TETRAPYRROLE COMPLEXES OF COBALT AS CATALYSTS OF THE OXIDATION OF EPINEPHRINE

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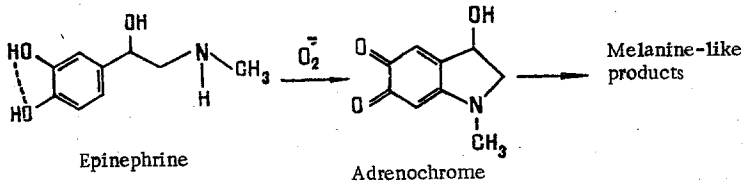
A scheme is proposed for the qualitatively different catalytic effects of metalloporphyrins in reactions involving the oxidation of epinephrine. A metallocomplex of porphyrin may accelerate the oxidation of some substances and inhibit the oxidation of others. The small value of the constant of regeneration of the catalyst gives a similar effect, i.e., the oxidation of a given substance may be accelerated by one metallocomplex and inhibited by others. The catalytic oxidation of epinephrine in the presence of the cobalt complex of porphyrin at biological pH values that we have detected is of undoubted interest for understanding the regulatory role of metalloporphyrins in living organisms.

The modeling of metal-containing biological objects, and also of processes taking place in living organisms with the participation of metal ions, with the aid of simple coordination compounds and organometallic compounds may be considered as a new modern level of the study of the biological role of trace elements. In this connection the study of the biological activity of tetrapyrrole metallocomplexes (TPMSs), the natural prototypes of which are heme, hemin, vitamin B_{12}, and chlorophyll, is of interest.

In recent years, the attention of many workers has been attracted by the catalytic properties of metallocomplexes of porphyrins in an aqueous medium. This is explained by the fact that redox reactions of organic compounds in aqueous solutions model biological systems and an explanation of the mechanism of the catalytic activity of metalloporphyrins in such reactions would give the key to such understanding enzymatic processes taking place in the living organisms. The information available on this question is extremely contradictory. Different metalloporphyrins do not behave in the same way in different reaction, either inhibiting oxidation-reduction or accelerating it. Hitherto, it has not been possible to find general laws controlling the behavior of metalloporphyrins in such systems.

One of the most important aspects of the problem under consideration is the catalysis of processes of oxidation by molecular oxygen. It may be assumed that the porphyrin complexes of transition metals are capable of activating molecular oxygen by the transfer of electrons from the metal atom to a O_2 molecule. This forms a superoxide anion radical of O_2 which possesses strong oxidizing properties and plays an important role in biological processes.

As a model, we have selected the autooxidation of epinephrine, for which the participation of a superoxide radical has been accurately established. In an alkaline medium, epinephrine is readily oxidized by atmospheric oxygen at room temperature. The first comparatively stable oxidation product is adrenochrome, which is then oxidized to melanin-like products. The reaction is effectively inhibited by superoxide dismutase, which confirms the participation of superoxide. It may be assumed that the O_2^- ion detaches H from the epinephrine molecule, forming a free epinephrine radical which is then converted into products (Fig. 1).



Epinephrine — Adrenochrome

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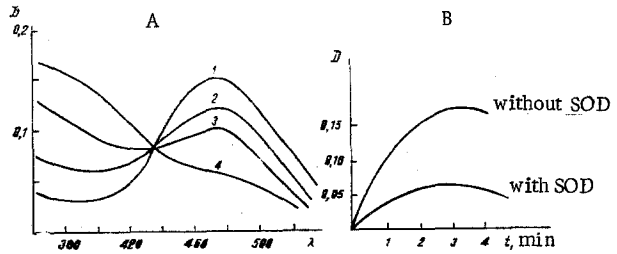

Fig. 1. Oxidation of epinephrine in an aqueous medium: A) change in the spectrum; B) inhibition by superoxide dismutase. 1-4) 1, 2, 3, and 4 min, respectively.

The kinetics of epinephrine was studied by a spectrophotometric method from the rate of formation of adrenochrome, which has an absorption maximum at λ 485 nm. The index of the catalytic action of the complexes studied was the initial rate of the reaction – the slope of the curve in plots of optical density versus time ($\Delta D/\Delta t$).

Below is shown the influence of various porphyrins and their metallocomplexes and other tetrapyrrole complexes on the oxidation of epinephrine. It can be seen that a catalytic effect is given only by the cobalt complexes of the porphyrins, i.e., both the presence and nature of the metal and the nature of the ligand are significant for catalysis.

Additive	Coefficient of acceleration of the oxidation of epinephrine
Cobaloxime dipyridinate	1.0
Co complex of mesoporphyrin IX, disodium salt	8,57
Co complex of tetra(p-sulfophenyl)porphine, sodium salt	8,85
Ni complex of tetra(p-sulfophenyl)porphine	1,0
Tetra(p-sulfophenyl)porphine, sodium salt	1,3
Di(α-propoxyethyl)deuteroporphyrin IX, disodium salt	1,2
Di(α-methoxyethyl)deuteroporphyrin IX, dipotassium salt	1,2

The results given relate to pH 10: With a lowering of the pH the autooxidation of the epinephrine slows down sharply, and at pH 8 the reaction scarcely proceeds. However, the addition of catalytic amounts of a cobalt complex under these conditions causes a rapid oxidation with the formation of the same products as in a strongly alkaline medium.

The change in the absorption spectrum in the course of the reaction is shown in Fig. 2. It can be seen that together with the formation of the oxidation products the intensity of the Soret band of the cobalt porphyrin complex (λ 435 nm) decreases. However, the rate of the consumption of the Co-porphyrin is substantially lower than the rate of formation of oxidation products: For each molecule of the porphyrin complex that decomposes about 10 molecules of epinephrine are consumed. Stoichiometric calculation give grounds for regarding the cobalt complex of porphyrin not as an oxidant but as a catalyst of the oxidation of epinephrine. In fact, the consumption of the porphyrin must be regarded as a side reaction ("poisoning" of the catalyst).

The accumulation of adrenochrome in the presence of the cobalt complex of porphyrin, both at pH 10 and at pH 8.0, is inhibited more rapidly. This inhibition may be connected with the passage of adrenochrome into products of further oxidation, and also with the poisoning of the catalyst. The addition of a fresh portion of cobalt-porphyrin accelerates the accumulation of adrenochrome. The reaction is also accelerated by additions of 10^{-3} M hydrogen peroxide.

The kinetic characteristics of processes at the two pH values are given below. At pH 8, the initial rate of accumulation of adrenochrome is directly proportional to the concentration of cobalt-porphyrin and does not depend of the concentration of epinephrine in the interval from 10^{-4} to $5 \cdot 10^{-3}$ M. The same orders with respect to the reactants are shown by the decomposition of the cobalt complex of porphyrin:

	pH 10	*pH 8*
Coefficient of acceleration at $[\Delta AH]/[\Delta CoP] = 10^2$	2,85	10^3
Order with respect to epinephrine	1,0	0
Order with respect to catalyst	1,0	1,0
Number of molecules of AH oxidized per molecule of catalyst	10^3	10–15

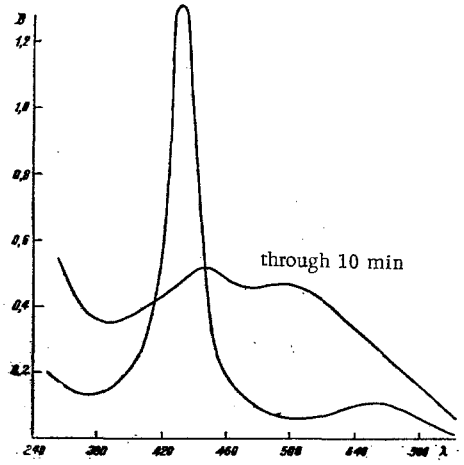

Fig. 2. Change in the absorption spectrum of a $2 \cdot 10^{-3}$ M solution of epinephrine + 10^{-5} M Co-porphyrin on oxidation.

In the absence of a catalyst at pH 10 and pH 8 $d[AH]/dt = 0$.

To explain the observed kinetic laws it is possible to put forward the following mechanism of the process:

$$\left[\overset{+}{\text{MP}} \cdots O_2^-\right] \underset{k_1^-}{\overset{k_1^+}{\rightleftharpoons}} \text{MP} + O_2^-, \tag{1}$$

$$AH + O_2^- \xrightarrow{k_2} \dot{A} + HO_2^-, \tag{2}$$

$$AH + HO_2^- \xrightarrow{k_3} \dot{A} + \dot{O}H + \overline{OH}, \tag{3}$$

$$AH + OH^- \xrightarrow{k_4} \dot{A} + H_2O, \tag{4}$$

$$\overset{+}{\text{MP}} + HO_2^- \xrightarrow{k_5} \text{MP} + O_2^- + \overset{+}{H}, \tag{5}$$

$$\text{MP} + O_2 \rightarrow \left[\overset{+}{\text{MP}} \cdots O_2^-\right].$$

As already mentioned, the autooxidation of epinephrine takes place through the participation of a superoxide. On the other hand, it is known that Co-porphyrin readily forms superoxide complexes $CoP^+ \ldots O_2^-$. It may be assumed that the stage of the formation of the epoxide radical limits the autooxidation and is accelerated in the presence of the cobalt porphyrin through the formation and subsequent dissociation of the oxygen adduct.

In the presence of epinephrine, the possibility of the formation of the ternary complexes $AH \ldots CoP \ldots O_2^-$ is not excluded, either, although these complexes apparently play no fundamental role in oxidation. If they participated in the formation of the superoxide radical, the reaction would be of the first order with respect to epinephrine, while experiment shows zero order.

The subsequent interaction of epinephrine with the superoxide takes place by a complex scheme in which the first stable product is adrenochrome. It is natural to assume that the first stage of oxidative conversions is the detachment by the superoxide of a hydrogen atom from the epinephrine molecule with the formation of a free radical (2). We assumed that the subsequent transformations of epinephrine take place rapidly and that reaction (2) limits the formation of adrenochrome.

The peroxide radicals HO_2^- formed in this reaction possess oxidizing properties and it may be assumed that they also oxidize epinephrine, in the final account reducing the superoxide to water (3 and 4). On the other hand, the HO_2^- ion also possesses reducing properties and in reaction with the strong oxidizing agent CoP^+ it may regenerate the catalyst (5).

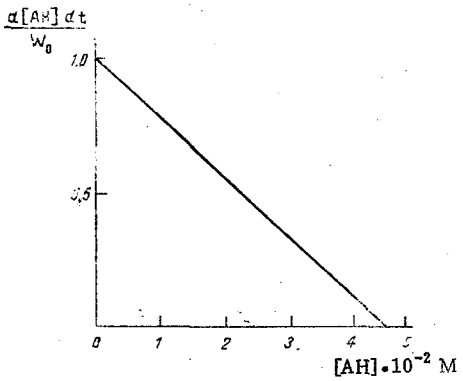

Fig. 3. Rate of the catalytic oxidation reaction [log P] = $2 \cdot 10^{-6}$ M at high concentrations of epinephrine [AH] = 10^{-2} M.

Attention must be directed to the fact that in the last reaction the second superoxide radical in the catalytic cycle is formed, which ensures stoichiometric relationships. The regenerated CoP rapidly adds a molecule of oxygen and begins a new catalytic cycle by reaction (1).

The calculation of this scheme under the condition that the concentration of all the intermediate products (radicals and ions) are quasistationary gives the following expression for the rate of oxidation

$$-\frac{d[\mathrm{AH}]}{dt} = \frac{4k_1 + [\mathrm{MP}]}{2\alpha\beta + 1}\left(1 - \beta\frac{[\mathrm{AH}]}{[\mathrm{MP}]}\right),$$

where

$$\alpha = \frac{k_1}{k_2};\ \beta = \frac{k_3}{k_5}.$$

Let us analyze the expression obtained. At sufficiently low concentrations of epinephrine, [AH]/[MP] $\ll 1/\beta$, the right member in parentheses can be neglected, and the rate of the reaction is directly proportional to the concentration of catalyst and independent of the concentration of epinephrine,

$$\frac{d[\mathrm{AH}]}{dt} = W_0 = 4\frac{k_1^+[\mathrm{MP}]}{2\alpha\beta + 1},$$

which agrees with experiment. At higher concentrations of epinephrine, [AH]/[MP] = $1/\beta$, the right member in parentheses becomes equal to unity and a kind of critical phenomenon must be observed – a linear fall of the rate to zero with an increase in the concentration of epinephrine

$$\frac{d[\mathrm{AH}]}{dt} = W_0\left(1 - \beta\frac{[\mathrm{AH}]}{[\mathrm{MP}]}\right).$$

As can be seen from Fig. 3, this phenomenon is actually observed experimentally. The value of the parameter β was calculated from the experimental results as $5.2 \cdot 10^{-5}$, and the lower limit of the rate constant of the generation of the superoxide anion of cobalt porphyrin was determined as $2.4 \cdot 10^{-3}$ sec^{-1}.

Thus, the experimental results are described satisfactorily by the proposed scheme and confirm the hypothesis of the capacity of metallocomplexes of porphyrin for catalytically generating a superoxide ion radical.

From the proposed scheme of catalysis follows the possibility of a qualitatively different action of metalloporphyrins in oxidation reactions. As can be seen from the equation obtained, the process is governed by the parameter β, which is equal to the ratio of the rate constant of the reaction of the substance being oxidized with the HO_2^- ion, i.e., with hydrogen peroxide, to the rate constant of the oxidized form of the catalyst with the same peroxide. If the substance being oxidized reacts readily with hydrogen peroxide and β is large, the right-hand member in parentheses may prove to be less than unity. Then the expression in parentheses becomes negative, i.e., instead of catalytic acceleration catalytic retardation of the oxidation process will be observed. Thus, one and the same metallocomplex of porphyrin may accelerate the oxidation of some and inhibit the oxidation of others. A similar effect is given by a small value of the catalyst regeneration constant k_5, i.e., the oxidation of a given substance may be accelerated by some metal complexes and inhibited by others. It is possibly just this circumstance which explains the contradictory nature of the results given in the literature [1-3] on the influence of metalloporphyrins on oxidative processes in an aqueous medium.

The mechanism of the poisoning of cobalt-porphyrin cannot be established unambiguously from the results obtained. Apparently, CoP is destroyed on interaction with one of the oxidation products of epinephrine, since in the absence of epinephrine it does not decompose.

EXPERIMENTAL

The kinetics of the catalytic oxidation of epinephrine was recorded on a Lomo SF-26 spectrophotometer and the absorption spectra of a solution of epinephrine on oxidation in the presence of porphyrin on Varian (Cary-219) spectrophotometer in cells 1 cm long at room temperature.

Sigma epinephrine was used. Its solution was prepared by a method described in the literature [4]. The epinephrine buffers and porphyrin solutions were prepared in double-distilled water. SOD was obtained from bovine erythrocytes [5, 6]. The enzyme obtained by this method had a spectral index D_{260}/D_{280} of 25.5. The porphyrin and the cobaloxine dipyridinate were synthesized in the Institute of Biophysics of the Ministry of Health of the USSR.

SUMMARY

A scheme has been proposed to explain the qualitatively different catalytic effects of metalloporphyrins in epinephrine oxidation reactions. A metallocomplex of porphyrin may activate the oxidation of some substances and inhibit the oxidation of others.

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