Crystalline Leghemoglobin

VIII. The Hemin of the Two
Main Components

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The hemin of Leghemoglobin (Lhb) was first investigated by Kubo. He studied the unresolved pigments of different leguminous plants. From spectroscopical studies of their alkaline pyridine hemochromogens with bands at 557 \( \mu \)m and 550 \( \mu \)m, he characterized their hemin derivative as protohemin. These results have been essentially confirmed by Virtanen et al. However, no studies have been performed with the resolved components of Lhb.

Investigating the hemins, extractable from effective root nodules, Falk et al. found two components upon chromatography with pyridine hemochromogen \( \alpha \)-bands at 557 \( \mu \)m and 550 \( \mu \)m, 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., 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 and split with acid:acetone as described before. The precipitated apoprotein was dissolved in cold water, dialyzed against distilled water and freeze-dried. The acetone solution containing the splited 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. 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.

The alkaline pyridine hemochromogen determination was made according to Paul et al. Free porphyrins were prepared by a method of Morell and Stewart. 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. 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. 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 \( \mu \)m and \( \beta \)-band equal to 526 \( \mu \)m).

The two preparations showed on paper in a water:propanol:pyridine system, which separates, e.g., proto-, hemato-, and mesohehmins, 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>
<thead>
<tr>
<th>Origin of porphyrin</th>
<th>Solvent</th>
<th>Absorption maxima, ( \mu )m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>Chloroform</td>
<td>630</td>
</tr>
<tr>
<td></td>
<td>25 % HCl</td>
<td>603</td>
</tr>
<tr>
<td>Fast component</td>
<td>Chloroform</td>
<td>630.5</td>
</tr>
<tr>
<td></td>
<td>25 % HCl</td>
<td>603.5</td>
</tr>
<tr>
<td>Slow component</td>
<td>Chloroform</td>
<td>629.0</td>
</tr>
<tr>
<td></td>
<td>25 % HCl</td>
<td>602</td>
</tr>
</tbody>
</table>

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SHORT COMMUNICATIONS

impurities all showed a pyridine hemo- 
chromogen spectrum identical to that of 
protoporphyrin. It seems that these represent 
some kind of degradation products. No 
hemin with a pyridine hemochromogen 
\( \alpha \)-band at 550 \( \mu m \) 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 protoporphyrin, the apoprotein of Lhb 
was recombined with the protoporphin. 
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 
protoporphyin. An additional confirmation 
to this was obtained by a recombination 
study of protoporphyrin with apoprotein of 
Lhb. There were no indications of another 
main hemin derivative forming a part of 
Lhb except protoporphin. The hemin derivative with a pyridine hemochromogen 
\( \alpha \)-band at 550 \( \mu m \) found in the root 
nodules, is not a constituent of Lhb.

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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 be-
tween 300–700°C and pressures of 2000 
atm, a reaction occurs resulting in the for-
mation 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 pow-
der pattern were: \( a = 11.742 \; \text{Å}; \; b = 3.067 \; \text{Å}; \; c = 8.738 \; \text{Å}; \; \beta = 90.48° \).

An X-ray spectroscopic analysis re-
vealed considerable silver content in the 
compound. The crystal structure was de-
crmination of the Patterson projection 
on to (010). The atomic arrangement thus 
obtained was refined by means of electron 
density projections and indicated the for-
mula to be \( Ag_2V_2O_7 \). The \( h0l \) and \( hhl \) data 
were then processed by a full-matrix least-
squares refinement using the Busing-Levy