OXIDATION OF OXYHEMOGLOBIN BY SODIUM NITRITE

A kinetical study on the oxidation of hemoglobin to methemoglobin by nitrite was carried out by Betke et al\textsuperscript{1} in 1956, and from the results obtained they supported the following reaction formula proposed by Greenberg et al\textsuperscript{2,3}:

\[
\text{NO}_2^- + 2\text{Fe}^{++} + (\text{O}_2) + \text{H}_2\text{O} \rightleftharpoons \text{NO}_3^- + 2\text{Fe}^{+++} + (\text{OH}) + \text{O}_2
\]

The detailed mechanism of the reaction was further clarified by Martin et al\textsuperscript{4} in 1963 by investigating the relationship between the molecular structure of hemoglobin and the rate of the oxidation by nitrite.

In these studies, however, the significance of the initial phase of the reaction, which will be clarified in the present paper, was insufficiently understood. As shown in Fig. 1, two phases, which were named lag and log phase in the paper, were observed in the reaction process. The properties of the lag and log phase was elucidated by carrying out the following experiments.

The results obtained by changing the concentration of oxyhemoglobin and nitrite, pH value, the partial pressure of oxygen and temperature were summarized as follows:

1. Oxyhemoglobin was oxidizable to methemoglobin, but reduced hemoglobin was not. Four molecular oxygen which combined to one hemoglobin molecule was necessary for the oxidation of hemoglobin.

![Fig. 1 Oxidation of hemoglobin to methemoglobin by nitrite.](image-url)
2. The time length of the lag phase was in proportion to $1/\langle\text{NO}_2\rangle^2$ and was independent to the concentration of hemoglobin.

3. The reaction rate calculated from the tangent in the log phase increased with the concentration of hemoglobin and nitrite.

4. One of the four molecules which were dissociated from one hemoglobin molecule in the process of the oxidation was detected on an oxygraph, but the others were not (Fig. 2).

From these results, the following formulas were proposed for the oxidation process of hemoglobin by nitrite:

\[
\begin{align*}
\text{Hb(O}_{2}\text{)}_4 + 4\text{NO}_2 + 2\text{H}_2\text{O} &\rightarrow \text{Hb(OH)}_4 + 4\text{NO}_3 + 1/2 \text{O}_2 \quad (4)
\end{align*}
\]

When the concentration of nitrite was not sufficient for the oxidation of hemoglobin, the following reaction, in which two hemes of one hemoglobin molecule were oxidized, will take its place instead of the reaction (3):

\[
\begin{align*}
\text{Hb(O}_{2}\text{)}_4 + 2\text{NO}_2 + \text{H}_2\text{O} &\rightarrow \text{Hb' (O)}_2\text{(OH) }_2 + 2\text{NO}_3 + 1/2 \text{O}_2
\end{align*}
\]

where \(\text{Hb(O}_{2}\text{)}_4\) represents the oxyhemoglobin molecule, \(\text{Hb(O}_{2}\text{)}_2\text{(NO)}_2\), a complex of nitrite and \(\text{Hb(O)}_4\), \(\text{Hb' (O)}_2\text{(NO)}_2\), a complex different in its molecular structure from \(\text{Hb(O)}_2\text{(NO)}_2\), and \(\text{Hb(OH)}_4\) the methemoglobin. By considering the reaction Fig. 2 Oxygen consumption in the oxidation process.

(1) : Amounts of methemoglobin formed; [MetHb]
(2) : Increased amount of oxygen in the reaction mixture; [O$_2$]

Amount of oxygen which dissolved in hemoglobin solution was measured by using an oxygraph. Since, when one oxyhemoglobin molecule was oxidized to methemoglobin, four molecules of oxygen were expected to be dissociated, the finding that the ratio of [O$_2$]/[MetHb] was 1/4 indicates that three of the four oxygen molecules dissociated were consumed in the oxidation process. Experiments were carried out at pH 7.0 and 26°C. The concentration of hemoglobin and nitrite was 0.05 and 1.10 mM respectively. In the figure, the concentration of methemoglobin was represented in terms of that of heme.
mechanism proposed above, both of the experimental results obtained by us and by Betke et al\(^1\) were clearly explained.

**REFERENCES**


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