EXPERIMENTAL

The 2,6-dichlorophenolindophenol (2,6-DPIP) reduction reaction was studied in detail by kinetic methods. The reaction was followed by monitoring the decrease in absorbance at 600 nm using a spectrophotometer. A 2,6-DPIP stock solution was prepared in ethanol and added to a reaction mixture containing substrate and enzyme. The reaction was initiated by adding the enzyme. The decrease in absorbance was monitored at regular intervals. The rate of reaction was calculated from the slope of the absorbance vs. time plot.

RESULTS

The rate of the reaction was found to be first-order with respect to the substrate concentration. The Michaelis-Menten equation was used to fit the data. The kinetic parameters were calculated from the equation. The activation energy for the reaction was found to be 12.4 kJ/mol. The reaction was found to be inhibited by heavy metals such as copper and iron.

DISCUSSION

The results obtained from this study suggest that the 2,6-DPIP reduction reaction can be used as a model system for studying the kinetics of enzyme-catalyzed reactions. The reaction is first-order with respect to the substrate concentration, indicating that the enzyme is not limiting. The activation energy for the reaction is relatively low, suggesting that the reaction is not highly exergonic. The inhibition by heavy metals suggests that the reaction may be affected by environmental factors.

REFERENCES


KINETIC METHOD

Reaction between thiocyanate and 2,6-dichloroquinone-4-chloro-imide. Aliquots in the reaction mixture were analyzed by iodimetric method. 50 ml of the reaction mixture containing 2,6-dichloroquinone-4-chloro-imide was pipetted out at various intervals of time into a mixture of iodate free KI solution containing dilute sulphuric acid (2N) in a carbon dioxide atmosphere. The liberated iodine was titrated against standard thiosulphate solution to the disappearance of the blue starch-iodine end point.

Reaction between iodide and 2,6-dichloroquinone-4-chloro-imide. The liberated iodine is estimated by the standard thiosulphate solution to the disappearance of blue starch-iodine end point.

Some kinetic runs were followed by analysis with a spectrophotometer. The rate constants obtained by both methods were with an accuracy of \(+2\%\).

STOICHIOMETRY

With iodide. In stoichiometric experiments employing $[\text{oxidant}]_0>[\text{substrate}]_0$ (example: $[\text{ox}]_0=0.001 \text{ mol dm}^{-3}$, $[\text{S}]_0=0.0005 \text{ mol dm}^{-3}$ potassium iodide; HOAc and water 20:80 %, NaOAc is 0.2 \text{ mol dm}^{-3} and temperature 30°C) the liberation of iodine is monitored until concentration of liberated iodine becomes constant. It was found that two moles of substrate required one mole of 2,6-dichloroquinone-4-chloro-imide (equivalent to one mole of HOCl formed due to hydrolysis of one mole of 2,6-dichloroquinone-4-chloro-imide), in accordance with the overall reaction as follows:

$$\text{I}^- + \text{HOCl} \rightarrow \text{HOCl} + \text{Cl}^-$$

$$\text{I}^- + \text{HOCl} \rightarrow \text{I}_2 + \text{OH}^-$$

With thiocyanate. In stoichiometric experiments employing $[\text{oxidant}]_0>[\text{substrate}]_0$ (example: $[\text{ox}]_0=0.002 \text{ mol dm}^{-3}$; $[\text{S}]_0=0.00025 \text{ mol dm}^{-3}$, HOAc and water 20:80 %; $[\text{HClO}_3]=0.05 \text{ mol dm}^{-3}$ and temperature 30°C) the disappearance of the oxidant was monitored until a constancy in the value of left over oxidant was found. It was found that two moles of substrate required one mole of 2,6-dichloroquinone-4-chloro-imide, in accordance with the overall reaction as follows:

$$2\text{SCN}^- + \text{HOCl} + \text{H}^+ \rightarrow (\text{SCN})_2 + \text{H}_2\text{O} + \text{Cl}^-$$

RESULTS AND DISCUSSION

Reaction between thiocyanate and 2,6-dichloroquinone-4-chloro-imide. It was of interest to study the kinetics of oxidation of thiocyanate by a new oxidizing agent, i.e. 2,6-dichloroquinone-4-chloro-imide. This reagent gives rise to two oxidizing species HOCl and 2,6-dichloroquinone with diverse redox potentials in subsequent stages of hydrolysis. Hence two reaction sequences occur: (1) with HOCl and (2) with 2,6-dichloroquinone. When large excess of the oxidant is used the main reaction is between the substrate and HOCl. On the contrary, when large excess of the substrate is used till the equality of the concentrations of the substrate and the oxidant the two reaction sequences occur: the first is a reasonably fast step involving thiocyanate and HOCl, and the second one is a slow reaction involving thiocyanate and 2,6-dichloroquinone. Approximately the rate of the second oxidation reaction by quinone is about 50 times slower compared to the first oxidation reaction by HOCl. This is quite in accordance with the redox potentials of HOCl and 2,6-dichloroquinone, respectively. This is the first example being reported in literature regarding the oxidation of SCN\(^-\) by consecutive sequences of oxidation by an oxidizing agent involving N-halo derivatives.

The sequences of reaction are:

1. \[
\begin{align*}
\text{Cl} &\quad \text{O} &\quad \text{Cl} \\
\text{N} &\quad \text{Cl} &\quad \text{N} \\
\text{H}_\text{2}\text{O} &\rightarrow &\text{Cl} &\quad \text{O} &\quad \text{Cl} \\
\end{align*}
\]

2. \[
\begin{align*}
\text{Cl} &\quad \text{O} &\quad \text{Cl} &\quad \text{N} &\quad \text{H}_\text{2}\text{O} &\rightarrow &\text{Cl} &\quad \text{O} &\quad \text{Cl} \\
\end{align*}
\]

3. \[
\begin{align*}
\text{NH}_3 &\quad \text{H}_\text{2}\text{O} &\rightarrow &\text{NH}_4\text{X} \\
\end{align*}
\]

4. \[
\begin{align*}
\text{SCN}^- &\quad \text{HOCl} &\quad \text{H}^+ &\rightarrow &\text{SCN}_2 &\quad \text{Cl}^- &\quad \text{H}_2\text{O} \\
\end{align*}
\]

5. \[
\begin{align*}
\text{SCN}^- &\quad \text{HOCl} &\quad \text{H}^+ &\rightarrow &\text{SCN}_2 &\quad \text{Cl}^- &\quad \text{H}_2\text{O} \\
\end{align*}
\]

Reaction (c) is the main oxidation reaction involving SCN\(^-\) and the hydrolyzed species of 2,6-dichloroquinone-4-chloro-imide, namely HOCl, whereas reaction (d) is a consecutively very slow reaction involving SCN\(^-\) and 2,6-dichloroquinone. The relevant kinetic data is given in Table I. The reported kinetic rate constants belong to the oxidation sequence by the hydrolyzed HOCl as the consecutive sequence of oxidation by 2,6-dichloroquinone is very slow.

The following facts emerge from the above data:
1. The reaction is mainly between the hydrolyzed species of 2,6-dichloroquinone-4-chloro-imide and SCN\(^-\). The consecutive reaction of oxidation of SCN\(^-\) by 2,6-dichloroquinone is very slow, about 60 to 70 times slower than the former reaction.
2. The reaction is first order in the oxidant, in the substrate and in H\(^+\) leading to an independence at higher concentrations of [H\(^+\)]. The reaction is catalyzed by Mn\(^2+\). The increase of percentage of AcOH increases the kinetic rate. The addition of perchlorate ion enhances the rate. The addition of chloride ion also enhances the rate.
Table 3. Kinetic data in case of oxidation of iodide with dichloroquinone-4-chloro-imide (oxidant) at temperature 30°C

<table>
<thead>
<tr>
<th>Variant</th>
<th>Concentration of non-variant</th>
<th>Concentration of variant (mol dm⁻³)</th>
<th>kₙ × 10⁴ (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidant</td>
<td>[I⁻] = 0.0015 mol dm⁻³</td>
<td>0.00025</td>
<td>6.722</td>
</tr>
<tr>
<td></td>
<td>[AcOH] = 5%</td>
<td>0.0005</td>
<td>8.528</td>
</tr>
<tr>
<td></td>
<td>[NaOAc] = 0.01 mol dm⁻³</td>
<td>0.00075</td>
<td>7.573</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.001</td>
<td>7.612</td>
</tr>
<tr>
<td>Oxidant</td>
<td>[I⁻] = 0.0005 mol dm⁻³</td>
<td>0.00025</td>
<td>7.123</td>
</tr>
<tr>
<td></td>
<td>[AcOH] = 20%</td>
<td>0.0005</td>
<td>7.772</td>
</tr>
<tr>
<td></td>
<td>[NaOAc] = 0.02 mol dm⁻³</td>
<td>0.001</td>
<td>7.854</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0015</td>
<td>7.461</td>
</tr>
<tr>
<td>I⁻</td>
<td>[oxidant] = 0.0003 mol dm⁻³</td>
<td>0.0005</td>
<td>2.378</td>
</tr>
<tr>
<td></td>
<td>[AcOH] = 5%</td>
<td>0.001</td>
<td>6.77</td>
</tr>
<tr>
<td></td>
<td>[NaOAc] = 0.01 mol dm⁻³</td>
<td>0.0015</td>
<td>8.528</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.002</td>
<td>13.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.004</td>
<td>27.838</td>
</tr>
<tr>
<td>I⁻</td>
<td>[oxidant] = 0.0005 mol dm⁻³</td>
<td>0.0005</td>
<td>0.399</td>
</tr>
<tr>
<td></td>
<td>[AcOH] = 5%</td>
<td>0.001</td>
<td>0.6525</td>
</tr>
<tr>
<td></td>
<td>[NaOAc] = 0.1 mol dm⁻³</td>
<td>0.005</td>
<td>4.332</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01</td>
<td>11.677</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.02</td>
<td>25.045</td>
</tr>
<tr>
<td>AcOH</td>
<td>[I⁻] = 0.0005 mol dm⁻³</td>
<td>5%</td>
<td>1.918</td>
</tr>
<tr>
<td></td>
<td>[oxidant] = 0.0005 mol dm⁻³</td>
<td>10%</td>
<td>4.4999</td>
</tr>
<tr>
<td></td>
<td>[NaOAc] = 0.02 mol dm⁻³</td>
<td>20%</td>
<td>7.772</td>
</tr>
<tr>
<td></td>
<td>[oxidant] = 0.0005 mol dm⁻³</td>
<td>30%</td>
<td>9.077</td>
</tr>
<tr>
<td>AcOH</td>
<td>[I⁻] = 0.001 mol dm⁻³</td>
<td>10.25%</td>
<td>8.0259</td>
</tr>
<tr>
<td></td>
<td>[oxidant] = 0.0005 mol dm⁻³</td>
<td>13.75%</td>
<td>12.244</td>
</tr>
<tr>
<td></td>
<td>[NaOAc] = 0.02 mol dm⁻³</td>
<td>25.75%</td>
<td>18.09</td>
</tr>
<tr>
<td></td>
<td>[oxidant] = 0.0005 mol dm⁻³</td>
<td>38.75%</td>
<td>26.715</td>
</tr>
<tr>
<td>NaOAc</td>
<td>[I⁻] = 0.001 mol dm⁻³</td>
<td>0.01</td>
<td>6.77</td>
</tr>
<tr>
<td></td>
<td>[AcOH] = 5%</td>
<td>0.025</td>
<td>1.2281</td>
</tr>
<tr>
<td></td>
<td>[oxidant] = 0.0005 mol dm⁻³</td>
<td>0.1</td>
<td>0.6525</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2</td>
<td>0.3838</td>
</tr>
</tbody>
</table>

This explains the observed kinetic orders. The complex decomposition may be visualised as follows:

slow  complex → I⁻ + Cl⁻ + H₂O  
fast  I⁻ + I⁻ → I₂  

rate = \( k_K [K]_2 [S][H^+] [oX] \)

If the concentration of dichloroquinone term in the denominator is small and negligible when compared to other terms the kinetic rate is given by:

rate = \( k_K [K]_2 [S][oX][H^+] \) / \( (1 + K_H[S]) \)

CONCLUSION

The consideration of the observed kinetic data of both the thiocyanate and iodide with 2,6-dichloroquinone-4-chloro-imide shows that this new N-halo derivative is a mild oxidizant which is capable of affecting oxidations smoothly of many inorganic systems, like hydroxylamine and hydrazine. Preliminary experiments with both systems indicate that the rate measurements are very smooth compared to the earlier studies of these nitrogen derivatives and metallic oxidizing agents like Cr(VI), Ce(IV), V(V) and even with oxy anions, like bromate, periodate and iodate.

REFERENCES


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