PORPHYRINS DERIVED FROM THE PROSTHETIC GROUP OF MYELOPEROXIDASE.

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Summary. Treatment of heat-denatured myeloperoxidase with methanol-HCl results in a 20% yield of protein-free hemins. The resulting porphyrins have been identified as 2(or 4)-(1'-methoxyethyl)-4(or 2)-methoxycarbonyl-hydroxymethyldeuteroporphyrin IX dimethyl ester (Ib) and 2(or 4)-(1'-methoxyethyl)-4(or 2)-methoxycarbonyldeuteroporphyrin IX dimethyl ester (IIb). The possible formation of these porphyrins from 2(or 4)-vinyl-4(or 2)-glyoxyloyldeuteroporphyrin IX (III) is discussed.

Myeloperoxidase, the peroxidase of neutrophil leucocytes, is believed to have a role in the destruction of phagocytosed bacteria (McRipley and Sbarra, 1967; Klebanoff, 1968). Unlike hemoproteins which have protoheme as their prosthetic group and show absorption maxima in the region of 555-570 mμ, myeloperoxidase, in the reduced form, has a strong absorption maximum at 640 mμ. The similarity between the spectrum of this derivative and that of ferrochlorin-globin complexes has led to the suggestion that the prosthetic group might have the electronic conjugation of a chlorin (Morell et al, 1967). Treatment of myeloperoxidase with pyridine results in the slow formation of a hemochrome having an absorption maximum at 595 mμ. This appears to be the hemochrome corresponding to a porphyrin with one or more strongly electrophilic substituents (Schultz and Shmukler, 1964; Newton et al, 1965). Attempts to remove the prosthetic group from myeloperoxidase by methods such as treatment with acetone-HCl or $AgSO_4$ in acetic acid have failed (Agner, 1943, 1958). Low yields of porphyrins have been obtained by treatment of the enzyme and peptides derived from it with strong acids (Schultz et al, 1962; Newton et al, 1965). This communication describes an improved method for the preparation of

porphyrins from myeloperoxidase and determination of the structures of two products.

Methods and Results. Myeloperoxidase was prepared from the Shay rat chloroma by the method of Newton et al (1965). Concentrations were determined from the ε_{mM} at 640 mμ per heme of 26 (Agner, 1958). The enzyme was reduced with ascorbic acid. Cerefoil silica gel sheets were used for thin-layer chromatography in light petroleum (b.p. 60-80°)-acetone (7:3). For paper chromatography of the porphyrin esters, Whatman No. 1 paper was used with chloroform-kerosine (2.6:4) and kerosine:n-propanol (5:1) as ascending solvents. For porphyrin free acids the descending 2,6-lutidine: water system of Falk et al (1956) was used. Mass spectra were determined at 300° and 70 eV on an AEI MS9 spectrometer.

Preparation of prosthetic group porphyrins was carried out by the following method. Myeloperoxidase (2.7×10^{-5}M, 50 ml) in 0.3 M pH 9.0 phosphate buffer was heated at 100° for 1 hr. Acetone (250 ml) was added and the suspension centrifuged. The precipitate was washed twice with methanol, suspended in methanol saturated with HCl and stirred overnight at 4°. The suspension was filtered and the residue washed with methanol. The filtrate was diluted with ether and washed with water followed by 20% HCl to remove porphyrins not bound to iron. The ether solution showed a typical hemin spectrum with maxima at 410, 507, 537 and 640 mμ. Assuming ε_{mM} at 410 mμ was about 100 the yield of hemin was 20%. After removing the iron by the method of Morell, Barrett and Clezy (1961) an ether solution of the porphyrins could be separated into two fractions by extraction with 7% HCl followed by 12% HCl. After recovery into chloroform the former fraction showed an aetio-type spectrum with band III at 540 mμ while the latter showed a rhodo-type spectrum with band III at 550 mμ (Falk, 1964). (Fig. 1). Derivatives of the two porphyrins were prepared by standard methods (Falk, 1964). The chromatographic results are summarised in Table 1. Hydrolysis to the free acid was accomplished in the case of the 7% HCl-porphyrin by standing in 10 N HCl overnight. In the case of the 12% HCl-

Table 1. R_F values of myeloperoxidase porphyrins and derivatives.

Methyl esters	$CHCl_3$:kerosine	Kerosine:nPrOH	T.L.C.
Protoporphyrin	0.67	0.85	0.56
Hematoporphyrin	0.01	0.35	0.42
2(or 4)-hydroxyethyl-4(or 2)-vinyldeuteroporphyrin	0.07	0.71	0.49
7% HCl-porphyrin	0.08	0.63	0.44
Acetylated 7% HCl-porphyrin	0.19	0.84	0.50
12% HCl-porphyrin	0.44	0.73	0.49
2(or 4)-vinyl-4(or 2)-methoxycarbonyldeuteroporphyrin	0.52	0.80	0.51
2(or 4)-methoxyethyl-4(or 2)-methoxycarbonyldeuteroporphyrin	0.43	0.72	0.49

No change in R_F occurred on reaction of either of the myeloperoxidase porphyrins with hydroxylamine, sodium borohydride or periodic acid.

Free acids	2:6 Lutidine:water (Falk, 1956)
Protoporphyrin	0.82
Tricarboxylic acid porphyrin (Falk *et al*, 1956)	0.68
Coproporphyrin III	0.56
7% HCl-porphyrin	0.68
12% HCl-porphyrin	0.70
Hematoporphyrin	0.83

porphyrin hydrolysis by this method was incomplete and was achieved by boiling for 5 min in 30% methanolic KOH.

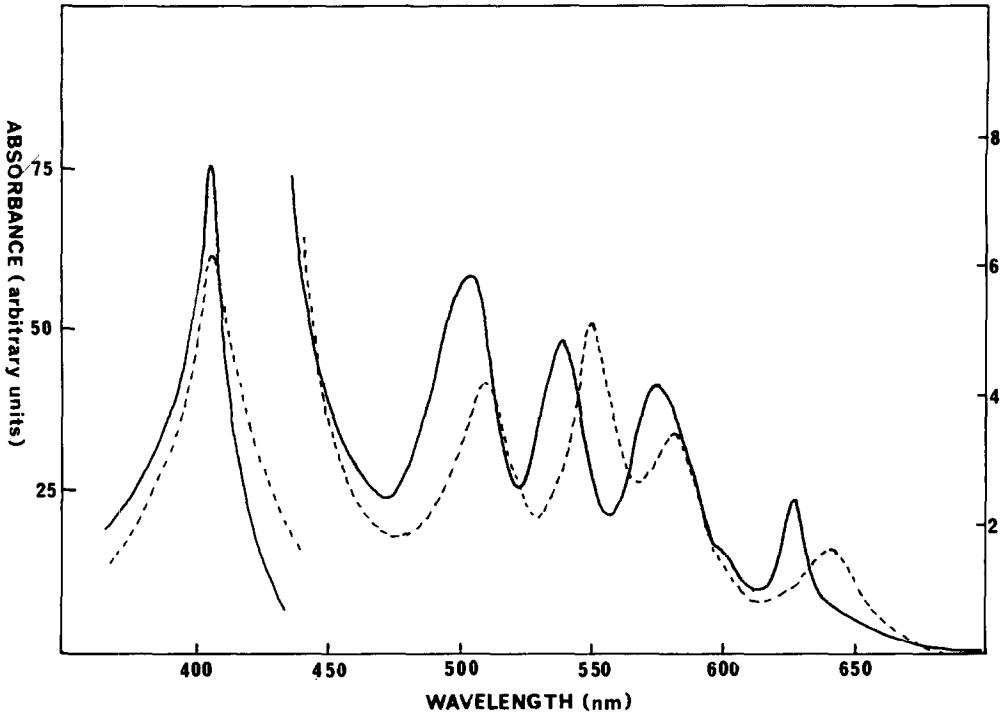

Figure 1. Absorption spectra of 7% HCl-porphyrin (——) and 12% HCl-porphyrin (----) in chloroform.

The results suggest that both porphyrins are tricarboxylic acids or esters thereof. In the case of the 12% HCl-porphyrin conjugation of one of these groups could account for its rhodo spectrum. While no other carbonyl functions could be detected, an increase in R_F on acetylation in the case of the 7%-HCl porphyrin suggests the presence of an OH group. Neither porphyrin showed a shift in absorption spectrum on treatment with ethyl diazoacetate, with HBr in acetic acid or on brief hydrogenation in formic acid with a palladium-charcoal catalyst, indicating the absence of carbon-carbon unsaturated side chains.

Several porphyrins with conjugated methoxycarbonyl side chains were prepared and the spectra compared with that of the 12% HCl-porphyrin. Of these 2(or 4)-vinyl-4(or 2)-methoxycarbonyldeuteroporphyrin, prepared by the method of Barrett and Clezy (1959) showed a very similar though not identical spectrum

to that of the 12% HCl-porphyrin. After treatment of this porphyrin with HBr in acetic acid followed by methanol, the product showed an identical spectrum and R_F values to that of the 12%-HCl porphyrin. The mass spectrum of the 12% HCl-porphyrin which was precipitated from a small volume of chloroform by adding methanol showed a molecular ion of 654 mass units confirming it to be 2(or 4)-(1'-methoxyethyl)-4(or 2)-methoxycarbonyldeuteroporphyrin IX dimethyl ester (Ib).

The mass spectrum of the 7% HCl-porphyrin showed M^+ at 684. This information together with the chromatographic results suggest that it is 2(or 4)-(1'-methoxyethyl)-4(or 2)-methoxycarbonylhydroxymethyldeuteroporphyrin IX dimethyl ester (IIb).

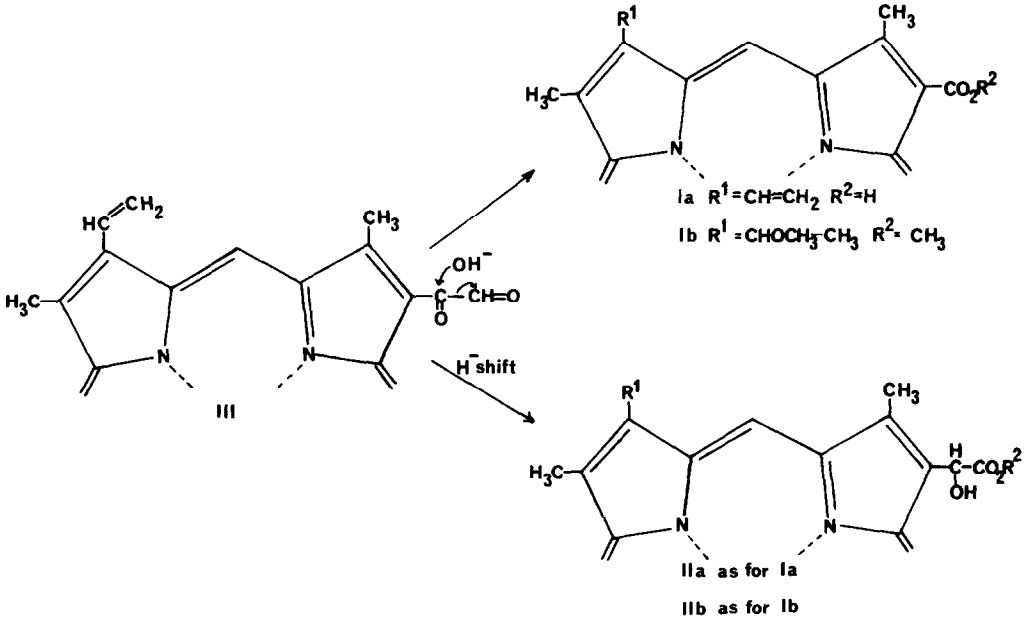

Figure 2. Partial structures of porphyrins isolated from the prosthetic group of myeloperoxidase and their mechanism of formation from a possible precursor. Hydroxide ion attack as shown applies only to the formation of Ia.

Discussion. Formation of the methoxyethyl side chain of the two prosthetic group porphyrins presumably occurs by solvation of a vinyl group during cleavage with methanol-HCl. This reaction occurs readily with small amounts

of protohemin but only slowly with protoporphyrin (Nichol, unpublished observation). It is suggested that the spectrum which finally appears on standing myeloperoxidase in pyridine having a maximum at 565 mμ (Schultz and Shmukler, 1964; Newton et al, 1965) is due to a mixture of the two vinyl-bearing hemochromes from which the 7% and 12% HCl-porphyrins are formed (hemochrome λ max 555 mμ and 566 mμ respectively).

A possible precursor of both of these compounds is 2(or 4)-vinyl-4(or 2)-glyoxyloyldeuteroporphyrin IX (III). A base-catalysed Cannizzaro reaction would lead to (IIa) while deformylation would lead to (Ia). The strongly electrophilic nature of the glyoxyloyl residue might account for the compound whose hemochrome has absorption at 595 mμ. The preparation of (III) is at present being undertaken and its possible formation from chlorin-like precursors studied.

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