6.6	24 ± 5	7 ± 2
			1000	13	25 ± 5	20 ± 2
		dried[c]	500	6.6	60 ± 10	11 ± 4
			1000	13	9 ± 3	15.8 ± 1.0

[a]Values shown are the average ± standard error of the mean for three trials. Inequalities are given in cases where the uncertainty in k'_{obs} values exceeds the mean values. [b]Residual toxin detected after 5 min of treatment. [c]Significant effect of NaClO or PAA concentration on k'_{obs}.

Table 5. Pseudo First-Order Rate Constants (k'_{obs}) for Sodium Hypochlorite- and Peracetic Acid (PAA)-Dependent Inactivation of Ricin in the Presence of Pancake Mix Slurries or Food Residues Dried on the Surface of Stainless Steel Coupons[a]

toxin	method of analysis	matrix (solution or dried)	NaClO (ppm)	NaClO (mM)	k'_{obs} ($10^{-3} \times s^{-1}$)	residual toxin[b] (%)
ricin in pancake mix	ELISA	slurry[c]	500	6.7	13.3 ± 1.6	2.9 ± 0.1
			1000	13	56 ± 2	1.5 ± 0.1
			2000	27	98 ± 3	0 ± 0
		dried[c]	500	6.7	27 ± 2	5.8 ± 0.1
			1000	13	19.3 ± 0.5	2.3 ± 0.1
			2000	27	67 ± 2	0 ± 0
	cytotox	slurry	500	6.7	>140	0 ± 0
			1000	13	100 ± 40	0 ± 0
			2000	27	120 ± 40	2.1 ± 0.6
			5000	67	>140	0 ± 0
		dried	500	6.7	>140	0.6 ± 0.6
			1000	13	75 ± 16	0 ± 0
			2000	27	>140	0 ± 0
			5000	67	>140	1.1 ± 0.1
toxin	method of analysis	matrix (solution or dried)	PAA (ppm)	PAA (mM)	k'_{obs} ($10^{-3} \times s^{-1}$)	residual toxin[b] (%)
ricin in pancake mix	cytotox	slurry	500	6.6	>140	0 ± 0
			1000	13	>140	3.8 ± 0.3
		dried	500	6.6	>140	0 ± 0
			1000	13	>140	5.4 ± 0.2

[a]Values shown are the average ± standard error of the mean for three trials. Inequalities are given in cases where the uncertainty in k'_{obs} values exceeds the mean values. [b]Residual toxin detected after 5 min of treatment. [c]Significant effect of NaClO concentration on k'_{obs}.

higher for crude abrin treated with 1000–2000 ppm sodium hypochlorite (13–27 mM) as compared to trials with ricin under these conditions (Tables 5–7).

Effects of Industrial Detergents and Sanitizers on Ricin Stability in the Absence and Presence of Food Matrixes. ELISA, which provided the most conservative estimates of toxin inactivation rates, was used to monitor the inactivation of ricin in PBS, foods, and dried food residues using industrial detergents and sanitizing agents (Figure 6A–F, Tables 8–10). The levels of industrial agents used in this study were selected to cover ranges that approximated the manufacturer's recommendations for food contact surfaces, where appropriate, through levels that might be justifiable in the extreme case of remediation following a bioterrorism attack.

Table 6. Pseudo First-Order Rate Constants (k'_{obs}) for Sodium Hypochlorite- and Peracetic Acid (PAA)-Dependent Inactivation of Ricin in the Presence of Peanut Butter Slurries or Food Residues Dried on the Surface of Stainless Steel Coupons[a]

toxin	method of analysis	matrix (solution or dried)	NaClO (ppm)	NaClO (mM)	k'_{obs} ($10^{-3} \times s^{-1}$)	residual toxin[b] (%)
ricin in peanut butter	ELISA	slurry	1000	13	36 ± 1	21.5 ± 0.6
			2000	27	85 ± 9	4.3 ± 0.7
			3000	40	140 ± 60	0 ± 0
		dried[c]	1000	13	6.2 ± 0.4	41.1 ± 1.2
			2000	27	100 ± 13	9.7 ± 0.4
			3000	40	68 ± 7	5.5 ± 0.3
	cytotox	slurry	1000	13	>140	0 ± 0
			2000	27	>140	0.9 ± 0.9
			3000	40	>140	0.6 ± 0.3
		dried	1000	13	>140	0 ± 0
			2000	27	>140	2.6 ± 0.8
			3000	40	>140	0.6 ± 0.3
toxin	**method of analysis**	**matrix (solution or dried)**	**PAA (ppm)**	**PAA (mM)**	**k'_{obs} ($10^{-3} \times s^{-1}$)**	**residual toxin (%)**
ricin in peanut butter	ELISA	slurry[c]	3000	39	10 ± 2	14.6 ± 0.7
			5000	66	87 ± 15	10.2 ± 0.5
			10 000	130	105 ± 15	0 ± 0
		dried[c]	3000	39	23 ± 6	15.2 ± 0.7
			5000	66	91 ± 13	17.2 ± 0.4
			10 000	130	nd	nd
	cytotox	slurry	3000	39	>140	2.0 ± 0.1
			5000	66	19 ± 3	0 ± 0
			10 000	130	>140	0 ± 0
		dried[c]	3000	39	43 ± 15	0 ± 0
			5000	66	nd	nd
			10 000	130	>140	0 ± 0

[a]Inequalities are given in cases where the uncertainty in k'_{obs} values exceeds the mean values. Values shown are the average ± standard error of the mean for three trials. [b]Residual toxin detected after 5 min of treatment. [c]Significant effect of NaClO or PAA concentration on k'_{obs}.

Table 7. Pseudo First-Order Rate Constants (k'_{obs}) for Sodium Hypochlorite-Dependent Inactivation of Abrin in the Presence of Food Slurries or Food Residues Dried on the Surface of Stainless Steel Coupons[a]

toxin	method of analysis	matrix (solution or dried)	NaClO (ppm)	NaClO (mM)	k'_{obs} ($10^{-3} \times s^{-1}$)	residual toxin[b] (%)
abrin in pancake mix	ELISA	slurry	1000	13	15.2 ± 1.3	36 ± 2
			2000	27	14.8 ± 0.8	5.4 ± 0.4
		dried	1000	13	6.6 ± 0.7	29.9 ± 1.5
			2000	27	6.9 ± 0.9	13.1 ± 1.6
abrin in peanut butter	ELISA	slurry	1000	13	10.2 ± 1.0	44.2 ± 1.9
			2000	27	10.4 ± 0.7	5.9 ± 0.6
		dried	1000	13	5.7 ± 1.0	45.0 ± 0.8
			2000	27	5.8 ± 1.8	18.6 ± 1.8

[a]Values shown are the average ± standard error of the mean for three trials. [b]Residual toxin detected after 5 min of treatment.

It must be noted that the manufacturers of the quaternary ammonium- and peracetic acid-based sanitizers we tested recommend that their products be used on precleaned food contact surfaces, for example, free of food residues. Our experiments were designed to evaluate the effectiveness of the chemical agents tested to inactivate protein toxins present in dried food residues, a use distinct from their typical applications in food manufacturing.

The phosphoric acid-based detergent was the least effective of the industrial products evaluated for inactivating ricin, showing no significant decay in detectable toxin (Figure 6A,B). This finding is not surprising because ricin is known to be fairly stable at the low pH values associated with this acidic detergent (pH < 2.5).

Inactivation of ricin using quaternary ammonium sanitizer is shown in Figure 6C,D and Table 8. Residual ricin detectable on food contact surfaces decayed to 8.4 ± 0.4% of the original level after 5 min of contact using 1000 ppm quaternary ammonium sanitizer, a concentration higher than the 400 ppm level recommended by the manufacturer for food contact surfaces. This agent is recommended for use at 800−1200 ppm for entryway sanitation systems, however. Figure 6D shows nearly complete inactivation of dried ricin residues on stainless steel after 5 min of contact using 2000 ppm quaternary ammonium sanitizer.

Table 9 and Figure 6E show the effects of a peracetic acid-based sanitizer on ricin inactivation. Under typical conditions, the manufacturer recommends a 0.13−0.26% solution of this product with at least 1 min of contact time to sanitize food contact surfaces in the absence of food residues. However, 5.0% peracetic acid-based sanitizer solutions and 10 min of contact can be used for sterilization purposes. Dried ricin was

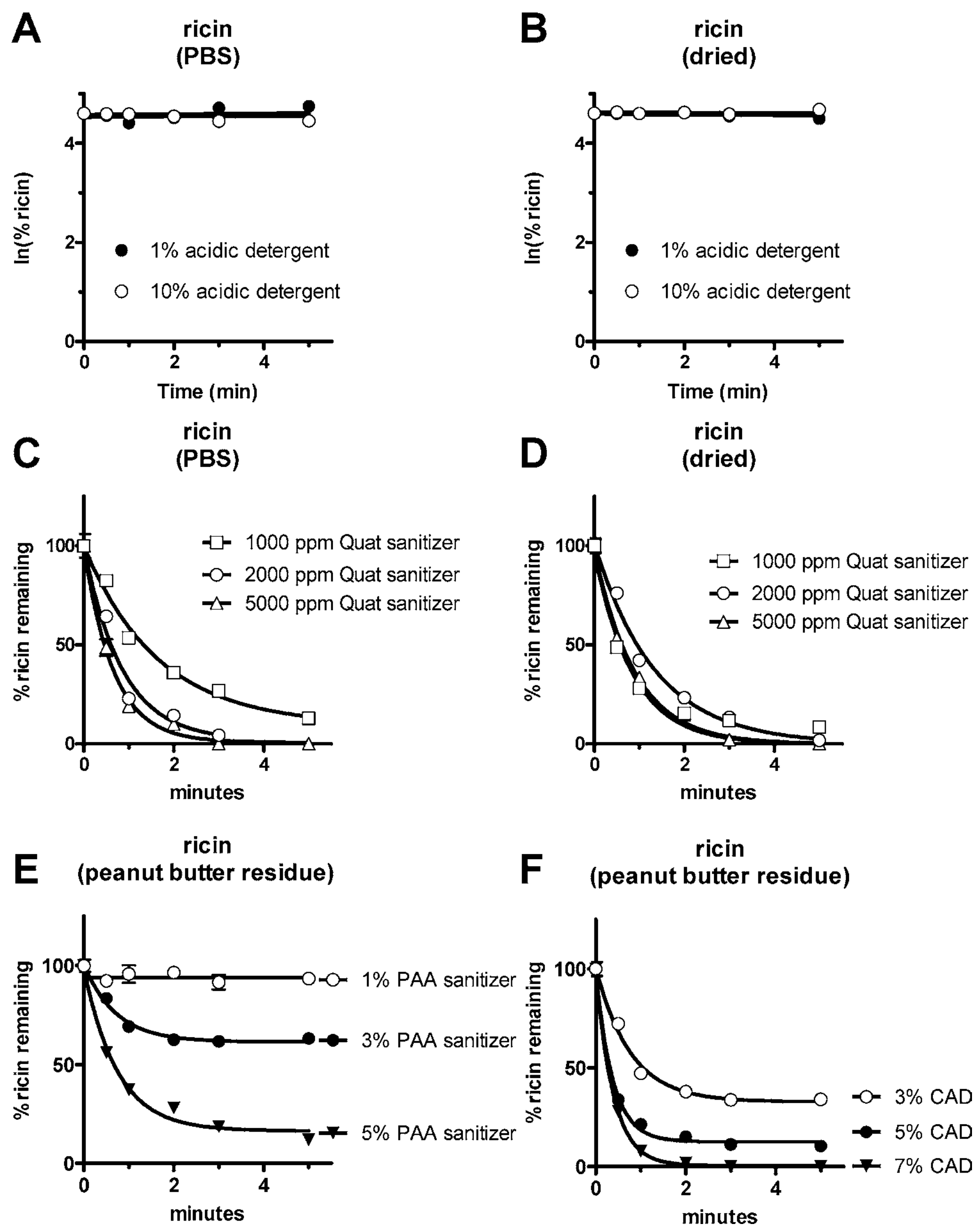

Figure 6. Inactivation of ricin at room temperature using commercial detergents and sanitizers detected using ELISA. (A,B) Ricin dissolved in PBS or dried onto stainless steel coupons and treated with acidic phosphoric acid-based detergent or (C,D) quaternary ammonium (Quat) sanitizer. Ricin in dried peanut butter residue was treated with (E) peracetic acid (PAA)-based sanitizer or (F) chlorinated alkaline detergent (CAD). Each point represents three trials. Error bars indicate standard error of the mean. The effect of phosphoric acid detergent concentration on the rate of loss of ricin epitopes was insignificant. Significant effects ($p < 0.05$) of concentration were detected on the rate of loss of ricin epitopes with treatments using Quat sanitizer, PAA-sanitizer, and CAD.

Table 8. Effect of Quaternary Ammonium (Quat) Sanitizer on Pseudo First-Order Rate Constants (k'_{obs}) and for the Loss of Detection (ELISA) of Ricin in PBS or Food Slurries or Dried Residues on Stainless Steel Coupons[a]

toxin	method of analysis	matrix (solution or dried)	Quat (ppm)	k'_{obs} ($10^{-3} \times s^{-1}$)	residual toxin[b] (%)
ricin	ELISA	solution[c]	1000	10.1 ± 1.0	12.8 ± 0.8
			2000	19 ± 2	1.4 ± 1.4
			5000	25.4 ± 1.5	0 ± 0
		dried	1000	27.5 ± 1.1	8.5 ± 0.4
			2000	12 ± 1	1.7 ± 0.9
			5000	19.1 ± 1.0	0 ± 0

[a]Values shown are the average ± standard error of the mean for three trials. [b]Residual toxin detected after 5 min of treatment. [c]Significant effect of Quat concentration on k'_{obs}.

inactivated effectively by 0.1−0.5% solutions of this agent in the absence of foods. Yet significantly higher concentrations were needed in the presence of peanut butter or pancake mix residues. For example, Figure 6E shows that 12.0 ± 0.5%

Table 9. Effect of Peracetic Acid (PAA)-Based Sanitizer on Pseudo First-Order Rate Constants (k'_{obs}) for the Loss of Detection (ELISA) of Ricin in PBS or Food Slurries or Dried Residues on Stainless Steel Coupons[a]

toxin	method of analysis	matrix (solution or dried)	PAA-based sensitizer (%)	pH	k'_{obs} ($10^{-3} \times s^{-1}$)	residual toxin[b] (%)
ricin in PBS	ELISA	slurry[c]	0.1	6.2	17.7 ± 0.9	53.0 ± 0.8
			0.5	5.7	29.7 ± 1.1	0 ± 0
			1.0	5.0	102 ± 8	0 ± 0
		dried[c]	0.1	6.2	6.6 ± 0.7	45.3 ± 0.7
			0.5	5.7	17.4 ± 0.6	0 ± 0
			1.0	5.0	108 ± 13	0 ± 0
ricin in peanut butter	ELISA	slurry[c]	1.0	5.0	22 ± 4	70 ± 2
			3.0	4.4	11.3 ± 0.6	41.7 ± 0.8
			5.0	3.9	105 ± 1	0 ± 0
		dried[c]	1.0	5.0	0.15 ± 0.17	93.5 ± 0.9
			3.0	4.4	22.9 ± 1.9	63.1 ± 1.2
			5.0	3.9	22.8 ± 1.5	12.0 ± 0.5
ricin in pancake mix	ELISA	slurry[c]	1.0	5.0	13.6 ± 1.0	29.6 ± 0.6
			3.0	4.4	81.3 ± 1.2	0 ± 0
			5.0	3.9	>140	0 ± 0
		dried[c]	1.0	5.0	4.8 ± 0.9	53.0 ± 0.9
			3.0	4.4	81.3 ± 1.2	0 ± 0
			5.0	3.9	105 ± 7	0 ± 0

[a]Inequalities are given in cases where the uncertainty in k'_{obs} values exceeds the mean values. Values shown are the average ± standard error of the mean for three trials. [b]Residual toxin detected after 5 min of treatment. [c]Significant effect of PAA-based sanitizer concentration on k'_{obs}.

Table 10. Effect of Chlorinated Alkaline Detergent on Pseudo First-Order Rate Constants (k'_{obs}) for the Loss of Detection (ELISA) of Ricin in PBS or Food Slurries or Dried Residues on Stainless Steel Coupons[a]

toxin	method of analysis	matrix (solution or dried)	chlorinated alkaline detergent (%)	pH	k'_{obs} ($10^{-3} \times s^{-1}$)	residual toxin[b] (%)
ricin in PBS	ELISA	slurry[c]	0.5	8.8	10.8 ± 0.9	31.8 ± 1.0
			2.0	9.7	25.0 ± 0.9	0 ± 0
			5.0	11.0	>140	0 ± 0
		dried[c]	0.5	8.8	6.8 ± 0.9	22.1 ± 0.2
			2.0	9.7	22.3 ± 1.7	0 ± 0
			5.0	11.0	>140	0 ± 0
ricin in peanut butter	ELISA	slurry[c]	3.0	10.2	37.6 ± 1.4	0 ± 0
			5.0	11.0	83 ± 4	0 ± 0
			7.0	12.1	>140	0 ± 0
		dried[c]	3.0	10.2	21.4 ± 0.4	34.0 ± 1.2
			5.0	11.0	44.7 ± 1.9	10.5 ± 0.3
			7.0	12.1	42.9 ± 1.6	0 ± 0
ricin in pancake mix	ELISA	slurry[c]	3.0	10.2	36.4 ± 1.9	0 ± 0
			5.0	11.0	>140	0 ± 0
			7.0	12.1	>140	0 ± 0
		dried[c]	3.0	10.2	28.0 ± 1.3	0 ± 0
			5.0	11.0	36.7 ± 1.3	0 ± 0
			7.0	12.1	63 ± 3	0 ± 0

[a]Inequalities are given in cases where the uncertainty in k'_{obs} values exceeds the mean values. Values shown are the average ± standard error of the mean for three trials. [b]Residual toxin detected after 5 min of treatment. [c]Significant effect of chlorinated alkali detergent concentration on k'_{obs}.

residual ricin was detected after 5 min of exposure to 5% peracetic acid-based sanitizer in the presence of dried peanut butter residues.

Chlorinated alkaline detergent was the most effective of the commercial agents tested for inactivating ricin in dried pancake mix and peanut butter food residues, considering both the kinetics of inactivation and residual toxin detected after 5 min of treatment (Figure 6A–F, Tables 8–10). Although chlorinated alkaline detergent is not considered a sanitizing agent, a 1.6–3.1% solution is recommended as a foam-type cleaner for dairy, food, and beverage processing equipment. The high pH and the presence of sodium hypochlorite in the chlorinated alkaline detergent (ca. 4.0–55 mM in our experiments) are likely to have contributed to the loss in ricin detection. Previous work[31,32] showed that ricin is less stable in neutral to alkaline buffers.

In summary, our studies showed that in some applications, such as ricin in dried infant formula, sodium hypochlorite was more potent than peracetic acid for destroying toxin epitopes detectable by ELISA and for inactivating the biological activity of these toxins. We found that chlorinated alkaline detergent was the most effective industrial agent for destroying toxin epitopes. Moreover, peroxyacetic acid-based sanitizer was less effective than chlorinated alkaline detergent for destroying toxin epitopes, but it was much more effective than phosphoric acid-based detergent. Overall, we found that sodium hypochlorite

and hypochlorite-containing products were most effective for inactivating protein toxins in dried food residues on food contact surfaces.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Studies were performed to evaluate kinetic models to describe reactions between toxins and chemical inactivation agents. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

Safety: Ricin and abrin are potent protein toxins and must be handled with care. Regulations and guidelines for research involving Select Agents (see CDC National Select Agents Registry Web site: http://www.selectagents.gov/index.html) were followed carefully. All work with these toxins was done in a laminar flow hood or biological safety cabinet, and personal protective gear (lab coats, safety glasses, gloves, etc.) was worn. Glassware and materials exposed to the toxins were detoxified by autoclaving at 121 °C for 60 min. Toxin solutions were mixed with an equal volume of >10% hypochlorite solution prior to disposal.

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The contents of this manuscript do not necessarily reflect the views and policies of the U.S. Food and Drug Administration, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

■ ABBREVIATIONS USED

CAD, chlorinated alkali detergent; PAA, peracetic acid; PBS, phosphate buffered saline; Quat, quaternary ammonium sanitizer

■ REFERENCES

(1) Audi, J.; Belson, M.; Patel, M.; Schier, J.; Osterloh, J. Ricin poisoning: a comprehensive review. *JAMA, J. Am. Med. Assoc.* **2005**, *294*, 2342−2351.

(2) Olsnes, S. The history of ricin, abrin and related toxins. *Toxicon* **2004**, *44*, 361−370.

(3) Winder, C. Toxicity of ricin. *J. Toxicol., Toxin Rev.* **2004**, *23*, 97−103.

(4) Hanson, D. Ricin toxin: Law enforcement's new challenge. *Police and Security News*; Quakertown, PA, 2004.

(5) Severson, K.; Brown, R. Georgia men held in plot to attack government. *NY Times*; New York, 2011.

(6) Investigation of a ricin-containing envelope at a postal facility-South Carolina. *MMWR Morb Mortal Weekly Rep.* **2003**, *52*, 1129−1131.

(7) Norton-Taylor, R.; Hopkins, N.; Henley, J. Poison suspect trained at al-Qaida camp. *The Guardian (London)*; London, 2003.

(8) Keteyian, A. Latest terror threat in US aimed to poison food. *CBS News*, 2010.

(9) Jackson, L. S.; Tolleson, W. H.; Chirtel, S. J. Thermal inactivation of ricin using infant formula as a food matrix. *J. Agric. Food Chem.* **2006**, *54*, 7300−7304.

(10) Jackson, L. S.; Zhang, Z.; Tolleson, W. H. Thermal stability of ricin in orange and apple juices. *J. Food Sci.* **2010**, *75*, T65−71.

(11) Jackson, L. S.; Tolleson, W. H. Thermal stability of ricin in orange juice. *IFT Annual Meeting*; New Orleans, LA, 2008.

(12) Hilgren, J.; Swanson, K. M.; Diez-Gonzalez, F.; Cords, B. Inactivation of Bacillus anthracis spores by liquid biocides in the presence of food residue. *Appl. Environ. Microbiol.* **2007**, *73*, 6370−6377.

(13) Mackinnon, P. J.; Alderton, M. R. An investigation of the degradation of the plant toxin, ricin, by sodium hypochlorite. *Toxicon* **2000**, *38*, 287−291.

(14) Cole, K. D.; Gaigalas, A.; Almeida, J. L. Process monitoring the inactivation of ricin and model proteins by disinfectants using fluorescence and biological activity. *Biotechnol. Prog.* **2008**, *24*, 784−791.

(15) Gaigalas, A. K.; Cole, K. D.; Bykadi, S.; Wang, L.; DeRose, P. Photophysical properties of ricin. *Photochem. Photobiol.* **2007**, *83*, 1149−1156.

(16) Hegde, R.; Maiti, T. K.; Podder, S. K. Purification and characterization of three toxins and two agglutinins from Abrus precatorius seed by using lactamyl-Sepharose affinity chromatography. *Anal. Biochem.* **1991**, *194*, 101−109.

(17) Walker, G. T.; Allen, S. A. Standardisation of sodium thiosulphate solutions by means of potassium iodate. *Chem. Ind.* **1953**, 568−569.

(18) Peters, D. G.; Hayes, J. M.; Hieftje, G. M. *Chemical Separations and Measurements: Theory and Practice of Analytical Chemistry*; W.B. Saunders: Philadelphia, 1974; p 749.

(19) Hussain, A.; Trudell, P.; Repta, A. J. Quantitative spectrophotometric methods for determination of sodium hypochlorite in aqueous solutions. *J. Pharm. Sci.* **1970**, *59*, 1168−1170.

(20) Jackson, L. S.; Tolleson, W. H. Stability of ricin and abrin in yogurt. *ACS Semiannual Meeting*; New Orleans, LA, 2008.

(21) Rutenber, E.; Katzin, B. J.; Ernst, S.; Collins, E. J.; Mlsna, D.; Ready, M. P.; Robertus, J. D. Crystallographic refinement of ricin to 2.5 A. *Proteins* **1991**, *10*, 240−250.

(22) Tahirov, T. H.; Lu, T. H.; Liaw, Y. C.; Chen, Y. L.; Lin, J. Y. Crystal structure of abrin-a at 2.14 A. *J. Mol. Biol.* **1995**, *250*, 354−367.

(23) Berman, H.; Henrick, K.; Nakamura, H. Announcing the worldwide Protein Data Bank. *Nat. Struct. Biol.* **2003**, *10*, 980.

(24) Jmol: an open-source Java viewer for chemical structures in 3D; http://www.jmol.org/, 2003.

(25) Akaike, H. Likelihood and the Bayes procedure. In *Bayesian Statistics*; Bernardo, J. M., DeGroot, M. H., Lindley, D. V., Smith, A. F. M., Eds.; University Press: Valencia, Spain, 1980; pp 143−166.

(26) Pattison, D. I.; Davies, M. J. Absolute rate constants for the reaction of hypochlorous acid with protein side chains and peptide bonds. *Chem. Res. Toxicol.* **2001**, *14*, 1453−1464.

(27) Kerkaert, B.; Mestdagh, F.; Cucu, T.; Aedo, P. R.; Ling, S. Y.; De Meulenaer, B. Hypochlorous and peracetic acid induced oxidation of dairy proteins. *J. Agric. Food Chem.* **2011**, *59*, 907−914.

(28) Finnegan, M.; Linley, E.; Denyer, S. P.; McDonnell, G.; Simons, C.; Maillard, J. Y. Mode of action of hydrogen peroxide and other oxidizing agents: differences between liquid and gas forms. *J. Antimicrob. Chemother.* **2010**, *65*, 2108−2115.

(29) Heywood, D. L.; Phillips, B.; Stansbury, H. A. Free radical hydroxylations with peracetic acid. *J. Org. Chem.* **1961**, *26*, 281.

(30) Rokhina, E. V.; Makarova, K.; Golovina, E. A.; Van As, H.; Virkutyte, J. Free radical reaction pathway, thermochemistry of peracetic acid homolysis, and its application for phenol degradation: spectroscopic study and quantum chemistry calculations. *Environ. Sci. Technol.* **2010**, *44*, 6815−6821.

(31) Tolleson, W. H.; Triplett, O. A.; Nguyen, K. T.; Jackson, L. S. Influence of pH on the kinetics of chemical inactivation for abrin and ricin. *Annual Meeting of the Society of Toxicology*; Salt Lake City, UT, 2010.

(32) Bushueva, T. L.; Tonevitsky, A. G. The effect of pH on the conformation and stability of the structure of plant toxin-ricin. *FEBS Lett.* **1987**, *215*, 155−159.

(33) Hawkins, C. L.; Pattison, D. I.; Davies, M. J. Hypochlorite-induced oxidation of amino acids, peptides and proteins. *Amino Acids* **2003**, *25*, 259−274.
(34) Pattison, D. I.; Hawkins, C. L.; Davies, M. J. Hypochlorous acid-mediated protein oxidation: How important are chloramine transfer reactions and protein tertiary structure? *Biochemistry* **2007**, *46*, 9853−9864.
(35) Nagy, P.; Ashby, M. T. Reactive sulfur species: kinetics and mechanisms of the oxidation of cysteine by hypohalous acid to give cysteine sulfenic acid. *J. Am. Chem. Soc.* **2007**, *129*, 14082−14091.
(36) Armesto, X. L.; Canle, M.; Fernandez, M. I.; Garcia, M. V.; Santaballa, J. A. First steps in the oxidation of sulfur-containing amino acids by hypohalogenation: Very fast generation of intermediate sulfenyl halides and halosulfonium cations. *Tetrahedron* **2000**, *56*, 1103−1109.
(37) Peskin, A. V.; Winterbourn, C. C. Kinetics of the reactions of hypochlorous acid and amino acid chloramines with thiols, methionine, and ascorbate. *Free Radical Biol. Med.* **2001**, *30*, 572−579.
(38) Davies, M. J. The oxidative environment and protein damage. *Biochim. Biophys. Acta* **2005**, *1703*, 93−109.
(39) Silverstein, R. M.; Hager, L. P. The chloroperoxidase-catalyzed oxidation of thiols and disulfides to sulfenyl chlorides. *Biochemistry* **1974**, *13*, 5069−5073.
(40) Pereira, W. E.; Hoyano, Y.; Summons, R. E.; Bacon, V. A.; Duffield, A. M. Chlorination studies. II. The reaction of aqueous hypochlorous acid with alpha-amino acids and dipeptides. *Biochim. Biophys. Acta* **1973**, *313*, 170−180.
(41) Drozdz, R.; Naskalski, J. W.; Sznajd, J. Oxidation of amino acids and peptides in reaction with myeloperoxidase, chloride and hydrogen peroxide. *Biochim. Biophys. Acta* **1988**, *957*, 47−52.
(42) Pattison, D. I.; Davies, M. J. Kinetic analysis of the role of histidine chloramines in hypochlorous acid mediated protein oxidation. *Biochemistry* **2005**, *44*, 7378−7387.