

## Crystalline Leghemoglobin

VIII. The Hemin of the Two  
Main Components

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The hemin of Leghemoglobin (Lhb) was first investigated by Kubo.<sup>1</sup> He studied the unresolved pigments of different leguminous plants. From spectroscopical studies of their alkaline pyridine hemochromogens with bands at 557  $m\mu$  and 530  $m\mu$ , he characterized their hemin derivative as protohemin. These results have been essentially confirmed by Virtanen *et al.*<sup>2</sup> However, no studies have been performed with the resolved components of Lhb.

Investigating the hemins, extractable from effective root nodules, Falk *et al.*<sup>3</sup> found two components upon chromatography with pyridine hemochromogen  $\alpha$ -bands at 557  $m\mu$  and 550  $m\mu$ , respectively. Effective root nodules evidently contain different types of hemins. Additional interest to the hemin of Lhb was given by the observations of Abel *et al.*<sup>4</sup> who showed it capable of binding molecular nitrogen. Hence, it was decided to reinvestigate the hemin of Lhb and in particular those of the two main components resolved.

*Experimental.* The two main components of soya bean Lhb were prepared chromatographically as described previously<sup>5</sup> and splitted with acid:acetone as described before.<sup>6</sup> The precipitated apoprotein was dissolved in cold water, dialyzed against distilled water and freeze-dried. The acetone solution

containing the splitted hemin was rapidly evaporated to dryness *in vacuo* by rotation. The homogeneity of the hemin preparations was checked by chromatography on silicon treated paper in a water:propanol:pyridine (5.5:0.1:0.4) system.<sup>7</sup> In the case of inhomogeneity the components were separated preparatively on an alumina (Brockman) column in an acetone:propionic acid:water (87:3:10) system.<sup>8</sup>

The alkaline pyridine hemochromogen determination was made according to Paul *et al.*<sup>9</sup> Free porphyrins were prepared by a method of Morell and Stewart.<sup>10</sup> The porphyrins obtained were further purified by partition between ether and aqueous acid solutions. From the ether the porphyrins were extracted into 5% (w/v) hydrochloric acid solution from which the porphyrins were further extracted with chloroform. The chloroform solution was washed with water until a neutral reaction and evaporated to dryness. The methyl ester of the porphyrins was prepared according to Falk.<sup>11</sup> The identity and purity of the esters were checked by chromatography on paper in a propanol:kerosene (1:5) and in a chloroform:kerosene (13:20) system.<sup>12</sup> A commercial protohemin preparation (Sigma) was used as a standard.

*Results and discussion.* The hemin preparations from the two main components of Lhb exhibited alkaline pyridine hemochromogen spectra identical with those of the protohemin standard ( $\alpha$ -band equal to 557  $m\mu$  and  $\beta$ -band equal to 526  $m\mu$ ).

The two preparations showed on paper in a water:propanol:pyridine system, which separates, *e.g.*, proto-, hemato-, and mesohemins, a main component common for the two preparations and with an  $R_F$  value identical with that of the protohemin standard. However, the chromatograms revealed some small inhomogeneities and therefore the two preparations were purified on an alumina column. The small

Table 1. Absorption maxima of porphyrins prepared from Lhb.

Origin of porphyrin	Solvent	Absorption maxima, $m\mu$				
Standard	Chloroform	630	604	575	540	506
»	25 % HCl		603	557		
Fast component	Chloroform	630.5	603	575	540.5	505
»	25 % HCl		603.5	557		
Slow component	Chloroform	629.0	604	575	540	506
»	25 % HCl		602	556.5		

impurities all showed a pyridine hemochromogen spectrum identical to that of protohemin. It seems that these represent some kind of degradation products. No hemin with a pyridine hemochromogen  $\alpha$ -band at  $550\text{ m}\mu$  was observed. The methyl esters of the two purified components showed on chromatography identical  $R_F$  values with that of standard protoporphyrin in the chloroform:kerosene system as well as in that of propanol:kerosene.

The porphyrins of the two components of Lhb both showed an etio-type spectrum in neutral solvents. The absorption maxima of these porphyrins in neutral and acidic solvents are given in Table 1.

In order to illustrate the definite identity of the hemin derivative of Lhb and protohemin, the apoprotein of Lhb was recombined with the protohemin. Apoprotein was used in excess to obtain as quantitative a recombination of hemin as possible: 0.1 ml of 1.5 mM hemin in 0.01 N NaOH was rapidly mixed into 3 ml of 0.15 mM apoprotein of the slower component of Lhb in a pH 7.0 phosphate buffer ( $\mu$  equal to 0.05). A slight precipitation of apoprotein occurred and was centrifuged off. The spectrum of the recombined slow component of Lhb was identical with that of the intact native slow component of Lhb at pH 7.0.

All results obtained, the spectroscopical as well as chromatographic studies of different derivatives of the hemin of the two main components of Lhb indicate the hemin of the two components to be protohemin. An additional confirmation to this was obtained by a recombination study of protohemin with apoprotein of Lhb. There were no indications of another main hemin derivative forming a part of Lhb except protohemin. The hemin derivative with a pyridine hemochromogen  $\alpha$ -band at  $550\text{ m}\mu$  found in the root nodules, is not a constituent of Lhb.

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Received January 19, 1965.

## Hydrothermally Grown Crystals of Silver Vanadium Oxide Bronzes

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When vanadium pentoxide is heated with 5–40 weight percent of water in a sealed silver capsule at temperatures between 300–700°C and pressures of 2000 atm, a reaction occurs resulting in the formation of blue-black rod-formed or plate-like crystals up to one mm in size. X-Ray powder patterns of the rods and the plates were identical.

The crystals were found to be monoclinic from X-ray single-crystal studies. The cell dimensions derived from the Guinier powder pattern were:  $a = 11.742\text{ \AA}$ ;  $b = 3.667\text{ \AA}$ ;  $c = 8.738\text{ \AA}$ ;  $\beta = 90.48^\circ$

An X-ray spectrographic analysis revealed considerable silver content in the compound. The crystal structure was determined by means of the Patterson projection on to (010). The atomic arrangement thus obtained was refined by means of electron density projections and indicated the formula to be  $\text{Ag}_{1-x}\text{V}_2\text{O}_5$ . The  $h0l$  and  $h1l$  data were then processed by a full-matrix least-squares refinement using the Busing-Levy