Part Two The Isolation And Kinetics of Enzyme
Action of The Catalase of The Liver of Giant Black Marlin

by

Hwoang Hwa Yang*

Present paper reports some results on the isolation of the catalase from the liver of the giant black marlin by the method of Sarker and Summer (1). This simple procedure has been found to give good yields of active crystals. The enzyme stands shaking with alcohol-chloroform mixture which denatures hemoglobin. The dark green crystals obtained with 0.45 saturated ammonium sulfate are extremely stable.

Results of the studies on kinetics of enzyme action of the fish liver catalase are also briefly discussed.

Experimental

Method of Isolation

Grind fresh fish liver in a meat grinder, putting the material through the grinder four times. To every kilogram of ground material add 1500 ml. of distilled water and mix well. Mix 1 vol. of 95% alcohol with 1 vol. of C.P. chloroform, and add 480 vol. of this mixture to every 1000 vol. of the liver suspension. Shake violently for 30 seconds and filter through folded filter papers at room temperature. When most of the liquid has filtered through, for every kilogram of liver pour about 500 ml. of distilled water on the residue to wash more catalase through. Cautiously bring the filtrate to pH 5.7 by adding glacial acetic acid, centrifuge, and remove protein impurity. (Fortunately no protein impurity was found in the preparation of the fish liver.) Add 250 ml. of tricalcium phosphate suspension which is about pH 5.7 (30 to 35 mg./ml.) to every liter of filtrate, and mix for 10 to 15 minutes. At once centrifuge down the adsorption complex, and discard the supernatant. Elute the catalase from the adsorption complex with 200 ml. of 0.1 M. phosphate buffer of pH 8.0 (for about 2 kg. of liver used at the start, followed by centrifuging. Repeat this elution once more, using 200 ml. of the pH 8.0 phosphate buffer. To combined eluates add solid ammonium sulfate

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using 30g. for every 100 ml. Mix, and allow to stand in the ice chest overnight. Next day centrifuge the catalase down at high speed in a refrigerated centrifuge and discard the supernant. Mix the sediment with just enough water to make a mushy suspension, transfer to a dialyzing parchment, and dialyze in the ice chest against several changes of distilled water. If a whitish or buff colored precipitate forms, it should be centrifuged down and discarded.

The dialyzate is chilled, saturated ammonium sulfate added cautiously until a haziness appears. The catalase crystallizes almost immediately. The material is kept in the ice chest and more ammonium sulfate is added until a good crop of crystals is obtained. However, it is inadvisable to add a great excess of ammonium sulfate since catalase crystals are difficult to centrifuge down from solutions of high specific gravity.

Recrystallization

Because catalase crystals were uncontaminated with protein impurity the recrystallization was easy. After centrifuges down the crystals pour off the supernatant liquid and dissolve the crystals in a small amount of water and the least possible volume of 9.6 per cent phosphate buffer of pH 7.4. If protein impurity present which is in form of particles shaped like footballs, does not dissolve. The solution is centrifuged until clear, decanted, and brought to about pH 5.3 by adding the necessary amount of acid potassium phosphate solution with rotation. The precipitation is cooled and saturated ammonium sulfate is added slowly, until most of the catalase has crystallized out. It is necessary to add from $\frac{1}{4}$ to $\frac{1}{2}$ volume of saturated ammonium sulfate to $1$ volume of catalase solution for practically complete crystallization of the enzyme.

It frequently happens that a portion of the catalase crystals is found to have become insoluble in phosphate buffer of pH 7.4. Such crystals have a diminished activity and apparently consist of denatured catalase.

The residue obtained from first extraction was extracted a second time, but did not give satisfactory yield of the active crystals.

The method of preparation of the active crystal of the fish liver catalase can be summarized in the following chart.

The Titration By Potassium Permanganate

Under specified conditions, the activities of the catalases in samples were followed by titration in H$_2$SO$_4$ with KMnO$_4$.

Results and Discussions

Properties of Crystalline Catalase

The crystals usually separate first as very minute crystals, which are shown in Fig. 1. After having grown to a larger size, upon recrystallizing as already described, they separated either as needles (Fig. 2) thin plates (Fig. 3) or cube
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(Fig. 4). (We wish to express our gratitude to professor C. I. Chen for his advice in taking the photomicrographs and for the use of his equipment.)

Individual crystals, if sufficiently large, are yellow under the microscope. In the test tube the crystals look black greenish yellow, or silvery according to the illumination. The crystals stain very well with methyl violet as described by Summer and Dounce (3) for beef liver catalase. Dilute solutions of catalase are yellow, while concentrated solutions are practically black.

The crystalline catalase shows high catalase activity by the manometric determination. (4)

**Chart 1. Procedure For Isolation of Crystalline Liver Catalase**

- **Fish liver** 2.5 kg
- Pass grinder 4 times, add 3.75 l. H₂O, 600 ml of 95% CH₃CH₂OH and 600 ml of HCCl₅, shake for 30 second

  - **Residue 1**
    - Wash with 1.25 l. of H₂O

  - **Filtrate 1**
    - Adjust to pH 5.7 with CH₃COOH and centrifuge

  - **Residue 2**
    - Use for second extraction

  - **Filtrate 2**
    - Add into Filtrate 1.

  - **Protein impurity**
    - No protein impurity was found in the fish liver preparation

  - **Filtrate 3**
    - Add 625 ml Ca₃(PO₄)₂ (pH 5.7, concentration 30 to 35 mg/ml). mix for 10-15 minutes

  - **Adsorption complex 1**
    - Elute catalase with 250 ml of 0.1 M phosphate buffer (pH 8.0) and centrifuge

  - **Filtrate 4**
    - discard

  - **Adsorption complex 2**
    - Elute catalase with 250 ml of 0.1 M phosphate buffer for 2nd time and centrifuge

  - **Filtrate 5**
    - Add 150 g. (NH₄)₂SO₄, mix. allow to stand in ice chest overnight and centrifuge

  - **Residue 3**
    - Add into Filtrate 5.

  - **Filtrate 6**
    - (Crude catalase)

  - **Sediment**
    - Add H₂O to make a mushy suspension and dialyze

  - **Precipitate 1**
    - (Denatured catalase)

  - **Filtrate 7**

  - **Filtrate 8**
    - Add 9.6% phosphate buffer (pH 7.4) and centrifuge

  - **Precipitate 2**
    - (Denatured catalase)

  - **Filtrate 9**
    - Adjust to pH 5.3 with KH₂PO₄, add 1/4 - 1/2 volume (NH₄)₂SO₄

  - **Crystalline catalase**
We must not let consideration of its outer appearance make us jump to conclusions about the arrangement of the particle inside a large crystal. Limited space or some other reason may cause a crystal to form by growing more in one direction than another.

Fig. 1 - 4 show how a minute crystal may develop into one of three other possible shapes - a cube, a flat plate or a long needle-like structure. All three of these solids have the same crystal lattice, but their habits, or characteristic forms, differ. Such a single substance which can exist in two or more crystal forms (under different conditions) is said to be polymorphous.

Absorption Bands of Catalase Solutions

Zeile and Hellström (6) found that their purified catalase solutions gave absorption bands at 629, 540, and 536 μm. For crystalline catalase we have observed absorption bands at 627 and 536 μm, our apparatus is not suitable for investigation in the shorter end of the spectrum.

Kinetics of Enzyme Action of The Fish Lish Liver Catalase

Filtrate 3 was used for convenience as the samples of the following studies
on kinetics of enzyme action of the fish liver catalase.

The Determination of The First-Order Rate Constant

The conventional assay for catalase activity involves determination of the first-order rate constant under specified conditions.

$0.024 \text{mole } H_2O_2 \text{ and } 0.0067 \text{ M. phosphate pH } 6.3 \text{ at } 0^\circ C.$ One milliter of enzyme is added to 50 ml. of reaction mixture and 5 ml. samples are titrated in $H_2SO_4$ with KMnO$_4$. The reaction constant is evaluated:

$$K' = \frac{2.303}{t} \log \frac{a}{a-x}$$

in which a represents the initial concentration, x represents the peroxide concentration (which, of course, may be expressed in terms of the permanganate used in the titration). The rate constant is a measure of the catalase activity in the test solution. The results are shown in Table 1 and depicted in Figure 5.

<table>
<thead>
<tr>
<th>Time (minute)</th>
<th>0.084 N.KMnO$_4$ (ml.)</th>
<th>$H_2O_2$ decomposed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>13.5</td>
<td>51.79</td>
</tr>
<tr>
<td>10</td>
<td>9.0</td>
<td>67.86</td>
</tr>
<tr>
<td>20</td>
<td>5.0</td>
<td>82.14</td>
</tr>
<tr>
<td>30</td>
<td>3.0</td>
<td>89.29</td>
</tr>
<tr>
<td>40</td>
<td>1.6</td>
<td>94.30</td>
</tr>
</tbody>
</table>

At 5 minutes, $K'$ may be calculated:

$$K' = \frac{2.303}{5} \log \frac{100}{(100-51.79)}$$

$$K' = 0.13 \text{ min}^{-1}$$

At 50% decomposition or time, $t\frac{1}{2}$,

$$t\frac{1}{2} = \frac{2.303 \log 2}{K} = \frac{2.303 \times 0.30103}{0.13} = 5 \text{ (min.)}$$

This is method of testing for the first-order equation.

The results of the experiment suggest that if the initial substrate concentration is lower and the incubation time is shorter will give more sharp determination.

Effect of Enzyme Concentration on Rate of Enzyme Action

The initial rates of the enzyme-catalyzed reactions are directly proportional to the enzyme concentrations. At high enzyme concentrations, the substrate concentrations limit the reactions and the rates of the reactions gradually decrease.

The results of the tests of effect of the enzyme concentration on the rates of the catalases activities are shown in Table 2, and depicted in Fig. 6.
Table 2. Effect of enzyme concentration on rate of enzyme action

<table>
<thead>
<tr>
<th>Volume of enzyme (ml.)</th>
<th>Volume of Buffer (ml.)</th>
<th>0.084 N. KMnO₄ (ml.)</th>
<th>H₂O₂ decomposed (%)</th>
<th>Volume of enzyme (ml.)</th>
<th>Volume of Buffer (ml.)</th>
<th>0.084 N. KMnO₄ (ml.)</th>
<th>H₂O₂ decomposed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>44.4</td>
<td>43.20</td>
<td>7</td>
<td>13</td>
<td>1.8</td>
<td>98.64</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>15.0</td>
<td>79.74</td>
<td>8</td>
<td>12</td>
<td>1.8</td>
<td>98.36</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>4.5</td>
<td>93.67</td>
<td>9</td>
<td>11</td>
<td>1.8</td>
<td>96.02</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>1.5</td>
<td>97.83</td>
<td>10</td>
<td>10</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>0.9</td>
<td>98.53</td>
<td>11</td>
<td>9</td>
<td>2.4</td>
<td>93.71</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>2.4</td>
<td>95.79</td>
<td>12</td>
<td>8</td>
<td>3.0</td>
<td>93.03</td>
</tr>
</tbody>
</table>

Experimental conditions:
Initial concentration of H₂O₂, 0.173 mole
Buffer solution, phosphate buffer pH 6.3
Incubation time, 10 minute
Temperature, 25°C.
After 10 minutes, 10 ml. of 7.5N. H₂SO₄ is added to 20 ml. of reaction mixture and 5 ml. samples are titrated in H₂SO₄ with KMnO₄.

Effect of Substrate Concentration on Rate of Enzyme Action

When the enzyme concentration is kept at a constant value and the initial substrate concentration is varied between wide limits in a reaction in which one component is undergoing change, the variation in initial reaction velocity \( V = -ΔS/Δt \), expressed as the amount of substrate converted per unit of time) may be described by means of the curve in Fig. 7. It will be seen that the curve at first rises linearly, then slopes off, and finally reaches a constant maximum value. Occasionally, a diminution in velocity may be caused by further increase
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in substrate concentration. It will be noted from the graph that at low substrate concentrations, the first-order equation $-dS/dt = KE(S)$ applies, and that the initial velocity is directly proportional to the substrate concentration ($S$). However, as the substrate concentration is increased, there is attained a maximal reaction velocity that is independent of substrate concentration. Here the relationship $-dS/dt = KE$ applies; this is the differential equation of a zero-order reaction. The results are shown in Table 3.

Experimental conditions:
Volume of enzyme, 5 ml.
Buffer solution, phosphate buffer pH 6.3
Incubation time, 10 minutes
Temperature, 15°C.
Volume of substrate ($H_2O_2$), 15 ml.
After 10 minutes, 10 ml of 7.5 N H$_2$SO$_4$ is added to 20 ml of reaction mixture and 15 ml samples are titrated in H$_2$SO$_4$ with KMnO$_4$.

Table 3. Effect of $H_2O_2$ concentration on its velocity of decomposition by catalase.

<table>
<thead>
<tr>
<th>Concentration of $H_2O_2$ (M)</th>
<th>Decomposition of $H_2O_2$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05370</td>
<td>79.1</td>
</tr>
<tr>
<td>0.02015</td>
<td>79.4</td>
</tr>
<tr>
<td>0.01218</td>
<td>65.5</td>
</tr>
<tr>
<td>0.00924</td>
<td>63.6</td>
</tr>
<tr>
<td>0.00756</td>
<td>58.8</td>
</tr>
<tr>
<td>0.00504</td>
<td>38.15</td>
</tr>
</tbody>
</table>

The estimation of $V$ (maximum velocity) and hence the Michaelis Constant ($K_m$) is exceedingly cumbersome from the data in Table 3. This is occasioned by the fact that $v$ never appears to reach a maximum but is still creeping up even after relatively concentrated substrate solutions are offered to the enzyme. In our experiments, in which we studied the decomposition of $H_2O_2$ by fish liver catalase, the highest $[S]$ was 0.0537 M. This is a relatively concentrated solution in which to ask the enzyme to perform. Even when the data are plotted, as in Fig. 7., we might guess that $V$ is in the range of 70~80%, but it is impossible to say what the actual figure may be.

The Michaelis-Menten equation is related to the Henderson-Hasselbach treatment of the dissociation of weak electrolytes. Rearranging the usual form of the Michaelis equation, we obtain

\[
K_m + [S] = \frac{V(S)}{v} \quad \text{[v]}
\]

\[
K_m = \frac{V(S)}{v} - [S] = \frac{[S]}{(-\frac{V}{v} - 1)}
\]

\[
[S] = \frac{K_m}{(V/v) - 1} = \frac{K_m}{(V/v) - (V/v)} = \frac{K_m}{V - v}
\]
Fig. 7. Effect of increasing substrate concentration on the velocity of decomposition of $\text{H}_2\text{O}_2$ by catalase

or

$$\log [S] = \log \text{Km} + \log \frac{V - v}{V}$$

and

$$-\log [S] = -\log \text{Km} + \log \frac{V - v}{V}$$

when $v = 50\%$, $\log \frac{V - v}{V} = 0$ and $[S] = \text{Km}$.

Table 4 contains the same data as Table 3 except that the substrate concentration has also been expressed as $-\log [S]$ and the velocity of decomposition of the substrate expressed as $v/V$. Michaelis and Menten (6) actually employed these sigmoid curves for the estimation of Km, corresponding to the maximum slope of the curve. It should be cautioned however that the ionization constant in the Henderson-Hasselbach equation is a thermodynamic equilibrium constant whereas
Km is derived from kinetic data.

The data in Table 4 are plotted in Fig. 8.

In 1934 Lineweaver and Burk (7) pointed out that these constants could be more accurately determined if the data were presented in the form of a straight line. If one takes the reciprocal of the Michaelis-Menten equation, the following equation is obtained:

\[
\frac{1}{v} = \frac{Km + (S)}{V(S)} = \frac{Km}{V} \left( \frac{1}{(S)} \right) + \frac{1}{V}
\]

### Table 4. Effect of the negative logarithm of H\textsubscript{2}O\textsubscript{2} concentration on its velocity (v/V) of decomposition of H\textsubscript{2}O\textsubscript{2} by catalase.

<table>
<thead>
<tr>
<th>v/V</th>
<th>1.00</th>
<th>0.97</th>
<th>0.83</th>
</tr>
</thead>
<tbody>
<tr>
<td>- log [S]</td>
<td>1.27</td>
<td>1.70</td>
<td>1.91</td>
</tr>
<tr>
<td>Relative velocity measured from decomposition of H\textsubscript{2}O\textsubscript{2} (%)</td>
<td>79.1</td>
<td>76.4</td>
<td>65.5</td>
</tr>
<tr>
<td>v/V</td>
<td>0.80</td>
<td>0.74</td>
<td>0.48</td>
</tr>
<tr>
<td>- log [S]</td>
<td>2.03</td>
<td>2.12</td>
<td>2.30</td>
</tr>
<tr>
<td>Relative velocity measured from decomposition of H\textsubscript{2}O\textsubscript{2} (%)</td>
<td>63.6</td>
<td>58.8</td>
<td>38.15</td>
</tr>
</tbody>
</table>

Fig. 9. shows our data, rearrange as in Table 5, plotted in this so called "double reciprocal" manner.

The intercept 1/V is 0.012, and so V = 84. The slope Km/V is 0.00004, and hence Km = 0.0033 M.

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![Fig. 9. Plot of 1/v against 1/(S) according to method of Lineweaver and Burk.](image1)

![Fig. 10. Plot of (S)/v against (S) according to method of Lineweaver and Burk.](image2)
Another graphical procedure for the calculation of $K_m$ and of $V$ from experimental data on $v$ as a function of $[S]$ involves the multiplication of both sides of the Lineweaver-Burk equation by $[S]$ to give

$$\frac{(S)}{v} = \frac{K_m}{V} + \frac{(S)}{V}$$

<table>
<thead>
<tr>
<th>Table 5. Relationship between reciprocal of $H_2O_2$ concentration and reciprocal of its velocity of decomposition by catalase</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[S]$, M</td>
</tr>
<tr>
<td>$1/[S]$</td>
</tr>
<tr>
<td>Relative velocity measured from decomposition of $H_2O_2$ (％)</td>
</tr>
<tr>
<td>$1/\text{Relative Velocity}$</td>
</tr>
<tr>
<td>$[S]$, M</td>
</tr>
<tr>
<td>$1/[S]$</td>
</tr>
<tr>
<td>Relative velocity measured from decomposition of $H_2O_2$ (％)</td>
</tr>
<tr>
<td>$1/\text{Relative Velocity}$</td>
</tr>
</tbody>
</table>

If $(S)/v$ is plotted against $(S)$(cf. Fig. 10 and Table 6), a straight line results; the slope is $1/V$ and the intercept $K_m/V$. For most purposes this is a more satisfactory method of plotting the data and of determining $V$ and $K_m$. The intercept $K_m/V$ is 0.00004, and so $V = 84$. The slope $1/V$ is 0.012, and hence $K_m = 0.0033$ M.

In the Michaelis-Menten equation there is an obvious dependence of the velocity on the relative magnitude of $K_m$ and $[S]$. If $[S]$ is very large in relation to $K_m$, the expression becomes $v = \frac{V[S]}{[S]}$, and the velocity is maximum, independent of $[S]$, and therefore one of zero order. If the converse is true, and $K_m$ is large contrasted to $[S]$. The relation becomes $v = \frac{V[S]}{K_m}$, $v$ depends on $[S]$, and the reaction is first order. $K_m$ is a constant for the enzyme only under rigidly specified conditions.

The $K_m$ is useful in estimating the substrate concentration necessary to give maximal velocity, i.e., it is the substrate concentration at half maximum velocity.

For enzymes which attack a variety of substrates, those with the smallest $K_m$ have often been regarded as the “natural” substrate.

The $K_m$ for enzymes isolated from different sources may be compared in order to provide information on their possible identity. In the previous report (8), the $K_m$ of the catalase was described to be 0.025 M while the $K_m$ of the fish...
liver catalase was found under the experimental conditions to be 0.0033 M. Therefore these enzymes are not identical.

In any event, the Km measured by kinetic studies of the activity must always be at least equal to or larger than the dissociation constant.

Effect of Temperature on The Rate of Enzyme Action

In the determination of the temperature coefficient of an enzyme-catalyzed reaction, it is important that the substrate concentration be chosen so that the over-all reaction is of zero order. Otherwise the temperature coefficient found will be descriptive of the combination of enzyme and substrate rather than of the decomposition of the enzyme-substrate compound.

The conclusions discussed above apply under conditions where the enzyme is stable; this limits the experimental investigation of the effect of temperature on the rate of the enzyme-catalyzed reaction to a relatively narrow range. In general, if a wider temperature range is studied, it is found that the rate of enzyme action first increases with increasing temperature, and then decreases abruptly, as shown by Table 7 and Fig. 11.

Table 7. Effect of temperature on the rate of enzyme action.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>0.084 N KMnO₄ (ml.)</th>
<th>H₂O₂ decomposed (%)</th>
<th>Temperature (°C)</th>
<th>0.084 N KMnO₄ (ml.)</th>
<th>H₂O₂ decomposed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18.9</td>
<td>69.30</td>
<td>25</td>
<td>13.8</td>
<td>77.67</td>
</tr>
<tr>
<td>5</td>
<td>16.2</td>
<td>73.78</td>
<td>30</td>
<td>24.6</td>
<td>60.18</td>
</tr>
<tr>
<td>10</td>
<td>9.0</td>
<td>85.31</td>
<td>35</td>
<td>38.5</td>
<td>57.66</td>
</tr>
<tr>
<td>15</td>
<td>5.7</td>
<td>90.77</td>
<td>40</td>
<td>54.6</td>
<td>11.63</td>
</tr>
<tr>
<td>20</td>
<td>5.4</td>
<td>91.15</td>
<td>45</td>
<td>60.6</td>
<td>1.91</td>
</tr>
</tbody>
</table>

Experimental conditions:
Initial concentration of H₂O₂, 0.173 Mole
Buffer solution, phosphate buffer pH 6.3
Incubation time, 10 minutes
Volume of enzyme, 5 ml.
volume of substrate, 15 ml.

After 10 minutes, 10 ml. of 7.5 N H₂SO₄ is added to 20 ml. of reaction mixture and 5 ml. samples are titrated in H₂SO₄ with KMnO₄.

If one studies the rate of an enzyme catalyzed reaction as a function of temperature, one may express the relationship between rate constant K₁ at temperature T₁ to the rate constant K₂ at temperature T₂ by means of the Arrhenius equation:

$$\log \frac{K_2}{K_1} = \frac{A}{4.58} \left( \frac{1}{T_1} - \frac{1}{T_2} \right)$$

where K is the reaction velocity constant, R is the gas constant, T is the absolute temperature, and A is a constant i.e. activation energy. A can be calculated from the slope of the line obtained on plotting log K against 1/T. In biological studies, it has been occasionally the practice to substitute for A, the term $\mu$.

Our procedure in studying the effect of heat on an enzyme preparation is to subject a solution of the enzyme to 0° - 45°C. for a 10 minutes of time, to cool the solution and to determine the residual enzyme activity. Under these conditions the rate of enzyme inactivation usually follows the kinetics of a first-order reaction. The composite effect of temperature on the rate of an enzyme-catalyzed reaction may be illustrated by means of the data shown in Table 8. (cf. Fig. 12). It will be noted that the plot of log K against 1/T shows a value of $\mu = 4,400$ cal in the temperature region where the rate of H₂O₂ decomposition is increasing, and a value of $\mu = 55,000$ in the temperature region where the rate is decreasing.

When temperature is 10°C, K₁ can be calculated:

$$K_1 = \frac{2.303 \log \frac{100}{(100 - 85.31)}}{10} = 0.19159/\text{min}.$$
Fig. 12. Effect of temperature on the rate of decomposition of $\text{H}_2\text{O}_2$ by catalase

When temperature is 20°C, $K_2$ can be calculated:

$$K_2 = \frac{2.303}{10} \log \frac{100}{(100 - 91.15)} = 0.2448/\text{min}.$$  

When temperature is increased from 10°C to 20°C, $\mu$ can be calculated:

$$\log \frac{0.2448}{0.19159} = \frac{\mu(293 - 283)}{283 \times 293 \times 4.58} \quad \mu = 4,400 \text{ cal}.$$

If temperature becomes 35°C, $K_1$ will be:

$$K_1 = \frac{2.303}{10} \log \frac{100}{(100 - 37.66)} = 0.0472/\text{min}.$$  

If temperature is 45°C, $K_3$ will be:

$$K_3 = \frac{2.303}{10} \log \frac{100}{(100 - 37.66)} = 0.00193/\text{min}.$$  

When temperature is increased from 35°C to 45°C, $\mu$ can be calculated:

$$\log \frac{0.00193}{0.0472} = \frac{\mu(318 - 308)}{308 \times 318 \times 4.58} \quad \mu = -56,000 \text{ cal}.$$

According to the data of Sizer (9) on catalase, however, $\mu$ is 4200 cal. in the temperature region where the rate of $\text{H}_2\text{O}_2$ decomposition is increasing, and a value of $\mu$ is 51,000 in the temperature regions where the rate is decreasing. The value of $\mu=4400$ for catalase is too high, as shown by R. K. Bonnischen et al. (9). Their more careful measurement with purified catalase were conducted under conditions where the enzyme is not destroyed in the course of the reaction. The correct value of $\mu$ is probably near 1700 cal. Careful studies of the effect of temperature on the rate of inactivation of a variety of purified enzymes have...
Table 8. Effect of temperature on the rate of decomposition of \( \text{H}_2\text{O}_2 \) by catalase

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>( 1/T \times 10^4 )</th>
<th>log rate of ( \text{H}_2\text{O}_2 ) decomposition (%)</th>
<th>Temperature (°C)</th>
<th>( 1/T \times 10^4 )</th>
<th>log rate of ( \text{H}_2\text{O}_2 ) decomposition (%)</th>
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<tr>
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<td>0.28103</td>
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Table 8 shows the effect of temperature on the rate of decomposition of \( \text{H}_2\text{O}_2 \) by catalase. The data indicate that the rate constant, optimum concentration of enzyme, \( \text{Km} \), the activation energy, and the temperature optimum are significantly influenced by the presence of impurities, the \( \text{pH} \) of the solution, and other factors.

Given values of \( \mu \) ranging between 40,000 and 100,000 cal. Generally speaking in the decomposition of \( \text{H}_2\text{O}_2 \) by catalase, the value of \( \mu \) is about 2000 cal.; when catalyzed by colloidal palladium, \( \mu = \text{ca.} 12,000 \) cal, and, when no catalyst is added, \( \mu = \text{ca.} 18,000 \) cal.

Occasionally, reference will be found to the “temperature optimum” of an enzyme; under the conditions of our experiments this is 18°C. However under the conditions of Sizer’s experiments, temperature optimum of catalase is 53°C. The reason of this difference is probably due to the fact that temperature optimum is near body temperature for enzymes of animal origin. Since the temperature optimum is a resultant of two effects, both of which are profoundly influenced by the presence of impurities, the \( \text{pH} \) of the solution, and other factors, this temperature value is dubious significance in the characterization of an enzyme.

Summary

(1) Catalase has been isolated in crystalline form and in pure condition from the liver of the giant black marlin by the method of Sarker and Summer.

(2) For crystalline catalase we have observed absorption bands at 627 and 536 m\( \mu \).

(3) Under the experimental conditions, the first-order rate constant, optimum concentration of enzyme, \( \text{Km} \), the activation energy and the temperature optimum are 0.13 min\(^{-1}\), 5ml., 0.0033 M., 4,400 cal, and 18°C respectively.

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Abstract

1. According to Sarker and Summer's methods, the enzyme catalase has been isolated from the肝脏 of the giant black marlin. It is a pure crystalline form.

2. The catalase solution at a concentration of 627 and 536 m/µ has absorption peaks at 627 and 536 m/µ.

3. Under the experimental conditions, the liver catalase shows a first-order reaction rate of 0.13 per minute, with an apparent activation energy of 4,400 calories and an active concentration of 18 degrees Celsius.

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