CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

Inorganic Chemistry of Defensive Peroxidases in the Human Oral Cavity

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ABSTRACT

The innate host response system is comprised of various mechanisms for orchestrating host response to microbial infection of the oral cavity. The heterogeneity of the oral cavity and the associated microenvironments that are produced give rise to different chemistries that affect the innate defense system. One focus of this review is on how these spatial differences influence the two major defensive peroxidases of the oral cavity, salivary peroxidase (SPO) and myeloperoxidase (MPO). With hydrogen peroxide (H_2O_2) as an oxidant, the defensive peroxidases use inorganic ions to produce antimicrobials that are generally more effective than H_2O_2 itself. The concentrations of the inorganic substrates are different in saliva *vs.* gingival crevicular fluid (GCF). Thus, in the supragingival regime, SPO and MPO work in unison for the exclusive production of hypothiocyanite ($OSCN^-$, a reactive inorganic species), which constantly bathes nascent plaques. In contrast, MPO is introduced to the GCF during inflammatory response, and in that environment it is capable of producing hypochlorite (OCl^-), a chemically more powerful oxidant that is implicated in host tissue damage. A second focus of this review is on inter-person variation that may contribute to different peroxidase function. Many of these differences are attributed to dietary or smoking practices that alter the concentrations of relevant inorganic species in the oral cavity (*e.g.*: fluoride, F^-; cyanide, CN^-; cyanate, OCN^-; thiocyanate, SCN^-; and nitrate, NO_3^-). Because of the complexity of the host and microflora biology and the associated chemistry, it is difficult to establish the significance of the human peroxidase systems during the pathogenesis of oral diseases. The problem is particularly complex with respect to the gingival sulcus and periodontal pockets (where the very different defensive stratagems of GCF and saliva co-mingle). Despite this complexity, intriguing *in vitro* and *in vivo* studies are reviewed here that reveal the interplay between peroxidase function and associated inorganic chemistry.

KEY WORDS: salivary peroxidase, myeloperoxidase, hypochlorite, hypothiocyanite.

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INTRODUCTION

The oral cavity contains a plethora of specific and non-specific defense factors. The non-specific factors include some mucins, proline-rich proteins, salivary glycoproteins, lactoferrin, lysozyme, histatins, cystatins, and peroxidases. This review focuses on the role of peroxidases in the context of oral health and disease, with an emphasis on the relevant inorganic chemistry. Particular attention is paid to the antimicrobial properties of the inorganic chemicals of the oral cavity that are associated with the peroxidases, and to the inter-person differences in the inorganic chemistry of the oral cavity that may influence peroxidase function. For further information on the structures and origins of human oral peroxidases, the reader is referred to the recent review by Tenovuo and co-workers (Ihalin *et al.*, 2006). There are two principal defensive peroxidase systems in the oral cavity, salivary peroxidase (SPO) and myeloperoxidase (MPO). SPO is structurally and catalytically similar to lactoperoxidase (LPO) (Ihalin *et al.*, 2006). *In vivo*, the SPO and LPO systems essentially use only the pseudohalide SCN^- as a substrate to produce $OSCN^-$ (Pruitt *et al.*, 1988). Such defensive peroxidases are commonly found in regions of the human body that are controlled by the mucosa: *e.g.*, breast milk (Shin *et al.*, 2000), lachrymal fluid (Van Haeringen *et al.*, 1979; Tenovuo *et al.*, 1985), and the mucosal lining of the lungs (Gerson *et al.*, 2000). LPO and SPO are coded for the same gene (Ueda *et al.*, 1997). In contrast to peroxidases that essentially employ only SCN^- as a substrate (*e.g.*, LPO and SPO), the MPO system is also capable of oxidizing Cl^- to produce hypochlorite (OCl^-) (Arnhold *et al.*, 2006). Hypobromite (OBr^-) can also be generated by the MPO system (Thomas *et al.*, 1995), but only in minor amounts in the oral cavity. All of the human defensive peroxidases can also utilize iodide (I^-) as a substrate. However, because of sequestration in the thyroid, the environmentally rare halide I^- is not abundant in most physiologic fluids, including the fluids of the oral cavity (Anttonen and Tenovuo, 1981). Accordingly, the limited bioavailability of I^- precludes its significant involvement in host defense.

SPO is a normal, non-inducible component of the saliva of the parotid and submandibular glands (Riva *et al.*, 1978), whereas MPO is an offensive mechanism of neutrophilic polymorphonuclear leukocytes (PMNs). Leukocytes are not normal components of the saliva of healthy individuals, but rather are introduced to the oral cavity by gingival crevicular fluid (GCF) during inflammatory responses (Kowolik and Grant, 1983). The leukocytes in the GCF are comprised of *ca.* 90% PMNs (Ebersole, 2003), and MPO accounts for about 5% of the total PMN protein (Pullar *et al.*, 2000). PMNs degenerate in saliva due to osmotic lysis, thereby releasing the content of the azurophilic granules (including MPO). It has been estimated that *ca.* 75% of the peroxidase activity in mixed saliva is due to MPO, with the remaining activity attributed to SPO (Thomas *et al.*, 1994a). Most of the SPO activity is associated with the soluble portion of the saliva, whereas most of the MPO activity is associated with the sediment (Thomas *et al.*, 1994a). Note that, in contrast to other regions of the mucosa—for example, the lungs—eosinophils are not usually recruited into

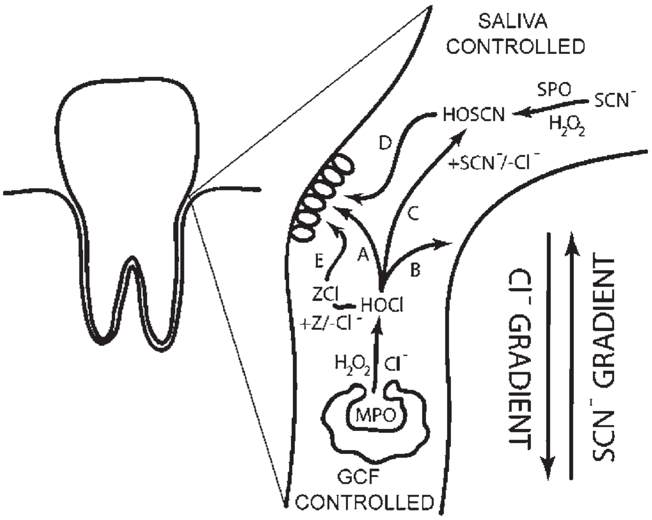

Figure 1. Spatial relationship between the inorganic host defense factors of the oral cavity and the ion gradients that influence their relative abundance. Refer to the text for an explanation of pathways A-E and the meaning of the variable Z.

Table 1. Two-electron Redox Couples for X^- (E°, pH = 7 *vs.* SHE), Apparent Rate Constants (k) of MPO Compound I (x $10^{-4}M^{-1}s^{-1}$) with X^-,[a] Reference Range Values (RRV) of X^- in Physiologic Fluids (μM or mM), and Specificities (S)[b] for Oxidation of X^- by MPO (consensus substrates in **bold**)

X^-	E°	k	RRV in GCF	GCF S	RRV in Saliva	Saliva S
Cl^-	1.08	2.5	**90 mM**	**6**	25 mM	1
SCN^-	0.77	960	40 μM	1	**1 mM**	**15**

[a] Furtmueller *et al.* (1998).

[b] $S = k^{X^-}_{maj}[X^-_{maj}]/k^{X^-}_{min}[X^-_{min}]$.

the oral cavity, although they can be introduced to saliva *via* the sputum of individuals who have asthma eosinophilia (Spahn, 2007) and from eosinophilic ulcers (rare lesions of the oral mucosa) (Mezei *et al.*, 1995; Hirshberg *et al.*, 2006). Consequently, there is no evidence that eosinophil peroxidase (another common defensive peroxidase that has properties somewhat different from those of LPO, SPO, and MPO) plays a significant role in oral fluids. Accordingly, this review focuses on SPO and MPO.

PEROXIDASES: ORAL HEALTH AND DISEASE

The two disease states of the oral cavity that we consider here are caries and periodontal diseases. The etiology of caries is clear: Acidogenic bacteria cause damage to tooth enamel in the presence of fermentable carbohydrates (*e.g.*, sucrose, fructose, and glucose) (Featherstone, 2000). When the pH at the surface of the tooth falls below 5.5, demineralization proceeds faster than remineralization, and decay ensues. The role of inorganic chemistry in this process is multifaceted: *e.g.*, the (de)mineralization process largely involves the inorganic mineral hydroxyapatite [*ca.* 96% for enamel and 70% for dentin (with some amorphous calcium phosphate)], and the aforementioned peroxidase-derived reactive inorganic species are involved in controlling the microbial growth ($OSCN^-$ in particular, although other inorganic chemical species have been proposed to be significant, *vide infra*).

Like caries, periodontal diseases are also caused by microbial infection (Smalley, 1994; Genco, 1996; Mombelli, 2003). Although the primary cause of periodontal diseases is the accumulation of dental plaque at the gingival margin and the consequential host response (Azuma, 2006), numerous factors affect the severity of the diseases, include smoking (Bergström, 2004), poorly controlled diabetes (Mealey and Oates, 2006), and genetic susceptibility (Baker and Roopenian, 2002; Shapira *et al.*, 2005). Both soft tissues (gingival and periodontal ligaments) and hard tissues (alveolar bone and cementum, which are both largely hydroxyapatite) are affected, but the cause of this tissue damage is a complex and as-yet-unresolved matter. It appears likely that inflammatory agents (including OCl^-) produced by the host (Pullar *et al.*, 2000; Klebanoff, 2005) and virulence factors produced by the infectious agents (Graves *et al.*, 2000) are both responsible for the tissue damage. The supragingival environment in which caries develops and the subgingival environment of periodontal diseases exhibit different chemistries that have a marked influence on the functions and activities of the human defensive peroxidases, which will be discussed next (Fig. 1, Table 1).

SPATIAL NATURE OF PEROXIDASE SYSTEMS AND THEIR CONSENSUS SUBSTRATES

The oxidation reactions that are catalyzed by the peroxidase systems of the oral cavity are governed by the amount of available hydrogen peroxide (H_2O_2), the limiting chemical reagent. A dual-oxidase system from the salivary glands is an endogenous source of H_2O_2 (Geiszt *et al.*, 2003; Donko *et al.*, 2005; Ris-Stalpers, 2006). Oral bacteria also produce H_2O_2 during anaerobic glycolysis (Carlsson *et al.*, 1983). A third source of H_2O_2 is derived from activated neutrophils during oxidative bursts (Dahlgren and Karlsson, 1999; Quinn, 2005). The amounts of OCl^- and $OSCN^-$ that are produced by the MPO system are related to the relative concentrations of Cl^- and SCN^- (van Dalen *et al.*, 1997; Arnhold *et al.*, 2006). At equal concentrations of (pseudo)halide, MPO catalyzes the oxidation of SCN^- about 1000 times faster than Cl^-, but Cl^- is about 1000 times more abundant in most physiologic fluids [*e.g.*, plasma and GCF (Anttonen and Tenovuo, 1981)]. Consequently, comparable amounts of $OSCN^-$ and OCl^- are produced by the MPO system in such fluids. However, SCN^- is essentially the only substrate of MPO in saliva, where the concentration of SCN^- is higher than in most other extracelluar fluids (Tenovuo and Makinen, 1976), as a consequence of its active transport (Fragoso *et al.*, 2004). While analysis of the data in Table 1 suggests that OCl^- should also be generated in saliva, albeit in a minor amount with respect to $OSCN^-$, it can be estimated that the half-life of the OCl^- in saliva is less than 15 μsec, as a consequence of its very fast non-enzymic reaction with SCN^- (Ashby *et al.*, 2004). The reaction of OCl^- and SCN^- yields $OSCN^-$ (Nagy *et al.*, 2006a). Thus, in effect, the only hypohalite of the SPO and MPO systems in saliva is expected to be $OSCN^-$, but a continuum of products is expected at the gingival margin, where a gradient of concentration of Cl^- and SCN^- exists. The spatial relationship between these gradients and the areas of the oral cavity that are respectively controlled by the OCl^- and $OSCN^-$ defense factors is illustrated in Fig. 1:

- Extraphagosomal OCl^- is cytotoxic to oral bacteria (Path A) (Briseno *et al.*, 1992; Webb *et al.*, 1995; Yesilsoy *et al.*, 1995; Barnard *et al.*, 1996; Winniczuk and Parish, 1997; Calas *et al.*, 1998; D'Arcangelo *et al.*, 1998; D'Arcangelo and Varvara, 1998; Huque *et al.*, 1998; Ferreira *et al.*, 1999; Wunder and Bowen, 1999; Spratt *et al.*, 2001; Mikami *et al.*, 2003; Sassone *et al.*, 2003a,b; Moller *et al.*, 2004; Nagayoshi *et al.*, 2004; Radcliffe *et al.*, 2004; Vianna *et al.*, 2004; Carson *et al.*, 2005; Fang *et al.*, 2006; Sena *et al.*, 2006; Ozok *et al.*, 2007) and gingival tissue (Path B) (Schraufstatter *et al.*, 1990; Vissers *et al.*, 1999; Hidalgo and Dominguez, 2000; Pullar *et al.*, 2000; Vile *et al.*, 2000; Hidalgo *et al.*, 2002). Importantly, nearly all of the investigations of the efficacy of OCl^- on oral bacteria have been carried out for single species in planktonic cultures. However, a recent study has focused on the effects of OCl^- on single- and dual-species biofilms of *Fusobacterium nucleatum* and *Peptostreptococcus micros* (Ozok *et al.*, 2007).
- Alternatively, OCl^- can react with SCN^- to produce HOSCN (Path C) (Ashby *et al.*, 2004). HOSCN is also produced by the SPO-catalyzed oxidation of SCN^- by H_2O_2 (Ihalin *et al.*, 2006 ; Nagy *et al.*, 2006a). HOSCN is antimicrobial toward oral bacteria (Path D) (Clem and Klebanof, 1966; Hoogendoorn, 1976; Pruitt *et al.*, 1979; Carlsson *et al.*, 1983; Thomas *et al.*, 1983, 1994b; Ellen *et al.*, 1988; Lopatin *et al.*, 1991; Lumikari *et al.*, 1991; Courtois *et al.*, 1992; Lenander-Lumikari *et al.*, 1993, 1997; van der Hoeven and Camp, 1993; Kirstila *et al.*, 1994; Jones *et al.*, 1998; Fadel and Courtois, 1999, 2001; Yu *et al.*, 2000; Ihalin *et al.*, 2001, 2003; Korpela *et al.*, 2002; Garcia-Graells *et al.*, 2003; Vannini *et al.*, 2004), but relatively non-injurious to the host (Bjoerck and Claesson, 1980; Marshall and Reiter, 1980; White *et al.*, 1983; Carlsson *et al.*, 1984; Carlsson, 1987).
- In addition to the reaction of OCl^- with SCN^-, it may react with other small molecules (Path E) to produce secondary antimicrobials [*e.g.*, when Z is an amine, a cytotoxic chlora–mine is produced, *vide infra* (Abia *et al.*, 1998; Hawkins and Davies, 1998; Hawkins *et al.*, 2003; Davies, 2005)].

The corresponding relevance of OCl^- *vs.* $OSCN^-$ in the oral cavity is related to the aforementioned spatial heterogeneity of the peroxidase defense systems and the corresponding chemistry. For example, the median concentration of $OSCN^-$ in freshly collected whole saliva is *ca.* 10 μM, although the concentration increases when the saliva is incubated at 37°C (Thomas *et al.*, 1980). However, direct measurement of the concentrations of these hypohalite species is problematic, because they are chemically reactive, and consequently the abundance of free ions does not necessarily reflect their significance *in vivo*. The fluxes of the hypohalites (the rates at which these reactive species are produced and consumed) are difficult to define in the context of the oral cavity. An even more complex issue is the relationship between these fluxes with respect to oral health and disease. This topic will be revisited in the concluding section of this review.

ANTIMICROBIAL PROPERTIES OF INORGANIC COMPOUNDS

Chemical Basis of Cytotoxicity

In contrast to antibiotics that typically target a single chemical step in a biosynthetic pathway, inorganic antimicrobials are generally biocides (they have a propensity to cause wholesale disruption of cellular processes) (Ashby, 2007; Zhu, 2007). Accordingly, these inorganic species tend to be cytotoxic, to greater or lesser degrees, to both eukaryotes and prokaryotes. Thus, any discussion of the antimicrobial properties of inorganic compounds toward infectious agents goes hand-in-hand with a related discussion of host tissue damage. It is fascinating to the author that the human body has found generally effective ways of harnessing the potentially indiscriminant cytotoxic properties of some of these compounds for defensive purposes. The cytotoxic properties of the inorganic compounds that are discussed herein can ultimately be traced to their chemistry, which can be roughly divided into two categories: (1) compounds that engage in one-electron (radical) chemistry, and (2) compounds that engage in two-electron chemistry (generally electrophilic, with eventual oxygen atom transfer). An example of one-electron chemistry is the reduction of O_2 by NADPH oxidase to give $O_2^{\cdot -}$ (a reaction that is carried out by PMN NADPH oxidase):

$$\mathrm{NADPH} + 2\,O_2 \rightarrow \mathrm{NADP}^+ + 2\,O_2^{\cdot -} + H^+$$

Note that NADPH is a two-electron reductant, so the chemical stoichiometry requires one NADPH to react with two oxygen molecules. However, the chemistry in fact involves one-electron steps, *vis-à-vis* enzyme intermediates. The reaction of HOCl with reduced glutathione (GSH) is an example of a two-electron (O-atom transfer) reaction (GSH is the principal cytoplasmic oxidative defense mechanism of eukaryotes, *vide infra*). The reaction occurs *via* a multistep mechanism, because the intermediate sulfenic acid (GSOH) is unstable (Nagy and Ashby, 2007; Nagy *et al.*, 2007b):

$$\begin{array}{l} \mathrm{GSH} + \mathrm{HOCl} \rightarrow \mathrm{GSOH} + H^+ + Cl^- \\ \underline{\mathrm{GSOH} + \mathrm{GSH} \rightarrow \mathrm{GSSG} + H_2O} \\ 2\,\mathrm{GSH} + \mathrm{HOCl} \rightarrow \mathrm{GSSG} + H_2O + H^+ + Cl^- \end{array}$$

Note that the first equivalent of GSH that reacts with HOCl involves a two-electron reaction (O-atom transfer, albeit probably *via* the hydrolysis of a sulfenyl chloride intermediate), as does the second reaction, even though the stoichiometry of the net reaction makes it appear that GSH is a one-electron reductant. In fact, the thiyl radical ($GS^{\cdot}$) is never involved in the reaction. While the distinction between one- and two-electron processes may appear to be a superfluous detail, in fact the difference distinguishes radical processes from non-radical processes. Radicals tend to target unsaturated functional groups in lipids, nucleotides, and aromatic amino acids (Buettner, 1993). In contrast, the hypohalites tend to target the nucleophiles in proteins (Hawkins *et al.*, 2003; Davies, 2005).

Unsaturated organic compounds (*e.g.*, aromatic amino acids and nucleotides) are particularly susceptible to derivation by high-energy radical species; hence, nuclear damage and mutagenesis are frequently the result of one-electron chemistry

Table 2. Major Inorganic (Reactive) Oxygen Species in the Oral Cavity

Name	Symbol	Major Sources in the Oral Cavity
Triplet oxygen	$^3O_2$	The atmosphere
Singlet oxygen	$^1O_2$	Peroxidase-catalyzed reactions of H_2O_2
Superoxide	$O_2^{\cdot -}$	NADPH reductase and leakage from peroxidases
Hydrogen peroxide	H_2O_2	Human dual oxidases (Duox) and aerobic metabolism of glucose
Hydroxyl radical	$^{\cdot}OH$	Metal-catalyzed homolysis of H_2O_2 (Fenton chemistry)
Ozone	O_3	Catalytic decomposition of $^1O_2$ by SIgA

Table 3. Major Inorganic (Pseudo) Hypohalites in the Oral Cavity

Name	Symbol	Major Sources in the Oral Cavity
Hypochlorite	OCl^-	Myeloperoxidase
Hypobromite	OBr^-	Myeloperoxidase (eosinophil peroxidase?)
Hypothiocyanite	$OSCN^-$	Myeloperoxidase and salivary peroxidase

(Box *et al.*, 2001; Marnett, 2002; Wang, 2008). Because radical chemistry tends to be very facile and comparatively indiscriminant from a chemical perspective, it is difficult for cells to mount an effective defense against radical species. In contrast, the reactivities of two-electron oxidants are typically related to the nucleophilicities of their reaction partners. Consequently, the chemistry of two-electron oxidants is usually well-defined (*cf.* radical chemistry). Cysteine (Cys) and methionine (Met) are usually the most reactive amino acid residues toward two-electron oxidants (because sulfur-containing compounds tend to be good nucleophiles) and are therefore often the first targets of two-electron oxidants (Hawkins *et al.*, 2003). It is not a coincidence that the cytoplasms of eukaryotes (Meister, 1988; Fernandes *et al.*, 2007) and many prokaryotes (Fahey *et al.*, 1978; Smirnova and Oktyabrsky, 2005) contain high concentrations of GSH (a tripeptide containing Cys, which is used to combat oxidative stress by two-electron oxidants that operate by the aforementioned electrophilic mechanism) (Meister, 1988). It should be noted that glutathione also erects a significant defense against radical species (Sitte and Von Zglinicki, 2003; Djordjevic, 2004) (Table 2).

Oxygen Derivatives

Water (H_2O) and molecular oxygen (O_2) represent limiting extremes in the oxidation state of the element oxygen (O) in an aqueous environment. From a thermodynamic perspective, O exists as O_2 in an overall aerobic (oxidative) environment, whereas it exists as H_2O in an anaerobic (reductive) environment. The oral cavity contains microenvironments that represent these extremes. Molecular oxygen is itself "antimicrobial" toward strict anaerobes (which are routinely found in mature supragingival plaques and are abundant in subgingival plaques). However, ground-state O_2 ($^3O_2$, triplet oxygen, a di-radical) is not generally included among the so-called "reactive oxygen species" (ROS, Table 2). The ROS of Table 2 can be divided into the radical species ($O_2^{\cdot -}$, $^{\cdot}OH$) and the "closed-shell" species ($^1O_2$, H_2O_2, and O_3). Hydroxyl radical ($^{\cdot}OH$) is not produced in large quantities by the defensive peroxidases of the oral cavity, so it will not be further discussed here, but we refer the reader to reviews of the role of ROS in periodontal tissue destruction for more information (Waddington *et al.*, 2000; Chapple and Matthews, 2007). In contrast, $O_2^{\cdot -}$ is pertinent in that it is produced by NADPH oxidase during neutrophilic respiratory bursts (*vide supra*). Despite being a radical, $O_2^{\cdot -}$ is relatively chemically unreactive, and mammalian cells [and some oral bacteria (Amano *et al.*, 1986)] contain superoxide dismutase that catalyzes the disproportionation of $O_2^{\cdot -}$ to give H_2O_2 and O_2 (Packer, 2002). It is noteworthy that the conjugate acid $HOO^{\cdot}$ (hydroperoxyl or perhydroxyl radical) is considerably more reactive than the conjugate base $O_2^{\cdot -}$. It is conceivable that $HOO^{\cdot}$ (pK_a *ca.* 4.8) (Bielski *et al.*, 1985) plays a role in the oral cavity (*e.g.*, during the development of acidogenic plaques). However, since $O_2^{\cdot -}$ and $HOO^{\cdot}$ are in rapid acid/base equilibrium, no distinction will be made between the two species in this review (*cf.* HOCl *vs.* OCl^-, *vide infra*). Of the closed-shell species, only H_2O_2, $^1O_2$, and O_3 are relevant to our discussion.

Hydrogen peroxide is a powerful oxidant that is produced in the oral cavity by the aforementioned mechanisms (Duox, anaerobic glycolysis, disproportionation of $O_2^{\cdot -}$, etc.). Like $O_2^{\cdot -}$, H_2O_2 is relatively chemically inert. For example, it takes more than an hour for H_2O_2 to react with millimolar concentrations of Cys (Ashby and Nagy, 2006a,b). Nonetheless, H_2O_2 is cytotoxic toward mammalian cells (Ward, 1991) and most prokaryotes (Asad *et al.*, 2004). Singlet oxygen has been detected in saliva (Takahama, 1993; Kou and Takahama, 1995; Sun *et al.*, 2006). However, many of the reported measurements of $^1O_2$ remain controversial, because the probes that are used to detect it tend to be insensitive and frequently non-specific (Martinez *et al.*, 2000). Nonetheless, it is believed that human peroxidases produce $^1O_2$ during their decomposition of H_2O_2 (Kanofsky, 1991). In addition to the use of therapeutic O_3 (Azarpazhooh and Limeback, 2008), it has been suggested that $^1O_2$ is converted to O_3 *via* an immunoglobulin-catalyzed reaction (Wentworth *et al.*, 2000, 2002). Secretory IgA (SIgA), the most abundant immunoglobulin in saliva, is also proposed to catalyze the reaction (Uehara *et al.*, 2006). However, the involvement of O_3 remains controversial, because the probes that have been used are not specific (Kettle *et al.*, 2004; Smith, 2004; Kettle and Winterbourn, 2005).

As a consequence of their reactive nature, all of the ROS exhibit cytotoxic properties. The relative importance of ROS as defensive agents in the oral cavity is difficult to assess, because various amounts act on microcosm plaques in diverse environments. There are likely synergisms in multi-species plaques. For example, pure cultures of oral streptococci produce H_2O_2 (they are catalase-negative), but H_2O_2 is not found in dental plaque or salivary sediment, despite streptococci being major components of their mixed bacterial populations. This is presumably due to the fast consumption of free H_2O_2 by catalase-positive species of bacteria (*e.g.*, *Neisseria, Haemophilus, Actinomyces*, and *Staphylococcus* spp.) (Ryan and Kleinberg, 1995). Furthermore, the SPO and MPO systems of the oral cavity may protect H_2O_2-sensitive bacteria (Adamson and Carlsson, 1982). In addition to synergism between bacterial species, there are likely to be additive and/or cooperative effects between defensive agents. For example, there is some evidence that $O_2^{-\cdot}$ can act synergistically with OCl^- (*vide infra*) to induce oxidative damage (Hawkins *et al.*, 2002) (Table 3).

(Pseudo) Hypohalites

The archetypal example of a biocide is hypochlorite (OCl^-, the principal component of household bleach). In sufficient concentrations, it is toxic to all life. Note that the reactive form of OCl^- (and the other hypohalites as well) is the corresponding conjugate acid, hypohalous acid (HOCl, $pK_a = 7.4$). As a neutral species, HOCl is presumably membrane-permeable, and therefore more cytotoxic. However, since the acid-base equilibrium between HOCl and OCl^- is exceedingly fast, the issue of which species is actually active is academic. Under acidic conditions (and in the presence of excess Cl^-), OCl^- comproportionates to give Cl_2, the corresponding halogen (Adam *et al.*, 1992). However, since the equilibrium between the hypohalites and the halogens is relatively fast, they are not generally treated as different biocides. Instead, the term "total active chlorine" is often used to describe the sum amount of hypohalous acid, hypohalite, and halogen. Furthermore, HOCl/OCl^- are the predominant species at physiologic pH. The relative ease with which the halides are oxidized is $I^- > Br^- > Cl^-$ (note that F^- is never oxidized in an aqueous environment), so the trend in oxidative strengths of the hypohalous acids is HOCl > HOBr >> HOI. As mentioned before, I^- is not abundant in most physiologic fluids, so only Cl^- and Br^- are relevant to this discussion.

HOCl and HOBr exhibit somewhat promiscuous reaction chemistry (the relative reactivities of HOCl toward proteinaceous groups are Cys ≈ Met >> cystine ≈ His ≈ α-amino > Trp > Lys >> Tyr ≈ Arg > backbone amides > Gln ≈ Asn, and a similar trend is observed for HOBr) (Pattison and Davies, 2001, 2004). However, the kinetics of some of the reactions of HOCl, and especially HOBr, approaches the diffusion limit (Nagy *et al.*, 2006b). As a consequence of their facile reaction, HOCl and HOBr probably exhibit poor chemical selectivity in a biological setting. In addition, secondary reactive species are produced during the reactions of HOCl and HOBr that likely contribute significantly to the overall toxicity—for example, haloamines (Grisham *et al.*, 1984; Abia *et al.*, 1998; Hawkins and Davies, 1998, 2003; Davies, 2005). From a chemical perspective, it is appropriate to view the reactions of HOX (X = Cl, Br) as a redox cascade (thermodynamically downhill) with the eventual production of chemically stable derivatives. Because of the labile nature of many of the intermediate species in such chemical cascades, and because of the fact that many of these species exhibit similar chemistries (*e.g.*, HOCl and chloroamines are both electrophilic chlorinating agents, albeit with different reactivities), it is difficult to chart the consequences of the damage that occurs. Accordingly, the complexity of the chemistry precludes a definitive assignment of the antimicrobial mechanism of HOX (X = Cl, Br). Nonetheless, there have been many studies of the effects of HOX (X = Cl, Br) on both eukaryotes (Hawkins and Davies, 2000; Hidalgo and Dominguez, 2000; Hawkins *et al.*, 2001; Hidalgo *et al.*, 2002; Soszynski *et al.*, 2002; King *et al.*, 2004) and prokaryotes (Albrich *et al.*, 1981, 1986; Albrich and Hurst, 1982; Barrette *et al.*, 1987, 1989; Hurst *et al.*, 1991; Palazzolo *et al.*, 2005). Although it is problematic to follow the reaction cascade that begins with the production of HOX (X = Cl, Br) *in vivo*, there is considerable interest in evaluation of the relevance of the HOX (X = Cl, Br) chemistry, particularly in the context of the host tissue damage that occurs with inflammatory disease. One approach to monitoring the chemistry of HOX (X = Cl, Br) *in vivo* is through the use of biomarkers, thermodynamically stable derivatives of HOX (X = Cl, Br). Promising biomarkers that appear to be specific for HOX (X = Cl, Br) include 3-halotyrosines (Winterbourn and Kettle, 2000) and a sulfonamide derivative of GSH (Harwood *et al.*, 2006).

In the field of inorganic chemistry, SCN^- is called a "pseudohalide", because its reaction chemistry frequently mirrors that of the halides (Lappert and Pyszora, 1966). This is reflected in the fact that defensive peroxidases use SCN^- (in addition to the halides) as a substrate. The oxidation potential of SCN^- falls between those of I^- and Br^-. Accordingly, the chemical properties of HOSCN are most similar to those of HOI (Nagy *et al.*, 2007a). In contrast to HOCl and HOBr (which react with a variety of functional groups), the only characterized reactions of HOSCN are with thiol moieties (which are among the most powerful nucleophiles), generally Cys and its derivatives (Ashby and Aneetha, 2004; Nimmo *et al.*, 2007; Lemma and Ashby, 2008). Given that SH groups are apparently the targets of the HOSCN, it is important to note that roughly 40% of all enzymes are rendered ineffective by chemical agents that are reactive toward thiols (Leung-Toung *et al.*, 2002). Thus, the destruction of functional SH moieties by HOSCN is one basis for its cytotoxicity. Importantly, the hypohalites can be interconverted through their reactions with other halides, but the process must be exothermic. For example, HOCl (Ashby *et al.*, 2004) and HOBr (Nagy *et al.*, 2006b) react with SCN^- to give HOSCN and the corresponding halides, but not *vice versa*. *In vivo*, these reactions are important because they restrict the lifetimes of the more powerful (and less discriminant) hypohalites, thereby limiting their propensity to cause collateral host tissue damage. In addition, the antimicrobial $OSCN^-$ is produced in the reaction (Nagy *et al.*, 2006a).

Halides

Fluoride is the only halide that is known to be antimicrobial without oxidation (Hamilton, 1990; Van Loveren, 1990; Marquis, 1995; Jenkins, 1999). Fluoride influences the metabolism of cariogenic and other bacteria *via* multiple mechanisms (Marquis *et al.*, 2003): F^- can bind directly to many enzymes (especially metalloenzymes) (Segal *et al.*, 1968; Wever and Bakkenist, 1980; Zgliczynski *et al.*, 1983; Thibodeau *et al.*, 1985; Ferrari *et al.*, 1997; Suzuki and Ohshima, 2003), thereby affecting their activities; catalase is inhibited by F^- [thereby affecting the ability of H_2O_2 to kill oral bacteria (Phan *et al.*, 2001)]; and some F^- complexes of metals (*e.g.*, AlF_4^- and $BeF_3^-\cdot H_2O$) can mimic phosphate, thereby affecting a variety of enzymes and regulatory phosphatases (Thongboonkerd *et al.*, 2002). The weak-acid property of HF ($pK_a = 3.15$), which is a transmembrane proton conductor, appears to be important for inducing the glycolytic inhibition of oral bacteria that is observed at low pH in dental plaque (Eisenberg and Marquis, 1981) (Table 4).

Nitrogen Derivatives

After many decades of angst about nitrates in our diet and their propensity to form potentially carcinogenic *N*-nitroso derivatives (Eichholzer and Gutzwiller, 2003), there is mounting evidence that NO_3^- is concentrated in saliva for beneficial purposes (McKnight *et al.*, 1999) (although the potential deleterious properties of NO_3^- on systemic health should not be discounted). The concentration of NO_3^- in saliva is proportional to the dietary intake (Eisenbrand *et al.*, 1980), it varies with the salivary flow rate (Granli *et al.*, 1989), and it is influenced by smoking (Tsuchiya *et al.*, 2002). Since NO_3^- is a relatively inert chemical species, the mechanism of antimicrobial action of NO_3^- probably involves a redox cascade. Facultative anaerobic bacteria in the oral cavity use NO_3^- as a terminal electron acceptor (*cf.* O_2 for aerobic bacteria) to produce nitrite (NO_2^-). Acidified NO_2^- inhibits the growth and affects the survival of cariogenic bacteria (*e.g.*, *Streptococcus mutans, Lactobacillus casei*, and *Actinomyces naeslundii*) (Silva Mendez *et al.*, 1999). Acidified NO_2^- has a similar effect on periodontal bacteria (*e.g.*, *Fusobacterium nucleatum, Eikenella corrodens*, and *Porphyromonas gingivalis*) (Allaker *et al.*, 2001). Importantly, while the growth of periodontal bacteria is known to be inhibited by acid in the absence of NO_2^-, there is a dose-dependent decrease in these bacteria in the presence of NO_2^- (Allaker *et al.*, 2001). Nitrous acid (HNO_2, $pK_a = 3.4$) is unstable and will spontaneously disproportionate: $3\ HNO_2 \rightarrow H_3O^+ + NO_3^- + 2\ {}^{\cdot}NO$. Some bacteria possess nitrite reductase (*e.g.*, *S. mutans*), an enzyme that is capable of accelerating the disproportionation of NO_2^- (Choudhury *et al.*, 2007). It appears that nitric oxide (${}^{\cdot}NO$) is the antimicrobial component of the $NO_3^-/NO_2^-/{}^{\cdot}NO$ redox cascade (Fang, 1997; Smith *et al.*, 1999; Sato *et al.*, 2008). The mechanism by which ${}^{\cdot}NO$ induces cell death is the subject of ongoing investigation. Alternative models include "oxidative stress" and "nitrosative stress" [*e.g.*, nitrosylation of proteins without a major alteration in cellular redox state (Eu *et al.*, 2000)]. Nitric oxide also reacts with $O_2^{\cdot-}$ to produce peroxynitrite ($ONOO^-$), which may also contribute to collateral host tissue damage in the oral cavity (Lohinai and Szabo, 1998; Lohinai *et al.*, 2001; Barley *et al.*, 2004).

INTER-PERSON DIFFERENCES

There is considerable variability in the physiological concentrations of many of the chemically stable inorganic ions that have been discussed herein. In most cases, these differences can be attributed to diets or smoking. Given the influence of these ions on the activity and function of the defensive peroxidases, there has been some interest in correlating interperson differences to oral disease. As a caveat, it is important to note that many of the relevant inorganic ions are chemically reactive, and consequently the abundance of free ions may not reflect their relevance *in vivo*. Some ions may exist as transient species (*e.g.*, OCl^-) or as their conjugates with reaction partners (*e.g.*, CN^- and OCN^-). In some cases, steady-state concentrations of reactive species may accumulate (*e.g.*, $OSCN^-$), but measured concentrations may not reflect the time-dependent flux of such species.

Thiocyanate

The main source of SCN^- *in vivo* is CN^-, *vide infra*. Cyanide is principally introduced by the digestion of glucosinolate-containing vegetables (*e.g.*, the *Brassica*) (Weuffen *et al.*, 1984). However, as a consequence of detoxification of hydrogen cyanide (HCN, $pK_a = 9.2$, which is known to be present in microgram amounts *per* cigarette), the level of SCN^- in smokers is considerably elevated relative to that in non-smokers. Indeed, this difference is routinely used as a biomarker for the evaluation of smoking behavior (Morabia *et al.*, 2001). While most of the CN^- that is converted to SCN^- *in vivo* is exogenous in origin, endogenous sources contribute the sulfur *vis-à-vis* a multitude of reactions, some of which are enzyme-catalyzed (Wood, 1975). It has been shown that higher concentrations of SCN^- in saliva can contribute to an enhancement of peroxidase activity (Tenovuo, 1976; Lamberts *et al.*, 1984; Fonteh *et al.*, 2005; Tahboub *et al.*, 2005). Furthermore, as noted earlier, SCN^- is a potent sequestering agent for some reactive oxidants (Ashby *et al.*, 2004; Nagy *et al.*, 2006b). Accordingly, one might conclude that higher SCN^- (either as a consequence of diet or through smoking) should result in a suppression of oral disease. However, while smokers have elevated SCN^- and $OSCN^-$ levels in their saliva, no corresponding correlation with dental caries has been observed (Lamberts *et al.*, 1984). Interestingly, although there is not an association between smoking and caries among adults, there is a positive correlation between environmental (second-hand) tobacco smoke and primary tooth caries in children (Shenkin *et al.*, 2004). In contrast to caries, there is a strong correlation between smoking (and consequently SCN^- levels) and some periodontal diseases (Rivera-Hidalgo, 2003). Since there is also a correlation between smoking and SCN^- levels in GCF, SCN^- levels presumably exhibit a positive correlation with periodontal diseases. However, SCN^- is only one of many inorganic and organic chemicals that are elevated by smoking. Two other inorganic ions are CN^- and cyanate (OCN^-), *vide infra*.

Table 4. Major Inorganic (Reactive) Nitrogen Compounds in the Oral Cavity

Name	Symbol	Major Sources in the Oral cavity
Nitrate	NO_3^-	Diet
Nitrite	NO_2^-	Reduction of nitrate by oral microflora
Nitric oxide	${}^{\cdot}NO$	Acidification of nitrite and by enzymic reactions
Peroxynitrite	$ONOO^-$	Reaction of ${}^{\cdot}NO$ and $O_2^{\cdot-}$

Cyanide

It addition to dietary sources (*e.g.*, cyanogenic glucosides, *vide supra*) and tobacco smoke, other sources of CN^- *in vivo* include micro-organisms (in particular certain pseudomonads) and industrial exposure (*e.g.*, *vis-à-vis* organonitriles) (Wong-Chong *et al.*, 2006). There appear to be no studies that have determined the normal concentration of CN^- in the fluids of the oral cavity. However, there is a statistical correlation between blood and salivary SCN^- levels (Tsuge *et al.*, 2000). As noted earlier, the concentrations of SCN^- in physiological fluids (including GCF and saliva) in smokers are substantially higher than those for non-smokers. Therefore, it follows that the oral cavity is exposed to higher concentrations of CN^- for smokers compared with non-smokers. HCN contributes to the loss of peroxidase activity in saliva upon exposure to cigarette smoke (Klein *et al.*, 2003). This is due to the strong complexation of CN^- to the iron-active sites of the peroxidases (fundamentally the same mechanism that renders CN^- toxic to the respiratory system *vis-à-vis* the complexation of hemoglobin and myoglobin). It is noteworthy that there is no correlation between CN^- in plasma and the concentration of HCN in breath (Lundquist *et al.*, 1988). Furthermore, the concentrations of HCN measured in breath are higher than expected for blood concentrations, which suggested a local production of HCN in the oropharynx. Under some circumstances, $OSCN^-$ can decompose to give CN^- (Aune and Thomas, 1977). While the major cyanide-derived product of the decomposition of $OSCN^-$ is OCN^-, we have observed the formation of substantial amounts of CN^- during the hydrolysis of thiocyanogen [$(SCN)_2$, analogous to a halogen] at neutral pH (unpublished observations). It is conceivable that the latter reaction is the source of HCN in breath.

Cyanate

Cyanate (OCN^-) is produced by the oxidation of CN^-. Thus, OCN^- levels are higher in smokers. Under some conditions, the decomposition of $OSCN^-$ also produces OCN^- (Oram and Reiter, 1966). MPO is inhibited by OCN^- (Qian *et al.*, 1997). The inhibition could be caused by heme binding of OCN^- (thereby blocking the active site) or by carbamylation of the protein by OCN^-. It is noteworthy that the functional impairment of proteins through carbamylation by OCN^- is thought to promote human inflammatory diseases (Wang *et al.*, 2007). However, the possible relevance of carbamylation in the oral cavity has not been investigated. There is very limited information available regarding the effect of OCN^- on oral bacteria (Morita, 1977).

Fluoride

In the absence of supplementation by fluoride-containing dentrifices, the concentration of F^- in saliva (and presumably GCF) is somewhat lower than the concentration in plasma (Oliveby *et al.*, 1989). The normal concentration of F^- in saliva (*ca.* 1 μM) can increase markedly after the ingestion of F^-, and change dynamically thereafter (Dawes and Weatherell, 1990). An early study that used PMN granules (and not isolated enzymes) suggested that F^- does not influence the activity of MPO (which was determined by measurement of the iodination of zymosan) (Gabler and Leong, 1980). In contrast, the same study reported that the iodination of zymosan was inhibited by F^- for intact PMNs (Gabler and Leong, 1980). However, the pH-dependent competitive inhibition of isolated LPO (Segal *et al.*, 1968; Thibodeau *et al.*, 1985) and MPO (Zgliczynski *et al.*, 1983; van den Abbeele *et al.*, 1992) by F^- has been demonstrated. For example, half of the activity of bovine LPO occurs for < 0.05 mM at pH 4, 0.3 mM at pH 5, 4.0 mM at pH 6, and greater than 10 mM at pH 7, as assayed with 5 mM I^- and 150 μM H_2O_2 (Thibodeau *et al.*, 1985). It is noteworthy that SPO was found to have lower pH optima relative to LPO, but it also was inhibited by F^- at sufficiently low pH (Thibodeau *et al.*, 1985). A similar inhibitory effect of F^- on peroxidase activity has been observed in whole saliva (Thibodeau *et al.*, 1985; Hannuksela *et al.*, 1994; van den Abbeele *et al.*, 1995). These observations suggest that F^- in dental plaque may inhibit the peroxidase defense system. However, when F^- and $OSCN^-$ are added simultaneously at pH 5.0, an additive effect of growth inhibition of *S. mutans* was observed (Lenander-Lumikari *et al.*, 1997). Thus, the small inhibitory effect of F^- on the defensive peroxidase systems of the oral cavity may be offset by the combinatorial antimicrobial effects of F^- and $OSCN^-$.

Nitrate and Nitrite

Basal levels of NO_3^- in saliva are 10-20 times those found in plasma (and presumably GCF) (Duncan *et al.*, 1995; Benjamin and McKnight, 1999; Pannala *et al.*, 2003; Lundberg *et al.*, 2004). For the average diet in the US, *ca.* 80% of dietary NO_3^- is derived from vegetables (White, 1975). Cured meats, for example, typically represent a minor source of NO_3^- in most diets. However, urinary, plasma, and saliva NO_3^- concentrations increase markedly after the consumption of a high-nitrate meal (Pannala *et al.*, 2003). The maximum concentration is achieved within a few hours following the meal, and NO_3^- concentrations return to basal levels within 24 hours. The amount of NO_3^- that is excreted in the urine following the consumption of a high-nitrate meal suggests that the majority of urinary NO_3^- can be accounted for in dietary sources (Pannala *et al.*, 2003). In contrast to NO_3^-, an increase in NO_2^- is observed in saliva only after the consumption of a high-nitrate meal (Pannala *et al.*, 2003), which is consistent with the fact that NO_3^- is metabolized to NO_2^- by bacterial flora on the posterior surface of the tongue in rat models (Duncan *et al.*, 1995). Nitrite has been shown to enhance the reactivity of LPO (Reszka *et al.*, 1997, 1998, 1999; van der Vliet *et al.*, 1997; Gebicka, 1999; Bruck *et al.*, 2001) and MPO (Burner *et al.*, 2000). In addition to an enhancement in the rate of catalysis by NO_2^-, it has been suggested that NO_3^- reduces acidity in the oral cavity (Li *et al.*, 2007). Thus, NO_2^- and NO_3^- may play a beneficial role in the oral cavity. We note that there has apparently been no attempt to correlate oral health with inter-person differences in nitrate-reducing capacity.

PEROXIDE TOXICITY AND PEROXIDASE FUNCTION

At sufficient concentrations, H_2O_2 is cytotoxic to mammalian cell lines, including human epithelial cells (Mattana *et al.*, 1992) and gingival fibroblasts (Tenovuo and Larjava, 1984; Tipton *et al.*, 1995a). While H_2O_2 is a relatively chemically inert molecule, its homolysis to give $^{\cdot}OH$ radicals is catalyzed by transition metals, particularly iron, in a reaction that is referred to as Fenton chemistry (Prousek, 2007). Much of the cytotoxicity of H_2O_2 is attributed to Fenton chemistry (Winterbourn, 1995). In the presence of SCN^-, the LPO system protects cultured mammalian cells against H_2O_2 toxicity (Hänström *et al.*, 1983). This is consistent with the observation that $OSCN^-$ is not toxic toward mammalian cells (Bjoerck and Claesson, 1980; Marshall and Reiter, 1980; White *et al.*, 1983; Carlsson *et al.*, 1984; Carlsson, 1987). It has been previously suggested that one of the important roles of human peroxidases is to detoxify H_2O_2 to prevent host tissue damage (Carlsson, 1987; Tipton *et al.*, 1995b). Since many prokaryotes are also sensitive to H_2O_2, the human peroxidase systems may also protect certain bacteria by sequestering H_2O_2. However, the $OSCN^-$ that is produced is inhibitory toward most bacteria. Consequently, it is difficult to envisage the net effect of H_2O_2 sequestration *vs.* $OSCN^-$ on bacterial growth (Fig. 2).

THE ANTIMICROBIAL DEPLETION MODEL

The efficacy of chemically reactive antimicrobial agents can be diffusion-limited. This is one (but certainly not the only) explanation for the greater sensitivity of planktonic cultures to chemically reactive antimicrobials (Stewart, 1994;

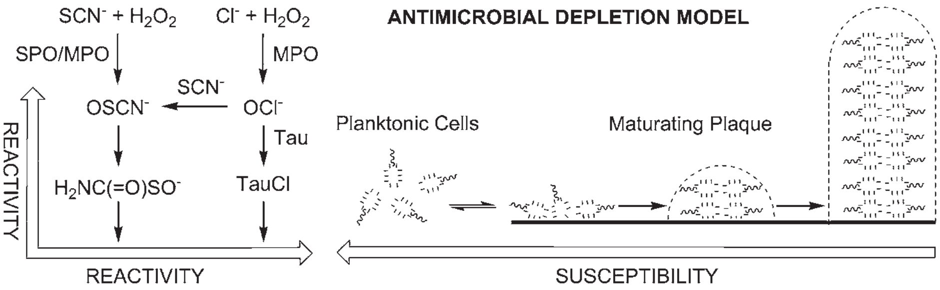

Figure 2. Relationship between the reactivities of inorganic defense factors of the oral cavity and maturation of plaque biofilms. The penetrability of the hypohalites and their derivatives is inversely related to their reactivities and the extent of maturation of the biofilm. See the text for a discussion of the possibility that the defensive factors could be generated within biofilms.

Stewart and Raquepas, 1995; Dodds *et al.*, 2000; Stewart *et al.*, 2001, 2004; Hunt *et al.*, 2005). Planktonic cultures are generally agitated, and consequently fluid flows by a convection mechanism that transports solutes rapidly. In contrast, fluid flow in biofilms can be highly restricted. For thick biofilms, such as the plaques of the oral cavity, the primary mechanism of solute transport is diffusion (unagitated flow). For highly reactive molecules such as HOCl, which also exhibit promiscuous reaction chemistry, the biomass of plaques offers a formidable barrier to penetration. While HOCl is an extremely effective antimicrobial toward planktonic cell suspensions, it is a comparatively inefficient killing agent with respect to biofilms. For example, a 600-fold increase in concentration of HOCl (*per* cell) is required to kill certain biofilms of *Staphylococcus aureus*, compared with planktonic cultures of the same species (Luppens *et al.*, 2002). More chemically selective antimicrobials, *e.g.*, $OSCN^-$, are more likely to penetrate thick biofilms before reacting. The relationship between chemical reactivity of the antimicrobial and biofilm penetrability forms the basis of the Antimicrobial Depletion Model (Fig. 2). There is presumably an inverse relationship between penetrability with respect to the antimicrobial reactivity. However, penetrability, of course, is not the only factor that determines the efficacy of the antimicrobial: $OSCN^-$ is generally considered to be bacteriostatic, whereas OCl^- is bactericidal. But, as mentioned before, there is a tradeoff for the higher reactivity/poorer selectivity of OCl^-: the potential of host tissue damage. In the oral cavity, it may be advantageous for the body to use a collection of antimicrobials that exhibit a continuum of reactivity. Thus, complementing HOCl is taurine chloroamine (TauCl), a less reactive (Carr *et al.*, 2001) and more selective (Peskin and Winterbourn, 2006) electrophilic chlorinating agent that may play a role in host defense in the oral cavity (Woldring, 1955; Mainnemare *et al.*, 2004). Similarly, we have discovered a corresponding less-reactive derivative of $OSCN^-$, thiocarbamate-S-oxide [$H_2NC(=O)\text{-S-}O^-$] (Nagy *et al.*, 2007c). Thiocarbamate-S-oxide, which is formed by the hydrolysis of $OSCN^-$, is one of the chemical species formed during the redox cascade that results in the decomposition of $OSCN^-$ (Nagy *et al.*, 2007c). Recently, we have learned that $H_2NC(=O)\text{-S-}O^-$ reacts with thiols *via* a mechanism that is analogous to the one that is observed for $OSCN^-$, albeit with slower kinetics (unpublished observations). While the effect of $H_2NC(=O)\text{-S-}O^-$ on bacterial physiology remains to be investigated, its discovery demonstrates that there is much to be learned about the inorganic antimicrobials produced by the defensive peroxidases.

The Antimicrobial Depletion Model does not explicitly include the possibility that defensive peroxidases might generate hypohalites within the biofilms, thereby obviating the need for reactive species to diffuse into biofilms. We note that the production of acid in salivary sediment is more effectively inhibited when $OSCN^-$ is produced by sediment-bound peroxidases than when salivary sediment is treated with exogenous $OSCN^-$ (Tenovuo, 1979). However, salivary sediment has a larger buffer capacity and "organic load" (*e.g.*, non-viable cells) than plaque (Singer *et al.*, 1983), so it is not clear whether the penetration of $OSCN^-$ into plaque is comparably diffusion-limited. The production of hypohalites from within plaques requires the transport of the components of peroxidase systems into the biofilm: peroxidase, (pseudo) halide, and H_2O_2. Relevant to the issue of diffusion of peroxidases into plaques, it has been previously observed that LPO binds to *S. mutans* (LPO is a cationic protein, and the outer membranes of Gram-positive bacteria are negatively charged) (Pruitt *et al.*, 1979). While cell-bound LPO remains catalytically active initially, it is eventually inactivated (Pruitt *et al.*, 1979). Thus, the electrostatic attraction of the peroxidases for cells and the subsequent inactivation of the cell-bound enzyme may preclude the generation of hypohalites within a thick plaque (Pruitt *et al.*, 1979). With regard to the availabilities of the other components of the peroxidase defense systems, it is probable that the concentrations of the (pseudo)halides in plaques reflect their concentrations in the physiologic fluids that surround them (*i.e.*, saliva or GCF), but it was noted earlier that H_2O_2 has not been found in dental plaques (Ryan and Kleinberg, 1995). The issue of whether or not the human defensive peroxidase systems are active in thick plaques remains unresolved.

CONCLUSION

The distinctive chemical environments of the supragingival and subgingival regions impose restrictions on the human peroxidase defense strategies of the oral cavity (*e.g.*, *vis-à-vis* substrate bioavailability). Inter-person differences may also influence the function and activity of the enzymes and the chemistry of the reactive species that the enzymes generate. The abilities of the defensive agents produced by the peroxidase systems to differentiate between host tissues and the microbiota are an unsettled issue. Oral diseases are accompanied by microbial shifts of dental plaque, so a more subtle issue is whether these agents differentiate between commensal and pathogenic microbiota. While it seems likely that ecological principles, as in the March ecological hypothesis (Marsh, 2003), can be applied to explain microbial shifts, it is not yet clear what ecological pressures are inducing these microbial shifts, nor is the root cause of host tissue damage completely clear. For dental caries in enamel, damage is clearly a direct effect of the plaque. In dentinal and root caries, some host response may be involved (as in periodontitis). The microbial shift during cariogenesis is toward acidogenic and acid-tolerant Gram-positive bacteria, which displace the acid-sensitive commensal microbiota that are associated with intact tooth tissues. Since supragingivally generated $OSCN^-$ targets Gram-positive bacteria (*S. mutans* may be particularly affected), it is possible that $OSCN^-$ may be important in modifying plaque cariogenicity. Other important health-maintaining functions of $OSCN^-$ may also occur, such as restricting supragingival intra-oral cross-infection by periodontal pathogens. During the development of periodontal diseases, an increase in disease-associated anaerobes occurs in conjunction with increased inflammation. The inflammatory response is primarily due to antigens that have been introduced by the bacteria. It is unclear whether the inflammation is due to changes in the antigens introduced by the microbial shift (Lamster and Novak, 1992), or whether the microbial shift has been induced by the inflammatory response, or both (Dalwai *et al.*, 2006). Is the host tissue damage of periodontal diseases solely the consequence of host mediators (*e.g.*, HOCl), or is there direct attack by bacterial virulence factors such as proteolytic enzymes? Definitive answers to these questions are critical for a rational approach to combating oral disease—Does one treat the inflammation, or does one target pathogenic bacteria, or is it necessary to treat both (Van Dyke, 2007)? Other risk factors being similar, is inter-individual variation in peroxidase activity a key factor in determining why some people develop oral disease and others do not? Whether inter-person differences between the inorganic chemistry of the oral cavity contribute to oral diseases is a topic that deserves further attention.

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