

Dedicated to Professor Victor-Emanuel Sahini
on the occasion of his 80th anniversary

MODELS FOR IRREVERSIBLE INACTIVATION IN THE ENZYMATIC DECOMPOSITION OF HYDROGEN PEROXIDE AND THEIR KINETIC TREATMENT BY MEANS OF GRAPHS

Adina RĂDUCAN,* Dumitru OANCEA and Eugen SEGAL

Dept. of Physical Chemistry, Faculty of Chemistry, University of Bucharest, Bulevardul Regina Elisabeta 4-12,
Bucharest, 030018, Roumania

Received May 16, 2007

The irreversible inactivation of catalase in the reaction with hydrogen peroxide appears at high substrate concentrations and low enzyme concentrations. The kinetics of this inactivation involves the formation of three complexes designated as X_1 , X_2 , and X_3 , the last one being responsible for the inactivation. Several models were proposed for the inactivation of catalase and the kinetic equations were obtained based on the quasi-steady state approximation and on the theory of graphs. The results showed that the theory of kinetic graphs can be applied for the kinetic treatment of these models.

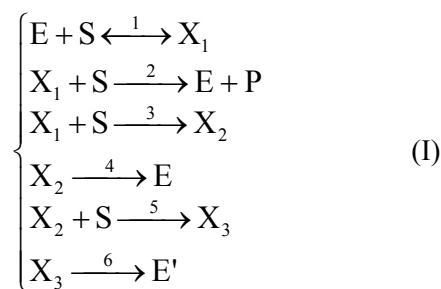
INTRODUCTION

In the presence of high concentration of hydrogen peroxide, catalase presents a gradual decrease of activity, due to the irreversible inactivation. Several kinetic equations were developed to account for this inactivation,^{1,2} in all models, some complexes between catalase and its substrate are assumed to be involved in the inactivation mechanism.

In some previous notes³⁻⁵ a number of hypothetical sequences for the irreversible inactivation of catalase in the decomposition of hydrogen peroxide at high values of substrate concentrations were analysed. By using graphs⁶⁻⁸ for the formal kinetic analysis we obtained identical results with those obtained by applying the quasi-steady state approximation. In the following we present some other sequences, obtained from a general model presented in literature² for the same transformation as well as their formal-kinetic treatment by means of kinetic graphs.

RESULTS AND DISCUSSION

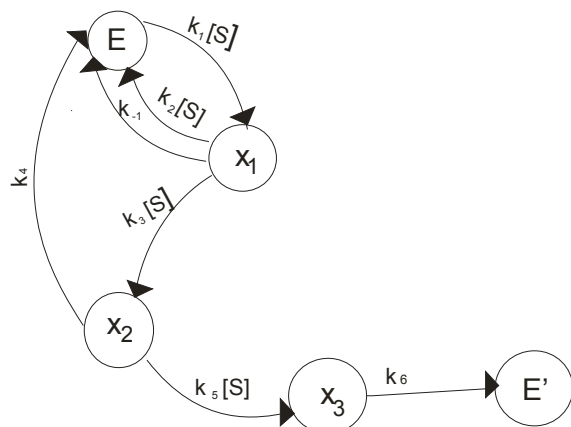
The most complete model for the irreversible inactivation of catalase in the decomposition of hydrogen peroxide² involves 6 steps and formation of three complexes enzyme-substrate and a form of inactive enzyme. The six step sequence is (I):



where E is the enzyme, S the substrate, X_i the complex between enzyme and substrate and E' an inactive form of catalase.

For this sequence, the representative kinetic graph is:

* Corresponding author: adina@gw-chimie.math.unibuc.ro



The corresponding basic determinants of the vertices are given by:

$$\begin{aligned} D_E &= k_{-1} + k_2[S] + k_1 k_3 k_4 [S]^2 \\ D_{X_1} &= k_1 [S] \\ D_{X_2} &= k_1 k_3 [S]^2 \\ D_{X_3} &= k_1 k_3 k_5 [S]^3 \\ D_{E'} &= k_1 k_3 k_5 k_6 [S]^3 \end{aligned}$$

The reaction rate is given by:

$$r = \frac{k_1 [S] (k_{-1} + k_2 [S] + k_1 k_3 k_4 [S]^2) - k_1 k_{-1} [S]}{k_{-1} + (k_1 + k_2) [S] + k_1 k_3 k_4 [S]^2 + k_1 k_3 [S]^2 + k_1 k_3 k_5 [S]^3 + k_1 k_3 k_5 k_6 [S]^3} [E]_0 \quad (1)$$

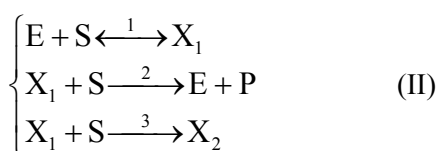
or

$$r = \frac{b[S] + c[S]^2}{a' + b'[S] + c'[S]^2 + d'[S]^3} [E]_0 \quad (2)$$

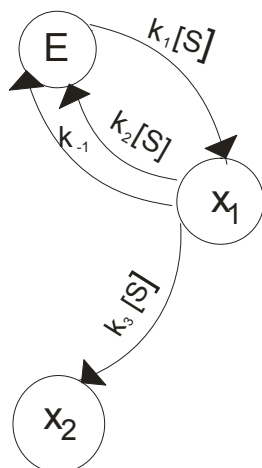
where where the meaning of constants b, c, a', b', c' and d' results from the comparison of relations (1) and (2).

Since this equation is too complex to be used for the estimation of kinetic constants, several simplified models are proposed here.

The simplest sequence (II) involves the formation of two complexes, from which X_2 is the inactive one:



and the representative kinetic graph is:



The corresponding basic determinants of the vertices are given by:

$$\begin{aligned} D_E &= k_{-1} + k_2 [S] \\ D_{X_1} &= k_1 [S] \\ D_{X_2} &= k_1 k_3 [S]^2 \end{aligned}$$

Correspondingly the reaction rate is given by:

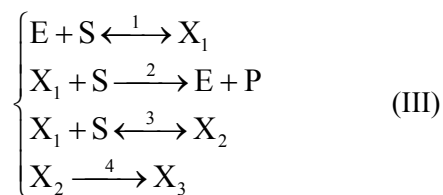
$$r = \frac{k_1 [S] (k_{-1} + k_2 [S]) - k_1 k_{-1} [S]}{k_{-1} + (k_1 + k_2) [S] + k_1 k_3 [S]^2} [E]_0 \quad (3)$$

or

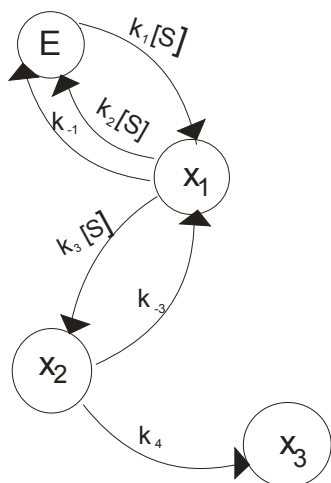
$$r = \frac{a[S]^2}{a' + b'[S] + c'[S]^2} [E]_0 \quad (4)$$

Let us further analyse other two sequences (III) and (IV) consisting of four elementary steps or stages with the formation of three complexes.

Sequence (III):



where X_3 is an inactive complex. The representative graph is:



with the following basic determinants of the vertices:

$$\begin{aligned} D_E &= k_{-3}(k_{-1} + k_2[S]) \\ D_{X1} &= k_1[S] + k_{-3} \\ D_{X2} &= k_1 k_3 [S]^2 \\ D_{X3} &= k_2 k_4 [S] \end{aligned}$$

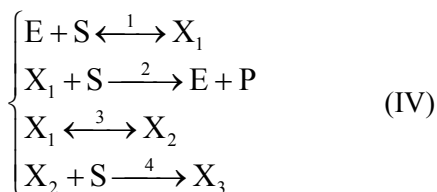
The classical formulae for the reaction rate lead to:

$$r = \frac{k_1 k_{-3} [S] (k_{-1} + k_2 [S]) - k_{-1} (k_1 [S] + k_{-3})}{k_{-3} (k_{-1} + k_2 [S]) + k_1 [S] + k_3 + k_2 k_3 [S]^2 + k_2 k_4 [S]} [E]_0 \tag{5}$$

or

$$r = \frac{a + b[S] + c[S]^2}{a' + b'[S] + c'[S]^2} [E]_0 \tag{6}$$

Sequence (IV):

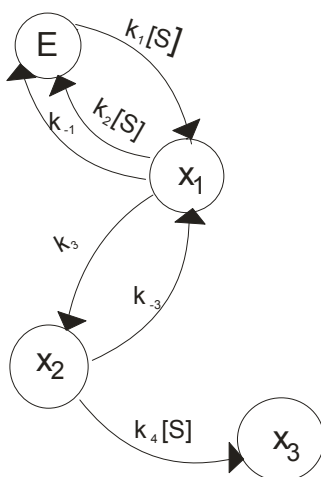


with the basic determinants:

$$\begin{aligned} D_E &= k_{-3} \{k_{-1} + k_2[S]\} \\ D_{X1} &= k_1[S] + k_{-3} \\ D_{X2} &= k_1 k_2 [S] \\ D_{X3} &= k_1 k_3 k_4 [S]^2 \end{aligned}$$

The reaction rate is given by:

when the representative graph is:

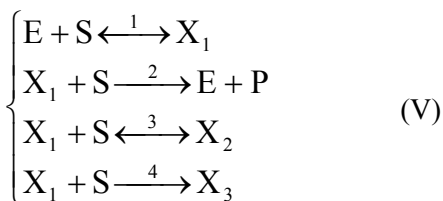


$$r = \frac{k_1 k_{-3} [S] (k_{-1} + k_2 [S]) - k_{-1} (k_1 [S] + k_{-3})}{k_{-1} k_{-3} + k_{-3} k_2 [S] + k_1 [S] + k_1 k_3 [S] + k_1 k_3 k_4 [S]^2 + k_{-3}} [E]_0 \tag{7}$$

or

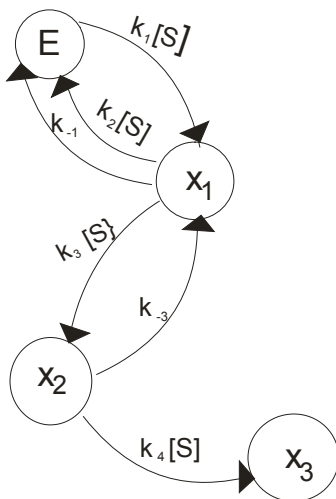
$$r = \frac{a + b[S] + c[S]^2}{a' + b'[S] + c'[S]^2} [E]_0 \tag{8}$$

For the sequence:



$$\begin{aligned} D_E &= (k_{-1} + k_2[S])k_{-3} \\ D_{X_1} &= k_1[S] + k_{-3} \\ D_{X_2} &= k_1k_3[S]^2 \\ D_{X_3} &= k_1k_4[S]^2 \end{aligned}$$

with the associated kinetic graph,

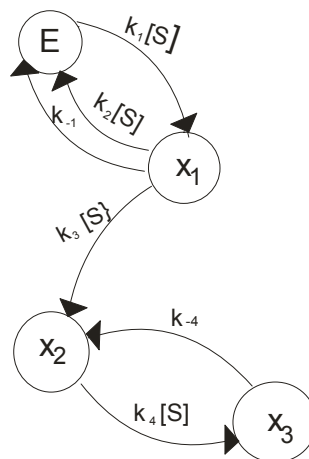
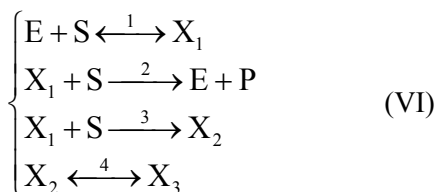


$$r = \frac{k_1k_{-3}[S](k_{-1} + k_2[S]) - k_{-1}(k_1[S] + k_{-3})}{(k_{-1} + k_2[S])k_{-3} + k_1[S] + k_{-3} + k_1k_3[S]^2 + k_1k_4[S]^2} [E]_0 \quad (9)$$

or

$$r = \frac{a + b[S] + c[S]^2}{a' + b'[S] + c'[S]^2} [E]_0 \quad (10)$$

For the similar sequence(VI):



$$\begin{aligned} D_E &= (k_{-1} + k_2[S])k_{-4} \\ D_{X_1} &= k_1[S] + k_{-4} \\ D_{X_3} &= k_1k_3[S]^2 \\ D_{X_4} &= k_1k_4[S]^2 \end{aligned}$$

with the corresponding graph:

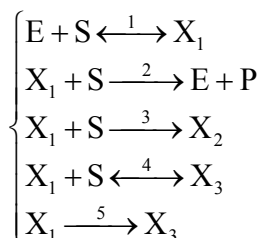
The reaction rate is given by:

$$r = \frac{k_1k_{-4}[S](k_{-1} + k_2[S]) - k_{-1}(k_1[S] + k_{-4})}{k_{-1}k_{-4} + k_2k_{-4} + k_1[S] + k_{-4} + k_1k_3[S]^2 + k_1k_4[S]^2} [E]_0 \quad (11)$$

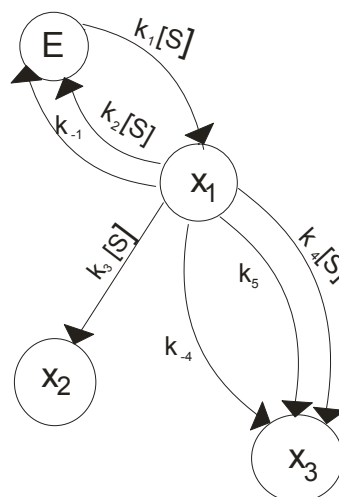
or

$$r = \frac{a + b[S] + c[S]^2}{a' + b'[S] + c'[S]^2} [E]_0 \quad (12)$$

Let us now consider the following five step mechanism(VII):



with the following associated graph:



The calculations of the basic determinants and of the reaction rate lead to:

$$\begin{aligned} D_E &= (k_{-1} + k_2[S])k_{-4} \\ D_{X_1} &= k_1[S] + k_{-4} \\ D_{X_2} &= k_1k_3[S]^2 \\ D_{X_3} &= k_1k_4[S]^2 + k_5 \end{aligned}$$

$$r = \frac{k_1k_{-4}[S](k_{-1} + k_2[S]) - k_{-1}(k_1[S] + k_{-4})}{(k_{-1} + k_2[S])k_{-4} + k_1[S] + k_{-4} + k_1k_3[S]^2 + k_1k_4[S]^2 + k_5} [E]_0 \quad (13)$$

or

$$r = \frac{a + b[S] + c[S]^2}{a' + b'[S] + c'[S]^2} [E]_0 \quad (14)$$

For all proposed sequences, the reaction rate depends linearly on the concentration of enzyme. As compared to equation (2), corresponding to the complete model, the other equations are simpler, containing the substrate concentrations at lower powers. The results obtained by using the kinetic graphs are similar with those obtained from the quasi-steady state approximation.

CONCLUSIONS

The application of the graph theory in order to obtain the rate equation leads to results which are similar with those obtained using the quasi-steady state approximation for all proposed sequences.

The obtained results show that the reaction rate depends linearly on the concentration of enzyme. Regarding the dependence of the reaction rate on the substrate concentration, this also depends on the values of the rate constants of the elementary steps contained by the considered sequence.

REFERENCES

1. S. P. O'Neill, *Biotechnol. Bioeng.*, **1972**, *14*, 201-205.
2. O. M. Lardinois, M. M. Mestdagh and P. G. Rouxhet, *Biochim. Biophys. Acta*, **1996**, *1295*, 222-238.
3. D. Oancea, A. Răducan and S. G. Ionescu, *Progress in Catalysis*, **1992**, *2*, 57.
4. A. Răducan, D. Oancea and E. Segal, *Progress in Catalysis*, **1997**, *6*, 139.
5. E. Segal, *Progress in Catalysis*, **1998**, *7*, 43.
6. K. B. Yatzimirskii, *Z. Chem.*, **1973**, *13*, 201.
7. E. L. King and C. Altman, *J. Phys. Chem.*, **1956**, *60*, 1375.
8. M. V. Volkeshtein and B. N. Goldstein, *Doklady Akad. Nauk. SSSR*, **1966**, *17*, 963.