The effect of amino acids on the rate of hydroxylation of cholesterol catalyzed by Mn and Fe porphyrinates

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The rate and extent of hydroxylation of cholesterol catalyzed by Mn and Fe porphyrinates in the presence of $NaBH_4$ increase with the addition of cysteine and histidine. This effect is more pronounced for cysteine. Leucine does not accelerate the process.

Key words: Mn and Fe porphyrinates, catalytic hydroxylation, cholesterol, amino acids.

Catalytic systems based on metalloporphyrins (MP) make it possible to mimic some stages in the functional cycle of heme-containing oxidases, for example, cytochrome (Cyt) P-450, and to reveal the main factors that govern their activity.[1,2]

Undoubtedly, one of the main problems in need of modeling is elucidation of the reasons for the high catalytic activity of the iron protoporphyrin (hemin)-containing prosthetic group of Cyt P-450, because, as has been demonstrated previously[3] in "metalloporphyrin—reducing agent" model systems, which catalyze regio- and stereoselective hydroxylation of steroid olefins, iron porphyrin complexes (PFe) are significantly inferior in activity to manganese porphyrinates (PMn).

It is usually believed that the enzymic activity of Cyt P-450 is associated with the amino acid composition of the protein surrounding of the active center. In fact, removal of histidine and cysteine from the polypeptide results in deactivation of the enzyme.[4] At the same time, the replacement of the methionine-80 incorporated in the active center of Cyt *c* by cysteine results in the appearance of properties similar to those of Cyt P-450.[5]

In model experiments, a substantial (5—10-fold) increase in the catalytic activity of PFe was observed after the introduction into hemin of covalently bound di- and tripeptides, even if they were devoid of cysteine and histidine.[6]

The present work demonstrates that the catalytic activity of Fe and Mn porphyrinates in the hydroxylation of cholesterol in the presence of $NaBH_4$ is increased by the addition of cysteine, histidine, and imidazole (which is a functional group of histidine, and determines the basic character of this amino acid).

Experimental

Mn and Fe tetraphenylporphyrinates (TPPMn and TPPFe) and Mn protoporphyrinate (PPMn) were prepared from the free porphyrin bases and metal acetates using conventional procedures.[7] Hemin, cholesterol, and amino acids were purchased from Sigma. Hydroxylation of cholesterol was carried out at constant temperature in a 1 : 1 chloroform—ethanol mixture in a closed reactor (20 mL volume) equipped with a magnetic stirrer and connected with a device for measuring small gas volumes.[8,9] A solution of cholesterol (0.5 mL, 0.04 *M*) in chloroform and an alcoholic solution (1 mL) of an amino acid or imidazole of the following concentrations: 10^{-3}, $2 \cdot 10^{-3}$, $3 \cdot 10^{-3}$, $4 \cdot 10^{-3}$, $5 \cdot 10^{-3}$ mol L^{-1} were added to a solution of MP (0.5 mL, $2 \cdot 10^{-3}$ mol L^{-1}) in chloroform to make molar ratios of MP and the amino acid of 1 : 1, 1 : 2, 1 : 3, 1 : 4, and 1 : 5. The resulting solution was stirred at ambient temperature for 5 min, $NaBH_4$ (0.8 mg) was added, and the reaction time count was begun. The reaction kinetics was monitored by following the consumption of oxygen by the reaction system using the device for measuring small gas volumes and by TLC on Silufol. The observed rate constant k_{obs} of hydroxylation of cholesterol was determined from the slopes of the kinetic curves of the consumption of oxygen at the initial time intervals. It has been shown previously[9] that hydroxylation of cholesterol in the presence of the MP—$NaBH_4$ system (M = Mn, Fe) proceeds through the intermediate formation of 5α-hydroperoxycholestan-3β-ol and affords a single product, *viz.*, 3β,5α-cholestanediol. The products were detected on TLC plates with a 5% ethanolic solution of phosphomolybdic acid. Complete disappearance of the substrate corresponds to 100% conversion. The yield of 3β,5α-cholestanediol was determined by isolating it using column chromatography (SiO_2, 100—250 mesh) in a 1 : 4 hexane—ethyl acetate system. The efficiencies of the catalytic systems were estimated from the molar ratio of the product and MP up to its complete degradation (the μ parameter in

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Table 1). The intermediate and the final products were identified by GLC-MS and TLC. The introduction of amino acids did not change the composition of the products.

Results and Discussion

The observed dependences of the rate constants of the hydroxylation of cholesterol on the concentrations of amino acids (cysteine and histidine) and imidazole in the presence of MP (PMn and PFe) and $NaBH_4$ are given in Figs. 1 and 2. The corresponding kinetic parameters are listed in Table 1. For comparison, analogous experiments were carried out in the presence of leucine. The introduction of leucine into the system did not affect the rate constant of the reaction. The dependence is extreme in the presence of cysteine, whereas the dependences of k_{obs} on C of histidine and imidazole are described by curves increasing monotonically to definite maximum values.

It should be noted that in the presence of manganese and iron tetraphenylporphyrinates, the extremum was observed at the 1 : 1 MP—amino acid molar ratio and for protoporphyrinates it was observed at a ratio of 1 : 2. The introduction of amino acids affects predominantly the activities of PPMn and PPFe. The change in activities of TPPMn and TPPFe in the presence of amino acids is smaller.

Table 1. Kinetic parameters of the hydroxylation of cholesterol ($[MP] = 5 \cdot 10^{-4}$ mol L^{-1}, [cholesterol] $= 10^{-2}$ mol L^{-1}, $[NaBH_4] = 10^{-2}$ mol L^{-1})

MP—amino acid system (molar ratio)	k_{obs}* $\cdot 10^5$ /mol L^{-1} s^{-1}	μ	Yield of cholestanediol (%)
TPPMn	6.9	~330	90
TPPMn—cysteine (1 : 1)	10.3	~330	90
TPPMn—histidine (1 : 1)	8.3	~330	92
TPPMn—imidazole (1 : 1)	8.0	~330	90
PPMn	1.3	~200	42
PPMn—cysteine (1 : 2)	2.8	~200	71
PPMn—histidine (1 : 1)	2.0	~250	75
PPMn—imidazole (1 : 1)	1.7	~230	72
PPFe	0.3	~100	23
PPFe—cysteine (1 : 2)	0.9	~250	54
PPFe—histidine (1 : 1)	0.5	~100	30

* Maximum k_{obs} values are presented.

These results altogether can be understood in the framework of the kinetic scheme of the hydroxylation of

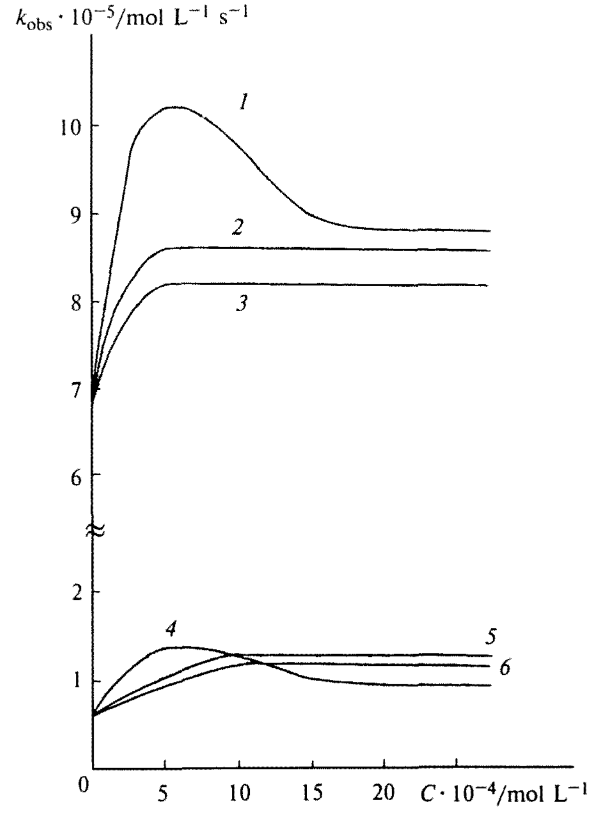

Fig. 1. Dependence of the observed rate constant k_{obs} of the oxidation of cholesterol in the presence of sodium borohydride and TPPMn (*1—3*) or TPPFe (*4—6*) on the concentration of cysteine (*1*, *4*), histidine (*2*, *5*), and imidazole (*3*, *6*). $[MP] = 5 \cdot 10^{-4}$ mol L^{-1}, $[NaBH_4] = 10^{-2}$ mol L^{-1}, [cholesterol] $= 10^{-2}$ mol L^{-1}.

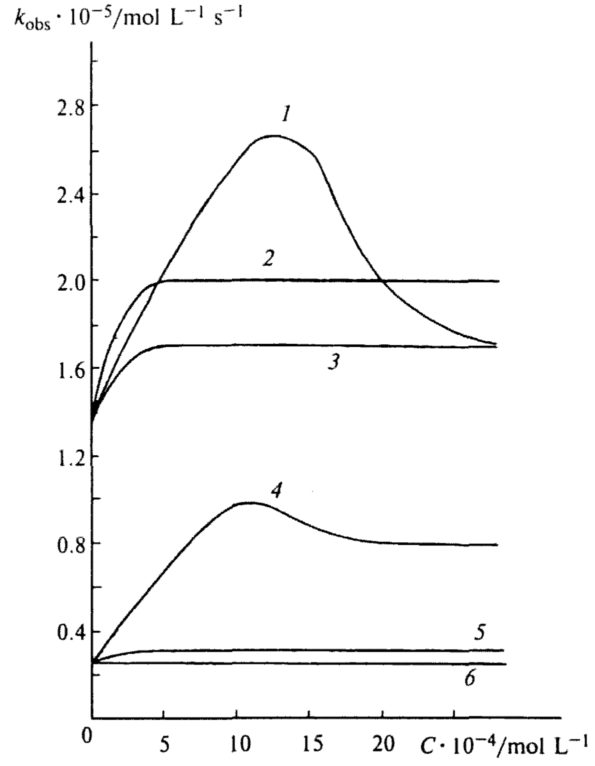

Fig. 2. Dependence of the observed rate constant k_{obs} of the oxidation of cholesterol in the presence of sodium borohydride and PPMn (*1—3*) or PPFe (*4—6*) on the concentration of cysteine (*1*, *4*), histidine (*2*, *5*), and imidazole (*3*, *6*). $[MP] = 5 \cdot 10^{-4}$ mol L^{-1}, $[NaBH_4] = 10^{-2}$ mol L^{-1}, [cholesterol] $= 10^{-2}$ mol L^{-1}.

olefins in the presence of an MP—$NaBH_4$ system suggested previously.[10—12] It was demonstrated[10—12] that the process of hydroxylation of aliphatic and steroidal olefins (ethene, propene, hex-1- and 2-enes, cholesterol, and androsterone) catalyzed by an MP—reducing agent system begins with the reversible reduction of the central ion (Mn or Fe) in the MP molecule and the formation of a catalytically active form of the catalyst PMn^{2+} or PFe^{2+}, which can bind molecular oxygen, activating it to a singlet-like state. In the presence of a substrate, a triple, intermediate complex forms (reduced metalloporphyrin $M^{2+}P$—oxygen—olefin), which decomposes later in the presence of $NaBH_4$ to yield the products and the initial PMn or PFe (Scheme 1, stages (1) and (2)). In this process, partial destruction of reduced MP and of the intermediate complex is possible (stages (3) and (4) in Scheme 1).

Scheme 1

$$P_1 \underset{k_{-1}}{\overset{[N]\,k_1}{\rightleftharpoons}} P_2 \qquad (1)$$

$$P_2 + S \underset{k_{-2}}{\overset{[O_2]\,k_2}{\rightleftharpoons}} P_3 \xrightarrow[k_3]{[N]} B + P_1 \qquad (2)$$

$$P_2 \xrightarrow[k_4]{} Q_1 \qquad (3)$$

$$P_3 \xrightarrow[k_5]{} Q_2 \qquad (4)$$

Here, P_1 is the initial MP, N is the reducing agent (sodium borohydride), P_2 is the reduced MP, S is the substrate, P_3 is the triple, intermediate complex $\{M^{2+}P—O—O—S\}$, B are the reaction products, Q_1 and Q_2 are the products of the decomposition of the active form of the catalyst and the triple complex, and k_1, k_{-1}, k_2, k_{-2}, k_3, k_4, and k_5 are the constants of the elementary stages of the process.

Analysis[10] of this kinetic scheme made it possible to determine several constants of the elementary stages of the process (for hydroxylation of cholesterol and hex-1-ene) and showed that the fact that the effective rate of the process is higher for cholesterol than it is for hex-1-ene is related to the increase in the rate constant of the formation of the intermediate, triple complex (k_2).

The k_1 value for this process was estimated using the known procedure[10] based on spectrophotometric data on the kinetics of the formation and decay of complexes P_2. It was found that this constant is independent of the introduction of amino acids. It is reasonable to assume that the influence of amino acids manifests itself at stage (2) of coordination of cholesterol and oxygen with the reduced central ion of MP. The coordination of cysteine and histidine, having electron-donating side groups, as outer-sphere ligands of PMn or PFe would favor additional polarization of the cholesterol double bond and, hence, according to the previously published data,[12] an increase in the rate constant for the catalytic transformation. The increase in the rate of the process in the presence of imidazole (see Fig. 1) and the absence of the effect upon the addition of leucine (an amino acid free of electron-donating functional groups) support the latter assumption.

The extremal character of the effect of cysteine on the rate constant of the process probably reflects competitive binding of dioxygen and the thiol group of cysteine at the central ion of MP (due to the similarity of the electronic configurations of oxygen and sulfur). If the cysteine : TPPM molar ratio exceeds 1 : 1, the partial displacement of O_2 from the inner coordination sphere of PM^{2+}, affording catalytically inactive MP—(cysteine)$_2$ complexes, is possible.

In the case of PPFe and PPMn with olefinic and ester groups in substituents at the porphyrin ring, it is possible that the additional coordination of the cysteine molecule near these groups occurs, which can result in displacement of the oxygen molecule from the position of the axial ligand and the appearance of the extremum of the $k_{obs}(C)$ dependence at the MP : amino acid ratio exceeding 1 : 2. The above peculiarities of the effect of cysteine on the kinetics of the process were not observed when histidine was introduced into the system, possibly because the imidazole group is less nucleophilic than the thiol group[13] and thus the interaction of histidine with MP molecules is much weaker.

The fact that the activity of TPPM in this process is greater than that of PPM is probably related[14] to the fact that the percentage of the high-spin state for manganese and iron porphyrin complexes having phenyl *meso*-substituents is greater than that for unsubstituted porphyrins. On this background, the electronic effects due to coordination of the outer-sphere ligands (amino acids and imidazole) are less noticeable for TPPM than for PPM and hemin. In fact, the rate constant of oxidation for PPMn and PPFe increases in the presence of cysteine by 2 and 4 times, respectively, whereas for TPPMn and TPPFe it increases by 1.4 and 1.9 times, respectively.

In connection with the irreversible destruction of catalysts in the processes considered,[11] which is reflected by the presence of stages (3) and (4) in catalytic Scheme 1, the efficiency of the system was estimated from the value of the rate of oxidation k_{obs} and from the μ parameter, which characterizes the stability of the catalytically active form. As can be seen from Table 1, the introduction of amino acids influences not only k_{obs}, but also μ, although in this case this effect is the same for histidine and cysteine.

Thus, in this work, the possibility of regulating the catalytic activity of MP in systems that mimic Cyt P-450 by introducting amino acids not bonded chemically with metalloporphyrin into the reaction system is demonstrated for the first time. The regularities found apparently make it possible to come closer in model experiments to elucidating the nature of the high activity of the heme group of native cytochromes. It is the presence of amino acids of the type studied in the neigborhood of heme that determines the enzymic activity of Cyt P-450.

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