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Formation of Chlorins by Oxidation of Deuteroporphyrin with Horseradish Peroxidase

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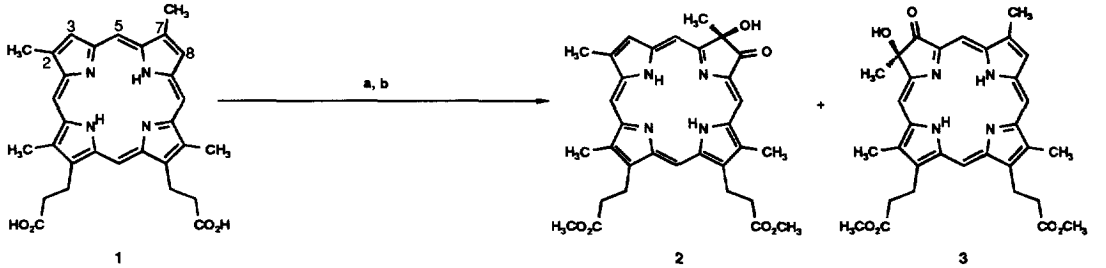
Abstract: Two novel chlorins **2** and **3** were isolated from the reaction of deuteroporphyrin **1** with horseradish peroxidase and glutathione. On incubation of uroporphyrin I, coproporphyrin III, protoporphyrin IX, hematoporphyrin IX and mesoporphyrin IX under the same conditions the formation of chlorins could not be detected. © 1997 Elsevier Science Ltd.

In mammals, some chemically induced uroporphyrias result from the inhibition of uroporphyrinogen decarboxylase leading to accumulation of large amounts of uroporphyrinogen which are subsequently oxidized to phototoxic uroporphyrins.[1] Studies to ascertain whether peroxidases play a role in the mechanisms by which these excess porphyrinogens are oxidized showed that some porphyrinogens are indeed converted into porphyrins by treatment with cytochrome P450 enzymes or peroxidases.[1, 2, 7] Jacobs et al.[3] studied the oxidation of uroporphyrinogen and deuteroporphyrinogen with horseradish peroxidase. Yields of porphyrin when oxidizing deuteropophyrinogen were lower than expected in comparison to the oxidation of uroporphyrinogen because a part of the porphyrinogen in this case is converted to green oxidation products with the spectroscopic characteristics of a chlorin. These compounds were also detected when deuteroporphyrin **1** was incubated under the same conditions.[3] The structure of these oxidation products is reported in this study.

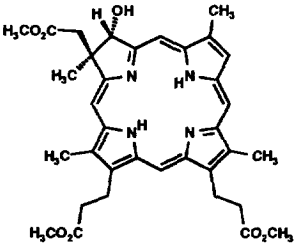


1 **2** **3**

Scheme 1: a) **1** (as hydrochloride in a 20% ethanol 0.01N KOH solution), Tris buffer (pH 7.5), EDTA, glutathione and horse radish peroxidase. b) 5% sulphuric acid in methanol.

After six hours of incubation of **1** with horseradish peroxidase (HRP) and glutathione as described previously,[3] the reaction mixture was lyophilized and esterified with methanol-sulphuric acid (5%). Analysis of the mixture by thinlayer chromatography showed a green pigment slightly more polar than deuteroporphyrin dimethylester which apart of some other brownish and much more polar products was also present in the mixture. The green product was purified *via* flash chromatography on a reversed phase column (RP18, methanol-water 95:5). We first expected the compound to be a dihydroxychlorin because porphyrins were often oxidized at a ββ pyrrolic double bond to yield diols.[4, 5] But the relatively low molecular extinction of the red band [λ 646nm; log ε = 4.64] in the UV/VIS spectrum suggested a chlorin system possessing a conjugated

carbonyl function. The comparison of the UV/VIS spectra of hydroxychlorin **4** and oxochlorin **5**,[8] both previously synthesized in our laboratory,[6] with the spectrum of the new chlorin[9] showed indeed a striking similarity between chlorin **5** and the enzymatically formed chlorins.



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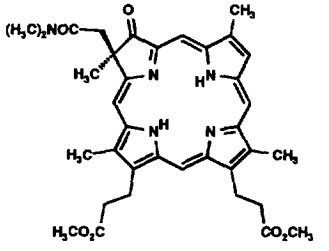

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Mass spectrometry which gave a molecular ion of m/z = 570 revealed that an hydroxy group and an oxofunction had been introduced into deuteroporphyrin **1** during enzymatic transformation. The NMR spectrum[8] confirmed that a mixture of two constitutionally isomeric chlorins **2** and **3**, having the oxo function at C- 3 or C-8 atom and the hydroxyfunction at C-2 or C-7 atom respectively were formed.

It has also been determined whether porphyrins other than deuteroporphyrin can be oxidized to chlorin derivatives by HRP and glutathione. Uroporphyrin I, coproporphyrin III, protoporphyrin IX, hematoporphyrin IX, and mesoporphyrin IX were incubated with HRP and glutathione using the same incubation conditions described above for deuteroporphyrin. Spectral scanning of the reaction mixture showed that none of these other porphyrins exhibited the spectral shifts characteristic of chlorin formation. From these results, it can be concluded that deuteroporphyrin is unique in being oxidized to a chlorin derivative by HRP and glutathione, possibly due to the missing substituents at position 3 and 8.

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8. Selected spectroscopic data of **4** and **5**: **4**: **UV/VIS** ($CHCl_3$): λ_{max} (lg ε) = 351 (4.39), 391 (5.16), 489 (3.94), 495 (3.94), 523 (2.30), 591 (3.20), 616 (3.15), 643 (4.57). **5**: **UV/VIS** ($CHCl_3$): λ_{max} (lg ε) = 387 (4.93), 404 (5.19), 485 (3.74), 504 (4.01), 540 (3.94), 585 (3.72), 642 (4.55).
9. Selected spectroscopic data of **2** and **3**: **UV/VIS** ($CHCl_3$): λ_{max} (lg ε) = 402 (4.72), 509 (3.53), 550 (3.5), 590 (3.35), 646 (4.14). **^{1}H NMR** (360 MHz, $CDCl_3$) : δ = -3.27 (1s, br., 2H, 2 NH); -2.93, -2.87 (2s, br.,2H, 2 NH); 3.321 (m, 8H, 13-, 17-$CH_2\underline{CH_2}COCH_3$); 3.23, 3.34 (2s, br, 2H, 2- or 7-OH); 3.44, 3.47 (2s, 6H, 12- or 18-CH_3); 3.56 (1s, 6H (2 Me), 12- or 18-CH_3); 3.63 (1d, ^2J = 0.78 Hz, 3H, 2- or 7-CH_3); 3.644 (1s, partially covered, br., 3H, 2- or 7-CH_3); 3.67 (1s, br., 3H, 2- or 7-CH_3); 3.641, 3.65, 3.665, 3.67 (4s, 12H, 13-, 17-$CH_2CH_2COC\underline{H_3}$); 3.69 (1d, ^2J = 1.16 Hz, 3H, 2- or 7-CH_3); 4.21 (*m*, 4 H, 13-, 17-$C\underline{H_2}CH_2COCH_3$); 4.37, 4.38 (2*m*, 4 H, 13-, 17-$C\underline{H_2}CH_2COCH_3$); 8.92, 9.01 (2s, br., 2H, 3- or 8-H); 9.34, 9.35, 9.74, 9.76, 9.82, 9.86, 9.87, 9.96 (8s, 8H, 5-, 10-, 15-, 20-H).

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