Peroxidase-Like Activity of Catalase Immobilized on Carbon Materials

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The immobilization of catalase was carried out from enzyme solutions in acid (pH < 3.5) and alkaline (pH > 11) medium on two kinds of soot differing by the average size of the particles. Under these conditions the peroxidase-like activity of immobilized catalase in the oxidation of phenol has been studied. The effect of the initial concentration of the substrate on the rate of the process catalysed by catalase immobilized on the soot of finer-grained structure has been studied. The relationships obtained are described by the equation of Michaelis-Menten. The kinetic parameters (the constant of Michaelis – K_m, the maximum reaction rate – V_{max}, the rate constant – k and the activation energy – E_a) of the process were calculated. It was found that catalase absorbed on the soot of larger globular particles does not take part in the peroxidase oxidation of phenol.

Introduction

In biocatalytic systems catalase is mainly used in immobilized state. Biocatalytic activity of catalase immobilized on cellulose was studied in nonaqueous solvents (J. 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. The effect of the polyacrylamide matrix for immobilization on the catalytic activity of immobilized catalase was studied in aqueous solutions (Jang and Zhang, 1993). Catalase is also used in co-immobilization with other enzymes such as glucose oxidase (Liu *et al.*, 1979; Wingard Jr. *et al.*, 1983), lactate dehydrogenase (Scheller *et al.*, 1985), or peroxidase (Tatsuma *et al.*, 1994) for creating membrane enzyme electrodes.

In acid (pH < 3.5) and alkaline (pH > 11) medium the catalase tetramer dissociates into monomers (Nelson, 1971). This process is accompanied by a certain loss of catalase activity and optic changes – a shift of absorbance maxima towards the short – wave region (Jones *et al.*, 1982).

It was found that catalase monomers show peroxidase-like activity *i.e.* it is possible to catalyse the oxidation of organic compounds (alkohols, phenols, amines) in the presence of hydrogen peroxide. Catalase monomers are stable in acid and alkaline solutions where enzyme peroxidase is deactivated.

The peroxidase-like function of catalase in solutions was well studied in the oxidation of ethyl alcohol and phenol (Jones *et al.*, 1982). However, there is no data about peroxidase-like activity of immobilized catalase.

To study the possibility for peroxidase-like function of immobilized catalase in acid and alkaline medium on the process of phenol oxidation is the object of the present work.

Materials and Methods

Catalase was (EC 1.11.1.6) from *Penicillium chrysogenum* 245 (Biovet – Peshtera, Bulgaria). The specific activity of the enzyme is 1000 $U \times mg^{-1}$ (International Enzyme Unit – 1U = moles of substrate reacting or product produced per minute). The reagents for the solutions, $Na_2HPO_4 \times 12H_2O$, KOH, H_3PO_4, citric acid, phenol, and H_2O_2, were of analytical grade qualification. The solutions were prepared with bidistilled water.

The carbon materials used were: soot "NORIT" and soot "PM-100". The two types of soot differ in their structure. The "NORIT" soot has a fine-grain structure, with an average size of particles of $5 \times 10^4 - 45 \times 10^4$ Å and the "PM-100" soot is built up of larger globular particles with an average size

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of $21\times10^4-340\times10^4$ Å. The two kinds of soot were kindly provided by the Institute of Electrochemistry in Moscow, Russia.

The adsorption of catalase on both types of soot was performed by an adsorption method under static conditions from 1 ml solution of catalase with concentration of the enzyme C = 100 μM in citrate buffer (pH = 3.02) and in phosphate buffer (pH = 11.21) per 10 mg soot in 24 hours.

The amount of catalase was determined spectrophotometrically by the decrease in the concentration of the catalase in the solution after the adsorption. The spectrophotometric measurements were carried out on a Specord UV VIS (Carl Zeiss, Jena, Germany). The amount of the catalase in the solution was determined on the basis of a calibration graph (Fig. 1) for the maxima at λ_{max} = 276 nm (pH = 3.02) and at λ_{max} = 286 nm (pH = 11.21). The values of the extinction coefficients were $\varepsilon_{276} = 0.9\times10^5$ $1\times mol^{-1}\times cm^{-1}$ and $\varepsilon_{286} = 1.083\times10^5$ $1\times mol^{-1}\times cm^{-1}$. The adsorption of catalase on soot was conducted at 4 °C.

The peroxidase activity of catalase in solution and in immobilized state on both types of soot was studied in the oxidation of phenol. The kinetics of the enzyme reaction was monitored spectrophotometrically by the decrease in the concentration of the substrate at λ_{max} = 270 nm.

Results and Discussion

For catalase immobilized on soot "NORIT" the dependence of the initial oxidation rate of phenol on the start concentration of the substrate was studied (Fig. 2). The obtained relationships are described by the equation of Michaelis-Menten (Stryer, 1981). The values obtained for catalase immobilized on "NORIT" are: at pH = 3.02, $K_m = 3.33\times10^2$ μM, V_{max} = 0.025 [absorbance$\times$min$^{-1}\times$mg^{-1}]; at pH = 11.21, $K_m = 11.76\times10^2$ μM, V_{max} = 0.055 [absorbance$\times$min$^{-1}\times$mg^{-1}].

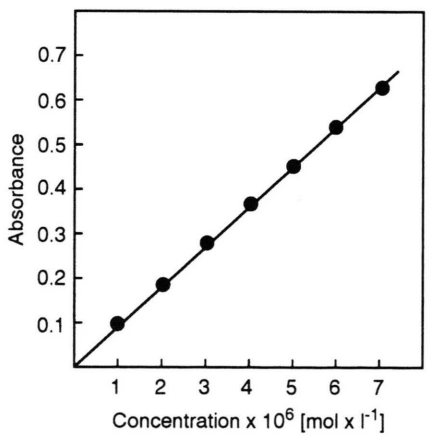

Fig. 1. Calibration graph for determination of catalase concentration in the solution; citrate buffer (pH = 3.02); 1 cm cell.

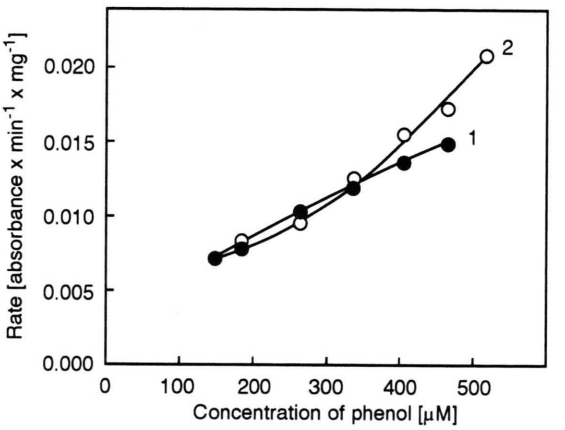

Fig. 2. Dependence of the rate of peroxidase oxidation of phenol on the start concentration of substrate. Catalase immobilized on "NORIT": 1 – at pH = 3.02 (citrate buffer); 2 – at pH = 11.21 (phosphate buffer). Concentration of $H_2O_2C = 2\times10^{-3}$ μM.

The study of phenol oxidation with catalase immobilized on soot "PM-100" showed that the catalase absorbed on the carrier did not indicate peroxidase activity. It follows that the catalase subunits obtained upon dissolving both in citrate buffer with pH = 3.02 and in phosphate buffer with pH = 11.21 are deactivated and lose their peroxidase activity when immobilized on that type of soot. This behaviour of catalase adsorbed on "PM-100" can be explained with the larger average size of the particles ($21\times10^4-340\times10^4$ Å) of that soot. Probably, upon the adsorption of the catalase monomers on those larger particles, favourable conditions for the bonding of monomers set in and recover the quaternary structure of the enzyme which only has a catalase function. The finer-grained structure of the "NORIT" soot (with an average size of the particles $5\times10^4-45\times10^4$ Å) on the one hand is conducive to the adsorption of the catalase subunits and on the other to a change of the conformation and the structure of the protein undissociated upon its immobilization. Thus, on the immobilization of catalase on "NORIT" soot,

there is a possibility of a partial liberation of catalase subunits which are adsorbet to the carrier. Therefore the "NORIT" soot samples with absorbed catalase show peroxidase activity both in acid and alkaline medium.

The rate constants (Table I) for the peroxidase oxidation of phenol at various temperatures were calculated by the kinetic equation for a first order reaction (Fig. 3). Depending on temperature, the immobilized catalase displays catalytic activity which is between 2 and 4 times higher in acid medium (pH = 3.02) than that at pH = 11.21. To explain this finding the rate of dissociation of catalase to subunits was investigated both at pH = 3.02 and pH = 11.21. The kinetic curves obtained (Fig. 4) show that in acid medium the complete catalase dissociation occurs after 1.5 min while this process at pH = 11.21 was found to be complete after 3 min. The absorbance changes with time in the process of catalase dissociation was registered at λ_{max} = 400 nm in acid medium and at λ_{max} = 364 nm in alkaline medium. Under these conditions calculated values of the dissociation rate constants are: at pH = 3.02, $k = 1.90\times10^{-3}$ s^{-1}; at pH = 11.21, $k = 1.60\times10^{-3}$ s^{-1}. The higher rate of catalase dissociation assume the greater number of monomers adsorbed on soot, which explain the higher catalytic activity of catalase subunits in peroxidase oxidation of phenol in extremely acid medium. In the other hand, the rate of an enzymatic reaction is proportional to the enzyme concentration, *i.e.* $V = k[E]$.

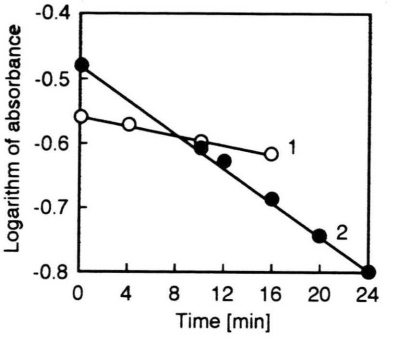

Fig. 3. Relationship logarithm of absorbance – time for peroxidase oxidation of phenol by catalase immobilized on "NORIT": 1 – at pH = 11.21 (phosphate buffer); 2 – at pH = 3.02 (citrate buffer). Temperature 30 °C.

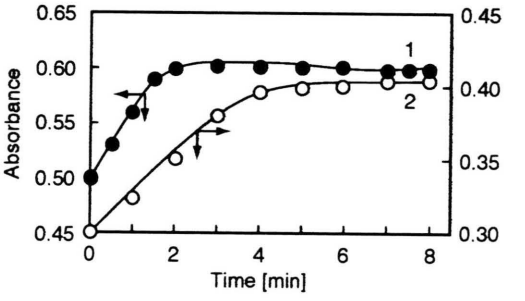

Fig. 4. Dependence of absorbance on time for the process of catalase dissociation into subunits: 1 – in acid medium (pH = 3.02); 2 – in alkaline medium (pH = 11.21). The arrows indicate the *X*- and *Y*-axes corresponding to each curve.

Table I. Kinetic and activation parameters of peroxidase oxidation of phenol by catalase and by peroxidase.

Catalyst	Kinetic parameters	Activation parameters
Catalase immobilized on "NORIT" pH = 3.02	$k = 2.53\times10^{-3}$ s$^{-1}\times$mg^{-1} $E_a = 10.95$ kJ×mol^{-1} $Z_o = 0.195$ s^{-1}	$\Delta S^* = -266.90$ J×K$^{-1}\times$mol^{-1} $\Delta H^* = 8.43$ kJ×mol^{-1} $\Delta G^* = 89.31$ kJ×mol^{-1}
Catalase immobilized on "NORIT" pH = 11.21	$k = 1.36\times10^{-3}$ s$^{-1}\times$mg^{-1} $E_a = 36.15$ kJ×mol^{-1} $Z_o = 2.320\times10^{3}$ s^{-1}	$\Delta S^* = -188.90$ J×K$^{-1}\times$mol^{-1} $\Delta H^* = 33.63$ kJ×mol^{-1} $\Delta G^* = 90.87$ kJ×mol^{-1}
Catalase immobilized on "PM-100" pH = 3.02 and pH = 11.21	does not show peroxidase activity	
Peroxidase immobilized on "NORIT" pH = 7.20	$k = 13.35\times10^{-3}$ s$^{-1}\times$mg^{-1} $E_a = 33.67$ kJ×mol^{-1} $Z_o = 8.514\times10^{3}$ s^{-1}	$\Delta S^* = -178.10$ J×K$^{-1}\times$mol^{-1} $\Delta H^* = 31.15$ kJ×mol^{-1} $\Delta G^* = 85.12$ kJ×mol^{-1}

The activation energy of the peroxidase oxidation of phenol (Table I) by immobilized catalase was calculated from the Arhenius plot in co-ordinates *ln k – 1/T*. The values obtained for E_a fully correspond to the values of the rate constants. For catalase adsorbed on "NORIT" the rate of the peroxidase oxidation of phenol is higher because the activation energy E_a of this process is lower.

On the basis of E_a values we can make a conclusion about the diffusion factors which are some of the most complicated points concerning catalysis with immobilized enzymes. The values for the activation energy in peroxidase oxidation of phenol by catalase immobilized on "NORIT" soot (at pH = 3.02, E_a = 10.95 kJ×mol^{-1}; at pH = 11.21, E_a = 36.15 kJ×mol^{-1}) and the temperature effect on the rate establish that in acid medium this process is limited by diffusion while in alkaline medium it takes place in the kinetic range of catalysis.

It is of interest to compare the kinetic characteristics of peroxidase oxidation of phenol by immobilized catalase with these of phenol oxidation by immobilized peroxidase (Table I). A comparison among the specific rate constants shows that the peroxidase activity of immobilized catalase at pH = 3.02 is 5 times lower and at pH = 11.21 is 10 times low than the catalytic activity of immobilized peroxidase towards the same subtrate – phenol. It should be mentioned that the reactions with these enzymes was realised at different values of pH. It is seen that catalase monomers immobilized on "NORIT" soot are a good catalyst for peroxidase oxidation of phenol. Although it catalytic activity is lower than this one of immobilized peroxidase, it has the unique ability to save relatively high peroxidase activity in strongly acid medium which deactivates enzyme peroxidase.

We used the basic equation of transition state theory: $k = \frac{k_B T}{h} e^{\Delta S^*/R} e^{-\Delta H^*/RT}$ (where k_B is the Bolzman constant; h is the Planck constant; ΔS^* is the activation entropy change), the relationship: $E_a = \Delta H^* + RT$ and the data for the rate constants at various temperatures and for E_a to calculate the activation parameters of the peroxidase oxidation of phenol (Table I). The data obtained indicate that the change of activation entropy in the peroxidase oxidation of phenol by immobilized catalase at pH = 11.21 is smaller than this one in the process of phenol oxidation by immobilized peroxidase. This finding explains the lower rate of phenol oxidation catalysed by immobilized catalase (alkaline medium) and the discrepancy between the values for the activation energy and the rate constants for the two enzymes.

Acknowledgement

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Jang V. and Zhang Y. (1993), Immobilization of catalase on crosslinked polymeric hydrogels – effect of anion on the activity of immobilized enzyme. Eu. Polymer. J. **29,** 1251–1254.

Jones M. N., Manley P., Midley P. J. W. and Wilkinson A. E. (1982), Dissociation of bovine and bacterial catalase by sodium n-dodecyl sulphate. Biopolymers **21,** 1435–1450.

Liu C. C., Wingard L. V., Wolfson S. K., Jao S. J., Drach A. L. and Schiller J. G. (1979), Quantitation of glucose concentration using glucose oxidase – catalase electrode by potentiostatic measurement. Bioelectrochem. Bioenerg. **6,** 19–26.

Nelson C. A. (1971), The binding of detergents to proteins I. The maximum amount of dodecyl sulphate bound to proteins and the resistance to binding of several proteins. J. Biol. Chem. **246** (12), 3895–3901.

Scheller F., Seigbah N., Danielsson B. and Mosbach K. (1985), High sensitivity enzyme thermistor determination of L-lactate by substrate recycling. Anal. Chem. **57,** 1740–1743.

Stein K. and Hain J. U. (1995), Catalase biosensor for the determination of hydrogen peroxide, fluoride and cyanide. Microchim. Acta **118,** 93–101.

Stryer L. (1981), Biochemistry (Freeman W., ed.). Publ. Freeman & Company, San Francisco, USA.

Tatsuma T., Watanabe T. and Tatsuma S. (1994), Substrate – purging enzyme electrodes – peroxidase catalase electrodes for hydrogen peroxide with an improved upper sensing limit. Anal. Chem. **66,** 290–294.

Wang J., Rivas G. and Liu J. (1995), A catalase electrode for organic – phase enzymatic assays. Anal. Lett. **28** (13) 2287–2295.

Wingard Jr. L. B., Candin L. A. and Castner J. F. (1983), Effect of enzyme-matrix composition on potentiometric response to glucose using glucose oxidase immobilized on platinum. Biochim. Biophys. Acta **21,** 748.