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A MICELLAR MODEL OF BLEOMYCIN ANTIBIOTICS

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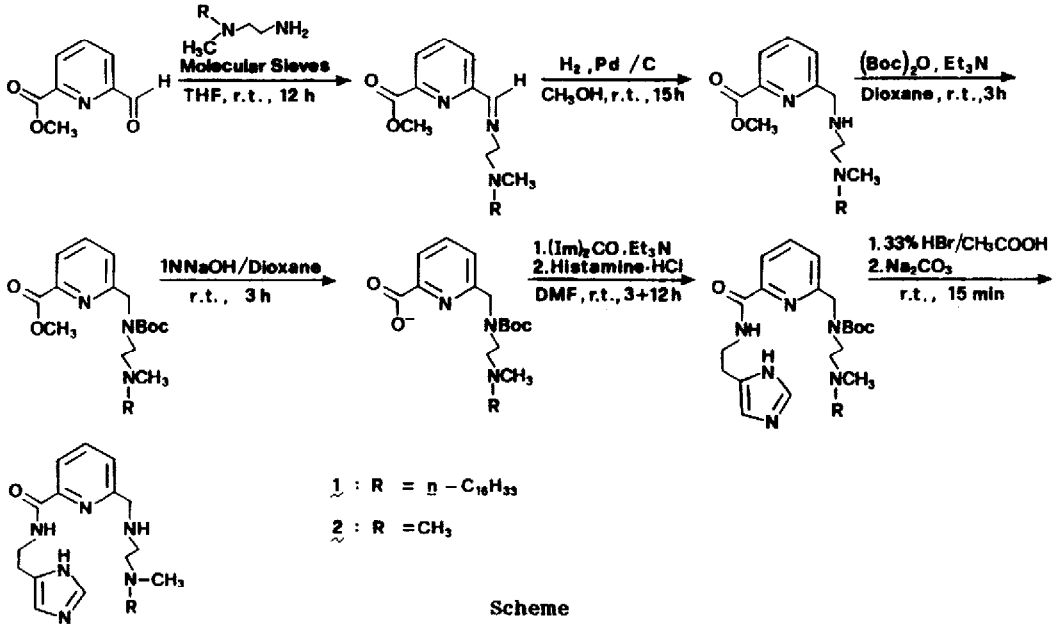
Abstract. The Fe^{III} complex of ligand 1 (6-N-[2-[N-methyl-N-*n*-hexadecyl)aminoethyl]aminomethyl-2-carbamoyl-[N-[2-(4-imidazolyl)ethyl]]-pyridine) as a micellar aggregate in water solution is a catalyst of the H_2O_2 oxidation of *p*-nitrophenyl methyl sulfide and behaves as a slower but otherwise close analogue of Bleomycin·Fe^{III}.

Bleomycins[1] (BLMs) are a family of glycopeptide-derived antibiotics with remarkable antineoplastic properties related to DNA degradation. Such a process is metal ion and oxygen dependent[2]. BLMs are also effective oxidizing agents for simple organic substrates[3], like olefins, either under aerobic conditions in the presence of Fe^{II} and a one electron donor or as a Fe^{III} or Cu^{II} complex using a monooxygen donor like iodosobenzene or a peroxide. The relevant biological role and the catalytic effectiveness of these molecules stimulated the interest for synthetic models of BLM[4].

The enhanced oxygen activation observed in the case of lipophilic BLM models[4c] and the reported[5] ability of some metallosurfactants to act as effective agents for dioxygen complexation were good premises for the synthesis and study of a ligand surfactant molecule containing a metal binding site resembling that of BLM as a catalyst for the oxidation of organic substrates. Accordingly we synthesized[6] the lipophilic ligand 1 and the analogous hydrophilic molecule 2. The synthetic procedure is reported in the Scheme.

Ligand 1 is soluble in water only when protonated (pH $\leq$ 3) or as the Fe^{III} complex (pH = 6÷7). Under these conditions it forms micellar aggregates. The critical micelle concentration (c.m.c.) is 5 x 10^{-5} M (pH = 3) and 1.2 x 10^{-5} M (as the Fe^{III} complex) from



1 : R = n-$C_{16}H_{33}$

2 : R = CH_3

Scheme

surface tension measurements. On the other hand, ligand 2 is soluble in water and does not form aggregates. A preliminary investigation of the ability of the Fe^{III} complex of micellized ligand 1 or model 2 to catalyze the oxidation of organic molecules was carried out using *p*-nitrophenyl methyl sulfide as a substrate. In 0.05 M 2-(N-morpholino)ethanesulphonate (MES) buffer, pH = 6.3, under aerobic conditions and in the presence of hydrogen peroxide as a monooxygen donor the pseudo-first-order rate constants reported in the Figure were evaluated by monitoring the decrease of the 350 nm absorption band of the substrate. From the rate *vs* concentration profile shown in the Figure for catalyst $1 \cdot Fe^{III}$ it is possible to evaluate[7] the approximate value of the binding constant of the substrate with the aggregate, 500±100 M^{-1}, and of the $k_{\psi max}$ (all substrate bound to the micelles), 3.5 x 10^{-4} s^{-1}. These data allow to estimate for $1 \cdot Fe^{III}$ a second-order rate constant which is 30 times smaller than that of the $BLM \cdot Fe^{III}$ complex[8] but *ca.* 20 times larger than that of the non micellar model[9] $2 \cdot Fe^{III}$.

Experiments performed in the presence of different amounts of H_2O_2 both with $1 \cdot Fe^{III}$ and $BLM \cdot Fe^{III}$ showed that the oxidation rate is not affected by the hydrogen peroxide concentration. The oxidation process stops after different amounts of substrate (depending on the initial $[H_2O_2]$) are oxidized; upon further addition of H_2O_2, the oxidation starts again with the same rate constant. At least qualitatively, the kinetic

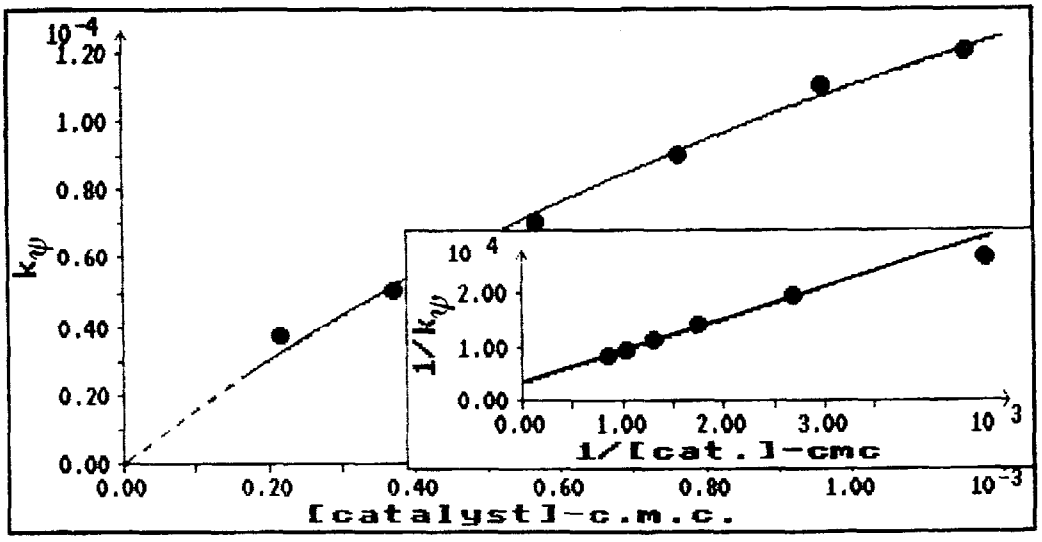


Figure. Observed rate constants, k_ψ, at 25°C for the oxidation of *p*-nitrophenyl methyl sulfide by the 1·Fe^{III} – H_2O_2 system. The solid line represents the computer-calculated curve assuming K_b = 500 M^{-1} and c.m.c. = 1.2 x 10^{-5} M. At zero catalyst concentration the rate it too slow and could not be determined. *Inset*: Lineweaver–Burk analysis of the data.

behavior of BLM·Fe^{III} and 1·Fe^{III} is remarkably similar and indicates that *both* catalysts not only transfer monooxygen to the substrate but also decompose the hydrogen peroxide. Decomposition of hydrogen peroxide[10] and peracids[11] has been reported for other Fe^{III} complexes, while recently BLM·Fe^{III} has been shown to catalyze the homolytic cleavage of alkyl hydroperoxides[12].

Being [substrate] = 1 x 10^{-3} M, [catalyst] = 1 x 10^{-4} M, [H_2O_2] = 1.5 x 10^{-2} M, after 12 h at 25°C, v.p.c. analyses of the reaction mixtures[13] indicated the following product conversion (%) and yield of sulfoxide and sulfone (within parentheses, %): with 1·Fe^{III}, 33 (22, 11) and with BLM·Fe^{III} 27 (18, 9)[14]. These results strengthen the analogy between the two systems which behave as truly catalytic oxidants and apparently react *via* a similar mode of action. The large relative yield of sulfone observed in each case rules out a purely electrophilic mechanism in the monooxygen transfer process[15], although the present data do not allow to define the detailed mechanism.

Work is in progress to better define the properties of 1·Fe^{III} and analogous metallomicellar systems.

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6. Ligand 1 ^{1}H-n.m.r. (CD_3OD): δ 0.89 (br t, 3H), 1.27 (br m, 26H), 1.48 (m, 2H), 2.25 (s, 3H), 2.39 (t J=7.32 Hz, 2H); 2.60 (t J=6.10 Hz, 2H), 2.76 (t J=6.10 Hz, 2H), 2.92 (t J=7.32 Hz, 2H), 3.67 (t J=7.32 Hz, 2H), 3.96 (s, 2H), 6.88 (d 0.92 Hz, 1H), 7.51 (dd J=7.63 and 1.53 Hz, 1H), 7.59 (d J=0.92 Hz, 1H), 7.91 (t J=7.63 Hz, 1H), 7.91 (dd J=7.63 and 1.53 Hz, 1H).
Ligand 2 ^{1}H-n.m.r. (CD_3OD): δ 2.34 (s, 6H), 2.63 (t J=6.10 Hz, 2H), 2.82 (t J=6.10 Hz, 2H), 2.97 (t J=7.32 Hz, 2H), 3.72 (t J=7.32 Hz, 2H), 4.02 (s, 2H), 6.93 (d J=1.22 Hz, 1H), 7.57 (dd J=7.63 and 1.53 Hz, 1H), 7.64 (d J=1.22 Hz, 1H), 7.96 (t J=7.63 Hz, 1H), 8.04 (dd J=7.63 and 1.53 Hz, 1H).
Satisfactory elemental analyses were obtained for both compounds

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8. Bleomycin, a mixture of bleomycin sulfate salts from Streptomyces verticillus, was a Sigma product used as received.

9. Note that a 90:10 methanol/buffer mixture has been used for $2{\cdot}Fe^{III}$ due to the very low solubility of the complex in purely aqueous buffer. The comparison is pertinent since no appreciable effect of added methanol on the reaction rate has been observed with $BLM{\cdot}Fe^{III}$.

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13. As carried out on a 3% FFAP over Chromosorb column.

14. The similarity in the product distributions indicates that $BLM{\cdot}Fe^{III}$ is a better catalyst than $1{\cdot}Fe^{III}$ in both substrate oxidation and H_2O_2 decomposition.

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