Catalytic Decomposition of 3-Chloroperoxybenzoic Acid by Immobilized Catalase in a Non-Aqueous Medium

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Catalytic activities of catalase (CAT) immobilized on graphite – GMZ™, soot – "NORIT" and "PM-100" to mediate decomposition of $3\text{-Cl-C}_6\text{H}_4\text{COOOH}$ (3-CPBA) in acetonitrile have been investigated. Under these conditions, the kinetic parameters K_m, k, E_a, V_{max}, and Z_0 were calculated. Conclusions on a probable mechanism of the catalytic process observed were drawn from the calculated values of ΔG^*, ΔH^*, and ΔS^*. A quantitative UV-spectrophotometrical approach was used as the basic analytical tool. The electrochemical reduction of oxygen generated in enzyme catalysed 3-CPBA decomposition was examined with polarization curves method.

Introduction

In biocatalytic and electrochemical systems catalase is mainly used in immobilized state. The biocatalytic activity of catalase was studied in non-aqueous solvents (Wang *et al.*, 1995). It was used for working out an organic-phase amperometric biosensor by immobilizing the enzyme in a polymeric film on a glass-carbon surface (Wang *et al.*, 1995). The thermal activity of immobilized catalase, using a polyacrylamide support for immobilization, was studied (Jang and Zhang, 1993). A catalase biosensor for hydrogen peroxide and for the inhibitors of the enzyme (fluorides, cyanides), was described (Stein and Hain, 1995). In co-immobilization with glucose oxidase, catalase is used for creating enzyme membranes for the electrochemical determination of glucose (Liu *et al.*, 1979). Addition of lactate oxidase and catalase to lactate dehydrogenase causes 1 000-fold increase in sensitivity of the determination of lactate (Scheller *et al.*, 1985). The sensitivity of the determination of H_2O_2 with a peroxidase electrode is increased by two orders of magnitude (Tatsuma *et al.*, 1994) when the electrode is coated with a film of catalase.

A biosensor for the determination of L-lysine was prepared, consisting of a Clark-type oxygen sensor and a convenient support with an immobilized enzyme system (catalase and L-lysine-alpha-oxidase) fixed on its surface is described (Vrbova *et al.*, 1992). The same author (Vrbova *et al.*, 1993) used catalase co-immobilized with holine oxidase for holine biosensor development. An on-line electrochemical sensor for the continuous measurements of gamma-aminobutyric acid (GABA), composed of a glutamate oxidase and catalase immobilized in a small-volume enzymatic reactor and a modified glass carbon electrode, was described (Niwa *et al.*, 1998).

A large part of investigations in studying biocatalytic and bioelectrochemical activity of immobilized catalase has been performed in neutral aqueous solutions. Therefore, many hydrophobic substrates are inaccessible for analysis in water solvents. The analysis of hydrophobic analytes has been discussed in current literature by a limited way.

To study biocatalytic activity of immobilized catalase in acetonitrile on 3-chloroperoxybenzoic acid decomposition and to examine the electrochemical reduction of oxygen produced in this process is the object of the present work.

Materials and Methods

Catalase (CAT) was (EC 1.11.1.6) from *Penicillium chrysogenum* 245 (Biovet – Peshtera, Bulgaria). The specific activity of the enzyme is 1000

U×mg^{-1} (International enzyme unit: 1U = mol of substrate reacting or product produced per minute). The reagents for the solutions, Na$_2$H-PO$_4$×12H$_2$O, KOH, H$_3$PO$_4$, citric acid, were of analytical grade qualification. The solutions were prepared with bidistilled water.

Acetonitrile for UV spectroscopy (Fluka), was used as reaction medium; 3-chloroperoxybenzoic acid (3-Cl-C$_6$H$_4$COOOH) (3-CPBA), with analytical grade qualification was purchased from Fluka.

The carbon materials used were: graphite GMZ^{TM} with a geometric surface S = 0.02 m^2×g^{-1}, density 1.62 ± 0.03 g×cm^{-3}, porosity 22%; soot "NORIT" and soot "PM-100". The two types of soot differ in their structure. The "NORIT" soot has a fine-grained structure, with an average size of particles of 5×10^3 – 45×10^3 nm and the "PM-100" soot are built up of larger globular particles with an average size of 21×10^3 – 340×10^3 nm. The two types of soot were kindly provided by the Institute of Electrochemistry in Moscow, Russia and the graphite (GMZ^{TM}) plates – by the State University of Moscow, Russia.

To study the catalase activity of the enzyme, the adsorption of catalase on both kinds of soot was performed under static conditions. 10 mg of adsorbent were added to 1 ml reaction volume including catalase with a start concentration of enzyme c = 10^{-4} M in phosphate-citrate buffer (pH = 7.00). The amount of the enzyme adsorbed was determined spectrophotometrically by the decrease of the catalase concentration in the solution after adsorption. The spectrophotometer used was Specord UV VIS (Carl Zeiss, Jena, Germany). The amount of the catalase in the solution was determined on the basis of a calibration graph for the maximum at λ_{max} = 280 nm. The adsorption of catalase on graphite was performed by the following procedure: Catalase is adsorbed on the electrochemically activated graphite (GMZ^{TM}) electrode surface. The electrochemical pretreatment of the graphite electrode was a cathode-anode cyclization (30 min) in the potential range of −0.2 – +0.6 V (Ag/AgCl). Just before immobilization, the graphite electrode was polarized for 2 minutes at E = 1.5 V. The adsorption of catalase was carried out by immersing the graphite electrode in the solution of the enzyme with a concentration c = 10^{-5} M, in phosphate buffer (pH = 7.0) for 30–60 min. After the adsorption the electrode was dried in the

air, at room temperature, for 45 min. Both on soot and on graphite the adsorption was conducted at room temperature.

The catalytic activity of catalase immobilized on both types of soot was estimated by the rate of 3-CPBA decomposition in acetonitrile. The enzyme reaction kinetics was monitored with a quantitative UV-spectrophotometrical approach at λ_{max} = 230 nm. All experiments were performed 3–5 times with a standard deviation RSD = 3%.

The electrochemical measurements on the reduction of oxygen generated from the enzymatic decomposition of 3-CPBA were carried out by the method of the stationary polarization curves in potentiostatic regime. The experimental system involved: a bipotentiostat, type BiPAD (TACUSSEL, Villeurbanne, France); a generator, type EG-20 (Elpan, Lubawa, Poland); a digital voltmeter, type 1AB105 (Priborostroitelen zavod, Pravets, Bulgaria).

The electrochemical measurements were performed in three electrode cell in acetonitrile. A silver-silver chloride electrode was used as a reference electrode and a platinum wire as counter electrode.

The experiments were repeated 3–5 times. Deviation from ten mean was about 3%.

For maintaining constant temperature a thermostat UH (VEB MLW Prufgerate Werk, Medingen, Sitz Freital, Germany) was used. A pH-meter OP-208 (Radelkis, Hungary) was used in the preparation of the buffer solutions.

Results and Discussion

Fig. 1 and Fig. 2 show the kinetic curves of 3-chloroperoxybenzoic acid decomposition in acetonitrile by catalase immobilized on "NORIT" and on "PM-100". These curves give the concentration of substrate (3-CPBA) changes with time in organic medium (acetonitrile). The rate of the process catalyzed by the immobilized enzyme depend specifically on the nature of the support used (Fig. 1), on temperature and 3-CPBA concentration (Fig. 2). The dependencies given have a hyperbolic trend typical for enzyme kinetics. The kinetic parameters of the enzyme process K_m and V_{max}, are calculated from the dependence of rate on concentration: for catalase immobilized on "NORIT" K_m = 714.29 μM and V_{max} = 18.1 μmol×
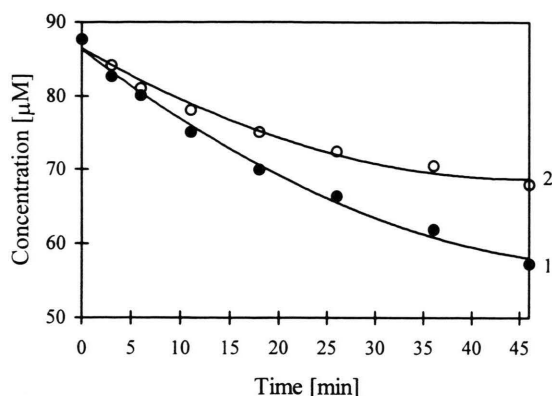
Fig. 1. Kinetic curves of catalytic decomposition of 3-chloroperoxybenzoic acid by catalase immobilised on: 1 – "NORIT"; 2 – "PM-100". Temperature 26 °C; concentration of substrate c = 90.9 μM.
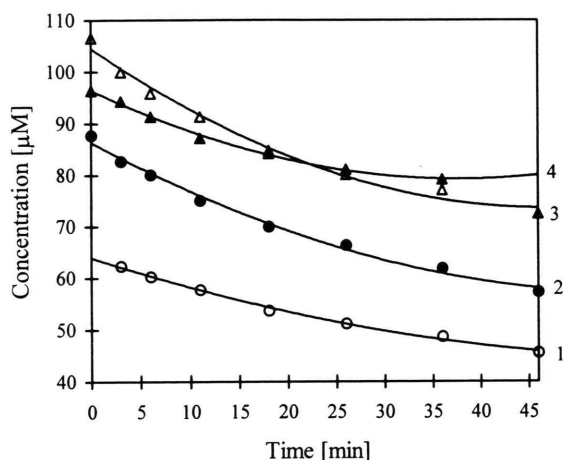

Fig. 2. Kinetic curves of catalytic decomposition of 3-chloroperoxybenzoic acid by catalase immobilized on "NORIT". Concentration of substrate, μM: 1–69.8; 2 and 4–90.9; 3–111.1; temperature, °C: 1, 2, 3–26; 4–10.

Fig. 3. Relationship logarithm of concentration – time for 3-chloroperoxybenzoic acid decomposition by catalase immobilized on: 1 – "NORIT"; 2 – "PM-100". Temperature 26 °C; concentration of substrate c = 90.9 μM.

min^{-1}×mg^{-1}; for catalase immobilized on "PM-100" K_m = 666.67 μM and V_{max} = 15.3 μmol× min^{-1}×mg^{-1}.

To clarify the basic laws of catalysis with catalase adsorbed on both types of soot in 3-CPBA decomposition the effect of temperature on the rate of the process was studied. The values of rate constants at various temperatures were calculated from the kinetic equation for a first order reaction, $k = (1/t) \times ln([c_o]/[c])$, where $[c_o]$ is the start concentration of the substrate; $[c]$ is the current concentration, given in coordinates $ln\ c - t$ (Fig. 3).
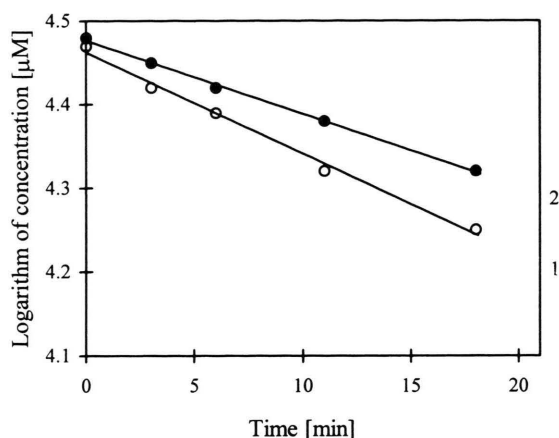
The effect of the temperature on the 3-CPBA decomposition rate was found stronger when catalase is immobilized on "NORIT" (for T = 283 K, k = 6.48×10^{-4} s^{-1}×mg^{-1}; for T = 293 K, k = 13.01×10^{-4} s^{-1}×mg^{-1}, i.e., with an increase of 10 °C the rate is doubled). For "PM-100", within the temperature range of 16 °C, the rate increased 1.7 times (for T = 283 K, k = 3.89×10^{-4} s^{-1}×mg^{-1}; for T = 299 K, k = 6.62×10^{-4} s^{-1}×mg^{-1}). From these data for the specific rate constants it follows that the rate of the process is higher when the enzyme is immobilized on "NORIT". The rate of 3-CPBA decomposition by catalase immobilized on "NORIT" is 1.7 times higher at 283 K compared with the same process catalyzed by catalase immobilized on "PM-100". The activation energy of 3-CPBA catalytic decomposition in acetonitrile by immobilized catalase was calculated by the Arrhenius equation: $ln(k_2/k_1) = (E_a/R) \times (1/T_1 - 1/T_2)$. For the process mediated by CAT/"PM-100" E_a = 23.39 kJ×mol^{-1}, and by CAT/"NORIT" E_a = 48.05 kJ×mol^{-1}. Based on the values for E_a and the temperature effect on the rate of the process it was established that the decomposition rate of 3-CPBA exhibited the typical pattern of a process which is either diffusion-limited (CAT/"PM-100") or located in the kinetic range (CAT/"NORIT") of catalysis. The difference in the rate setting stage of 3-CPBA decomposition, depending on the adsorbent for the immobilization of catalase, can

possibly be explained with the difference in the structure of the two kinds of soot. The rate of diffusion controlled reactions with immobilized enzymes decreases with the growth of the size of particles (Berezin *et al.*, 1987). For this reason on "PM-100" which is built up of coarse globular particles, the rate setting stage is the diffusion of the substrate.

The activation parameters of 3-CPBA decomposition by catalase adsorbed on soot were calculated (Table I) using the basic equation in the theory of the transition state (1), the relationship between E_a and ΔH^* (2), and the Gibbs equation (3).

$$k = \frac{k_B T}{h} \times e^{(\Delta S^*/R)} \times e^{(-\Delta H^*/RT)}, \qquad (1)$$

where k_B is the Bolzmann constant; h is Planck constant; ΔS^* is the activation entropy change, ΔH^* is the activation enthalpy change; $R = 8.314$ J×K^{-1}×mol^{-1} is the gas constant.

$$E_a = \Delta H^* + RT. \qquad (2)$$
$$\Delta G^* = \Delta H^* - T\Delta S^* \qquad (3)$$

ΔG^* is the Gibbs energy of activation change.

Table I. Kinetic and activation parameters of 3-CPBA decomposition by catalase immobilized on soot (T = 299 K).

Soot	Kinetic parameters	Activation parameters
NORIT	$k = 13.01 \times 10^{-4}$ s^{-1}×mg^{-1} $E_a = 48.01$ kJ×mol^{-1} Zo = 4.8×10^5 s^{-1}	$\Delta S^* = -144.33$ J×K^{-1}×mol^{-1} $\Delta H^* = 45.61$ kJ×mol^{-1} $\Delta G^* = 87.90$ kJ×mol^{-1}
PM-100	$k = 6.62 \times 10^{-4}$ s^{-1}×mg^{-1} $E_a = 23.39$ kJ×mol^{-1} Zo = 8.09 s^{-1}	$\Delta S^* = -256.86$ J×K^{-1}×mol^{-1} $\Delta H^* = 20.91$ kJ×mol^{-1} $\Delta G^* = 91.43$ kJ×mol^{-1}

The data for ΔS^* in Table I indicate that the decomposition 3-CPBA in acetonitrile by catalase immobilized on "PM-100" takes place with a smaller change in the entropy of activation than on "NORIT". This finding explain the lower rate of the process on this soot and the discrepancy between the values for the activation energy and the rate constants for the two kinds of soot.

The steric factor P (dimensionless) was calculated from the equation $P = e^{(\Delta S^*/R)}$. For catalase adsorbed on "NORIT", $P = 2.9 \times 10^{-8}$ and for cata-

lase on "PM-100", $P = 5.0 \times 10^{-13}$. For both types of soot $P \ll 1$, but for "PM-100" its value is much lower than for "NORIT". Obviously, the steric interferences for "PM-100" are much stronger.

The electrochemical reduction of oxygen generated from the enzymatic decomposition of 3-CPBA was found to be strictly quantitative through properly defined experimental conditions. This was further used for developing an amperometric enzyme electrode by immobilizing catalase on graphite (GMZ™). Fig. 4 shows polarization curves for oxygen reduction on graphite electrode. Curve 1 gives the current of the graphite electrode without enzyme adsorbed in 3-CPBA solution, curves 2 and 3 give the current of the enzyme electrode (CAT/GMZ™) in the solution with various substrate concentrations. The electroreduction of oxygen in acetonitrile does not take place at the potentials E > -0.3 V on pure graphite (curve 1). For the graphite electrode with catalase adsorbed with increase of the substrate concentra-
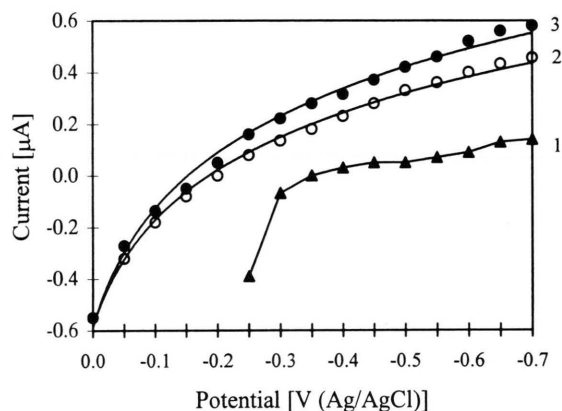
Fig. 4. Polarization curves of the electroreduction of oxygen generated on enzymatic decomposition of the substrate in acetonitrile: 1 – graphite (GMZ™) electrode in 3-chloroperoxybenzoic acid solution; 2 and 3 – graphite electrode with immobilized catalase in 3-chloroperoxybenzoic acid solution. Concentration of 3-CPBA, μM: 1, 2–17; 3–48; temperature 26 °C.

Table II. Electroreduction rate I [μA], of oxygen generated in 3-CPBA decomposition by immobilized on graphite catalase at various potentials and substrate concentrations.

[3-CPBA] [μM]	Potential of polarization [V]					
	−0.40	−0.45	−0.50	−0.55	−0.60	−0.65
17	0.23	0.28	0.33	0.36	0.40	0.46
48	0.28	0.35	0.42	0.46	0.50	0.56

tion (curves 2 and 3) the electroreduction rate also increases (Table II) which is clearly marked at potentials E < −0.4 V. The effect observed proves that catalase immobilized on graphite shows biocatalytic activity in the process of 3-CPBA decomposition and the oxygen produced undergoes an electrochemical reduction on graphite electrode.

In conclusion, the above results may have relevance to the development of amperometric biosensors applicable to measurements of 3-CPBA concentration under non-aqueous conditions.

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