# Coupling of L-Histidine Methyl Ester and L-Histidine-containing Peptide Esters to Ferric Protoporphyrin IX Chloride

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*Summary* The coupling of L-histidine methyl ester and L-histidine-containing peptide esters to ferric protoporphyrin IX chloride is performed by the mixed carboxylic–carbonic acid anhydride method to give the disubstituted products in good yield: spectroscopic evidence indicates that the degree of interaction between the imidazole ligands and the iron atom depends upon the length of the peptide chain.

As part of an investigation into the relationship between the colour of myoglobin and its structure,[1] we prepared a number of model compounds by coupling L-histidine methyl ester, and several peptide esters containing L-histidine, to the propionic acid side chains of ferric protoporphyrin IX chloride (hereafter abbreviated to protohemin). We investigated the dependence of the intramolecular complexation in the resulting disubstituted products on the length of the side chain. Lautsch *et al.*[2] prepared similar products as enzyme models using the phosphazo-method to couple histidine-containing peptides to mesoporphyrin, iron being inserted afterwards. Lautsch *et al.* were the first to consider intramolecular co-ordination, and stated that the stability of the complexes will depend upon the length of the side chain.[2,3] Losse and Müller described the direct coupling of protohemin with L-histidine methyl ester in dimethylformamide in the presence of dicyclohexylcarbodi-imide,[4] but we were unable to obtain pure product by this method. During the course of our work Warme and Hager[5a] published a method for coupling amino-acids to mesoheme using mesoheme sulphuric anhydrides as intermediates. The milligram-scale preparation which they describe is laborious, and moreover is unsatisfactory for protohemin derivatives since 'a side reaction which involved the vinyl side chains of protoheme occurred in the activation step.'

We prepared disubstituted protohemins in high yields on the gram scale by the well-known mixed carboxylic–carbonic acid anhydride method: histamine, the methyl esters of L-histidine, β-Ala-L-His, Gly-L-His, and L-Ala-L-His, and the ethyl ester of Gly-L-His-Gly were coupled with protohemin in dimethylformamide in the presence of triethylamine and ethyl chlorocarbonate. The crude product mixtures were filtered, evaporated to dryness under reduced pressure, and fractionated by gradient elution with $C_6H_6$–MeOH from an alumina column. As an example, bis-(L-histidine methyl ester)protohemin was obtained in 47% yield when 3·1 g of the crude product was separated on a 55 × 2·8 cm $Al_2O_3$ column (activity I, Woelm) using the following $C_6H_6$–MeOH mixtures (v/v ratios): 100/2 (612 ml), 100/5 (525 ml), 100/10 (1650 ml), 100/15 (460 ml), and 100/20 (2640 ml). The disubstituted product, which on evaporation of the solvent was obtained as an amorphous black-violet powder, left the column with the last indicated eluant mixture. T.l.c. of the product on silica gel plates with $C_6H_6$–MeOH (75/30 v/v) gave one spot ($R_F$ 0·23) with tailing, and with $Bu^tOH$–AcOH–$H_2O$ (4/1/1 v/v) one spot ($R_F$ 0·62), also with tailing. Elemental analysis was correct for $C_{48}H_{50}N_{10}O_6FeCl$: $\nu_{max}$ (KBr) 1739 (ester C=O), 1640 (amide I), 1550 (amide II), 381 $cm^{-1}$ (Fe–N); $\lambda_{max}$ (MeOH) 412 ($\epsilon$ 9·2 × $10^4$), 532 (8·6 × $10^3$), 562 nm (sh, 7·3 × $10^3$).

From a study of Corey–Pauling–Koltun molecular models we concluded that the length of the side chain should have a

pronounced effect upon the extent of interaction between the imidazole ligands and the iron atom. There is much more steric constraint in the side chains of bis-(L-histidine methyl ester)protohemin than in those of the corresponding dipeptide derivatives: the imidazole groups cannot approach closely to the iron atom, and the interaction between these entities will consequently be weak. Dipeptide chains, however, appeared to be sufficiently long and flexible to permit close approach of the imidazole rings to the iron atom, resulting in a stronger interaction.

We are studying these intramolecular complexes by spectroscopic techniques which give information about the environment of the iron atom: some preliminary results are presented here. The u.v. and visible spectra of the protohemin complexes in the oxidized and reduced states are similar to those published by Warme and Hager for mesoheme dipeptides.[5b] Absorption peaks in the region 530—565 nm in the spectrum of bis-(L-histidine methyl ester)-protohemin (I) in methanol are broader and of lower intensity than those in the spectra of dipeptide ester derivatives (II) under the same conditions. This indicates that in the latter case the effective concentration of the imidazole ligands in the vicinity of the protohemin iron is much higher than in compound (I). A similar phenomenon was observed in the spectra of heme groups embedded in a film of polystyrene (25%) and 1-(2-phenylethyl)imidazole (75%), the spectra being recorded before and after the material had been heated at 70—80° for 1 h.[6] The extinction coefficients were the same for dilute methanolic solutions of (I) as for concentrated solutions, indicating that no aggregation occurred in the concentrated solutions: we may thus eliminate the possibility of intermolecular co-ordination. Consequently the difference between the spectra of compounds (I) and (II) can only be due to the influence of peptide chain length on the extent of intramolecular co-ordination. Examination of the c.d. behaviour of (I) and (II) in MeOH–glycerol (9/1 v/v) from 220 to 600 nm showed the former to absorb more intensely over the whole range, especially at low temperature (−150°). This may indicate that at low temperature more unsymmetrical forms of (I) are favoured because of steric constraint; the position of the imidazole rings in (II) allows strong interaction with the iron atom, and any temperature effect is too small to be observed.

The i.r. spectra of the synthesized compounds were in accord with the suggested structures. A band of medium—weak intensity at 381 $cm^{-1}$ in the spectrum of (I) was ascribed to the stretching mode of the iron–ligand bond, which is in good agreement with recently published data.[7] In the spectra of the dipeptide derivatives this band appears at shorter wavelengths (385 $cm^{-1}$) indicating a stronger interaction. We concluded that none of our products formed oxygen-carrying dimers since none of them exhibited the i.r. bands at 840 and 903 $cm^{-1}$ found by Sadasivan *et al.*[8] and Brown *et al.*,[9] respectively, in the spectra of hemin derivatives, and attributed by them to the Fe–O–Fe stretching mode.

Details of the synthesis will be published later, together with the results of our study of the c.d. properties of these materials in the visible and u.v. regions, and the i.r., Mössbauer, and e.s.r. spectra.

(*Received, December 23rd*, 1970; *Com.* 2216.)

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