The Interconversion of Nucleic Acid Bases by Iron(III) Porphyrins and Nitric Oxide

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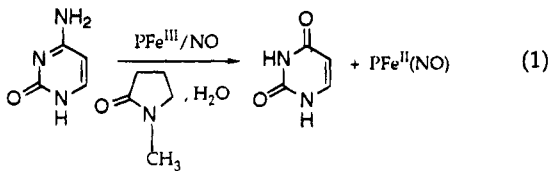
Summary: The conversion of cytosine and *N*-methylcytosine to the corresponding uracils and adenine to hypoxanthine can be accomplished with iron(III) porphyrins and nitric oxide under anaerobic conditions.

The *N*-nitrosation of proline with metmyoglobin and nitric oxide[1] prompted us to ask whether or not arylamines, and in particular nucleic acid bases, could be converted to diazonium ions by PFe^{III}/NO. If these ions were produced and subsequently hydrolyzed, an interconversion of the bases could occur. We report herein that iron(III) porphyrins and NO in argon-purged solutions of amides containing low percentages of water (a protein-like solvent) can indeed bring about these transformations.

In a typical reaction, 10 mL of an *N*-methylpyrrolidone–2% water solution of 4.0×10^{-3} M protoporphyrin IX dimethyl ester and cytosine was purged with argon for 1 h. NO gas, 2.4 mL (1.25 stoichiometric excess), was added to the sealed mixture, and stirring was continued for ~1 day. The visible spectrum of the dark cherry red solution (λ_{max} *398*, 479, 540, 564) was that of the Fe^{II}(NO) adduct. The mixture was purged with argon for 1 h to remove excess NO. One mL was removed for HPLC (reversed-phase ODS column, λ 254 nm detector) and nitrite analyses.[2] The rest was stripped to dryness at 60 °C. The dark solid was extracted with warm water and filtered. The lightly colored aqueous nucleic acid base fraction was concentrated to dryness and dissolved in Me_2SO-d_6 for ^{13}C NMR analysis. Uracil, δ 152, 165, 101, 143, and unreacted cytosine, δ 165.5, 173, 93, 162, were the only pyrimidines present in agreement with the HPLC analysis. The concentration of uracil and NO_2^- in the reaction mixture was $(1.5 \pm 0.1) \times 10^{-3}$ M (35–40% conversion) and $(2.2 \pm 0.2) \times 10^{-3}$ M (50–60%). Note, with adenine and *N*-methylcytosine HPLC analysis was confirmed by FTIR of the amide carbonyls of hypoxanthine (1667 cm^{-1}) and *N*-methyluracil (1695 cm^{-1}).

Because of solubilities and the ease of HPLC analysis, our best studied case is the conversion of cytosine (C) to uracil (U) (eq 1). Repeated runs in *N*-methylpyrrolidone



(1)

–2% H_2O show a consistent 35% conversion of cytosine to uracil and a 65% conversion to nitrite ion.

No influence of porphyrin substituent upon the course of the reaction was noted in the series protoporphyrin IX dimethyl ester, mesoporphyrin IX dimethyl ester, deuteroporphyrin IX dimethyl ester, octaethylporphyrin. That is, under the same conditions the conversion to uracil and NO_2^- was the same. A quantitative production of heme NO adduct is obtained in all cases, and neither uracil nor the porphyrin macrocycle is nitrosated. The influence of solvent and added reagents upon the C to U conversion by chloroiron(III) octaethylporphyrin and nitric oxide is given in Table 1. The addition of triethylamine stops the reaction completely. This amine does not ligate Fe^{III} octaethylporphyrin (Fe^{III}OEP),[3] but in the presence of water produces the μ-oxo dimer. The visible spectrum of the μ-oxo dimer of Fe^{III} OEP remained unchanged upon the addition of NO. The addition of an equivalent amount of NO_2^- to the standard reaction had no effect, and its presence in the absence of NO elicited no reaction. Thus, we conclude the μ-oxo dimer is inert and NO_2^- is not a nitrosating species under reaction conditions.

Table 1. Cytosine to Uracil Conversion by Chloroiron(III) Octaethylporphyrin and Nitric Oxide as a Function of Solvent and Added Reagents[a]

solvent	addn reagent	% convn[b] C → U
pyridine/15% H_2O		0
THF/0.5% H_2O		3
NMP/2%H_2O		35
NMP/2% H_2O	Et_3N[d]	0
NMP/2% H_2O	$NaNO_2$[c]	35
NMP/2% H_2O	$NaNO_2$ (without NO)	0

[a] $(PFe^{III})_0$ = (cytosine)$_0$ = 4.0×10^{-3} M, $(NO)_0 = 10 \times 10^{-3}$ M. [b] $(Et_3N)_0 = 5.0 \times 10^{-3}$ M. [c] $(NO_2^-)_0 = 4.0 \times 10^{-3}$ M. [d] Although the time for completion was shorter, all reactions were analyzed after 13 h.

We formulate the process as an attack by nucleic acid base (base-NH_2) upon a transient formally iron(III)–NO adduct[1] (PFe^{III} NO ↔ PFe^{II} NO^+) (Scheme 1, eq 2). In these reactions, water competes as a nucleophile for the equivalent of an iron-complexed nitrosonium ion.[1] We cannot unambiguously rule out NOCl as the nitrosating species in these reactions, but its rapid hydrolysis by water renders this unlikely.

The interconversion of other nucleic acid bases was also possible under these conditions. Thus, *N*-methylcytosine is cleanly converted to *N*-methyluracil (40%) by all of the porphyrin complexes under the same conditions. The conversion of adenine to hypoxanthine also proceeded smoothly, but the conversion was lower (12–15%). Guanine was not reactive, presumably due to its insolubility.

These results are significant because they demonstrate that point mutations brought about by PFe^{III}/NO species are possible[4] and they suggest the cross-linking of DNA[5]

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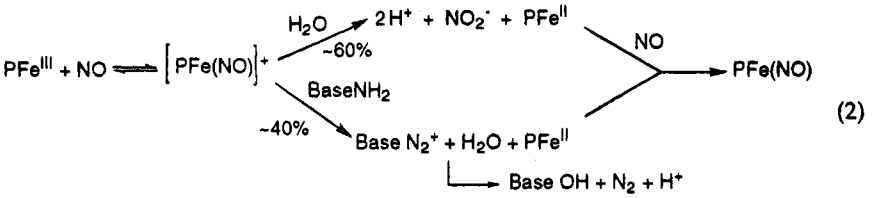
Scheme 1



(2)

by such reagents may be feasible. The significance of these results is heightened by the realization that NO is an air pollutant. Moreover, NO has recently been identified as a ubiquitous messenger in living systems.[6] At this time, NO is considered important in vasodilation, cytostasis, neural transmission, and in cell to cell communication. Its synthase has recently been identified as a cytochrome P-450 like enzyme.[7] In addition, the bioreceptor for NO is the enzyme guanylate cyclase .[8] It too contains an ironporphyrin, and it has been suggested that the conversion of GTP to cyclic GMP by this enzyme is activated by the binding of NO to the heme iron.[9] NO is also a toxic gas to many life forms, including mammals. Like CO, its toxicity is in part vested in its ability to quickly form very stable adducts with iron(II) hemoglobin and myoglobin, as well as a variety of hemeproteins in the respiratory sequence.[10] In addition, NO rapidly reacts with the oxy adducts of hemoglobin and myoglobin to produce the met (Fe^{III}) species.[11]

Clearly the chemistry of reaction of NO with iron porphyrins is at the center of both its toxic and beneficial action in vivo. The valence of iron, and the nature of its ligation, can have a dominant influence upon the process that occurs. As an example, the activation of guanylate cyclase noted above may, in addition to any effects associated with the binding to iron(II)porphyrin, entail a nitrosation in the protein (via PFe^{III}) that aids in unveiling or promoting the chemistry at the active site.[12] We believe the PFe^{III}/NO reagents have far reaching consequences for the role of nitrosation chemistry in biology at sites of low oxygen concentration.

The binding of certain cationic porphyrins to DNA is currently under intense scrutiny.[13] Our work suggests the use of the corresponding iron complexes followed by exposure to NO could be a means of inducing site specific mutations.

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