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Bactericidal Effect of Hydrogen Peroxide Is Prevented by the Lactoperoxidase-Thiocyanate System Under Anaerobic Conditions

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Streptococcus sanguis and *Peptostreptococcus anaerobius* were exposed to various combinations of the components of the lactoperoxidase-thiocyanate-hydrogen peroxide system. The bactericidal effect of hydrogen peroxide was prevented under anaerobic conditions by lactoperoxidase together with thiocyanate, but not by lactoperoxidase or thiocyanate alone. Thiocyanate was effective already at a molar ratio to hydrogen peroxide of 1:100.

Lactoperoxidase, thiocyanate ions, and hydrogen peroxide provide an antibacterial system in milk and saliva (6, 9, 11). The enzyme catalyzes an oxidation of thiocyanate ions into hypothiocyanite (1, 5), which oxidizes protein sulfhydryl groups to sulfenyl-thiocyanate derivatives (13). The antibacterial effect of these reactions can to some extent be reversed by sulfhydryl compounds such as thioglycolate, glutathione, and dithiothreitol, which reduce the sulfenyl derivatives and excess of hypothiocyanite (7, 8, 13).

The antibacterial effect of the lactoperoxidase-thiocyanate-hydrogen peroxide system has usually been studied under aerobic conditions. In the oral cavity there are aerobic as well as anaerobic microbial ecological niches, and the aim of the present study was to determine the efficiency of the lactoperoxidase-thiocyanate-hydrogen peroxide system under anaerobic conditions.

Streptococcus sanguis ATCC 10556 and *Peptostreptococcus anaerobius* ATCC 27337 were kept on blood agar plates at 4°C, and all experiments were performed in an anaerobic box with an atmosphere of 10% H_2 and 5% CO_2 in nitrogen (14). The composition of TSY broth and cysteine-free dilution blank solution was as previously described (3, 4). Hydrogen peroxide (30% [wt/wt]; Perhydrol) was from E. Merck AG, Darmstadt, Germany. Lactoperoxidase (L 2005; from milk) was from Sigma Chemical Co., St. Louis, Mo.

S. sanguis was exposed to various combinations of the components of the lactoperoxidase-thiocyanate-hydrogen peroxide system when it was in the exponential growth phase at 37°C in TSY broth. Samples (0.1 ml) were taken at various time intervals after the exposure, diluted, and spread over the surface of duplicate blood agar plates. The plates were incubated for 1 day at 37°C, and the numbers of surviving organisms were determined. Cultures of *S. sanguis* and *P. anaerobius* in exponential growth phase were also diluted in the cysteine-free dilution blank solution to a density of about 2×10^3 organisms per ml, stored in this solution for 1 h at 37°C, and then exposed to various combinations of the components of the lactoperoxidase-thiocyanate-hydrogen peroxide system. The numbers of surviving organisms after 2- or 10-min exposure to the components of the system were determined.

S. sanguis was rapidly killed when it was in exponential-growth phase in TSY broth and exposed to 8 mM hydrogen peroxide. The presence of 10 mM thiocyanate ions in the broth did not influence the growth rate or the bactericidal effect of hydrogen peroxide. Lactoperoxidase had no significant protecting effect against the hydrogen peroxide toxicity, whereas lactoperoxidase in the presence of thiocyanate protected the organisms from the bactericidal effect of hydrogen peroxide (Fig. 1). When *S. sanguis* or *P. anaerobius* was stored in the dilution solution for 1 h before exposure to the components of the lactoperoxidase-thiocyanate-hydrogen peroxide system, similar effects of the various combinations of components were found (Table 1). The lactoperoxidase-thiocyanate system was as effective in preventing the bactericidal effect of hydrogen peroxide at high as at low concentrations of hydrogen peroxide (Table 1). When various concentrations of thiocyanate were tested in the presence of 10 mM hydrogen peroxide and lactoperoxidase, 1 mM thiocyanate fully protected from the bactericidal effect of hydrogen peroxide and 10 μM thiocyanate already had a protective effect (Fig. 2).

Under aerobic conditions, the lactoperoxidase-thiocyanate-hydrogen peroxide system is most efficient at equimolar concentration of thiocyanate and hydrogen peroxide (2, 8), and the

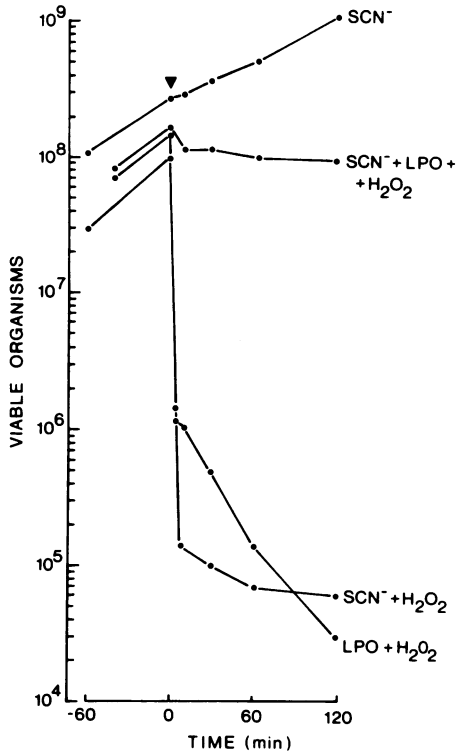

FIG. 1. *The killing of S. sanguis in exponential growth phase in TSY broth at 37°C when exposed under anaerobic conditions to various combinations of the components of the lactoperoxidase-thiocyanate-hydrogen peroxide system. The components were added to the culture at time zero in the sequence: thiocyanate (10 mM), lactoperoxidase (LPO; 100 μg/ml), and hydrogen peroxide (8 mM).*

TABLE 1. *The effect of lactoperoxidase-thiocyanate-hydrogen peroxide on S. sanguis and P. anaerobius stored in dilution blank solution for 1 h at 37°C*[a]

Species	Time of exposure (min)	Additions	% Survival
S. sanguis	10	16 mM H_2O_2	12
		16 mM H_2O_2 + LPO	29
		16 mM H_2O_2 + LPO + 16 mM KSCN	101
		16 mM H_2O_2 + 16 mM KSCN	10
P. anaerobius	2	50 μM H_2O_2	1
		50 μM H_2O_2 + LPO	6
		50 μM H_2O_2 + LPO + 100 μM KSCN	95
		50 μM H_2O_2 + 100 μM KSCN	1

[a] The concentration of lactoperoxidase (LPO) was 100 μg/ml.

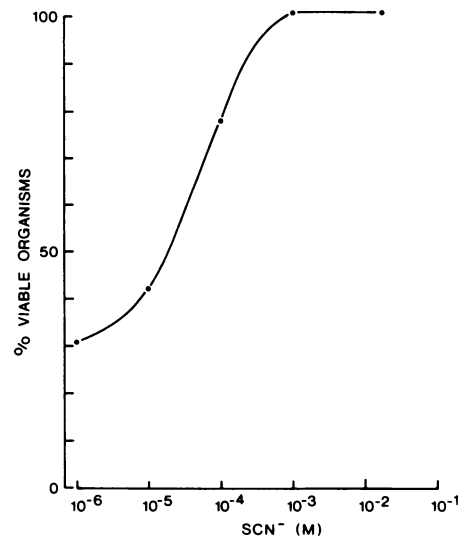

FIG. 2. *Percent surviving organisms of S. sanguis in cysteine-free dilution solution (37°C) after 10-min exposure to 10 mM hydrogen peroxide in the presence of lactoperoxidase (100 μg/ml) and various amounts of potassium thiocyanate. The organisms were stored in the cysteine-free dilution solution for 1 h before exposure.*

extent of the antimicrobial action seems to be dependent on the amount of hypothiocyanite formed, the time of exposure to hypothiocyanite, and the ability of cells to repair oxidative damage (13). Under anaerobic conditions, the lactoperoxidase-thiocyanate system appeared to catalyze a reaction which protected the cells from the bactericidal effect of hydrogen peroxide. This system has to be considered as a protection against hydrogen peroxide toxicity in the anaerobic microbial niches of the oral cavity. Of specific interest is that the lactoperoxidase of saliva is readily adsorbed to the bacteria colonizing the oral surfaces and that the adsorbed enzyme is catalytically active (8, 10, 12).

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