Mode of action of hydrogen peroxide and other oxidizing agents: differences between liquid and gas forms

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Objectives: Antimicrobials such as chlorine dioxide, peracetic acid and hydrogen peroxide (H2O2) share a basic mechanism of action (chemical oxidation of cellular components), but profound differences arise in their efficacy against microorganisms. Optimization of activity requires an understanding of their interaction with microbial targets and a clear differentiation between the chemical efficacies of each oxidative biocide. This study aimed to elucidate the biochemical mechanisms of action of oxidizing biocides at a macromolecular level, using amino acids, protein and an enzyme as model substrates for the action of each biocide.

Methods: The interactions of a number of oxidising agents (liquid and gaseous H2O2, ClO2, peracetic acid formulations) with amino acids, proteins (bovine serum albumin and aldolase) and enzymes were investigated by spectrophotometry, SDS-PAGE and alkaline phosphatase activity measurements.

Results: Biocide reactions yielded different types of oxidative structural change and different degrees of oxidation to amino acids and proteins, and differences in activity against a microbial enzyme. In particular there was a marked difference in the interactions of liquid H2O2 and gaseous H2O2 with the macromolecules, the latter causing greater oxidation; these results explain the dramatic differences in antimicrobial efficacy between liquid and gas peroxide.

Conclusions: These results provide a comprehensive understanding of the differences in interactions between a number of oxidizing agents and macromolecules commonly found in microbial cells. Biochemical mechanistic differences between these oxidative biocides do exist and lead to differential effects on macromolecules. This in turn could provide an explanation as to their differences in biocidal activity, particularly between liquid and gas peroxide.

Keywords: antimicrobial, proteins, amino acids, interactions

Introduction

Biocides, at a high concentration, cause massive cellular damage at a macromolecular level, with disparate mechanisms of action depending on the chemical nature of the biocide.1 Oxidative biocides [such as chlorine and hydrogen peroxide (H2O2)] remove electrons from susceptible chemical groups, oxidizing them, and become themselves reduced in the process. At a cellular level, low levels of oxidation can be a highly reversible process and prokaryotic organisms have evolved many defences against these effects.2 At higher biocide concentrations, these defence mechanisms can be overwhelmed, with significant surface, cell wall and intracellular damage. Oxidizing agents are usually low-molecular-weight compounds and are considered to pass easily through cell walls/membranes, whereupon they are able to react with internal cellular components, leading to apoptotic and necrotic cell death.3 Alternatively, they can severely damage microbial structure causing the release of intracellular components, which are then oxidized. The concentration exponent (n) of oxidizing agents is found to be in the low (<2) group suggesting that they interact strongly with their target by chemical, and not physical, means.3

Although biochemical mechanisms of action may differ between oxidative biocides, the physiological actions are largely similar.1 Oxidative biocides are proposed to have multiple targets within a cell as well as in almost every biomolecule; these include peroxidation and disruption of membrane layers, oxidation of oxygen scavengers and thiol groups, enzyme inhibition, oxidation of nucleosides, impaired energy production, disruption of protein synthesis and, ultimately, cell death.4−6 There are, however, marked differences in bactericidal activity between oxidizing agents; e.g. treatment with 2% H2O2 has been found to be more effective than treatment with 10% peracetic acid (PAA) at controlling Pseudomonas aeruginosa and Stenotrophomonas...
maltophilia contamination in output water from microfiltered water dispensers. H₂O₂ or PAA washing has been found to be more effective than chlorine dioxide (ClO₂) at preventing microbial growth on ‘Galía’ melons and PAA has been found to be more effective than ClO₂ at disinfecting urban waste water. Although some of these differences might be attributed to variation in test methods, exposure conditions (concentration, formulation, pH, etc.) and microbial defence mechanisms, it is also possible that the type of interaction with microbial macromolecules could explain this disparate activity.

In this study, liquid and gaseous (or vaporized) H₂O₂, ClO₂ and PAA formulations were tested for their ability to oxidize an array of amino acids and proteins. Both ClO₂ and vaporized H₂O₂ have been described as highly efficacious biocidal agents. Liquid H₂O₂ and PAA also have a well-proven efficacy against microorganisms. As well as investigating the ability of different chemicals to oxidize amino acids and proteins at comparable molarities, this study also explored the effect of the physical state (liquid or gas) and the effect of different formulations (which includes the addition of buffers, stabilizers and enhancers) on the oxidative efficacy of biocides.

Materials and methods

Materials

All amino acids were purchased from Sigma (Dorset, UK). Alkaline phosphatase was purchased from Sigma (St Louis, MO, USA). BSA, aldolase and sodium bicarbonate were obtained from Fisher Scientific (Loughborough, UK).

Solutions of liquid H₂O₂ [diluted from Vaprox™, 35% (v/v) in water] and vaporized H₂O₂ (VHP™, gas generated from Vaprox using a VHP100P generator; STERIS Ltd, Basingstoke, UK), ClO₂ in formulation (Tristel; Tristel Solutions Ltd, Snailwell, UK), unformulated PAA, PAA in formulation [HAMO PAA (HAMO) and STERIS 20 (S20); STERIS Ltd] and a combination of H₂O₂ and PAA in formulation [SPOR-KLENT™ (SK); STERIS Ltd] were used.

Preparation of biocidal solutions

Solutions of liquid H₂O₂, ClO₂, neat PAA, PAA in various formulations (HAMO and S20) and SK were all prepared/diluted in deionized water to concentrations from 0.1 to 50 mM and stored at room temperature. These solutions were freshly prepared daily to eliminate loss of efficacy due to degradation.

Interactions of oxidizing agents with amino acids

Solutions of cysteine, methionine, histidine, glycine, tryptophan and lysine were freshly prepared in deionized water to concentrations of 1 and 10 mM and stored at 4°C until use. The oxidation of amino acids caused by biocide exposure was measured spectrophotometrically (Helios alpha spectrophotometer; Thermo Scientific, Loughborough, UK). A fresh solution of amino acid was mixed with a fresh solution of the biocide at room temperature and changes in absorbance spectrum were immediately measured using Vision 32™ software (Thermo Scientific). The wavelength peak shifts were recorded and interpreted as oxidation of the amino acid, with different oxidation products peaking at different wavelengths due to changes in chemical composition. A range of concentration ratios of amino acid:biocide was investigated. Measurements were conducted against a blank consisting of deionized water and biocide, and two controls were performed for each experiment derived from pre-oxidized and unoxidized amino acid solutions. Experiments were conducted at least in triplicate.

Interactions of oxidizing agents with proteins

BSA and aldolase were dissolved in deionized water to a stock concentration of 1 mg/mL and stored at −4°C until use. Stock aldolase solution (250 μL) was mixed with 250 μL of deionized water (control) or a 2.5% (w/v) solution of each biocide for 1 min. In order to neutralize the acidity of the protein/biocide solution (i.e. acidity interfered with the test), 50 μL of a saturated solution of sodium bicarbonate was added. A preliminary test demonstrated that 50 μL of a saturated solution of sodium bicarbonate was appropriate to raise the pH of a 250 μL solution of each biocide tested (at a concentration of 2.5%) to neutrality or up to pH 8 in the case of H₂O₂. All samples were treated and analysed by SDS–PAGE.

Tests on enzyme activity

Alkaline phosphatase was dissolved in deionized water to a concentration of 500 μg/mL. An aliquot of 100 μL of the enzyme solution was diluted in 100 μL of 5% (w/v) sodium thiosulphate and 100 μL of deionized water. The activity of the enzyme was determined at room temperature (22°C) by the addition of 100 μL of the enzyme solution to 900 μL of phosphate buffer (50 mM, pH 10.25) containing magnesium chloride (20 mM) and p-nitrophenol phosphate (0.4 μM). The reaction was measured spectrophotometrically at 405 nm in a Helios alpha spectrophotometer for 14 min. To determine the effect of each biocide on alkaline phosphatase activity, 100 μL of alkaline phosphatase solution was mixed with 100 μL of the oxidizing biocide (at various concentrations from 0.33 to 330 mM) for 5 min at room temperature and the biocide neutralized by the addition of 100 μL of 5% (w/v) sodium thiosulphate. A preliminary experiment demonstrated that the neutralizer quenched the activity of the highest concentration of the biocides investigated (data not shown). An aliquot of 100 μL of this mixture was then added to 900 μL of the substrate solution and A₅₀₀ was measured for 10 min. All data were plotted using Statview™ software (SAS Institute, Cary, NC, USA).

Exposure to vaporized H₂O₂

Fifty microlitres of sample, amino acids (100 mM), BSA or aldolase (1 mg/mL), was applied to a steel disc (18 × 7 mm; Grade 2B finish; provided by STERIS Ltd) and dried for 30 min at room temperature in a laminar air flow cabinet. Steel discs were washed with acetone to eliminate any residual protein, rinsed with deionized water and sterilized prior to use. Vaporized H₂O₂ exposures were conducted in a sealed isolator with a vaporized H₂O₂ generator (VHP100P; STERIS Ltd) according to the manufacturer’s instructions. A typical decontamination cycle was conducted, using an injection rate of 2 mg/L H₂O₂ for vaporized H₂O₂ generation for up to 120 min before aeration. It should be noted that all exposures were conducted to ensure the presence of gas-phase H₂O₂ only, with no liquid or condensed peroxide. For amino acid analysis, exposed discs were removed and flushed with 50 μL of running buffer (0.112 M acetate/0.112 M Tris pH 6.5) and the gross effect of vaporized H₂O₂ on protein was analysed by SDS–PAGE.

If oxidation of amino acids did not occur when dried on the stainless steel support, an alternative support was used. Filter paper was soaked in a solution of 100 mM amino acids and the excess liquid removed. The filter paper was placed directly in a solution of liquid H₂O₂ for 10 s–10 min or in the VHP (1.8 mg/L H₂O₂ for 15 min and 2.0 mg/L for 90 min). Oxidation of amino acid resuspended in solution was measured spectrophotometrically as described above.
**SDS–PAGE analysis**

One hundred microlitres of BSA or aldolase sample was mixed with 100 μL of each biocide for 1 min at room temperature. The reaction was then neutralized with 100 μL of 5% sodium thiosulphate and 50 μL of the Phast-running buffer (1 M Tris–HCl pH 6.8/5% SDS/2 M glycine) was added. The mixture was brought to the boil for 5 min and then cooled down. Fifty microlitres of loading buffer [1 M Tris–HCl pH 6.8/20% SDS/glycerol (100%) /2 M β-mercaptoethanol/0.06% Bromophenol Blue] was added and 10 μL of the samples loaded to a pre-cast high-density gel. The gel was run using the Phast system according to the manufacturer’s instructions (PhastSystem separation technique file No. 112; Pharmacia AB). After electrophoresis the gels were silver stained and scanned as described above.

**Results**

**Interactions of oxidizing agents with amino acids**

The oxidative efficacy of different concentrations of each biocide was established by the ability to form a given oxidative product at a low stoichiometric ratio. Each amino acid has a different oxidative product or products (Table 1), which were clearly identifiable. For example, the oxidation of cysteine to sulphenic, sulphinic and sulphonic acids, respectively, depended upon the concentration and nature of the biocide (Figure 1). Liquid H₂O₂ proved to be a good oxidant against all amino acids tested except tryptophan, although it was not as powerful as unformulated PAA and the HAMO formulation, the only two biocides to oxidize all amino acids. In addition, these two biocides were observed to completely oxidize cysteine and glycine at a very low (100:1) amino acid:biocide ratio. Higher ratios were necessary for liquid H₂O₂ where lower amounts of oxidation products were observed except for lysine. The PAA formulation S20 (at room temperature) was also shown to oxidize all amino acids with the exception of tryptophan, although the PAA formulation did not demonstrate as high an oxidation potential as unformulated PAA and HAMO. In contrast, amino acid exposure to SK or ClO₂ did not result in any observable oxidation products at the highest ratio tested (1:5). Vapour phase H₂O₂ also did not result in any observable oxidation of amino acids when they were dried on a stainless steel surface (Table 1). However, when cysteine was soaked on a filter paper, both H₂O₂ (100 mM; 10 s exposure) and vaporized H₂O₂ (2.0 mg/L; 90 min exposure) oxidized cysteine to cysteine sulphonic acid, methionine to methionine sulphonic acid, lysine to aminoadipic semialdehyde, histidine to di-histidine and glycine to aminomalonate. Tryptophan was, however, not oxidized by these oxidizers in these conditions.

**Interactions of oxidizing agents with proteins**

From SDS–PAGE results, H₂O₂, even at the highest concentration tested of 2.5%, did not result in any visible degradation of the

**Table 1. Oxidation of amino acids on a stainless steel surface**

<table>
<thead>
<tr>
<th>Amino acid/oxidation product</th>
<th>H₂O₂</th>
<th>vH₂O₂</th>
<th>ClO₂</th>
<th>PAA</th>
<th>HAMO</th>
<th>SK</th>
<th>S20</th>
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<tr>
<td>Cysteine</td>
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<tr>
<td>sulphenic acid</td>
<td>1:2</td>
<td>_b</td>
<td>_a</td>
<td>2:1</td>
<td>100:1</td>
<td>_a</td>
<td>_a</td>
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<tr>
<td>sulphinic acid</td>
<td>1:3</td>
<td>_b</td>
<td>_a</td>
<td>_a</td>
<td>1:1</td>
<td>_a</td>
<td>1:3</td>
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<tr>
<td>sulphonic acid</td>
<td>_a</td>
<td>_b</td>
<td>_a</td>
<td>1:2</td>
<td>1:2</td>
<td>_a</td>
<td>_a</td>
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<tr>
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<tr>
<td>sulphoxide</td>
<td>1:3</td>
<td>_b</td>
<td>_a</td>
<td>2:1</td>
<td>10:1</td>
<td>_a</td>
<td>_a</td>
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<tr>
<td>sulphonic acid</td>
<td>_a</td>
<td>_b</td>
<td>_a</td>
<td>1:2</td>
<td>1:1</td>
<td>_a</td>
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<td>_b</td>
<td>_a</td>
<td>2:1</td>
<td>10:1</td>
<td>_a</td>
<td>1:3</td>
</tr>
<tr>
<td>2′ oxo-lysine</td>
<td>1:1</td>
<td>_b</td>
<td>_a</td>
<td>10:1</td>
<td>_a</td>
<td>_a</td>
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<td>aminoadipic semialdehyde</td>
<td>1:3</td>
<td>_b</td>
<td>_a</td>
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<td>_b</td>
<td>_a</td>
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<tr>
<td>aminomalonate</td>
<td>1:3</td>
<td>_b</td>
<td>_a</td>
<td>1:2</td>
<td>1:5</td>
<td>_a</td>
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<tr>
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</table>

vH₂O₂, vaporized H₂O₂.

The molar amount of biocide required to oxidize each amino acid to its specified product is represented by the amino acid:biocide ratios in the grid. Experiments were performed at least in triplicate.

*No oxidation observed at a 1:5 ratio.

*aNo oxidation observed at 100 mM amino acid and 2 g/L vH₂O₂.
Figure 1. Oxidation of cysteine to sulphenic acid by (a) H₂O₂, and cysteine to (b) sulphinic acid and (c) to sulphonic acid by PAA. The graphs are representative of results obtained on at least three separate occasions.
protein (Figure 2a). However, BSA was completely degraded by ClO₂ at a concentration of 2.5% (Figure 2b). PAA 2.5% and vaporized H₂O₂ (2 mg/L; 10 min) also resulted in the complete degradation of BSA in a similar manner to ClO₂, while HAMO produced a partial fragmentation of BSA (data not shown).

While the time of exposure to vaporized H₂O₂ was 60 times greater than to liquid H₂O₂ (i.e. 10 min exposure with vaporized H₂O₂ and 10 s with H₂O₂), the concentration of the liquid biocide was >10000 times greater at the highest test condition, therefore this difference in protein effect seems unlikely to be due to the different exposure times. Low concentrations of ClO₂ (0.5% and 1.2%) showed alterations in protein profile and partial fragmentation of the BSA (Figure 2b). A low concentration of unformulated PAA (0.5% and 1.2%) showed partial fragmentation similar to that obtained with ClO₂ (Figure 2b).

Aldolase did not stain as well as BSA with silver staining, although a clear, identifiable band (65 kDa) was observed (Figure 2c and d). Different results from those of aldolase oxidation were observed. Liquid H₂O₂ and vaporized H₂O₂ showed a similar effect with complete aldolase degradation achieved (no observable band) at a concentration of 1.2% H₂O₂ (Figure 2c) and after 10 min exposure to vaporized H₂O₂ (2 mg/L), whereas partial fragmentation of aldolase was observed following exposure to ClO₂ (1.2%) (Figure 2d) and PAA (0.5%) (in a similar way to ClO₂).

**Effect of oxidizing agents on enzyme activity**

Alkaline phosphatase activity was assessed by the presence of the product p-nitrophenol, measurable by an increase in absorbance at 405 nm. Normal activity is characterized by an increase in concentration over time (data not shown). Incubation of the enzyme with 5% (w/v) sodium thiosulphate did not result in any decrease in activity (Figure 3a - d, control). The oxidizing agents were tested at a range of concentrations from 0.33 to 330 mM. There were marked differences in activity between the different oxidizers (Figure 3a - d) especially at low concentrations. ClO₂ was the most efficient inhibitor of enzyme activity with some effects at 3.3 mM and complete inhibition of the enzyme at 10 mM (Figure 3a). H₂O₂ and HAMO completely inhibited alkaline phosphatase at a concentration of 100 mM (Figure 3b and c), whereas PAA produced this effect at a concentration of 50 mM (Figure 3d). Lower concentrations of PAA produced a reversible effect on enzyme activity, which was
proportional to the concentration. At a concentration of 3.3 and 33 mM the enzyme resumed activity after 4 and 10 min, respectively (Figure 3d). The PAA formulation HAMO increased enzymatic activity at a low concentration (3.3 mM). However, a concentration of 50 mM showed some reversible inhibitory effect against enzyme activity (Figure 3c).

**Discussion**

Oxidizing agents have been thought to react strongly with thiol groups in enzymes and proteins, DNA and the bacterial cell membrane. The redox potentials of the biocides used in this study differed, with PAA having the highest (1.81 V), H$_2$O$_2$ next (1.76 V) and ClO$_2$ the lowest (1.27 V). Higher redox potentials indicate a greater tendency to acquire electrons and thus be reduced, with the electron donor species being oxidized, so one would expect that the substance with the highest redox potential would be the most effective oxidant. This was reflected in the oxidation of amino acids by these liquid agents, where PAA produced the most oxidation and ClO$_2$ the least (Table 1). Though the mechanisms of the biocidal action of PAA are largely unknown it is thought that PAA denatures proteins in a similar manner to H$_2$O$_2$, increasing cell wall permeability by disrupting sulphhydryl (-SH) and sulphur (-S) bonds.

Although ClO$_2$ has been reported to react readily with the amino acids cysteine, tryptophan, histidine and tyrosine in water, this was not the case in this study. It should be noted, however, that these studies used exposure times of up to 24 h, in contrast to the short spectrophotometer scan time, which may explain this discrepancy. Despite the apparent lack of interaction with amino acids, ClO$_2$ was shown to have a strong effect on complex protein degradation and enzyme inhibition, which confirms some results of an early investigation into the physiological effects of ClO$_2$ by Benarde et al. That study found that killing of *Escherichia coli* by ClO$_2$ was very rapid; no release of protein and nucleic acid from cells could be detected by measuring absorbance at 260 and 280 nm. Although protein synthesis was disrupted by the addition of ClO$_2$, no direct reaction between ClO$_2$ and free amino acids could be measured. Benarde et al. hypothesized that the primary mechanism for inactivation of the *E. coli* cell was therefore disruption of the protein synthesis pathway by inhibition of

![Figure 3. Activity of oxidizing agents against alkaline phosphatase. (a) ClO$_2$: control (open squares), 0.33 (closed circles), 3.3 (closed triangles), 10 (open circles), 20 (asterisks), 33 (open diamonds), 50 (closed squares) and 330 mM (closed diamonds). (b) H$_2$O$_2$: control (open squares), 0.33 (closed circles), 3.3 (closed triangles), 10 (open circles), 50 (closed squares), 100 (dashes) and 330 mM (closed diamonds). (c) HAMO: control (open squares), 3.3 (closed triangles), 33 (open diamonds), 50 (closed squares), 100 (dashes) and 330 mM (closed diamonds). (d) PAA: control (open squares), 0.33 (closed circles), 3.3 (closed triangles), 33 (open diamonds), 50 (closed squares), 100 (dashes) and 330 mM (closed diamonds). Experiments were performed in triplicate. Error bars represent the standard deviation of the mean.](image-url)
enzymes or interference with nucleic acid–amino acid com-
plexes. However, a later study by Roller et al. reported that
the inhibition of protein synthesis may not be the primary inac-
tivation mechanism as the percentage of E. coli cells killed was
higher than the percentage of protein synthesis inhibited. This
study also found that dehydrogenase activity was quickly inhib-
ited by addition of ClO₂, leading Roller et al. to suggest that
ClO₂ inactivation was due to disruption of many cellular processes
by irreversible damage to proteins. Another study reported that
ClO₂ disrupted the permeability of the membrane. Potassium
glycinate, but no significant loss of large molecules from cells,
was observed, suggesting that gross damage to the membrane
did not occur and it was hypothesized that damage to proteins in
the membrane could lead to loss of permeability control.

Ogata also hypothesized that, due to the ability of ClO₂ to
oxidize tryptophan and tyrosine residues, protein denaturation
was the major mechanism of ClO₂ biocidal activity. It should be
noted that all these, and our investigations, studied the
effects of liquid ClO₂ solutions and may not reflect gaseous
mechanisms of action.

The ability of ClO₂ to denature (or degrade) the proteins studied
(BSA, aldolase) was matched only by vaporized H₂O₂, which
could cause complete degradation of BSA; this is perhaps surprising
given that ClO₂ had the lowest reduct potential of the substances
tested. Similar results had previously been published in a compar-
isn of the effects of liquid and gaseous peroxide against prions,
which are proposed infecting proteins. Like vaporized H₂O₂,
ClO₂ may provide a more ‘gaseous’ model for protein oxidation.
We speculate that as these gases have higher kinetic energies and
are uncharged, they can surround and penetrate the
three-dimensional protein structures more easily, oxidizing
buried cysteine residues and breaking vulnerable bonds between
subunits. In contrast, fully dissolved (liquid) biocides might not be
able to penetrate three-dimensional structures, although this
may be facilitated by formulation effects (formulation being a
mixture of chemicals in addition to the biocide). Further evidence
is provided by contrasting results between the liquid and vaporized
H₂O₂, with the latter considerably outperforming the liquid
system in terms of protein oxidation and their known antimicro-
bial activities. Similarly, the specific effects of these biocides in
liquid form may be further modified by various formulation
effects that can enhance their antimicrobial activity; this may
explain a previous report on the effects of oxidizing agent-based
disinfectants in protein removal from surfaces, which showed
that some PAA-based disinfectants showed cross-linking of pro-
teins on surfaces while others showed removal effects.

Biocides such as H₂O₂, ClO₂ and PAA are generally thought to
oxidize protein side chains, of which there are 21 different types
(excluding unusual amino acids and any post-translational
modifications). These reactions include hydrogen abstraction,
which occurs primarily with aliphatic residues, and addition,
which occurs with aromatic residues and usually results in the
formation of altered side chains.

Very strong oxidizing agents that can result in localized free
radical formation may oxidize the protein backbone, primarily
by hydrogen atom abstraction at the α-carbon, which can
result in backbone fragmentation. This multistep oxidation
process results in the formation of a carbon radical via direct
or indirect reaction, detectable by electron spin resonance
(ESR) trapping. Subsequent formation of the peroxyl radical in
the presence of O₂ is essential to backbone cleavage, and little
fragmentation occurs in the absence of O₂.

Reversible effects of ClO₂ (3.3 mM), PAA (3.3 and 33 mM) and
HAMO (33 mM), where the activity of alkaline phosphatase was
observed to be delayed following neutralization of the biocide,
may indicate the occurrence of reversible side chain oxidation in
the enzyme, which may affect the tertiary structure of the
protein and reduce the efficacy of the enzymatic active site, ren-
dering the enzyme functional but at lower capacity. Non-specific
oxidation of susceptible biomolecular groups such as thiols,
amines and alkynes has been shown to cause widespread desta-
bilization of protein tertiary structure.

It was interesting to note that among the oxidizers tested, only H₂O₂ showed truly
concentration-dependent inhibition of alkaline phosphatase
(Figure 3b). All oxidizing agents at a concentration of 100 mM
showed complete irreversible inhibition of alkaline phosphatase
and may indicate the oxidative breakdown of disulphide bridges,
known to be integral to E. coli alkaline phosphatase structure.

The increase in alkaline phosphatase activity in the presence of
a low concentration of HAMO, but more notably H₂O₂, may be
explained by the fact that sodium thiosulphate, a strong chemical
reductant, had some inhibitory effects on the activity of alkaline
phosphatase (data not shown). Weak solutions of oxidative bio-
cides neutralized the reducing effect of the sodium thiosulphate,
leading to an increase in enzyme activity. In the case of HAMO,
the buffer in the formulation stabilized the pH at 5.5 (whereas
reactions with other oxidizers took place in a pH range of 2–4)
and probably increased the activity of alkaline phosphatase,
which obviously exhibits optimal activity under alkaline conditions.

This study highlights that considerable differences exist in the
ability of a range of oxidizing agents to oxidize simple macromol-
eules, differences that are not directly related to the redox
potential of these agents. The interaction between these bio-
cides and simple macromolecules was also altered by the formu-
lation and the physical state of the oxidizing agents. Of particular
note, the mode of action of H₂O₂ in liquid form is different from
that in gas form, and the action of the biocide can also vary
depending on its formulation (or mixture with other chemicals).
Further studies are required to understand similar mechanisms
differences of action against other biomolecules, such as
lipids and nucleic acids.

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Transparency declarations
G. M. is an employee of STERIS Ltd and the industrial supervisor of
M. F. and E. L. All other authors: none to declare.

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