An O\(^{18}\) Study of the Hemoglobin Degradation to Biliverdin in the Model Reaction*  

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(Received for publication, February 28, 1961)

The mechanism of oxidative degradation of hemoglobin (Hb)-hem to biliverdin is known to involve a series of oxidative reaction steps. At least two intermediates are formed in the in vitro experiments; they have been characterized mainly by their spectroscopic properties, and are the oxy(porphyrin)-hem-Hb** and verdohem-globin** (Lemberg, 1956 (1)).

The O\(^{18}\) isotopic experiments were carried out to study the chemical mechanism of biliverdin formation from Hb-hem by model reaction(s) which, however, may differ from the in vivo or TPN-dependent enzymatic oxidation (Nakajima (2)).

Reaction mixtures consisted of 80 ml. of Hb solution (100 \(\mu\)moles) at pH 7.6 (0.1M phosphate buffer) and 20 ml. of neutralized ascorbate solution (10 moles) in 0.1M phosphate buffer, pH 7.6. The ascorbate was added in 4 portions at 30 minute intervals from a separatory funnel, the contents of which were being gassed with hydrogen. At the end of the incubation period (2 hours) at 50°C, the mixture was frozen, lyophilized, and biliverdin isolated by the method of Lemberg et al. (3). For the O\(^{18}\) experiments the gas phase was repeatedly evacuated and filled with O\(^{18}\) from a reservoir***.

The purity of labelled biliverdin isolated (5) and purified according to the Lemberg's method (4) was 90 to 95 per cent when determined spectrophotometrically at 655 nm (the absorption maximum). The shape of the absorption curve of O\(^{18}\)-labelled compounds were identical with that of a standard biliverdin****. Contamination of the oxidized derivatives of biliverdin (bilipurpurins and biliviolins) was nil so far as the fluorescent test upon adding alcoholic Zn reagent (Kench (5)).

The O\(^{18}\) content of the biliverdin samples was determined by Unterzaucher analyzer followed by mass spectroscopy***** of the recovered carbon dioxide (6). Atom per cent excess of oxygen in a reservoir obtained electrolytically from H\(_2\)O\(^{18}\) was determined by the direct mass spectroscopic analysis******.

The results thus indicate approximately 1 atom of net incorporation of O\(^{18}\) from molecular oxygen. Since the biliverdin iso-

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* This work was supported by a grant from the United States Public Health Service.

** Oxy(porphyrin)-hem is used for the C\(_{34}\)-intermediate having \(-\text{OH}\) or \(=\text{O}\) at \(\alpha\)-methine carbon of hem. Verdohem stands for the C\(_{32}\) compound, in which \(\alpha\)-methine carbon is replaced by \(-\text{O}^-\), but practically the crude cholehem obtained at the incubation product in the model experiment is accompanied by biliverdin-iron globin, containing \(-\text{O}^-\text{-H} \cdots \text{O}=\) between pyrrole rings I and IV.

*** O\(^{18}\)-enriched water was electrolyzed after metallic Li being added, and molecular oxygen generated was stored in an aspirator-type bottle filled with pure liquid paraffin and water. Hydrogen evolved was collected in two aspirator bottles of the same size as oxygen reservoir. The levels of liquid paraffin overlying on water were balanced from time to time by arranging the barrel connected to each bottle through their outlet mouth.

**** Prepared by the method of Lemberg (4) from bilirubin. The fresh biliverdin was used for standardization of its absorption curve which was consistent with that in the references.

****** Determined by Dr. C. C. Delwiche at the University of California, Berkeley.
Analysis of O¹⁸ Incorporated in Biliverdin

<table>
<thead>
<tr>
<th>(A) O¹⁸ used</th>
<th>(B) O¹⁸ detd. as CO₂¹⁸</th>
<th>(C) O¹⁸ incorpd.</th>
</tr>
</thead>
<tbody>
<tr>
<td>atom % excess</td>
<td>atom % excess</td>
<td>atoms per mole</td>
</tr>
<tr>
<td>9.4₉</td>
<td>0.68₉</td>
<td>0.8₉</td>
</tr>
<tr>
<td>8.9₇</td>
<td>0.77₇</td>
<td>1.0₇</td>
</tr>
<tr>
<td>8.9₇</td>
<td>0.88₇</td>
<td>1.1₈</td>
</tr>
</tbody>
</table>

Average .......... 1.0₇

O¹⁸ incorporation was calculated according to the following equation:

\[ x = \frac{B}{A} \times 2^n \]

whereby \( n \) stands for the number of oxygen atom in the compound tested.

Table I

The system is under further study by electron spin resonance spectrometry.

**SCHEME I**

Proposed Mechanism of Autoxidation

```
H | \( \text{O}_{\text{H}} \) |
---|-------------------------|
C  | | C-Mes
\( \text{Mes-C}=\text{C-Mes} \)
OH | 
O₂ | 
O₂ | 
C | | C \( \text{C}=\text{C} \) |
\( \text{O}-\) | O₂ | | O₂ |
\( \text{O}-\) | O₂ | | O₂ |
\( \text{O}-\) | O₂ | | O₂ |
\( \text{O}-\) | O₂ | | O₂ |
\( \text{O}-\) | O₂ | | O₂ |
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REFERENCES

(1) Lemberg, R., Rev. Pure & Appl. Chem., 6, 12 (1956)
(8) Fuson, R.C., Byers, D.J., Rachlin, A.I., and

