ACTIVATION OF HEMATIN CATALASE FUNCTION BY ETHYLENEDIAMINE

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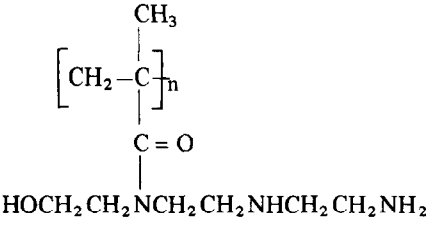
1. Introduction

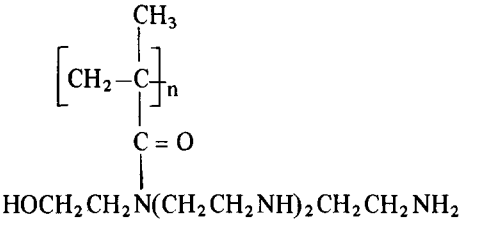
It is of interest to see if there are catalysts which simulate the action of an enzyme which consist of active centres of an enzyme, or similar compounds, fixed on a polymeric support. Hematin, which is a part of some redox enzymes (catalase, peroxidase, cytochrome *c* etc.), is just such a catalytic centre. However, attempts to use different forms of hematin for catalysis have shown that its catalytic activity, e.g. in the catalase reaction, is incomparably lower than that of the enzyme [1–4]. We have shown [5,6] that ethylenediamine (EDA) speeds up decomposition of hydrogen peroxide (HP) on synthetic polymeric catalysts, containing Fe^{3+} ions complexed with polyacids such as polyacrylic acid (PAA) and polymethacrylic acid (PMAA). These catalysts simulate to a certain extent the action of catalase. The activating effect of EDA may be due to the intermediate Fe–PAA–EDA–HP complex, in which HP is activated by EDA [6].

To find out whether this effect can be used to activate decomposition of HP on hematin, we have studied the effect of EDA and other amines on the rate of HP decomposition by hematin in aqueous solutions.

2. Materials and methods

Hematin was of a pure grade from Koch Light. Its concentration in aqueous solution was determined spectrophotometrically at $\lambda = 393$ nm, $\epsilon = 5.0 \times 10^4$ $M^{-1}sm^{-1}$ [7]. Butylamine (BA) and EDA were of analytical grade and were rectified. Imidazole of analytical grade and histamine from Fluka AG, T_m 80–83°C, were used without further purification. Polyvinylamine, with a mol. wt of 20 000, contained not not more than 2% of phthalimide radicals. Modification of PMAA (mol. wt 50 000) by trimer and tetramer ethyleneimine (EI_3 and EI_4) was carried out in aqueous solution at 20°C by adding to 1 M PMAA solution stoichiometric amounts of EI oligomers. The monomer chains of the reaction products are supposed to have the following structures:





Hydrogen peroxide of a pure grade was twice distilled in a quartz device. HP decomposition was carried out in a thermostated vessel with intensive stirring. The pH's of hematin complexes with low molecular weight and polymeric amines were adjusted by addition of NaOH. pH's were measured with a pH-340 potentiometer. HP was added to the catalyst and the reaction rate determined by stopping the reaction with sulphuric

acid and titrating the unreacted HP with $KMnO_4$ titrant [8]. The degree of HP conversion into oxygen and water was measured gasometrically [9]. UV spectra of the hematin complexes with amines were taken in a 'Specord' recording spectrophotometer.

3. Results and discussion

Values of the catalase activity of hematin complexed with various low- and high-molecular weight amines are given in table 1.

Monoamines and polymeric amines only cause a few-fold increase in hematin activity. EDA increases the reaction rate by more than two orders of magnitude. It is noteworthy that in this process HP completely decomposes into oxygen and water, i.e. it is not spent on oxidation processes. The reaction cycle can be repeated several times with one catalyst (fig.1).

UV spectra of hematin in the presence and without EDA are shown in fig.2. At pH 6.0 (fig.2b), when both EDA groups are protonated, it does not react with hematin. If one amino group in EDA is deprotonated (pK_a = 7.0) (fig.2b, pH 8.0), it complexes hematin, which entails depolymerization of the latter. This is manifested by a 393 nm to 408 nm shift in the absorption band of hematin [7].

Analogous changes in the absorption spectrum also occur at pH's when 75% of EDA molecules exist as free base (fig.2a, pH 10.5). Hence, EDA is bound to hematin via one amino group. Spectral changes similar to those observed for EDA–hematin have also been

Table 1
Catalytic activities of hematin complexes

Catalytic system	Number of catalytic events sec^{-1}
1. Hematin	4.3
2. Hematin-butylamine	10.1
3. Hematin-imidazole	8.8
4. Hematin-histamine	11.4
5. Hematin-EDA	900
6. Hematin-PVA	95
7. Hematin-PMAA EI_4	22
8. Hematin-PMAA EI_3	36

Reaction conditions: pH 10–10.5, 21°C. Concentrations (M): hematin, 1.5×10^{-5}; HP, 5×10^{-2}, Amines, 10^{-1}; PVA, 0.88.

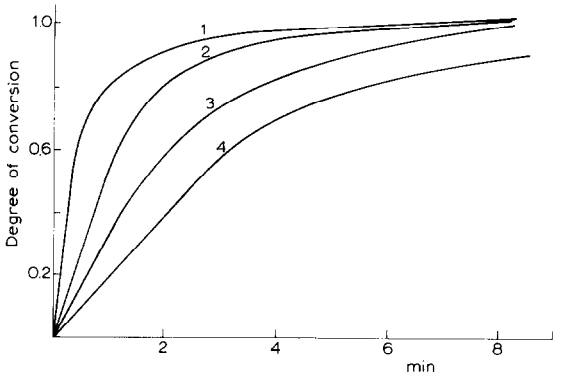
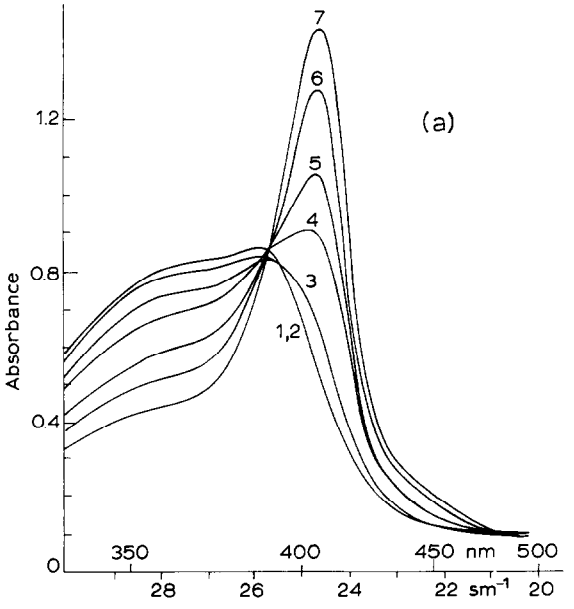


Fig. 1. Repeated catalytic cycles (1–4) of decomposition of HP on hematin in the presence of EDA (gasometric procedure) pH 10.5, 22°C. Concentrations (M): hematin 1.5×10^{-5}; EDA, 10^{-1}; HP, 10^{-1} each time.

obtained with other amines, butylamine, imidazole, histamine, for which λ_{max} shifts to 408 nm, 433 nm and 410 nm respectively. Thus, the binding of hematin in an amine complex and its depolymerization fail to bring about a significant increase in catalytic activity. For catalytic activity, a second non-protonated amino group is indispensable and it should be located in close proximity to the metal. However, if the basic group in question is not NH_2, but imidazole (histamine), catalytic activity is low (table 1).



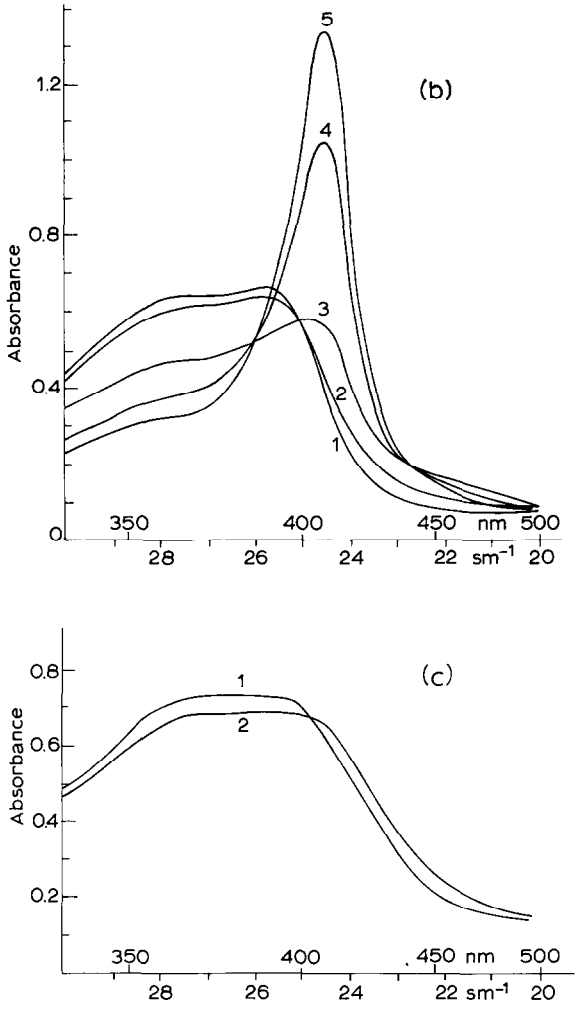


Fig. 2. UV spectra of hematin in the presence of EDA. Hematin 1.5×10^{-5} M, 25°C. a) pH 10.5; EDA, (M): 1, 0; 2, 5×10^{-2}; 3, 10^{-1}; 4, 2×10^{-1}; 5, 3×10^{-1}; 6, 5×10^{-1}; 7, 1.0. b) pH 8.0; EDA, (M): 1, 0; 2, 10^{-2}, 3, 10^{-1}, 4, 4×10^{-1}; 5, 1.0; c) pH 6.0; EDA (M): 1,0; 2, 1.0.

The idea that one deprotonated amino group of EDA, not bound to hematin, affects the catalase activity is supported by the pH dependence of the reaction rate shown in fig.3. The curve shows a maximum at pH 10.0, which coincides with pK_{a_2} of EDA [10] indicating that EDA, like Fe^{3+} ions, has a catalytic function. It may be assumed that a free amino group reacts with a molecule of HP via an unshared electron pair of a nitrogen atom. Hence, the reaction rate reaches maximum when the $-NH_3^+ \rightleftarrows -NH_2 + H^+$ equilibrium is shifted to the right, between pH's 9 and 11. The decrease in rate as the pH is increased further is probably due to the extrusion of EDA from the EDA–hematin complex by OH^- ions. It is known [8] that at pH 10.5 catalase activity decreases, which may also be due to OH^- ions binding to the active centre of the enzyme.

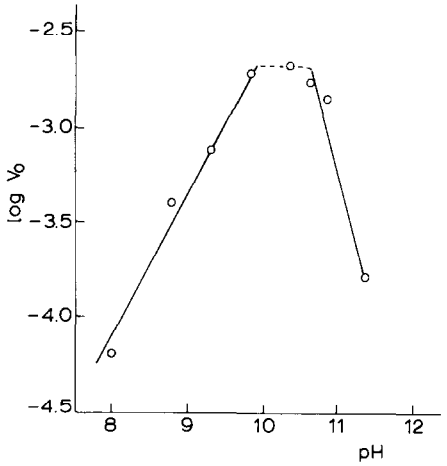


Fig. 3. pH dependence of the logarithm of the rate of HP decomposition on a hematin–EDA complex. 22°C. Concentrations (M): hematin, 3×10^{-6}; EDA, 0.94×10^{-1}; HP, 5×10^{-2}.

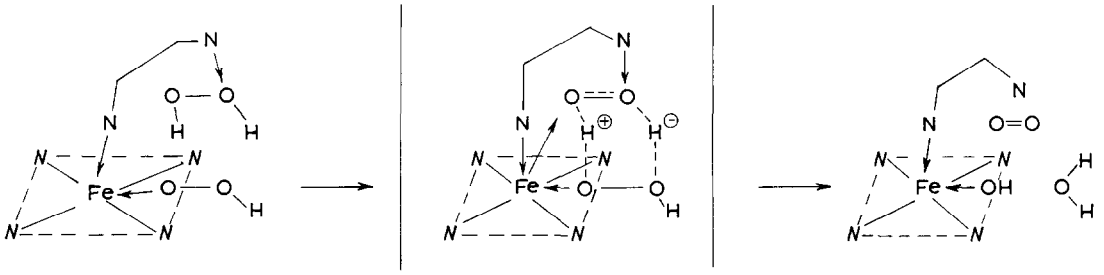


Fig. 4. A possible mechanism of decomposition of the catalytic hematin–EDA–2HP complex.

Fig.4 shows a possible structure of a hematin–EDA–2HP complex and a mechanism for its decomposition. It is to be expected that the effect described should not depend on whether the complex is formed by either of the following sequences: 1. hematin–EDA; 2. hematin–EDA + 2 HP or the stages: 1. EDA–HP, 2. EDA–HP + hematin + HP. The second HP molecule binds to the Fe^{3+} ion, no matter what is the method of hematin–EDA–HP complex formation.

Thus, the previously observed significant activation of the catalase function of a synthetic polymeric catalyst by EDA has been shown also to occur with the catalytic centre of the natural enzyme, hematin. It is likely that this effect will also occur with hematin fixed on a polymeric support, although this certainly requires investigation.

It is worth mentioning that Jones and Suggett's hypothesis [8] that the protein moiety of catalase has an activating effect due to the participation of acid-base groups in the enzymatic reaction is indirectly confirmed by the experiments with EDA described here.

Acknowledgement

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