PEROXIDASE ACTIVITY OF HEMOPEXIN, FERRIHEMALBUMIN, FERRIHEMOGLOBIN, AND FERRIMYOGLOBIN

Z. HRKAL and Z. VODRÁŽKA

Institute of Hematology and Blood Transfusion, 128 20 Prague 2

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The peroxidase activity of several hemoproteins was determined by the guaiacol method. The activity of all hemoproteins studied complied with the Michaelis–Menten equation and decreased in the order ferrimyoglobin > ferrihemoglobin ≫ ferrihemalbumin > heme-hemopexin.

The rate of hydrogen peroxide decomposition by ferri compounds significantly depends on the neighborhood of the Fe^{3+}-ions. Whereas the catalytic activity of the Fe^{3+}-ion is slightly increased after its incorporation into a complex with protoporphyrin *IX* or deuterioporphyrin[1] and depends on the oligomerous state of these molecules, the catalytic activity of a specific peroxidase, catalase, is 10^6-times higher than the activity of the ferriheme[2] monomer. As part of our studies on the physico-chemical[3] and functional[4] properties of hemopexin we compared the peroxidase activity of the heme-hemopexin complex with the activity of several hemoproteins differing in the neighborhood of the heme group.

EXPERIMENTAL

Material. Hemopexin was prepared in the form of the heme-hemopexin complex from fresh human serum by precipitation with ammonium sulfate[5], and purified by chromatography on DEAE-cellulose[6]. Human hemoglobin free of catalase was obtained by chromatography on Sephadex G-100 according to Aebi and coworkers[7]. Ferrihemoglobin was prepared from oxyhemoglobin by treatment with a 20% stoichiometric excess of potassium ferricyanide, followed by dialysis against 0·1M phosphate buffer at pH 7·0. The ferrihemalbumin complex was prepared by the addition of 1·2 equivalent of hematine to a solution of human serum albumin at pH 7·0; the complex was chromatographed on a column of DEAE-cellulose eluted by 0·1M phosphate buffer at pH 7·0. Horse myoglobin was a commercial preparation of Serva. The concentration of protein solutions was determined spectrophotometrically. The following absorption coefficients were used: at 540 nm, 11·5 cm^{-1} mM^{-1} for cyanomethemoglobin and 11·3 cm^{-1} mM^{-1} for cyanometmyoglobin, at 280 nm 37·2 cm^{-1} mM^{-1} for albumin and 185 cm^{-1} mM^{-1} for the heme-hemopexin complex.

The peroxidase activity was assayed by a modification of the method of Connel and Smithies[8]. The hemoglobin solution (62 μM, 0·1 ml) and subsequently hydrogen peroxide (0·1—10M, 0·1 ml) were added to 2·75 ml of 0·03M guaiacol in 0·1M phosphate buffer at pH 7·0. The change in absorbance at 470 nm of the reaction mixture as a function of time was recorded at 20°C in Uni-

cam SP 700 recording spectrophotometer. The rate of hydrogen peroxide decomposition was calculated from the initial slope value of the plot obtained; an absorption coefficient of 26·6 cm^{-1} mM^{-1} was used at 470 nm for tetrahydroguaiacol[9].

RESULTS AND DISCUSSION

The dependence of the rate of hydrogen peroxide decomposition on substrate concentration for the four hemoproteins studied by us is given in Fig. 1. The experimental data were analyzed by means of the Michaelis–Menten equation $v = k_2 E_0 S : (K_m + S)$, where v is the reaction rate, k_2 the rate constant of decomposition of the enzyme–substrate complex, K_m the Michaelis constant, E_0 the enzyme concentration,

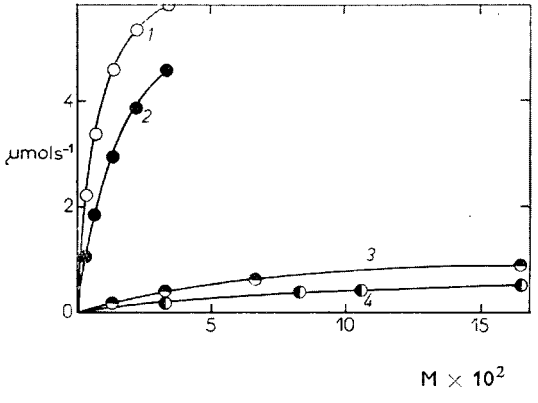

FIG. 1

Dependence of Initial Rate of Hydrogen Peroxide Decomposition on its Concentration

The experiments were carried out in 0·1M phosphate buffer at pH 7·0 and 20°C; the concentration of the hemoproteins was 2·20 μM (in heme equivalents). The theoretical curves were calculated by means of the Michaelis–Menten equation; the parameter values given in Table I were used. *4* heme-hemopexin (◐), *3* ferrihemalbumin (◓), *2* ferrihemoglobin (●), *1* ferrimyoglobin (○).

TABLE I

Peroxidase Activity of Hemoproteins at pH 7·0 and 20°C

Values of k_2 and K_m of the Michaelis–Menten equation.

Hemoprotein	k_2, s^{-1}	K_m, mol . l^{-1}
Heme-hemopexin	0·40	$1{\cdot}18 \cdot 10^{-1}$
Ferrihemalbumin	0·75	$1{\cdot}06 \cdot 10^{-1}$
Ferrihemoglobin	3·40	$1{\cdot}90 \cdot 10^{-2}$
Ferrimyoglobin	3·20	$7{\cdot}00 \cdot 10^{-3}$

and S the initial substrate concentration. By plotting S/v *versus* S, a linear dependence was obtained for all the hemoproteins studied and parameters k_2 and K_m were calculated from this dependence. The values of these parameters are given in Table I. The theoretical dependence of v on S calculated from these parameters, is shown in Fig. 1; as can be seen, all the experimental data comply with the Michaelis–Menten equation as regards all the hemoproteins studied. The maximum decomposition rate of hydrogen peroxide is lowest for the complex heme-hemopexin, and heme-albumin whereas the catalytic activity of ferrihemoglobin and ferrimyoglobin is approximately ten times higher.

The activity of the "real" peroxidase, *i.e.* of bovine liver catalase, is even 10^8-times higher[10], as follows from the value of $k_2 = 3{\cdot}8 \cdot 10^7\ s^{-1}$.

Our results support the views of Jones and Suggett[11] who postulate that the peroxidase activity of an enzyme depends on the stereospecificity of the neighborhood of its prosthetic group. Proteins which undergo analogous heme-protein interactions, like myoglobin and hemoglobin, show a similar peroxidase activity. The activity of the complex heme-hemopexin and heme-albumin is also comparable yet substantially lower. It is possible that the accessibility of their heme groups to ligands is worse or that the binding of hydrogen peroxide to Fe^{3+}-ions is limited as a result of the hemichromogen character of the heme-protein interactions as regards both the heme-hemopexin and heme-albumin complex. As evidenced unambiguously by the results of our measurements, the peroxidase activity of hemopexin is too low to be a functional property of this protein.

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