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INHIBITION OF MYELOPEROXIDASE BY QUERCETIN

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Abstract

Reaction mechanism of quercetin induced inhibition of myeloperoxidase isolated from human neutrophils was proposed by following peroxidase activity of the enzyme, using the *o*-dianisidine and H_2O_2 as substrates. The dependence of initial reaction rate *vs*. H_2O_2 concentration in the absence and presence of quercetin revealed the reaction mechanism that involved the enzyme inhibition by the excess of the substrate. The rate and equililbria constants for proposed reaction paths were determined

Introduction

The heam enzyme myeloperoxidase (MPO) is a major neutrophil protein and is also present in monocytes. Depending on substrate availability, this enzyme paths throught halogenation and/or the peroxidase cycle [1]. Halogenating agents, especially hypochlorous acid, promote the oxidative killing of micro-organisms by neutrophils and the inflammatory tissue damage that the cells cause. Quercetin (3,5,7,3,`4`-pentahydroxy flavon), one of the most prevalent member of flavonoids, exerts anticancer, antiviral, antioxidant and free-radical scavenging abilities [2]. In the present study the mechanism of quercetin induced inhibition of MPO was investigated.

Experimental

MPO was purified from human neutrophils to a purity index (A_{430}/A_{280}) > 0.70 as described previously [3]. Enzyme activity was determined using the *o*-dianisidine (*o*-D) assay in 3 mL 50 mM phosphate buffer (pH 6.0) containing 110 ng MPO at 25°C. The reaction rate was followed in the initial reaction phase in the absence or presence of inhibitor and H_2O_2, while maintaing concentration of *o*-D constant.

Results and Disscusion

MPO catalyses the oxidation of *o*-D by H_2O_2 [4]. Quercetin inhibites MPO activity with IC_{50}=(5.28 ± 0.36) μM, i.e. the inhibitor concentration that induced 50% of enzyme inhibition. The reaction mechanism between MPO and quercetin was investigated by measuring the initial reaction rate as the function of H_2O_2 in the concentration range from 2-700 μM. Two series of kinetic experiments were performed, using 0.53 mM and 1.5 mM *o*-D. The concentration of quercetin was varied from $2x10^{-6}$ to $8x10^{-6}$ M, since these concentrations significantly inhibited the enzyme activity. Fig. 1 shows the dependance of the initial rate of oxidation of *o*-D as

a function of H_2O_2 concentration in the absence (control) and the presence of $2x10^{-6}$ M quercetin in the reaction assay containing 1.5 mM *o*-D. The shape of the curves suggested the reaction mechanism that involved the substrate inhibition of the enzyme and is consistent with the reaction scheme presented below:

$$E + H_2O_2 \underset{k_-}{\overset{k_+}{\rightleftharpoons}} [H_2O_2E] \xrightarrow{k_1} EO + H_2O \quad (1)$$

$$EO + o\text{-}D \xrightarrow{k_2} E + o\text{-}D^+ \quad (2)$$

$$EO + H_2O_2 \overset{K_1}{\rightleftharpoons} EOH_2O_2 \quad (3)$$

$$QE \overset{K_i}{\rightleftharpoons} Q + E \quad (4)$$

$$[EOQ] \overset{K_i`}{\rightleftharpoons} EO + Q \quad (5)$$

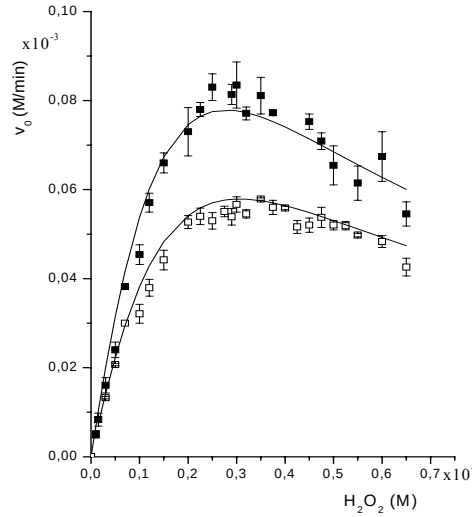

Fig. 1. Kinetics of H_2O_2 dependence of myeloperoxidase activity in the absence (solid simbols) and presence (open simbols) of 2 μM quercetin. *o*-D concentration was 1.5 mM. Symbols present the mean of at least three experiments ± S.E., while the solid lines present the reaction rate calculated from eq.6, using parameters from Table 1

where E - free enzyme in fero state, H_2O_2E - the complex between H_2O_2 and enzyme, EO - feryl state of the native enzyme, EOH_2O_2 - the complex between H_2O_2 and compound I, Q - quercetin, EOQ - the complex between compound I and Q. k_1 and k_2 are rate constants for the formation of EO complex and oxidised form of *o*-D. K_1 is the equilibrium constant for the complex formation between the EO complex and H_2O_2. K_i and $K_i`$ are the equililbria constants for dissociation of quercetin complexes with enzyme. Under the experimental conditions with 1.5 mM o-D, the concentration of MPO fulfilled the relation $E<<H_2O_2$+o-D, and the concentration of $H_2O_2<<$o-D. The change of [o-D] during the course of the reaction underwent minimal changes. Using this into account and applying the steady-state assumption with respect to H_2O_2E and EO, the expression for the initial reaction rate of o-D^+ generation as the function of H_2O_2 was obtained:

$$v_0 = \frac{k_1 k_2 K_M^{-1} [E]_0 [o-D]_0 [H_2O_2]}{\left(1+\frac{[Q]}{K_i}\right) k_2 [o-D]_0 + K_M^{-1} k_1 \left(1+\frac{[Q]}{K_i`}+\frac{k_2}{k_1}[o-D]\right)[H_2O_2] + K_M^{-1} K_1 k_1 [H_2O_2]^2} \quad (6)$$

Table 1. Kinetic constants for the oxidation of *o*-dianisidine (0.53 mM and 1.5 mM) by MPO in the presence of quercetin.

	[o-D] (mM)	
	0.53	1.50
$K`_i$(M)	(2.85 ±0.22) x 10^{-6}	(3.02 ±0.17) x 10^{-6}
k_2($M^{-1}min^{-1}$)	(3.05 ±0.15) x 10^{6}	(2.59 ±0.18) x 10^{6}
K_1(M)	(5.20 ±0.26) x 10^{-6}	(5.00 ±0.25) x 10^{-6}
K_M(M)	(2.41 ±0.12) x10^{-3}	(2.63 ±0.14) x10^{-3}
k_1(min^{-1})	(1.50 ±0.08) x 10^{4}	(1.70 ±0.10) x 10^{4}
K_I (M)	-	(8.00 ±0.40) x10^{3}

Eq.(6) enabled us to obtain the rate and equilibria constants for the reaction scheme presented above, by using its appropriate transformations. The treatment of the experimental data from Fig. 1 depended on weather the experimental points lied on the asceding or on the descending branch of v_o *vs.* [H_2O_2] curves. Eq. (6) was rerranged to the Line- weaver - Burk form in the non-inhibiting H_2O_2 concentration range (below 0.25 mM):

$$\frac{1}{v_0} = \frac{1}{k_2[E]_0[o-D]_0}\left(\left(1+\frac{[Q]}{K_i^{`}}+\frac{k_2}{k_1}[o-D]\right)+\frac{k_2[o-D]_0}{k_1K_M^{-1}}\left(1+\frac{[Q]}{K_i}\right)\frac{1}{[H_2O_2]}\right) \qquad (7)$$

In this way the reaction scheme was reduced to the Michaelis-Menten type with $K`_M$ beeng dependent on the concentration of inhibitor and *o*-D. For the analysis of the experimental data in the range of inhibiting H_2O_2 concentrations (above 0.25 mM) the linearized form of eq. (6) was applyed to the experimental results:

$$\frac{1}{v} = \frac{1+\frac{k_2}{k_1}[o-D]}{k_2[E]_0[o-D]}\left(1+\frac{[Q]}{K_i^{`}}+K_1[H_2O_2]\right) \qquad (8)$$

The rate and equilibria constants consistent with the reaction scheme (eqs. (1) – (5)) were obtained from the graphical presentation of $1/v_o$=f($1/H_2O_2$) and v_o =f(H_2O_2) and are given in Table 1. Besides, v_o was recalculated from eq. (6) using the obtained parameters and is presented in Fig.1 as solid line.

Conclusion

The results presented in Table 1 show good agreament of rate and equilibria constants obtained for two *o*-D concentrations. Calculated curves (Eq. (6)) fitted the experimental results in the range of experimental error and confirmed the proposed reaction mechanism.

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