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Metalloporphyrins as cytochrome P450 models for chlorhexidine metabolite prediction

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ABSTRACT

The catalytic oxidation of chlorhexidine (CHX, a strong microbicidal agent) mediated by ironporphyrins has been investigated by using hydrogen peroxide, *m*CPBA, *t*BuOOH, or NaOCl as oxidant. All of these oxygen donors yielded *p*-chloroaniline (pCA) as the main product. The higher pCA yields amounted to 71% in the following conditions: catalyst/oxidant/substrate molar ratio of 1:150:50, aqueous medium, FeTMPyP as catalyst. The medium pH also had a strong effect on the pCA yields; in physiological pH, formation of this product was specially favored in the presence of the catalysts, with yields 58% higher than those achieved in control reactions. This provided strong evidence that CHX is metabolized to pCA upon ingestion.

1. Introduction

Chlorhexidine (CHX, Fig. 1) is a bis-guanidine with bactericidal and fungicidal properties. It is commonly used in surgical [1–3], neonatal treatments, periodontal treatments [4,5], and oral rinses [6], and it is also employed as additive in chicken and pig food, among other applications. Although the skin absorption of CHX is not significant, as documented in the literature [7], the use of this compound as preservative in chicken meat or food as well as in rinse solutions might lead to the generation of toxic metabolites [8] such as *p*-chloroaniline (pCA) and *p*-chloronitrobenzene (pCNB) [8–12] when such food is consumed.

The oxidative metabolism of exogenous compounds in plants, animals, bacteria, and fungi is mediated by a super family of cytochrome P450 enzymes [13], which have an iron protoporphyrin IX as the prosthetic group (Fig. 2).

The iron(IV)-oxo porphyrin π-cation, a highly eletrophilic species, is assumed to be the most important catalytic intermediate in reactions catalyzed by cytochrome P450 enzymes. However, the general consensus nowadays is that this species may not be the only catalytic intermediate responsible for the large number of reactions mediated by the cytochromes P450, as reported in recent works [14,15]. The existence of different catalytic species enables the cytochromes P450 to carry out a wide variety of chemical transformations, including countless reactions like alkene epoxidation, *n*-dealkylation of secondary and tertiary amines, *o*-dealkylation, and hydroxylation of aromatic compounds, among others [13,16].

A number of biomimetic systems that are able to mimic the function of P450 enzymes have been developed, in order to contribute to a better understanding of the action mechanisms of these enzymes [13,17]. Synthetic metalloporphyrins have been successfully used as P450 models for the oxidation of many endogenous and exogenous compounds, mainly for comparison purposes and identification of the metabolites formed in *in vivo* systems and/or as an alternative method for the production of these metabolites.

The toxicity of the organochloride metabolites of chlorexidine [18–20] justifies studies involving CHX degradation or CHX metabolization by cytochrome P450 in living organisms. However, the great complexity inherent to the study of *in vivo* systems, the way metalloporphyrins successfully mimic the cytochrome P450 enzymes, and our ongoing interest in this field have prompted the present investigation on the use of metalloporphyrins as cytochrome P450 models for the prediction and identification of the possible metabolites generated from the antimicrobial agent CHX.

2. Experimental

2.1. Physical measurements

UV–vis spectra were obtained on a Hewlett–Packard 8452A diode array spectrometer. Analytical HPLC analyses were

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