STUDIA UNIVERSITATIS BABEŞ-BOLYAI, CHEMIA, LI, 1, 2006

THE INHIBITORY EFFECT OF THE ATENOLOL UPON THE ENZYME CATALYZED HYDROGEN PEROXIDE DECOMPOSITION

FLORINA POGACEAN, IOAN BALDEA* AND FLORIN TURBAT

ABSTRACT. The reaction of decomposition of hydrogen peroxide in the presence of catalase has been investigated by means of a Clark oxygen sensor, in the presence and absence of various concentrations of Atenolol – a β-blocker drug - having an inhibiting role. The Michaelis – Menten kinetic parameters have been determined from Lineweaver-Burk plots. The inhibition pattern we have deduced, suggested by the Lineweaver - Burk plots, corresponds to a fully mixed inhibition mechanism. Inhibition constants K_I and K_I' were determined. A kinetic method for the determination of Atenolol has been suggested.

Keywords: Atenolol, Catalase, Hydrogen peroxide decomposition, Inhibition mechanism, Kinetic methods.

Introduction

Catalase, an oxidoreductase EC 1.11.1.6, is a tetrameric haemin-enzyme consisting of a 4 identical tetrahedrally arranged subunits of 60000 g/mol each [1]. Catalase is widely distributed in nature It is found in all aerobic microorganism, in plant and animal cell [2]. The enzyme, when located in organelles, acts as a regulator of the H_2O_2 level, while in erythrocytes, catalase provides) a protection for hemoglobin against the oxidizing agents like H_2O_2 together with gluthathione peroxidase [3].

The enzyme - catalyzed reaction:

$$2H_2O_2 \xrightarrow{catalase} 2H_2O + O_2 \quad (1)$$

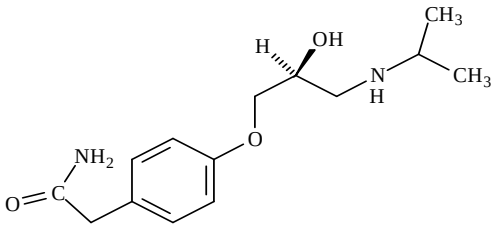
has been the subject of extensive investigations, both in regard to the kinetics and to the mechanism of the decomposition [4, 5, 6, 7, 8].

Similar reaction were also observed for other organic peroxides [9, 10] Well-known inhibitors of these reactions are ions like azide, cyanide and fluoride [11, 12, 13], or some organic compounds compounds like 3-amino-1,2,4-triazole [14] and atenolol[15].

Atenolol is a cardioselective β-adrenoceptor blocking agent (β-blocker).

* *Department of Chemistry and Chemical Engineering, Babes-Bolyai* University of Cluj, 11 Arany Janos Str.,Cluj-Napoca 400028

Atenolol ($C_{14}H_{22}N_2O_3$) according to European Pharmacopeia (±)-2-[4-(2-hydroxi-3-izopropylaminopropoxil)]-phenyl]-acetamide[16]



Atenolol has a chiral center, and is clinically used as a racemate [15]. B-adrenoceptor antagonists are a group of compounds that competitively inhibit the effects of catecholamines at β- adrenergic receptors [17]. These agents are used widely in clinical medicine for the treatment of varios deseases including hypertension, angina pectoris [18] cardiac arrythmias, hypothyroidism and glaucoma.

The main aim of this paper is the description of the inhibition mechanism. It would permit to develop a kinetic method of the determination of the drug Atenolol in very low concentration, based on the inhibitory effect on the rate of hydrogen peroxide decomposition.

Experimental

Equipment. The measurements were undertaken with a Clark oxygen sensor, attached to a Multiline P4 multimeter with automatic data acquisition on a PC. The sensors cover a measuring range from 0 up to 19.99 mg/L for the dissolved oxygen, with a resolution of 0.01 mg/L and an accuracy of ±0.5% from 5.30 ℃. All experiments were performed in a v essel provided with a water jacket. The temperature was maintained constant at 20±0.1 ℃, by means of a Falc 90 recirculatory water bath. The reaction mixtures were stirred with a magnetic stirrer, always with the same frequency.

Reagents and Solutions. We used bacterial catalase from *Micrococcus Lysodeikticus* 176340 U/ml (where the enzyme unit 1U is the amount of enzyme needed to transform 1 μmol of the substrate within 1 min, under standard conditions), from FLUKA with a purity index of 0.85, which came from the ratio of absorbances at 405 nm and 280 nm, $A_{405}/_{A280.}$

The molar concentration of catalase was determined spectrophotometrically at 407 nm, where the extinction coefficient is known to be $4x10^5$ mol^{-1} dm^3 cm^{-1} [19].

All the other reagents were of analytical reagent grade. The solutions were prepared with de-ionized, four-times distilled water in order to avoid the interference of heavy metals. Stock solutions of catalase ($9x10^{-10}$

mol/L), H_2O_2 (4.9 x 10^{-2} mol/L) and atenolol (10^{-5} mol/L) in phosphate buffer of pH=7.0 were freshly prepared before each set of experiments. Hydrogen peroxide has been standardized against potassium permanganate in acidic media.

Procedure

The reaction mixture of 10 mL volume was prepared directly in the reaction vessel connected to a thermostate, where the solutions of the reagents were kept. Measured volumes of buffer and atenolol stock solution were placed in the vessel, and the change in the concentration of oxygen was monitored while the mixture was continuously stirred. The oxygen concentration became constant after about 50 s, when a known volume of H_2O_2 stock solution was added to the mixture. After about 50 s the concentration of oxygen reached a constant value again, so that the catalyzed reaction was started by the injection of 2 ml of the stock solution of the enzyme. A typical oxygen concentration versus time curve obtained by monitoring the reaction with a Clark sensor is presented in Fig. 1.

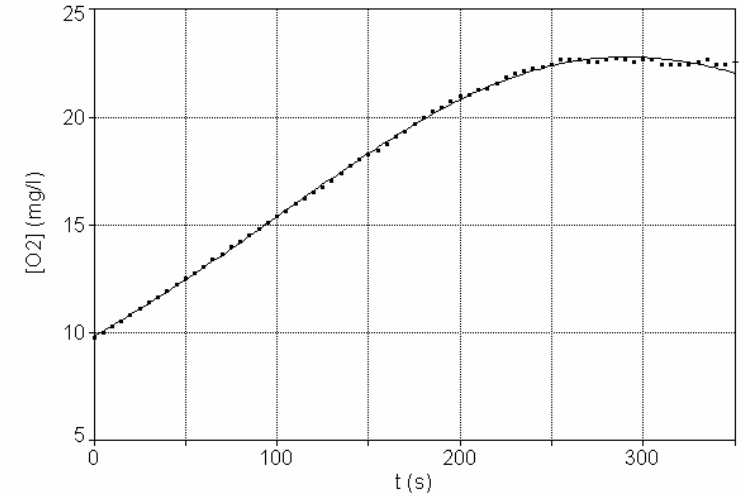


Fig. 1.
A typical curve for O_2 evolution, recorded with the Clark sensor in the reaction mixture pH=7 at 20^0C

The rate has been measured after the introduction of the enzyme solution into the mixture as the slope of the oxygen concentration increase with time. Only the starting period, within 25 – 30 % of reaction, with a linear dependence has been considered. The degree of H_2O_2 transformation was calculated from the experimental measured oxygen concentration as

$$X = \frac{2[O_2]_t - [O_2]_0}{[H_2O_2]_0} \quad (1)$$

where $[O_2]_t$ is the actual concentration of O_2, and $[O_2]_0$ is the constant value of $[O_2]$ obtained after the addition and consumption of all amount of H_2O_2.

Results and Discussion

A large amount of information is available in literature about the kinetics and the mechanism [5, 20- 22] of the reaction in the absence of inhibitor

The initial reaction rates, r_0, were determined from the slopes of the early part of the O_2 evolution curves, after catalase addition. Although the catalytic cycle seems to be quite complicate [23] because of the various oxidation states of Fe in heme, our data of initial rates fitted well to Michaelis-Menten equation [24]

$$r_0 = \frac{k[E_0][S]}{K_M + [S]} = \frac{r_{max}[S]}{K_M + [S]} \quad (2)$$

where $[E]_0$ and [S] stand for the initial concentration of the enzyme and for the substrate concentration respectively, K_M and r_{max} are the Michaelis-Menten parameters, and k is the rate constant of breakdown of the enzyme substrate complex to the product. This equation can be brought into the double-reciprocal form:

$$\frac{1}{r_0} = \frac{1}{r_{max}} + \frac{K_M}{r_{max}} \cdot \frac{1}{[S]} \quad (3)$$

and used to obtain the Lineweaver-Burk plots [25]. The value of the Michaelis-Menten constant K_M= 3.94×10^{-4} mol/L and the maximum velocity r_{max}=4.64×10^{-2} mol L^{-1} s^{-1}, obtained by us, are comparable to those mentioned in literature [26].

The Influence of Atenolol on the Enzyme-Catalyzed Decomposition of Hydrogen Peroxide

Several kinetic runs performed in the presence of different concentrations of atenolol proved the inhibitory effect of this compound. When the concentration of H_2O_2 was varied at several fixed concentrations of atenolol, Lineweaver-Burk plots for the inhibited reaction

$$\frac{1}{r_{o(I)}} = \frac{1}{r_{max(I)}} + \frac{K_{M(I)}}{r_{max(I)}} \cdot \frac{1}{[S]} \qquad (4)$$

resulted in a family of straight lines (Fig. 2), which have a common intersection point on the left side of the ordinate and below the abscissa.

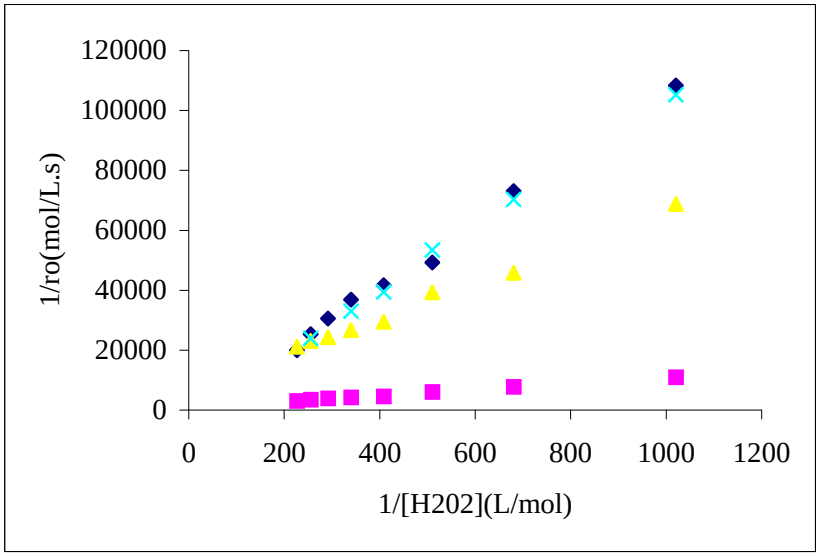


Fig.2.
Lineweaver Burk plots obtained for different concentration of atenolol

As the slopes of Lineweaver- Burk plots depend on the inhibitor concentration, a systematiuc study could lead to a kinetic method of determination of this inhibiting agent concentration.

The Michaelis-Menten parameters in the presence of the inhibitorI were determined from the parameters of the lines in figure 2. Their values are:

$$r_{max(I)} = r_{max} \frac{1 + \beta \frac{[I]}{K'_I}}{1 + \frac{[I]}{K'_I}} \qquad \text{and} \qquad K_{M(I)} = K_M \left(\frac{1 + \frac{[I]}{K_I}}{1 + \frac{[I]}{K'_I}} \right) \qquad (5)$$

where: $K_{M(I)}$ is the apparent Michaelis constant in the presence of inhibiting agent, K'_I and K_I are the dissociation constants of the enzyme – substrate - inhibitor complex, ESI, and the enzyme inhibitor complex, EI, respectively.

The constant β is equal to zero for full inhibition, while for partial inhibition 0< β≤1. Lineweaver-Burk plots of Fig. 2 correspond to the inhibition pattern of full or partial mixed inhibitors [26].

In order to distinguish between full and partial inhibition, the slopes and intercepts from the primary Lineweaver- Burk plots were re-plotted against the corresponding inhibitor concentration. They are presented in figure 3 Both

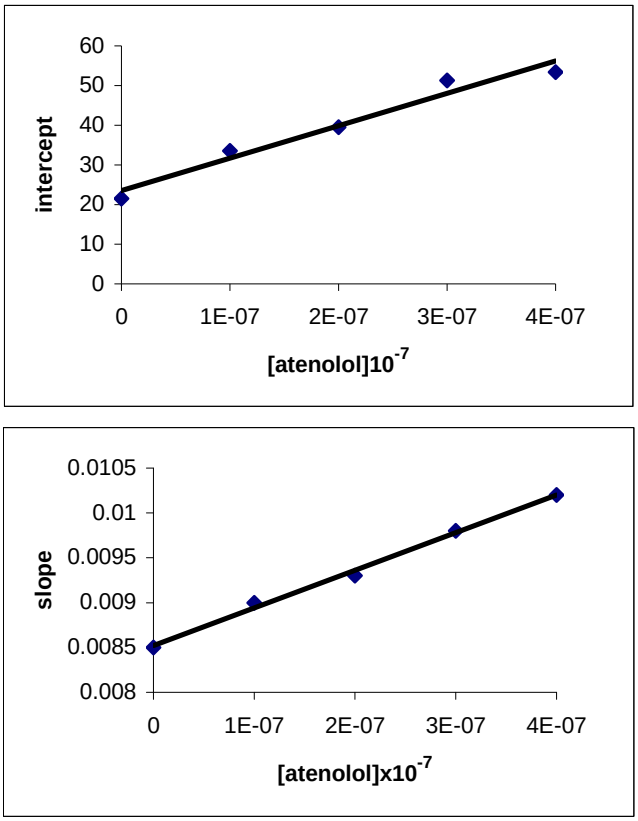


Fig 3.
The plots of intercepts and slopes versus the inhibitor concentration

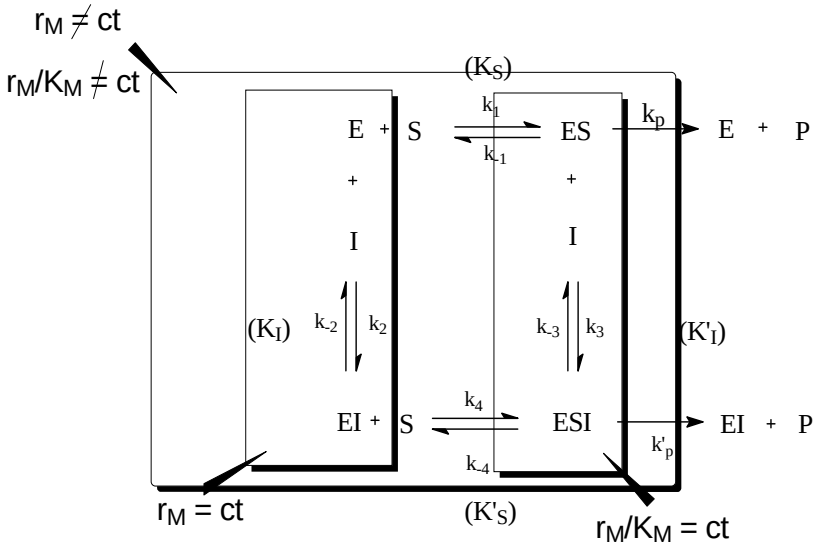
these plots gave straight lines with good correlation coefficients. The straight lines equations are intercept: $y = (8{,}0 \pm 2.07) \cdot 10^{7}\, x + (23{,}55 \pm 5{,}08)$, and slope: $y = (42 \pm 7.76) \cdot 10^{2}\, x + (85 \pm 1{,}9) \cdot 10^{-4}$. This behavior is considered as typical for full inhibition [26]. Considering the possibility of a

Table 1.
Mean values or the maximum velocity $r_{max(I)}$ and Michaelis Menten constant in the presence of the inhibitor $K_{M(I)}$

$[atenolol]_0 \times 10^7$ (mol/l)	$r_{max(I)} \times 10^2$ (mol/ls)	$K_{M(I)} \times 10^4$ (mol/l)
1.00	2.98	2.68
2.00	2.53	2.35
3.00	1.94	3.19
4.00	1.87	1.66

partial inhibition, a non-linear fit of the intercept $1/r_{max(I)}=f([I]$ and the slope $K_{M(I)}/r_{max(I)}=f$ [I] gave negative values for β.

Therefore it is very likely that atenolol acts as a reversible full mixed inhibitor according to the reaction scheme:



Scheme 1.
Mixed inhibition mechanism for atenolol, where E stands for catalase, S for hydrogen peroxide and I for atenolol.

The plot (Fig.3) of slopes and intercepts respectively against the inhibitor concentration were used to determine the dissociation constants mentioned in the scheme above, $K_I=(2.023\pm0,2)\times10^{-6}$ M of EI and $K'_I=(2.94\pm0,2)\times10^{-7}$ M Of ESI . The constant K_I was obtained from the slope of the $K_{M(I)}/r_{max(I)}$ versus [atenolol] graph:

$$K_I = \frac{K_M}{r_{max} \cdot slope} \quad (6)$$

while the K'_I constant was determined from the slope of the $1/r_{max(I)}$ versus [atenolol] graph:

$$K'_I = \frac{1}{r_{max} \cdot slope} \quad (7)$$

Conclusions

The catalase –catalyzed decomposition of hydrogen peroxide in phosphate buffer was studied in presence of atenolol. The atenolol acts like a fully mixed inhibitor, as observed from Lineweaver-Burk plots.

Michaelis Menten parameters were determined for the decomposotion of H_2O_2 in the presens of catalase yielding the values of K_M =3.94x10^{-4} mol/L and r_{max}=4.64x10^{-2}mol/$L^{-1}s^{-1}$

REFERENCES

1. B.Chance , H.Sies, A. Boveris, Hydroperoxide Metabolism in Mammalian Organs, *Physiol Rev*., **1979**, 59, 527-605.
2. A. Deisseroth, A.L.Dounce, Physical and Chemical Properties, Mechanism of Catalysis and Physiological Role*, Physiol Rev*. **1970**, 50, 319-375.
3. B. Chance H.Sies, A. Boveris, *Physiol Rev*., **1979**, 59, 527-605.
4. B.Chance, *Acta Chem Scand*, **1947**, 1, 236-267.
5. P. Jones, A Suggett, *Biochem J*., **1968**, 110, 617-620.
6. P.George, *Biochem J*., **1949**, 44, 197-205.
7. B. Chance , D. Herbert, *Biochem J*., **1950**, 46, 402-414.
8. OM. Lardinois, MM. Mestdagh, PG. Rouxhet, *Biochim Biophys Acta*, **1996**, 1295, 222-238.
9. K.Kikuchi, Y. Kawamura-Konishi, H.Suzuki, *Arch. Biochem. Biophys*, **1992**, 296, 88-94.
10. M.L.Kremer, *J.Chem. Soc., Faraday Trans*. **1985**, 1, 81,91-104.
11. M.L.Kremer, *J.Phys Chem*. **1981**, 85, 835-839.
12. . B.Chance, *J. Biol. Chem*., **1949**, 179, 1299-1309.
13. Y. Ogura, Y. Tonomura, S.Hino, H. Tamiya, *J. Biochem*-Tokyo, **1950**, 37, 153-177; Y. Ogura, I. Yamazaki , idem, **1983,** 94, 403-408.
14. E. Margoliash, A. Novogrodsky, *Biochem J*, **1958**, 468-475.
15. K. Yoshihiro, S.Yoshiyuki, H. Jingyi, K. Takahiro, *J. Chem.Soc. Perkin Trans*, **1995** ,2, 1749-1759

16. ***Pharmacopee Europeene,3-rd Ed. **1997**.
17. M.A. Barret, in Beta-adrenoceptive agonist;in Recut Advances in Cardiology , ed Churchill Livingstone , **1973**, p 289.
18. B.N. C. Pritchard, G.S. Avery, Cardiovascular Drug , β-AdrenoceptorBloking Drug, **1977**, p 85
19. P. Jones, A. Sugget*, Biochem J*., **1968**, 108, 833-838.
20. DP. Nelson, LA. Kiesow, *Anal. Biochem*, **1972**, 49, 474-478.
21. P.Jones, Wynnes-Jones WFK, *T.Faraday Soc*.,**1972**, 49, 474-478.
22. ML Kremer, *J. Chem. Soc Farad T* ,**1983**, 79, 2125-2131.
23. P.Nicholls, P. Loewen, I. Fita Enzymology and structure of catalases in Meme – Fe Proteins,Advances in Inorg. Chem. Ed A.G.Sykes and G. Mauk Vol 51, pp 52-106, **2001**
24. L. Michaelis ,M. Menten, *Biochem.Z*, **1913**, ,49, 33-369.
25. H.Lineweaver, D. Burke, J*. Am. Chem. Soc* ,**1934**, 56, 658-666.
26. JL. Gelpi, JJ.Aviles, M. Busguets, S.Imperial, A. Mazo, A. Cortes, *J. Chem Educ*. ,**1993,** 70,805-816.