N-Alkylporphyrin Formation during the Reactions of Cytochrome P-450 Model Systems

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Received December 20, 1984

We have recently found that the electronically substituted hemin 5, 10,15,20-tetakis(2,6-dichlorophenyl)porphino)iron(III) chloride (1) is an effective catalyst for the rapid oxidation of organic compounds with the oxidant iodosopentafluorobenzene (2). 1 2 Unlike all other hemin catalysts so far described, compound 1 is especially robust. We have, for instance, measured 10,000 turnovers, at room temperature, for the epoxidation of norborne! During our studies we noticed that the reaction solution was at first green. However, at the end of the reaction, the hemin was either destroyed (no or very resistant substrate), returned to its original spectrum (reactive substrate), or changed to a new species having a different spectrum. The latter observation occurred mostly with terminal olefins.

Recently, isolations of N-alkylporphyrins from the livers of animals treated with cytochrome P-450 inhibitors such as terminal olefins and acetylenes, monoalkylhydrazines, 4-alkyl-3,5-bis(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydropyridines, and 1-aminobenzotriazoles have been reported. 2 The N-alkyl hemes, derived from such suicide inhibitors, have been shown to result in hepatic porphyrias accompanied by the formation of the so-called "green pigment".

Since the hemin 1 can catalyze numerous turnovers, it might be used to explore the less frequent P-450 chemistry where suicide labeling of liver microsomes occurs approximately 1 in 200 turnovers. In this paper we report a reaction that closely mimics the self-catalyzed inactivation of P-450 enzymes.

In a typical experiment the oxidant 2 (1 g) was added in five portions, every 15 min, to a mixture of the hemin 1 (50 mg) and 4,4-dimethyl-1-pentene (3, 3 g) in dichloromethane (50 mL) and the mixture stirred at room temperature for 15 min. 3 The solution changed from brown to green-brown and the reaction was monitored by the disappearance of the Soret band of 1 (417 nm) coupled with the development of a new band at 435 nm (Figure 1). The remaining oxidant was destroyed with 10% aqueous sodium metabisulfite (10 mL), and the green product was demetalated in a mixture of concentrated HCl (1 mL) and acetic acid (20 mL). After chromatography the N-alkylporphyrin 4 was isolated in 53% yield.

Metalaletion of 4 with FeCl3 in refluxing THF 5 followed by workup in dichloromethane using 1% aqueous HCl and then H2O gave the aquoiron(III) complex 5. 6 Addition of the oxidant 2 to 5, in dichloromethane, gave virtually the same optical spectrum (λmax 435, 570, and 620 nm) as that of the initial green-brown reaction mixture. The analogous spectrum was obtained when a solution of 5, in dichloromethane, which had been previously treated with 1% aqueous HCl 7 or saturated aqueous NaHCO3 8 changed to green-brown and the reaction was monitored by the disappearance of the Soret band of 1 (417 nm) coupled with the development of a new band at 435 nm (Figure 1). The remaining oxidant was destroyed with 10% aqueous sodium metabisulfite (10 mL), and the green product was demetalated in a mixture of concentrated HCl (1 mL) and acetic acid (20 mL). After chromatography the N-alkylporphyrin 4 was isolated in 53% yield.

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Scheme I. Interconversions of the 4-Alkylporphyrin and Its Iron Complexes

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Species</th>
</tr>
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<tbody>
<tr>
<td>a</td>
<td>4,4-Dimethyl-1-pentene, C₆H₁₀/CH₂Cl₂</td>
</tr>
<tr>
<td>b</td>
<td>C₆H₁₀I/CH₂Cl₂</td>
</tr>
<tr>
<td>c</td>
<td>concentrated HCl/CH₂Cl₂</td>
</tr>
<tr>
<td>d</td>
<td>1% aqueous HCl/CH₂Cl₂</td>
</tr>
<tr>
<td>e</td>
<td>H₂O/CH₂Cl₂</td>
</tr>
<tr>
<td>f</td>
<td>aqueous NaHCO₃/CH₂Cl₂</td>
</tr>
<tr>
<td>g</td>
<td>AcOH/CH₂Cl₂</td>
</tr>
<tr>
<td>h</td>
<td>pyridine/CH₂Cl₂</td>
</tr>
<tr>
<td>i</td>
<td>FeCl₃/THF</td>
</tr>
</tbody>
</table>

was treated with 2. All of the species shown in Scheme I were interconvertible, though gradual decomposition of 8 and 9 was observed spectroscopically. These observations suggest that 8 and 9 may be the [chloro(pentafluorophenyl)iodoxo]iron(III) and the [hydroxo(pentafluorophenyl)iodoxo]iron(III) complexes. The ferric oxidation state in 8 and 9 is supported by their rhombic ESR spectra (g = 8.57, 5.35, 2.05, CH₂Cl₂, -196°C), which change to those of typical low-spin ferric complexes (g = 2.38, 2.14, 1.94) on the addition of pyridine.

In most respects hemins and iodosobenzene mimic the hydroxylations and epoxidations catalyzed by the cytochromes P₄₅₀. The additional parallels reported here, where an in vitro system mimics the suicide inhibitors characteristic of microsomal P₄₅₀, add further support to the validity of using the chemical to model the enzymic systems.

Acknowledgment. This work was supported by the NIH (GM 29198) and the NSF (CHE 81-20969).

Registry No. 1, 91042-27-2; 2, 827-15-6; 3, 762-62-9; 4, 96326-84-0; 5, 96326-85-1; 6, 96326-86-2; 7, 96326-87-3; 8, 96326-88-4; 9, 96326-89-5; 10, 96326-90-8; cytochrome P₄₅₀, 9035-51-2.

(8) We tentatively assigned the hydroxide structure 7 for the species generated by this treatment (Scheme I): UV λmax (CH₂Cl₂) 361, 444, 570, 585, 640 nm.

(9) The reversible formation of 8 and 9 from 6 and 5, coupled with the ESR results, suggests the structures shown. The coordination of iodoso-


(10) The structure assigned to 10 is most likely for the species formed in the presence of excess pyridine (Scheme I). UV λmax (CH₂Cl₂, 1 drop of pyridine) 432, 585 (sh), 618 (sh) nm. The N-alkyl group prevents coordination on both sides of the metal, a similar reaction, [Fe[II](N-MeOEP)Cl]⁺ + pyridine = [Fe[II](N-MeOEP)py]⁺ + Cl⁻ (K ~ 1.66 (where OEP = octaethylporphyrin), has been previously reported: Ogoshi, H.; Kitamura, S.; Toi, H.; Aoyama, Y. Chem. Lett. 1982, 495.