

Adsorption, Catalytic and Electrochemical Activity of Catalase Immobilized on Carbon Materials

E. Horozova*, N. Dimcheva and Z. Jordanova

Department of Physical and Theoretical Chemistry, University of Plovdiv 24, Tsar Assen St., Plovdiv 4000, Bulgaria

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The adsorption of catalase on two types of soot differing in their structure has been characterized. The adsorption of this enzyme obeys the Tyomkin adsorption isotherm. It has been established that the catalase immobilized on soot and graphite takes part in the electrochemical oxidation of phenol. The enzyme activity of catalase immobilized on both types of soot was studied on the decomposition of hydrogen peroxide. The kinetic and activation parameters of the processes studied have been determined.

Introduction

The catalase macromolecule consists of four subunits – each of them involving ferriporphyrin as a prosthetic group (Metelitsa, 1984). The gross molecular mass of catalase is $M_r=250\,000$. Catalase is a highly specific enzyme and its basic function is high performance catalysis of hydrogen peroxide decomposition with liberation of water and molecular oxygen. Besides that, catalase also shows a moderate peroxidase activity, i.e. it can speed up oxidation reactions with hydrogen peroxide (Artemchik *et al.*, 1985, 1986). It has been proved by spectroscopy that catalase like peroxidase forms three compounds when reacted with hydrogen peroxide. When catalase reacts with hydrogen peroxide compound I (an intermediate enzyme-substrate complex I) is formed. Furtheron it can oxidise the hydrogen peroxide. The loss of an oxidation equivalent in compound I leads to the formation of compound II. Compound III is formed on the oxidation of compound II with hydrogen peroxide and has three oxidation equivalents of Fe(III). (Dixon and Webb, 1966; Hughes, 1983). Compound I has high activity and takes part in the enzymatic process. Not only hydroperoxides but also other hydrogen donors can react

with compound I – ethanol for example. The activity of compound II is by 10^4 lower than that of compound I, while compound III has no enzymatic activity at all.

In biocatalytic and electrochemical systems catalase is mainly used in immobilized state. A high activity of adsorbed catalase was achieved by its sorption immobilization on cellulose (Eremin *et al.*, 1995), on silica gel modified with fatty acids or phospholipids (Veselova *et al.*, 1974), as well as on activated carbon fibres and tissues (Litvinchuk *et al.*, 1994). The biocatalytic activity of catalase was also studied in nonaqueous solvents (Eremin *et al.*, 1994, 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 pad 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). Additions of lactate oxidase and catalase to lactate dehydrogenase brings about a 1000 fold increase in sensitivity of the determination of lactate (Sheller *et al.*, 1985). The sensitivity of the determination of H_2O_2 with peroxidase electrode coated with a film of catalase

Reprint requests to Dr. E. Horozova.

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is increased by two orders of magnitude (Tatsuma *et al.*, 1985).

The objective of the present work is to study the adsorption, catalase and electrocatalytic activity of catalase immobilized on carbon materials.

Materials and Methods

The catalase used was (EC 1.11.1.6) from *Penicillium chrysogenum* 245 (Biovect – Bulgaria). The specific activity of the enzyme is $1000 \text{ U} \times \text{mg}^{-1}$. The reagents for the solutions, $\text{Na}_2\text{HPO}_4 \times 12\text{H}_2\text{O}$, KOH , H_3PO_4 , citric acid, KMnO_4 , phenol, and H_2O_2 , were with analytical grade qualification. The solutions were prepared with bidistilled water.

The carbon materials used were: graphite with a geometric surface $S = 1.6\text{--}1.8 \text{ cm}^2$. The structure characteristics of graphite are as follows: specific surface $S = 0.02 \text{ m}^2 \times \text{g}^{-1}$, density $1.62 \pm 0.03 \text{ g} \times \text{cm}^{-3}$, porosity 22%; soot "NORIT" and soot "PM-100".

The two types of soot differ in their structure. The "NORIT" soot has fine-grained structure, with an average size of particles of $5 \times 10^4 - 45 \times 10^4 \text{ \AA}$ and the "PM-100" soot are built up of larger globular particles with an average size of $21 \times 10^4 - 340 \times 10^4 \text{ \AA}$. The two kinds of soot were kindly provided by the Institute of Electrochemistry in Moscow, Russia.

To study the catalase activity of the enzyme the adsorption of catalase on both types of soot was performed by an adsorption method under static conditions in a 1 ml reaction volume including catalase with a start concentration of enzyme $C = 1 \times 10^{-4} \text{ M}$ in phosphate-citrate buffer ($\text{pH} = 7.02$), and 10 mg of soot. 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 $\lambda_{\text{max}} = 280 \text{ nm}$. The adsorption of catalase on graphite was performed by a procedure described by Horozova *et al.*, 1995. Both on soot and on graphite the adsorption was conducted at room temperature.

The catalase activity of dissolved catalase immobilized on soot was determined by the decomposition reaction of H_2O_2 . The amount of the

hydrogen peroxide was determined by permanganometry.

The electrochemical measurements on the oxidation of phenol by immobilized catalase were carried out by using the method of the stationary polarization curves in potentiostatic regime. The experimental system involved: Potentiostat $\Pi\text{A-5848}$ (Zavod izmeritel'nykh priborov, Gomel, Russia), recording device XY-Recorder (VEB Messapparatwerk, Schlotheim, Germany), Multimeter G-1004.500 (RFT VEB Mikroelektronik "Karl Marx", Erfurt, Germany). The electrochemical measurements were performed in three electrode cell in phosphate-citrate buffer ($\text{pH} = 7.02$). A silver-silver chloride electrode was used as a reference electrode and a platinum wire as counter electrode. Two kinds of working electrodes were used: a) tablets of hydrophobized soot with a deposited active layer (1–2 mg) of soot "NORIT" or soot "PM-100" and b) compact graphite electrodes with adsorbed catalase. The solution was purged with argon during the voltammetric measurements.

Results and Discussion

The kinetic curves for adsorption of catalase from $2 \times 10^{-4} \text{ M}$ solution of the enzyme with $\text{pH} = 7.02$ on both types of soot are shown in Fig. 1. The curves give the enzyme concentration change with the time in the solution from which the immobilization takes place. From the curves is seen that the adsorption proceeds at a higher rate on "PM-100" than on "NORIT". It is confirmed by the data for the adsorption rate constants calculated

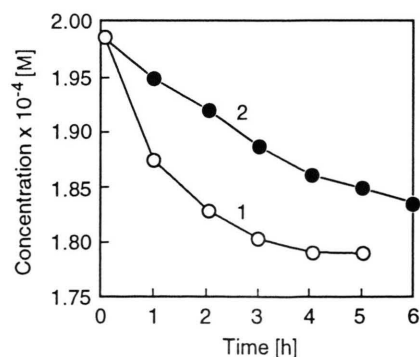


Fig. 1. Adsorption kinetic curves for catalase immobilized on soot: PM-100 (1) and NORIT (2). Start concentration of the enzyme $2 \times 10^{-4} \text{ M}$, $\text{pH} = 7.02$.

from the relationship $\ln A - t$, where A is the adsorption proportional to the current concentration of the enzyme in the solution. The linear course of the relationship shows that the adsorption of catalase on both types of soot obeys the kinetic equation for a first order reaction. On "PM-100" soot the adsorption of the enzyme is characterized by the rate constant $k = 9.33 \times 10^{-6} \text{ min}^{-1} \times \text{mg}^{-1}$, and on the "NORIT" soot by $k = 7.66 \times 10^{-6} \text{ min}^{-1} \times \text{mg}^{-1}$. The values for the specific rate constants show that on "PM-100" the catalase adsorption rate is 1.2 times higher than that on "NORIT". The difference in the rate of the enzyme adsorption can be explained with the difference in the structures of the two types of soot. The large globular particles in the structure of "PM-100" favour the higher adsorption rate of the enzyme. The maximum amount of adsorbed enzyme also depends on the type of the adsorbent. For adsorption from solutions of catalase with concentration $C = 10^{-4} \text{ M}$, with $\text{pH} = 7.02$, the maximum amount of catalase adsorbed on "NORIT" was 60 mg per gram of soot, and on "PM-100" – 38 mg per gram of soot. Besides on the type of the adsorbent the maximum amount of adsorbed protein depends on the pH of the catalase solution. On adsorption from catalase solution (10^{-4} M) with $\text{pH} = 3.02$, on "NORIT", the maximum amount of the adsorbed enzyme was $40 \text{ mg} \times \text{g}^{-1}$, and on "PM-100" – $28 \text{ mg} \times \text{g}^{-1}$. The decrease in the soot adsorption capacity in acid solutions of catalase is due to decomposition of the quaternary structure of the enzyme (Artemchik *et al.*, 1985).

The relationship between the amount of catalase adsorbed and its concentration in the solution was studied with "NORIT". With the increase in the concentration of the enzyme in the solution the amount of the adsorbed catalase increases. The relationship is linear up to a concentration of $1.25 \text{ mg} \times \text{ml}^{-1}$. At concentrations over $3 \text{ mg} \times \text{ml}^{-1}$ the adsorption value increases up to about $60 \text{ mg} \times \text{ml}^{-1}$ and then remains constant. In Fig. 2 the relationship $g_{\text{KAT}} = f(-\ln C_{\text{KAT}})$ is shown. It is seen that for an average amount of adsorbed compound the relationship is linear which confirms that the adsorption of catalase on "NORIT" obeys the Tyomkin adsorption isotherm, i.e. an adsorption on an energetically heterogeneous surface takes place.

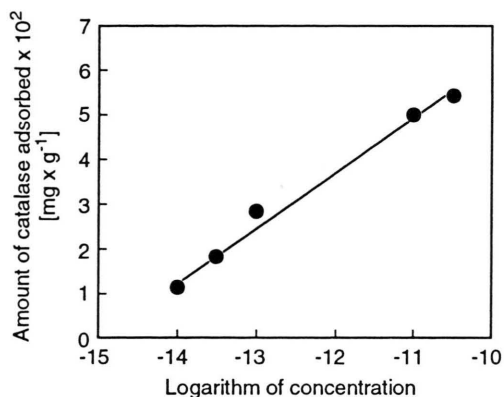


Fig. 2. Relationship $g_{\text{KAT}} = f(-\ln C_{\text{KAT}})$ at adsorption of catalase on NORIT soot.

The enzymatic activity of catalase on the decomposition of hydrogen peroxide was studied with the enzyme adsorbed on both kinds of soot – "NORIT" and "PM-100". The activity of the enzyme in solution was compared to that in immobilized state. It has been shown that the enzyme in immobilized state retains its activity and the dependence of the reaction rate on the concentration of the substrate has a hyperbolic character in both cases. The kinetic parameters of the enzyme reaction were calculated by the relationship between the decomposition rate of hydrogen peroxide and the concentration of the substrate. The kinetic parameters are as follows: for catalase in solution – $K_m = 2.0 \times 10^{-2} \text{ M}$ and $V = 7.14$; for catalase immobilized on the "NORIT" soot $K_m = 2.5 \times 10^{-2} \text{ M}$ and $V = 2.50$, and on the "PM-100" soot $K_m = 1.7 \times 10^{-2} \text{ M}$ and $V = 14.29$. From these data follows that the enzyme activity decreases after immobilization.

In order to clarify the kinetic laws of hydrogen peroxide decomposition with catalase immobilized on both kinds of soot, the effect of the temperature on the rate of the process was studied. By the kinetic equation for a reaction of first order, $k = \frac{1}{t} \ln \frac{[C_0]}{[C]}$, where $[C_0]$ is the start concentration of the substrate; $[C]$ is the current concentration, given in the coordinates $\ln C - t$ (Fig. 3), the rate constants of the catalytic process at various temperatures were calculated. The effect of the temperature on the decomposition rate of hydrogen peroxide was found stronger when the enzyme was

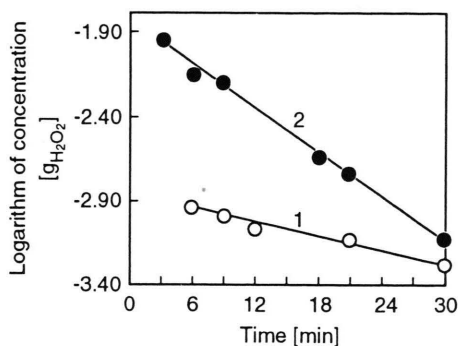


Fig. 3. Function $\ln C - t$ for H_2O_2 decomposition by catalase immobilized on NORIT at different temperatures [°C]: 1 – 20; 2 – 30.

adsorbed on “NORIT” (for $T = 288\text{ K}$, $k = 8.18 \times 10^{-3} \text{ s}^{-1} \times \text{mg}^{-1}$ and for $T = 303 \text{ K}$, $k = 18.63 \times 10^{-3} \text{ s}^{-1} \times \text{mg}^{-1}$, i.e., with an increase of 15°C the rate is doubled). For “PM-100”, within the temperature range of 10°C , the rate increased only 1.25 times (for $T = 288 \text{ K}$, $k = 9.19 \times 10^{-3} \text{ s}^{-1} \times \text{mg}^{-1}$, and for $T = 298 \text{ K}$, $k = 11.42 \times 10^{-3} \text{ s}^{-1} \times \text{mg}^{-1}$). From these data for the specific rate constants it follows that the process rate is higher when catalase is immobilized on the “NORIT” soot. The activation energy of the enzymatic decomposition of hydrogen peroxide with catalase in immobilized state was calculated by the Arrhenius equation drawn in coordinates $\log k - 1/T$. For the process on the “PM-100” soot $E_a = 16.5 \text{ kJ} \times \text{mol}^{-1}$ and on “NORIT” – $E_a = 41.3 \text{ kJ} \times \text{mol}^{-1}$. Based on the values for E_a and the temperature effect on the rate of the process it was established that on “PM-100” the process is limited by diffusion, and on “NORIT” it takes place in the kinetic range of catalysis. The difference in the rate setting stage of hydrogen peroxide 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 the diffusion controlled reactions with the immobilized enzyme decreases with the growth of the size of the particles (Berezin *et al.*, 1987). That is why on “PM-100” which is built up of coarse globular particles, the rate setting stage is the diffusion of the substrate.

From the data for the rate constants at various temperatures and for E_a , by the basic equation in the theory of the transition state $k =$

$\frac{k_B T}{h} e^{\Delta S^*/R} e^{-\Delta H^*/RT}$, where k_B is the Boltzmann constant; h is the Planck constant; ΔS^* is the activation entropy change and taking into consideration that $E_a = \Delta H^* + RT$, we calculated the activation parameters of the decomposition of H_2O_2 with catalase adsorbed on soot (Table I).

Table I. Kinetic and activation parameters of hydrogen peroxide decomposition by catalase immobilized on soot ($T = 298 \text{ K}$).

Soot	Kinetic parameters	Activation parameters
N	$k = 14.58 \times 10^{-3} \text{ s}^{-1} \times \text{mg}^{-1}$	$\Delta S^* = -92.46 \text{ J} \times \text{K}^{-1} \times \text{mol}^{-1}$
O		
R	$E_a = 41.20 \text{ kJ} \times \text{mol}^{-1}$	$\Delta H^* = 38.79 \text{ kJ} \times \text{mol}^{-1}$
I		
T	$Z_0 = 2.5 \times 10^5 \text{ s}^{-1}$	$\Delta G^* = 66.34 \text{ kJ} \times \text{mol}^{-1}$
	$k = 11.42 \times 10^{-3} \text{ s}^{-1} \times \text{mg}^{-1}$	$\Delta S^* = -177.73 \text{ J} \times \text{K}^{-1} \times \text{mol}^{-1}$
P		
M	$E_a = 16.46 \text{ kJ} \times \text{mol}^{-1}$	$\Delta H^* = 13.97 \text{ kJ} \times \text{mol}^{-1}$
100	$Z_0 = 8.9 \text{ s}^{-1}$	$\Delta G^* = 66.93 \text{ kJ} \times \text{mol}^{-1}$

The data for ΔS^* in Table I indicate that the decomposition of hydrogen peroxide by catalase immobilized on “PM-100” takes place with a smaller change in the entropy of activation than on “NORIT”. This fact explains 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 decomposition of H_2O_2 in the presence of enzymes which bear active centres (Fe)protoporphyrin IX, (similar to those of catalase) takes place by following of two competitive mechanisms (Metelitsa, 1984; Eremin *et al.*, 1995). The first one (ionic or “productive”) leads to the formation of H_2O and O_2 and the second (radical or “unproductive”) which comprises the formation of HO_2^\bullet and HO^\bullet radicals, responsible for the destruction of biocatalyst. The calculated ΔS^* values show that the ionic mechanism is predominant when catalase is immobilized on “NORIT” since the second one (radical) is more probable in the case when catalase is immobilized on “PM-100”. The steric factor P was calculated from $P = e^{\Delta S^*/R}$. For catalase adsorbed on “NORIT”, $P = 1.6 \times 10^{-5}$, and for catalase on “PM-100” – $P = 0.55 \times 10^{-9}$. 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. That confirms the above sugges-

tion for the predominant radical mechanism of decomposition of H_2O_2 by catalase adsorbed on the "PM-100" soot.

Fig. 4 shows the data of the catalytic stability of catalase in immobilized state. The catalytic stability of the enzyme is the degree of retaining of its catalytic activity in its repeated use in immobilized state. From the figure is seen that for 6 cycles the specific catalytic activity of catalase immobilized on "NORIT" decreases 4 times and of catalase immobilized on "PM-100" – 6 times, i.e. the catalytic stability of catalase immobilized on "NORIT" is bigger than when immobilized on "PM-100". These data confirm the above assumption for the dominant radical mechanism of decomposition of H_2O_2 by catalase adsorbed on the "PM-100" soot. By using the active complex theory the rest of the activation parameters, the enthalpy of activation ΔH^* and isobaric-isothermic potential of activation ΔG^* , were also calculated (Table I). The values for ΔG^* are the same for both adsorbents. This is explained by the compensation relationship between ΔH^* and $-T\Delta S^*$.

Catalase adsorbed on carbon materials undergoes a redox transformation affecting the iron in the heme of the enzyme (Horozova *et al.*, 1995). This fact brought about studies of the participa-

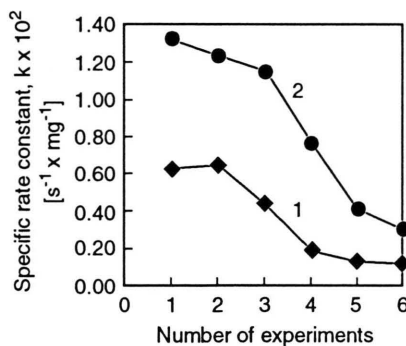


Fig. 4. Catalytic stability of catalase immobilized on NORIT (1) and on PM-100 (2).

tion of catalase immobilized on carbon materials in the acceleration of electrochemical reactions. Fig. 5 shows polarization curves for phenol electrooxidation by catalase immobilized on soot. It is seen that catalase immobilized on "NORIT" does not change its activity and speeds up the electrooxidation of phenol (curve 2). Electrooxidation of phenol was also carried out on an electrode of pure soot "NORIT", (without immobilized enzyme) (curve 1'). It is seen that curve 1' is much lower than curve 2', i.e. the process is considerably speeded up when catalase is adsorbed on "NORIT". An insignificant bioelectrocatalytic effect is observed on the electrooxidation of phenol by catalase adsorbed on "PM-100" soot (curve 2).

A more detailed study was carried out on the electrooxidation of phenol by catalase adsorbed

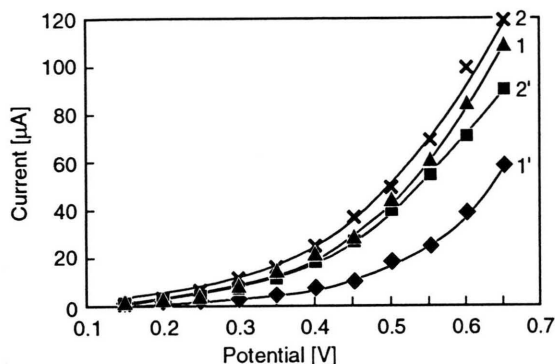


Fig. 5. Polarization curves of electrooxidation of phenol by catalase immobilized on NORIT soot (curves 1' and 2') and on PM-100 soot (curves 1 and 2); 1,1' – soot and phenol in background electrolyte; 2,2' – catalase immobilized on soot and phenol. Concentration of phenol, 2×10^{-5} M.

on graphite. The catalase adsorbed on graphite also speeds up the electrooxidation of phenol. The electrooxidation rate of phenol is increased with both the increase in the concentration of the substrate and with temperature. Reasons for this con-

Table II. Kinetic and activation parameters of electrooxidation of phenol by catalase adsorbed on graphite.

E [V]	I [μA]	E_{ef} [kJ×mol ⁻¹]	ΔG^* [kJ×mol ⁻¹]	$-\Delta S^*$ [J×K ⁻¹ ×mol ⁻¹]	ΔH^* [kJ×mol ⁻¹]
0.20	0.45	43.65	89.85	167.09	41.23
0.25	0.70	43.65	88.78	163.42	41.23
0.30	0.90	43.65	88.18	161.33	41.23

clusion are the values for the anode current higher than those in the electrooxidation curve for phenol on pure graphite (without adsorbed catalase).

The effective activation energy of the oxidation of phenol (Table II) was calculated from the basic equation in electrochemical kinetics $\ln I = -\frac{E_{ef}}{RT} + B$. The activation parameters ΔG^* , ΔS^* and ΔH^* of this process are also given in Table II.

From the data in Table II it is seen that the values of E_{ef} for various polarization potentials are equal. The values for E_{ef} and its independence on the polarization potential indicate that the rate of the electrooxidation of phenol on graphite electrode with immobilized catalase is limited by concentration polarization.

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