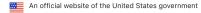
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Denaturation of protein by chlorine dioxide: oxidative modification of tryptophan and tyrosine residues

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Abstract

Oxychlorine compounds, such as hypochlorous acid (HOCI) and chlorine dioxide (ClO2), have potent antimicrobial activity. Although the biochemical mechanism of the antimicrobial activity of HOCI has been extensively investigated, little is known about that of CIO2. Using bovine serum albumin and glucose-6-phosphate dehydrogenase of Saccharomyces cerevisiae as model proteins, here I demonstrate that the antimicrobial activity of CIO2 is attributable primarily to its proteindenaturing activity. By solubility analysis, circular dichroism spectroscopy, differential scanning calorimetry, and measurement of enzymatic activity, I demonstrate that protein is rapidly denatured by CIO2 with a concomitant decrease in the concentration of CIO2 in the reaction mixture. Circular dichroism spectra of the CIO2-treated proteins show a change in ellipticity at 220 nm, indicating a decrease in alpha-helical content. Differential scanning calorimetry shows that transition temperature and endothermic transition enthalpy of heat-induced unfolding decrease in the CIO2treated protein. The enzymatic activity of glucose-6-phosphate dehydrogenase decreases to 10% within 15 s of treatment with 10 microM CIO2. Elemental analyses show that oxygen, but not chlorine, atoms are incorporated in the CIO2-treated protein, providing direct evidence that protein is oxidized by CIO2. Furthermore, mass spectrometry and nuclear magnetic resonance spectroscopy show that tryptophan residues become N-formylkynurenine and tyrosine residues become 3,4-dihydroxyphenylalanine (DOPA) or 2,4,5-trihydroxyphenylalanine (TOPA) in the ClO2treated proteins. Taking these results together, I conclude that microbes are inactivated by CIO2 owing to denaturation of constituent proteins critical to their integrity and/or function, and that this denaturation is caused primarily by covalent oxidative modification of their tryptophan and tyrosine residues.

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