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Kinetic Model of Biphasic Character of Catalase Inhibition

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Abstract. A mathematical model of kinetics of fungal catalase inhibition with hydroxylamine in presence of substrate (hydrogen peroxide) has been developed. The scheme includes an intermediate formation and slow reversible native enzyme production. The model is based on differential equations of nonstationary kinetics of the enzyme action. The computer simulation was carried out using adaptive Runge-Kuta method.

Good satisfactory is achieved if the kinetic constants calculated by modeling nonstationary state of hydrogen peroxide decomposition are used for calculations of inhibition of the catalases at the second phase of inhibition and for kinetics of the intermediate formation, that was determined with stop-flow spectrophotometer.

Keywords: catalase, hydrogen peroxide, hydroxylamine, kinetics, inhibition.

1 Introduction

Catalases are involved in the neutralization of hydrogen peroxide in living cell [1]. The catalases show high specificity to hydrogen peroxide and only some compounds inhibits catalases activity. During investigation of fungal catalase (*A.niger*) inhibition with hydroxylamine the biphasic character of hydrogen peroxide decomposition has been indicated [2]. The task of this investigation was to explain the unusual kinetics of this inhibitor action. For this purpose

the analysis of non stationary kinetics of catalases action has been performed and calculated results were compared with the experiment.

2 Mathematical Model

The typical scheme of catalase action involves Compound 1 formation and oxygen production (1), (2). We are suggesting that in presence of inhibitor (HA) Compound 1 interacts with the inhibitor (HA) following intermediate formation (3). Ferricatalase (native enzyme) is regenerating in reaction (4):

$$\mathrm{Ferricatalase} + \mathrm{H_2O_2} \rightarrow \mathrm{Compound\,I} + \mathrm{H_2O} \qquad k_1 \qquad (1)$$

$$\mathrm{Compound\,I} + \mathrm{H_2O_2} \rightarrow \mathrm{Ferricatalase} + \mathrm{O_2} + \mathrm{H_2O} \qquad k_2 \qquad (2)$$

$$\mathrm{Compound\,I} + \mathrm{HA} \rightarrow \mathrm{Intermediate} \qquad k_3 \qquad (3)$$

$$\mathrm{Intermediate} \rightarrow \mathrm{Ferricatalase} \qquad k_4 \qquad (4)$$

where k_1, k_2, k_3 and k_4 corresponds to the kinetic constants of the reaction (1), (2), (3) and (4), respectively.

The system of differential equations corresponding to this scheme can be written:

$$df/dt = -k_1 \cdot f \cdot p + k_2 \cdot c1 \cdot p + k_4 \cdot i \qquad (5)$$

$$dc1/dt = k_1 \cdot f \cdot p - k_2 \cdot c1 \cdot p - k_3 \cdot c1 \cdot ha \qquad (6)$$

$$dp/dt = -k_1 \cdot f \cdot p - k_2 \cdot c1 \cdot p \qquad (7)$$

$$dha/dt = -k_3 \cdot c1 \cdot ha \qquad (8)$$

$$di/dt = k_3 \cdot c1 \cdot ha - k_4 \cdot i \qquad (9)$$

where $f, p, c1, ha, i$ and t is a concentration of ferricatalase, hydrogen peroxide, compound 1, hydroxylamine, intermediate and time, respectively.

The system of differential equations (5)–(9) was solved by using adaptive Runge-Kuta method (Mathcad PLUS).

3 Fitting experimental data by the model

The analysis of suggested scheme permit to conclude that decomposition of hydrogen peroxide in *absence of inhibitor* ($ha = 0$) shows monotonic character as depicted in Fig. 1.

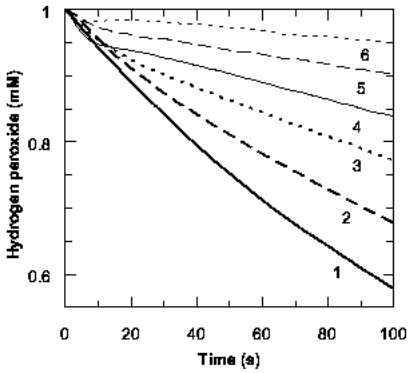

Fig. 1. Kinetics of hydrogen peroxide decomposition in presence of catalase at pH 7.2 and 30°C. Concentrations of HA: 0 μM (1), 0.1 μM (2), 0.2 μM (3), 0.5 μM (4), 1.0 μM (5), 2.0 μM (6).

The time of establishment of quasi steady state ($dc1/dt \approx 0$) that is typical for classical enzyme kinetics [3] can be calculated as $\ln(2)/k_1 \cdot p_0$ (k_1 value is taken from Table 1). At $p_0 = 10^{-3}$ M the quasi-steady state is settled during 0.5 ms. Therefore, the decrease of hydrogen peroxide concentration may be

Table 1. Kinetic constants of fungal catalase action at pH 7.2 and 30°C

$k_1 \cdot 10^{-6}$ $M^{-1}s^{-1}$	$k_2 \cdot 10^{-6}$ $M^{-1}s^{-1}$	$k_3 \cdot 10^{-6}$ $M^{-1}s^{-1}$	$k_4 \cdot 10^2$ s^{-1}
1.40 ± 0.01	5.7 ± 0.5	0.86 ± 0.03	2.6 ± 0.3

expressed by exponential function:

$$p = p_0 \cdot \exp(-k \cdot t), \tag{10}$$

where k is the least constant from k_1 and k_2.

In *presence of the inhibitor* the quasi-steady state is established at beginning of reaction, too. However, if the intermediate conversion rate (4) is low the new quasi-steady state is established during prolong substrate and inhibitor incubation. This consideration was confirmed by solving the system of differential equations and finding k_1 – k_4 by fitting the experimental data. The relative standard error (CV) of data fitting was 0.8%. The approximation of catalatic process (1), (2) at different hydrogen peroxide concentration and

in absence of HA gives k_1 and k_2 (Table 1). As it is possible to notice k_2 value is large than k_1. The approximation of kinetic curves of hydrogen peroxide decomposition in presence of HA gives the values of the rest of constants. The constant k_3 corresponding to Compound 1 interaction with HA is less in comparison to Compound 1 interaction with hydrogen peroxide (2). Moreover, the constant of the intermediate conversion (4) is very low. The low value of the constant k_4, in fact, determinates prolonged time of the establishment of the second phase. An apparent lifetime of the intermediates of the catalases, estimated as $\ln(2)/k_4$, is 26.7 s. This value fits experimental results (Fig. 2).

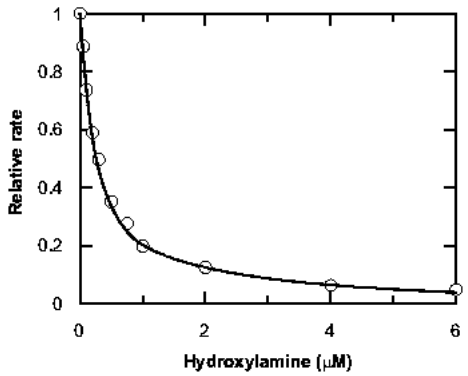

Fig. 2. The dependence of catalase inhibition with HA at the second phase of the reaction. Curves represent data approximation by the model.

The confirmation of the suggested scheme might be the calculation of catalase inhibition at the second phase (Fig. 2). The results depicted in Fig. 2 demonstrate good agreement of experimentally determined activity and calculated values if the constants have been derived from the kinetics of hydrogen peroxide decomposition (Table 1).

Next strong support of the suggested scheme comes from the calculations of intermediate formation rate (Fig. 3). If suggested scheme of the inhibitor action is valid, the constants, those are calculated at nanomolar concentration of the enzyme and the inhibitor, could be applied for the calculations of the intermediate concentration, that has been determined at micromolar concentrations of hydrogen peroxide and the inhibitor. The results depicted in the inset of Fig. 3 demonstrates good correlation (CV=1.9%) of experimentally

determined and calculated absorbance change.

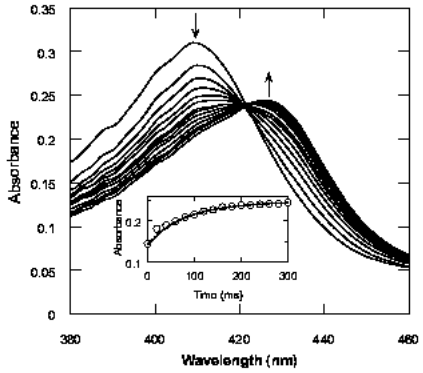

Fig. 3. The change of absorbance of *A.niger* catalase at Soret region during reaction with H_2O_2 and HA. The spectra were recorded every 20 ms. Concentrations: catalase 2.8 μM, H_2O_2 10μM, HA 2 mM. Insert approximation of kinetics of absorbance change at 428 nm.

The structure of the intermediate has not been determined. However, it was documented that during animal catalase treatment with azide and hydroxylamine in presence of hydrogen peroxide spectroscopically identical compounds were formed [4]. The structure of these compounds was attributed to NO-ferrocatalase. The formation of NO-ferrocatalase during fungal catalase and hydroxylamine action in presence of hydrogen peroxide may be explained by 3 electrons reduction of compound 1 with HA.

4 Conclusions

It was shown that biphasic character of decomposition of hydrogen peroxide in presence of HA proceeds through intermediate formation. Slow formation and decay of intermediate determinates biphasic character of inhibitor action. Analysis of literature data show that this type of catalases inhibition is not unique for HA. Slow inhibition of bovine liver catalase was demonstrated during the catalases inhibition by ascorbate, sulfite and azide in presence of hydrogen peroxide [4]–[7]. To our knowledge, performed non stationary kinetic analysis represents the first explanation of biphasic action of catalase inhibitors. The exploring this type of inhibition permits, among others, to make

practically useful schemes of catalases inhibition in biotechnological process and to build new bioanalytical systems.

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