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Hydroxylating Activity of a Water-soluble Manganese Porphyrin Associated with Potassium Hydrogen Persulfate : Formation of 8-hydroxyadenosine-5'-mono-phosphate from AMP.

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Summary. The association of potassium monopersulfate, $KHSO_5$, with a water-soluble manganese porphyrin complex has been recently reported as being a very efficient system for the oxidative cleavage of DNA (Fouquet et al., J. Chem. Soc., Chem. Commun., 1987, 1169) ; similar results are observed with RNA. As puzzle piece of the mechanism of this oxidative degradation of nucleic acids, we report in the present communication the identification of 8-hydroxyadenosine-5'-monophosphate as oxidation product of adenosine- 5'-monophosphate (AMP) by $KHSO_5$/$[Mn(Mepy)_4P](OAc)_5$. In the absence of manganese catalyst, the only detectable compound is the adenosine-N^1-oxide- 5'-monophosphate.

The mechanism of action of bleomycins, a family of potent antitumor antibiotics, has been intensively studied during the last decade at the molecular level[1] and these naturally occuring transition-metal chelating molecules are now considered as the prototypes of a new generation of synthetic DNA cleavers : e.g. methidium-propyl-EDTA-Fe(II)[2], hemin-intercalators[3]. Other simple transition metal complexes with a noticeable affinity for DNA are also able to cleave it by oxidation : copper phenanthroline[4], chiral cobalt complexes[5] or metalloporphyrins[6]. Among the latter ones, the readily available pentaacetate of *meso*-tetrakis-(N-methyl-4-pyridyl)porphyrinato-manganese(III) cleaves DNA and RNA[7] at nanomolar concentrations within one minute incubation time after being activated by potassium monopersulfate, $KHSO_5$ (an oxygen atom donor in epoxidation and hydroxylation reactions catalyzed by manganese porphyrin complexes[8]). In order to obtain more details at the molecular level on this efficient catalytic oxidation of nucleic acids, we have studied this reaction with model compounds like adenosine-5'-monophosphate (AMP, **1**). We report in the present communication the identification of 8-hydroxyadenosine-5'-monophosphate **2** as oxidation product of AMP by $KHSO_5$/$[Mn(Mepy)_4P](OAc)_5$.

Treatment of a 1 mM aqueous, buffered (phosphate 0.066 M, pH 6) solution of AMP **1** with 50 μM $[Mn(Mepy)_4P](OAc)_5$ associated to 3 mM $KHSO_5$ for 10 min led to the formation of the compound **2** (25% yield), along with unreacted AMP. The reacting mixture was separated into its component parts by semi-preparative C_{18} μBondapak

reverse phase HPLC. The compound 2 has been tentatively identified as 8-hydroxyadenosine-5'-monophosphate on th basis of the observed ^1H NMR and UV spectra[9]. Furthermore the alkaline phosphatase can cleave this compound leadin to the dephosphorylated compound 3 ; this product is identical (selected data of chromatographic and spectroscopi parameters in notes [10] and [11] respectively) to an authentic 8-hydroxyadenosine sample synthetized fron 8-bromoadenosine according to a published procedure[12].

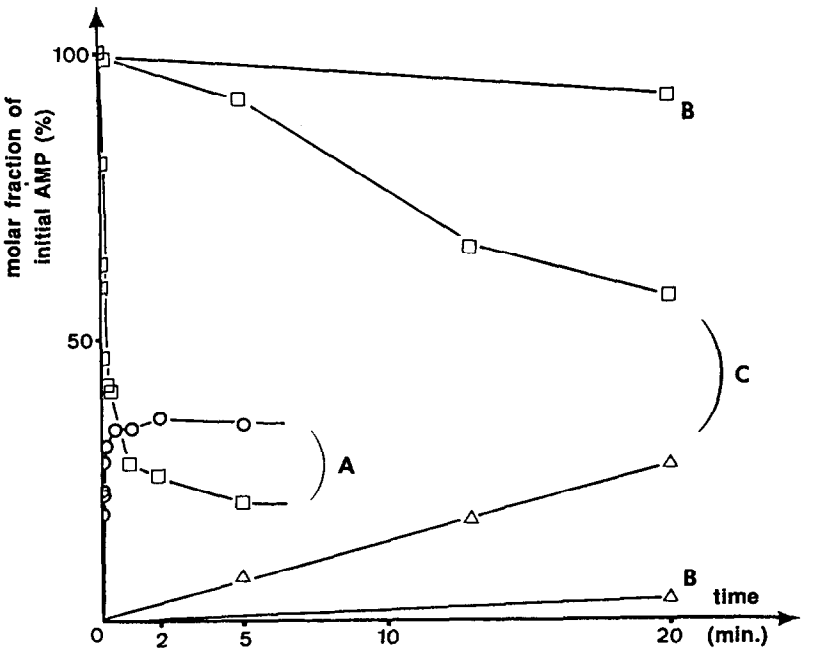


Figure. Reactivity of AMP with $[Mn(Mepy)_4P](OAc)_5$ plus $KHSO_5$ (A) or with $KHSO_5$ alone (B and C). Reaction conditions were : aqueous buffered (phosphate 0.066 M, pH 6) solutions of 0.5 mM AMP were treated by 25 µM $[Mn(Mepy)_4P](OAc)_5$ and 2.5 mM $KHSO_5$ at 25° C (conditions A) or by 2.5 mM $KHSO_5$ alone at 25° C (conditions B) or 60° C (conditions C). Quantitative analysis of 1 (□), 2 (○) and 4 (△) were performed by HPLC on a Waters chromatograph using a µßondapak C_{18} column and a mixture of methanol/5 mM ammonium acetate (8/92, v/v) as eluent after acidification to pH 4.5 with acetic acid, and using calibrated solutions of reference compounds.

*The data indicated on the ordinate axis correspond to the conversion of AMP in % of the starting AMP and the yield of the oxidation product 2 (or 4 for the reaction without catalyst) in % of the starting AMP. On conditions (A) only traces of 4 (<1%) were detected after 5 min.

So the formation of 2 is a strong evidence that $[Mn(Mepy)_4P](OAc)_5$ activated by $KHSO_5$ is able to hydroxylate a substrate in an aqueous medium. This is a very fast process as it is mentionned on the figure. The reaction has gone to completion after only a few seconds. It is also noteworthy that with $KHSO_5$ alone , an oxidative process could be observed but it is (i) very much slower in the same temperature conditions and (ii) the target is different : the isolated compound has been identified as the already known adenosine-N^1-oxide-5'-monophosphate 4[13] (see scheme).

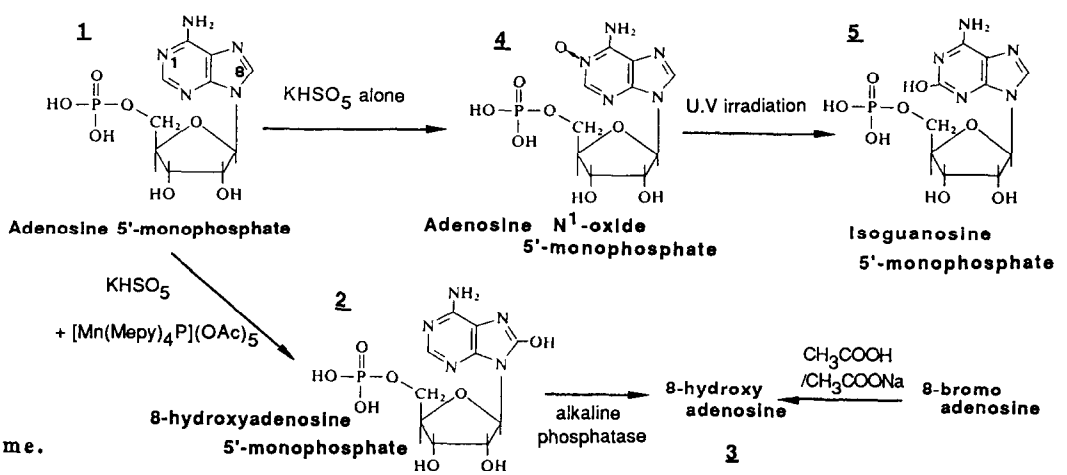
An other fact has to be underlined : in the adenine nucleos(t)ides family, only AMP gave a UV detectable oxidized derivative with a noticeable yield. In the same conditions no degradative product of adenine, adenosine, adenosine-2'-monophosphate, adenosine-3'-monophosphate, adenosine-2':3'-cyclic-monophosphate, adenosine- 3':5'-cyclic

-monophosphate or adenosine-5'-diphosphate have been obtained with a good yield, althought the disappearance of the starting adenine derivative was concomittantly observed ; the same behaviour has been observed for other nucleotides (guanosine-, cytidine- and uridine-5'-monophosphate).

The regiospecific hydroxylation of C_8-H bond to C_8-OH only in the case of AMP strongly argue for some stacking between the purine and the porphyrin rings conveniently oriented by an additional electrostatic interaction between the phosphate group of AMP and the cationic pyridinium structure of porphyrin.

On the mechanism of the oxygenation process of the C_8-H bond, we can propose a " P-450 like " route which involves a high valent porphyrin manganese-oxo complex. Such active species has already been proposed for epoxidation or hydroxylation reactions of hydrocarbons in organic medium catalyzed by iron or manganese porphyrin complexes associated to an oxygen atom donor like PhIO, NaOCl, ROOH or H_2O_2[15]. Epoxidation by $KHSO_5$ has never been observed in aqueous phase with water-soluble complexes and olefins, probably because bleaching of the catalyst is the main reaction. We think that, in water, a strong affinity is required between the catalyst and the substrat to allow the oxygen transfer. The known porphyrin affinities for nucleos(t)ides[16] may be an explanation of the results of the present note which describes the first example where a manganese porphyrin complex in the presence of $KHSO_5$ is able in an aqueous medium to activate a C-H bond and generates an hydroxylated product. An alternative mechanism for the C_8-H hydroxylation would be to consider the addition of an hydroxyl radical at the C_8 position. But recent pulse radiolysis studies have shown that HO· radicals add to an adenosine derivative at C_4, C_5, and not only at C_8[17]. These facts argue against an hydroxyl radical pathway for the hydroxylation at C_8 of AMP by $KHSO_5$/$[Mn(Mepy)_4](OAc)_5$.

Abbreviations. AMP, adenosine-5'-monophosphate ; $KHSO_5$, potassium monopersulfate, known as OXONE^R. $[Mn(Mepy)_4P](OAc)_5$, pentaacetate of *meso*- tetrakis -(N-methyl-4-pyridyl) porphyrinato- manganese (III).



Scheme.

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9 - Selected data for **2** : ^{1}H NMR (250 MHz, CD_3COOD) ; ∂=8.46 (1H, s, 2-H), 6.05 (1H, d, J = 4.5 Hz, H'_1), 5.12 (1H, t, J = 4.5 and 4.5 Hz, H'_2), 4.84 (1H, broad s, H'_3), 4.3 (3H, broad s, H'_4+H'_5+H''_5). UV [H_2O] λ_{max} 256 (shoulder), 268 nm.

10 - HPLC data : RRT (Relative retention time defined as Rt of oxidized derivative/Rt of AMP) = 0.56 (**4**), 0.68 (**2**) and 4.0 (**3**) ; for HPLC conditions, see caption of figure.

11 - Selected data for **3** : ^{1}H NMR (250 MHz, CD_3COOD) ∂= 8.27 (1H, s, 2-H), 6.09 (1H, d, J = 7.O Hz, H'_1), 5.04 (1H, dd, J = 5.4 and 7.0 Hz, H'_2), 4.59 (1H, dd, J = 1.8 and 5.4 Hz, H'_3), 4.34 (1H, d, J = 1.8 Hz, H'_4), 3.98 (1H, dd, J = 1.9 and 12.7 Hz, H'_5 or H''_5), 3.90 (1H, dd, J = 1.9 and 12.7 Hz, H''_5 or H'_5). UV [H_2O] λ_{max} 256 (shoulder), 268 nm ($\log \varepsilon$ = 4.1). MS chemical ionisation (NH_3) : m/z, 284 (MH), 152 (Ade-H_2).

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