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Effect of urate on the lactoperoxidase catalyzed oxidation of adrenaline

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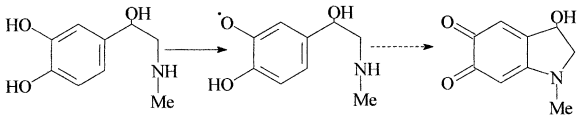
Abstract

Lactoperoxidase is an iron containing enzyme, which is an essential component of the defense system of mammalian secretary fluids. The enzyme readily oxidizes adrenaline and other catecholamines to coloured aminochrome products. A K_m-value of 1.21 mM and a catalytic constant ($k = V_{max}/[Enz]$) of 15.5×10^3 min^{-1} characterized the reaction between lactoperoxidase and adrenaline at pH 7.4. Urate was found to activate the enzyme catalyzed oxidation of adrenaline in a competitive manner, the effect decreasing with increasing adrenaline concentration. Lactoperoxidase was able to catalyze the oxidation of urate. However, urate was a much poorer substrate than adrenaline, and it seems unlikely that urate activates by functioning as a free, redox cycling intermediate between enzyme and adrenaline. The activation mechanism probably involves an urate-lactoperoxidase complex.

Introduction

Lactoperoxidase is a heme containing enzyme found in milk, lacrimal and salivary glands. It is an important component of mammalian antimicrobial defenses. The iron in the heme ring of the 'resting' enzyme is in the oxidation state Fe(III). The enzyme is activated by H_2O_2 to compound I enzyme intermediate (Fe(IV)=O), which can subsequently oxidize several organic compounds, including catecholamines. The catalytic mechanism of lactoperoxidase has recently been discussed by Ghibaudi & Laurenti (2003). Lactoperoxidase, as well as horseradish peroxidase, rapidly catalyze the transformation of catecholamines to coloured aminochrome products (Polis & Shmukler 1953, Løvstad 1979, Metodiewa *et al.* 1989, Ferrari *et al.* 1999); a reaction that is markedly enhanced in the presence of phenothiazine derivatives (Løvstad 1980). Adrenaline was found to be a better substrate than dopa and dopamine, since the methyl group on the side chain improves the substrate affinity to the protein through hydrophobic association forces (Ferrari *et al.* 1999). The primary oxidation product of adrenaline is proposed to be a semiquinone radical (Adak *et al.* 1998).



Adrenaline o-Semiquinone radical Adrenochrome

Urate is the end product of purine degradation in humans, who lack the enzyme, uricase (urate → allantoin). It has previously been demonstrated that urate interacts with horseradish peroxidase (Wrona & Dryhurst 1979, Maples & Mason 1988); an enzyme that has a biochemistry resembling that of lactoperoxidase. In the present communication the effect of urate on the lactoperoxidase catalyzed oxidation of adrenaline has been studied.

Materials and methods

Bovine milk lactoperoxidase (EC 1.11.1.7), adrenaline, uric acid, xanthine, hypoxanthine, and Tris buffer were purchased from Sigma Chemical Company (St. Louis, Missouri, USA), and H_2O_2 from E. Merck AG (Darmstadt, Germany).

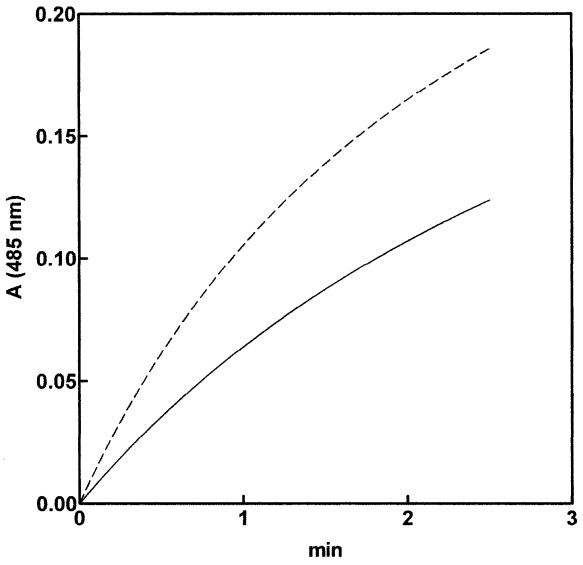


Fig. 1. Time course of adrenochrome formation in the absence (—) and presence (- - -) of 20 μM urate. The reaction solutions contained 0.4 mM adrenaline, 50 μM H_2O_2 and 6 nM lactoperoxidase in 40 mM Tris buffer, pH 7.4 (T = 30 °C).

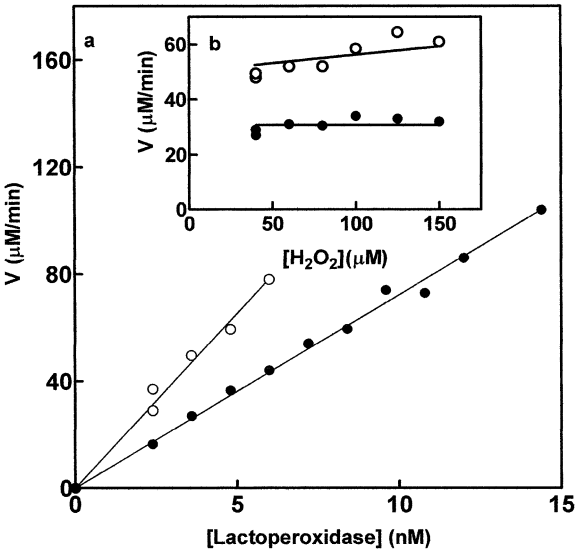


Fig. 2. a. Effect of lactoperoxidase concentration on the rate of adrenaline oxidation in the absence (●) and presence (○) of 0.1 mM urate. The reaction solutions contained 1 mM adrenaline, 50 μM H_2O_2 and 4.3 nM lactoperoxidase in 40 mM Tris buffer, pH 7.4 (T = 30 °C). b. Adrenaline oxidation rate at various H_2O_2 concentrations in the presence of 0.2 mM urate, keeping conditions otherwise as described above.

The formation of adrenochrome, as a result of lactoperoxidase action on adrenaline, was monitored spectrophotometrically at 485 nm. A molar absorption coefficient of 4020 M^{-1} cm^{-1}, determined according to a method described in a previous publication (Løvstad 1971), was used for calculating the enzyme activity. The rate of lactoperoxidase dependent urate oxidation was determined from the decrease of the 295 nm absorption band, characterizing the compound (ε = 11 000 M^{-1} cm^{-1}). The concentration of lactoperoxidase was estimated from the Soret band at 412 nm (ε = 114 000 M^{-1} cm^{-1}; Ghibaudi & Laurenti 2003). Measurements were performed in a Cecil 292 instrument connected to a Radiometer Rec 80 recorder.

Results and discussion

Figure 1 shows the time course of adrenochrome formation, recorded spectrophotometrically at 485 nm, after addition of lactoperoxidase to a solution of adrenaline and H_2O_2 at pH 7.4. When 20 μM urate was present in the reaction solution the rate of adrenaline oxidation was markedly enhanced. Initially, the adrenochrome concentration increased linearly with time. The enzyme activity was determined from this part of the time course curves. No significant adren-

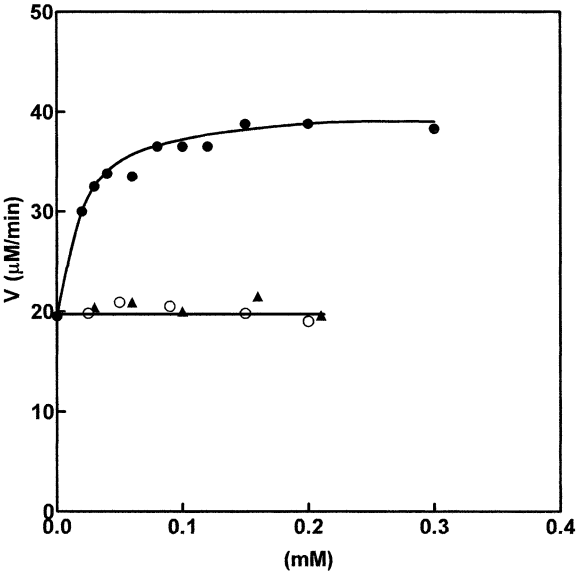
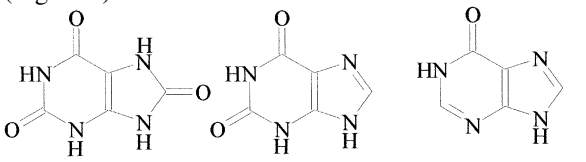


Fig. 3. Rate of adrenaline oxidation in the presence of various concentrations of urate (●), xanthine (○) and hypoxanthine (▲). The reaction solutions contained 0.4 mM adrenaline, 50 μM H_2O_2 and 6 nM lactoperoxidase in 40 mM Tris buffer, pH 7.4 (T = 30 °C).

aline oxidation took place in the absence of lactoperoxidase in these experiments.

The relationship between lactoperoxidase concentration and rate of adrenochrome formation, in the absence and presence of urate, was tested. As shown in Figure 2a a linear correlation between activity and enzyme concentration was obtained in both cases. The lactoperoxidase catalyzed oxidation of adrenaline was not much influenced by the concentration of H_2O_2 in the 40–150 μM region (Figure 2b). High concentrations of H_2O_2 were found to reduce the lactoperoxidase activity against catecholamine (Polis & Shmukler 1953). In the following experiments a H_2O_2 concentration of 50 μM was employed.

The rates of the enzyme dependent adrenaline oxidation was measured in the presence of various concentrations of urate (0.02–0.3 mM). As shown in Figure 3 a rapid increase in activity was obtained initially. When the urate concentration was increased further (> 0.1 mM) the curve started to level off. Xanthine and hypoxanthine, although closely structurally related to urate, had no significant effect on the lactoperoxidase catalyzed oxidation of adrenaline (Figure 3).



Uric acid Xanthine Hypoxanthine

Maple & Mason (1988) demonstrated, by means of the electron-spin-resonance technique, that horseradish peroxidase was able to interact with urate in the presence of H_2O_2. It thus seems likely that the activating effect of urate, observed in the present study, could be due to an interaction with the lactoperoxidase molecule. As discussed by Szent-Györgyi (1960), urate is a good electron donor, which may form a charge-transfer complex with other molecules. As shown in Table 1 lactoperoxidase reacts with urate, slowly oxidizing the compound in a catalytic manner. In this case an enzyme-urate complex probably breaks up into a reduced form of the enzyme and a urate free radical. Xanthine and hypoxanthine have a poorer electron donor ability than urate (Szent-Györgyi 1960), which may be the reason why they do not affect the enzyme catalyzed oxidation of adrenaline (Figure 3). In a previous study it was shown that chlorpromazine, a peroxidase substrate, increased the rate of the enzyme

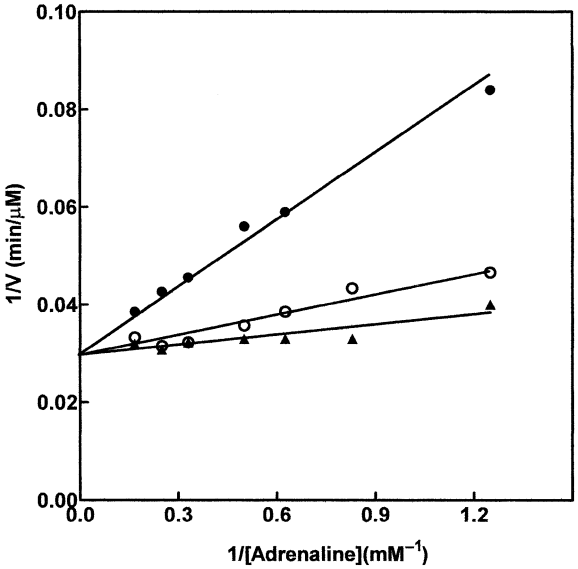


Fig. 4. The reciprocal rate of adrenaline oxidation plotted against the reciprocal adrenaline concentration in the absence (●) and presence of 0.05 mM urate (○) and 0.2 mM urate (▲). The reaction solutions contained adrenaline (0.8–6 mM), 50 μM H_2O_2 and 4.7 nM lactoperoxidase in 40 mM Tris buffer, pH 7.4. (T = 30 °C).

dependent oxidation of catecholamines by acting as a free, redox cycling intermediate between enzyme and catecholamine (Løvstad 1979). The possibility that urate might activate the reaction by a similar mechanism seems unlikely in view of the fact that adrenaline is a much better substrate than urate. As shown in Table 1 the catalytic constant (V/[Enzyme]) estimated with adrenaline was more than 100 times higher than the one obtained, when urate acted as substrate.

The rates of adrenochrome formation, as a result of adrenaline oxidation by lactoperoxidase at various substrate concentrations, were measured at 485 nm. A hyperbolical enzyme saturation curve was obtained. By means of a computer program published by Cleland (1967) a K_m-value of 1.21 mM (SE = 0.14 mM) and a catalytic constant, k = Vmax/[Enzyme], of 15.5 $\times$ 10^3 min^{-1} (SE = 0.6 $\times$ 10^3 min^{-1}), were estimated. The data are presented in the Lineweaver-Burk plot in Figure 4, together with activities calculated in the presence of 0.05 mM and 0.2 mM urate. The plot clearly demonstrates a competitive relationship between urate and adrenaline, the activating effect decreasing with increasing adrenaline concentration. A possible mechanism of urate activation could involve urate acting as a protein bound redox cycling intermediate between adrenaline and enzyme. However, other activator mechanisms, resulting in a competitive

Table 1. Lactoperoxidase dependent adrenaline- and urate oxidation rates

Lactoperoxidase (nM)	$V_{adrenaline}$ (μM Chrome/min)	V_a/[Enzyme] (min^{-1})	V_{urate} (μM urate/min)	V_u/[Enzyme] (min^{-1})
6	7.1	1183		
9	9.8	1090		
12	12.8	1067		
60			0.52	8.7
90			0.82	9.1
120			1.04	8.7

The reaction solutions contained 50 μM H_2O_2, 0.1 mM adrenaline, or 0.1 mM urate, in 40 mM Tris buffer, pH 7.4 (T = 30 °C).

type of Lineweaver-Burk plot, have been discussed by Dixon & Webb (1979). The mechanism of urate activation remains to be clarified.

Acknowledgements

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