Tetrahedron Letters,Vol.24,No.10,pp 995-998,1983 0040-4039/83/100995-04$03.00/0
Printed in Great Britain

NONHYDROLYTIC CHEMICAL CONVERSION OF OCTAETHYLVERDOHEMOCHROME TO OCTAETHYLBILIVERDIN

Takashi Hirota and Harvey A. Itano*
Department of Pathology, D-006, University of California, San Diego, La Jolla, California 92093

Summary: Octaethylverdohemochrome, a ferrous oxaporphyrin, was converted to octaethylbiliverdin with the incorporation of an oxygen atom from $^{18}O_2$ into the latter; this result showed that hydrolysis is not essential for this conversion and that an oxygen-bridged compound cannot be excluded as a precursor of biliverdin.

When hemin is oxidized in pyridine by O_2 in the presence of a reducing agent, a green intermediate convertible by hydrolysis to biliverdin is produced in a process known as coupled oxidation. This green compound, which is called verdohemochrome, has been postulated to be the pyridine complex of an iron oxaporphyrin, a product in which one of the methene bridges of protoporphrin has been oxidized, expelled as CO, and replaced by an oxygen bridge.[1] Biliverdin from the coupled oxidation of protohemin is a mixture of the four isomers that result from cleavage at the four different methene bridges of protoporphyrin IX.[2] To avoid this complication, Jackson et al.[3] used octaethylhemin, the symmetrical octaethyl analogue of protohemin, in their experiments on coupled oxidation by mixtures of $^{16}O_2$ and $^{18}O_2$ in the presence of ascorbate. Their results revealed that both terminal oxygens of octaethylbiliverdin (4) were derived from O_2 and, moreover, that the two oxygen atoms came from different molecules of O_2. Assuming that the formation of 4 from octaethylverdohemochrome (1) must be hydrolytic and therefore would result in the incorporation of an oxygen derived from water into 4, they ruled out the participation of an oxygen-bridged intermediate such as 1 in the formation of 4.

In order to resolve the apparent contradiction between the postulated structure of 1 and the incorporation of an oxygen atom from O_2 into 4, we looked for an alternative mechanism to hydrolysis. We first obtained a crystalline preparation of the green intermediate by the coupled oxidation of octaethylhemin and phenylhydrazine. This product yielded elemental analyses that agreed with the composition of the chloride of the dipyridine complex of 1 and an electron impact mass spectrum in which the base peak at m/e 591 corresponded to the ion derived from this compound less the chloride and the two pyridine ligands. The compound is labile to O_2 and light in common organic solvents other than pyridine. It does not bind CO in pyridine but does so in $CHCl_3$ under N_2 with change in its color from green to blue. Its color is restored by bubbling N_2 through the blue solution. The chemical and physical properties of the green compound are thus in accord with a ferrous oxaporphyrin structure for 1. Lagarias[4] isolated the green intermediate in the coupled oxidation of octaethylhemin and ascorbate as the tetrafluoroborate of the dipyridine complex and also assigned the structure of 1 to his product. Although the elemental analysis was unsatisfactory, spectral data were similar to those of our product. He suggested that an "oxidative hydrolysis" would explain the results of Jackson et al. as well as the assumed hydrolysis of 1 to 4 but did not suggest a possible mechanism for such a process.

Hydrolysis and demetallation of 1 by treatment with equimolar KOH followed by excess HCl yielded a yellow intermediate postulated as 2, then 4. Treatment of 1 with a 10-fold excess each of phenylhydrazine-HCl and $^{18}O_2$ in tetrahydrofuran (THF)-ethanol produced 4 in which 81.7% (78.2% in a duplicate experiment) of the product contained one ^{18}O. Incorporation in high yield of an oxygen atom from O_2 into 4 showed that 1 can be cleaved nonhydrolytically and therefore cannot be excluded as a precursor of 4. The mechanism of this reaction was studied by observing the effects of oxidants and reductants on 1. Treatment of the green solution of 1 in THF with H_2O_2 resulted in a yellow solution. Addition of phenylhydrazine-HCl to the yellow solution converted it to a blue solution of 4. Electronic absorption spectra corresponding to these changes are shown in Figure 1. When phenylhydrazine-HCl and $^{18}O_2$ were added to 1, the initial reaction presumably was the reduction of $^{18}O_2$ to $H_2{}^{18}O_2$. Reaction of $H_2{}^{18}O_2$ with 1 presumably produced an $^{18}O_2$-labelled yellow intermediate postulated as 3, and reduction of the latter by the excess of phenylhydrazine present in the reaction mixture produced ^{18}O-labelled 4. This cleavage process is the culmination of coupled oxidation, and this mechanism provides for the addition of the elements of water to 1 without the participation of water. The alternative pathways for the conversion of octaethylverdohemochrome to octaethylbiliverdin are shown in Figure 2. The same mechanism has been postulated in the cleavage of verdohemochrome IXα to biliverdin IXα.[5]

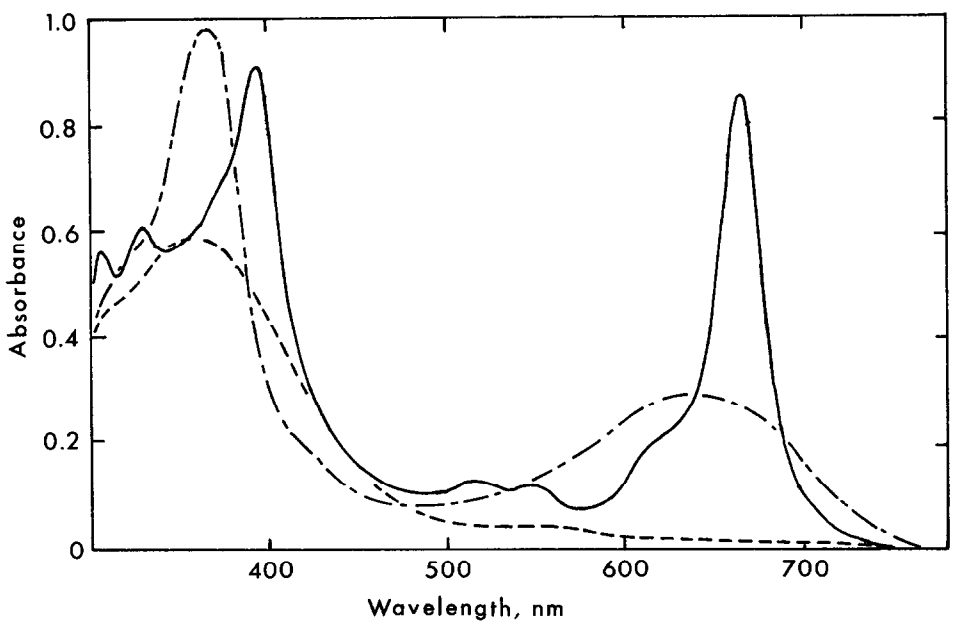

Figure 1. Electronic absorption spectrum of green solution of octaethylverdohemochrome (1) in THF under N_2 (——). Spectrum of yellow solution one hour after the addition of equimolar H_2O_2 to the green solution (----). Spectrum of blue solution 35 minutes after the addition of equimolar phenylhydrazine-HCl to the yellow solution (—·—).

To a solution of octaethylhemin (499 mg, 0.8 mmol) in one liter of 4:1 (v/v) pyridine/H_2O in a two-liter pear-shaped flask, phenylhydrazine-HCl (578 mg, 4 mmol) was added at once, and the flask was closed immediately with a rubber stopper to prevent the entrance of additional air. As the dark brown solution was stirred gently for one day at room temperature, it gradually became dark green. The solvent was removed under vacuum below 45 °C, and the residue was fractionated by TLC (Merck silica gel 60, 20 cm x 20 cm x 1.5 mm) with 2.0:6.5:0.8:0.7 (v/v) pyridine/benzene/ethanol/2-butanone as the solvent system. The major green zone (R_F ca 0.2-0.4) was scraped off and eluted with 1:1 (v/v) pyridine/ethanol under N_2 as quickly as possible. The resulting green solution was evaporated to dryness under vacuum below 45 °C, and the residue was chromatographed on a silica gel column (7 g, pre-saturated with argon) with 4:1 (v/v) benzene/pyridine under argon. After some colored products were eluted by this solvent system, the major green product was eluted with 7:2:1 (v/v) benzene/pyridine/ethanol, and the green eluate was

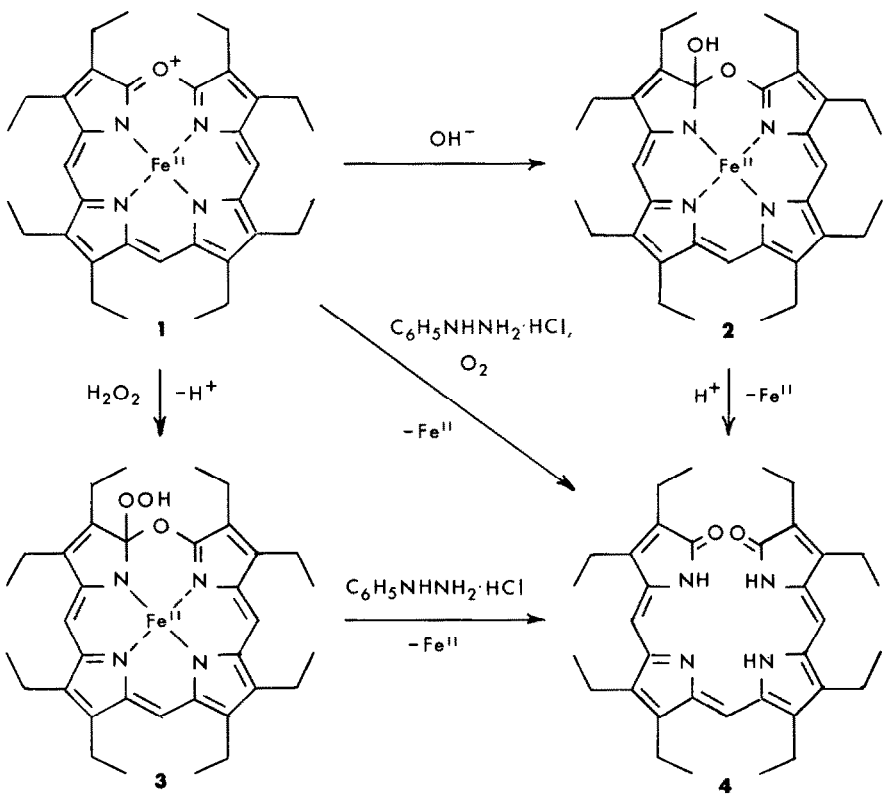

Figure 2. Conversion of octaethylverdohemochrome (1) to octaethylbiliverdin (4) by hydrolytic and nonhydrolytic routes. Yellow intermediates in the respective processes are postulated to be 2 and 3. The existence of a nonhydrolytic route was shown by the incorporation of one ^{18}O into 4 when 1 was treated with phenylhydrazine-HCl and $^{18}O_2$.

concentrated to about one-fifth of its volume under vacuum at around 40 °C. After the addition of approximately an equal volume of benzene to the concentrate, n-hexane was gradually added at around 40 °C under argon until fine crystals with a bronze luster began to form. After 3-4 hours in a refrigerator in a stoppered flask, the crystals were collected on a filter, washed with 3:2 (v/v) n-hexane/THF, and dried at 30 °C for one day under vacuum to obtain analytically pure dipyridine chloride of 1 (165 mg, 26.3%). This product sintered at around 110 °C and blackened but did not melt completely below 300 °C. Anal. Calcd for $C_{45}H_{53}N_6OClFe$: C, 68.83; H, 6.80: N, 10.70; Cl, 4.51; Fe, 7.11. Found: C, 69.00; H, 6.53; N, 10.66: Cl, 4.27; Fe, 6.94. Mass spectrum (EI 70 ev) m/e (relative intensity) 593 (16.2), 592 (55.6), 591 (M - Cl^- - $2C_5H_5N$, 100), 590 (6.8), 589 (9.4), 577 (6.9), 576 (4.3), 575 (7.3), 563 (8.6), 562 (4.3), 561 (9.5), 547 (5.2), 546 (3.0), 545 (3.9), 531 (4.7), 296 (8.1), 295.5 (12.9), 288.5 (6.5), 288 (13.8), 280.5 (3.5), 273 (10.4). Electronic absorption spectrum λ_{max}^{nm} (ε_{mM}) in pyridine 652 (45.9), 608 sh (14.4), 526 (15.0), 492 (9.0), 425 sh (20.1), 386 (58.0), 323 (21.3); in THF 665 (52.3), 620 sh (12.1), 549 (6.7), 516 (7.1), 394 (48.8), 375 sh (34.1), 330 (29.9). PMR spectrum (90 MHz, pyridine-d_5, Me_4Si) δ 1.63 (m, 24 H, 8 x CH_3), 3.52 (m, 16 H, 8 x CH_2), 9.04 (s, 1 H, -CH=), 9.50 (s, 2H, 2 x -CH=). Signals of the pyridine ligands of 1 overlapped those of the solvent. The ligand molecules probably exchanged rapidly with the large excess of solvent pyridine-d_5. PMR measurements in other solvents were unsuccessful owing to instability of 1. No significant absorption band was observed in the IR spectrum of 1.

To a mixture of 7.9 mg (10 μmol) of the dipyridine chloride of 1 in 30 ml of THF and 14.5 mg (100 μmol) of phenylhydrazine-HCl in 2 ml of ethanol under argon, 2.24 ml of $^{18}O_2$ was injected with a needle through a rubber stopper. The solution was stirred at room temperature for 10 hours, during which time a gradual change in its color from green to greenish blue occurred. The solvents were removed by evaporation, and the residue was subjected to TLC (Merck silica gel 60, 20 cm x 20 cm x 0.25 mm) with 5:4:1 (v/v) benzene/n-hexane/pyridine as the solvent system. The blue zone with R_F 0.7 was scraped off and eluted with 5:1 (v/v) chloroform/acetone. The residue obtained by evaporation of the eluate was recrystallized from methanol to give 2.3 mg (41%) of a product that was identical with an authentic sample[6] of 4. The ratio of intensities of the molecular ion peaks (m/e 554 and 556) in the mass spectrum of 4 was 20.3:81.7 (21.8:78.2 in a duplicate experiment).

Acknowledgement. This work was supported by National Institutes of Health Grant AM 14982.

References

1. R. Lemberg, Rev. Pure Appl. Chem. 6, 1-23 (1956).
2. R. Bonnett and A. F. McDonagh, J. Chem. Soc. Perkin Trans. 1, 881-888 (1973).
3. A. H. Jackson, M. G. Lee, R. T. Jenkins, S. B. Brown, and B. D. Chaney, Tetrahedron Lett. 5135-5138 (1978).
4. J. C. Lagarias, Biochim. Biophys. Acta. 717, 12-19 (1982).
5. S. Saito and H. A. Itano, Proc. Natl. Acad. Sci, U.S.A. 79, 1393-1397 (1982).
6. R. Bonnett and M. J. Dimsdale, J. Chem. Soc. Perkin Trans. 1, 2540-2548 (1972).

(Received in USA 22 September 1982)