Synthesis of Hemin C from Hemin

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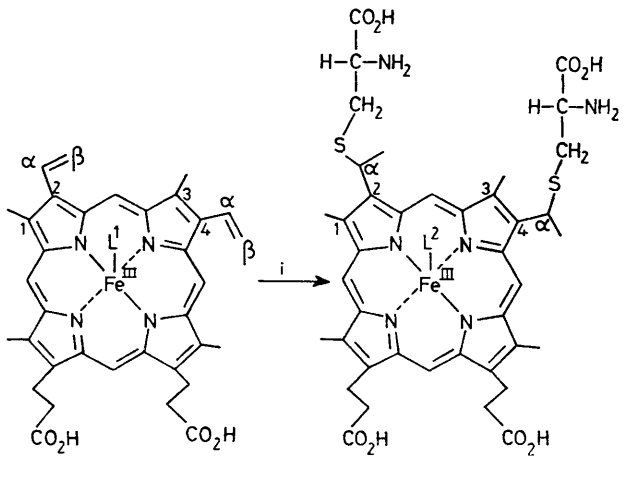
Summary Hemin C (**2**) was prepared in 45—50% yield by the reduction of a solution of iron protoporphyrin IX [hemin (**1**)] and L-cysteine with sodium borohydride in the presence of oxygen and cetyltrimethylammonium bromide micelle.

THE formation of a sulphide bond between the α-carbon of the vinyl group of iron protoporphyrin IX [hemin (**1**)] and cysteine to form iron 2,4-bis(α-*S*-cysteinylethyl)deuteroporphyrin IX [hemin C (**2**)] is the key reaction for an understanding of the biosynthetic mechanism of cytochrome c. One biosynthetic approach utilized protoporphyrinogen as starting material[1,2] to form porphyrin c [2,4-bis(α-*S*-cysteinylethyl)deuteroporphyrin IX]. This reaction was particularly useful for the reconstitution[3] as well as the chemical synthesis[4,5] of cytochrome c. However, enzymatic studies suggest that protohemin (**1**) is the precursor of cytochrome c.[6] Here, we report the first model reaction for the formation of hemin C (**2**) from hemin (**1**) and cysteine under physiological conditions.



(1) (2)

i; Cysteine, CTAB, room temp., O_2, $NaBH_4$, pH 8·1

Protohemin chloride (**1**) (10 mg) dissolved in 10 ml of 0·1 N NaOH solution was added to 200 ml of phosphate buffer (0·2 M; pH 8·1) containing 72 mg of cetyltrimethylammonium bromide (CTAB) with nitrogen bubbling. After the addition of 500 mg of cysteine, the total oxygen content was adjusted to *ca.* 15 μmol† and then sodium borohydride (20 mg) was added. The mixture was vigorously stirred for another 2 min. After purification according to the literature,[2] the yield of (**2**) was 45—50%. The structure of the product was determined by spectral data as well as chemical conversions into known compounds, hematohemin[2,5] (95% yield) and hematoporphyrin dimethyl ester dimethyl ether[7]‡ (85% yield) utilizing established procedures. Two cysteic acid residues formed by performic acid oxidation of the product (**2**) were determined by amino-acid analysis.

The iron atom of hemin played an important role in this reaction, because protoporphyrin IX did not undergo addition of cysteine under similar conditions. Oxygen was also crucial in the present reaction since formation of the sulphide bond was not observed under strictly deoxygenated conditions. Other reducing agents such as sodium dithionite, sodium sulphite, sodium ascorbate, or cysteine itself could not replace sodium borohydride. These facts may suggest that the intermediate of the present reaction is not a simple Fe^{II}–protoporphyrin IX.

CTAB micelle prevented the loss of iron from protoheme during the reduction with $NaBH_4$. In the absence of CTAB, the yield of hemin C was very poor. When the reaction was performed in a neutral micelle (triton X-100) or in an anionic micelle (sodium dodecyl sulphate), hemin C formation was negligible, suggesting that a cationic hydrophobic environment was important.

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† An equimolar amount of oxygen to (**1**) gave the best yield. The concentration of oxygen was monitored by the use of a Gilson oxygraph K-IC.

‡ The structure was identified by 220 MHz n.m.r. and visible spectroscopy, hydrochloric acid number, and chromatographic behaviour.

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