ENTHALPY OF DECOMPOSITION OF HYDROGEN PEROXIDE BY CATALASE AT 25°C (WITH MOLAR EXTINCTION COEFFICIENTS OF H₂O₂ SOLUTIONS IN THE UV)

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<table>
<thead>
<tr>
<th>KEY WORDS</th>
<th>LINK A</th>
<th>LINK B</th>
<th>LINK C</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ROLE</td>
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</tr>
<tr>
<td>1. Enthalpy</td>
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<td></td>
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<tr>
<td>2. Hydrogen Peroxide</td>
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<td></td>
<td></td>
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<tr>
<td>3. Catalase</td>
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<tr>
<td>4. Extinction</td>
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<td></td>
<td></td>
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<td>5. Coefficients</td>
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Enthalpy of Decomposition of Hydrogen Peroxide by Catalase at 25°C (with Molar Extinction Coefficients of H₂O₂ Solutions in the UV)¹

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Thermodynamic methods are becoming increasingly significant as research tools in biochemistry (1-3). The heat of reaction is generally a very specific indication of the extent of reaction. Heat measurements are of particular advantage for those systems in which spectrophotometric techniques are complicated by absorbance or light scattering of the sample.

Reported here are the ΔH values for the decomposition of H₂O₂ solutions by catalase (EC 1.11.1.6) at 25°C and the molar extinction coefficients of H₂O₂ at the wavelengths 245, 240, 235, and 230 nm. In pure solutions, peroxide concentrations can be determined directly from the optical absorbance. Solutions containing components which absorb light in this region, however, cannot be assayed by a direct spectrophotometric approach. In this case, a calorimetric assay based on the heat of decomposition of hydrogen peroxide is useful. Biochemical reactions that either utilize H₂O₂ as substrates or produce H₂O₂ as a reaction product can then be monitored calorimetrically in direct or coupled reactions. In addition, oxygenation reactions (4) that are concerned with the effect of changing partial pressures of O₂ can be studied in a closed system calorimetrically by generating oxygen gas in situ through the decomposition of H₂O₂ with catalase. The enthalpy change associated with the decomposition of H₂O₂, once it is known, can then be subtracted from the total enthalpy change.

MATERIAL AND METHODS

Hydrogen peroxide solutions (~0.02 M) were prepared using Matheson, Coleman & Bell 30% hydrogen peroxide solution containing 0.05%

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sodium pyrophosphate as a preservative. Solutions were prepared in 0.154 M NaCl solution, Pioneer Chemical, ACS reagent, with EDTA-disodium salt (0.001 M) Mallinckrodt, A.R., added as a final peroxide stabilizer. Catalase (from Aspergillus niger) was obtained from Calbiochem. Solutions of this enzyme were prepared in the concentration of 1 mg of catalase to 100 ml of the NaCl-EDTA stock solution.

Concentrations of the hydrogen peroxide solutions were initially determined by titration with KMnO₄-Mallinckrodt, A.R., which was standardized against oxalic acid. Titrisol pre-standardized ampules, E. Merck & Co. Optical densities of the H₂O₂ solutions were measured at 245, 240, 235, and 230 nm on a Zeiss model PMQ II spectrophotometer. Wavelength calibration of the spectrophotometer was accomplished using two emission lines of the hydrogen light source. A second independent determination of H₂O₂ was performed manometrically at 25°C in a Warburg apparatus, using catalase as the decomposition catalyst in the side arm and measuring the pressure change due to the evolved oxygen. The total volume of the Warburg vessels was ~20 ml with a 3 ml fluid volume. Approximately 25 μmoles H₂O₂ were decomposed in each run resulting in pressure changes of roughly 170 mm Brodie's solution. The final catalase concentration after mixing was 100 μg/3 ml. The extinction coefficients determined by the two independent methods agreed very well and the values obtained by both methods were averaged.

Calorimetric measurements were performed on a Beckman 190B microcalorimeter, Beckman Instruments Inc., Palo Alto, California, using standard glass vessels with two drop wells. 16 ml of catalase solution, prepared as above, were placed in the annular space of the reaction vessel and a precisely measured volume (0.2-0.4 ml) of H₂O₂ solution was placed in the drop wells. Peroxide concentrations were calculated using the measured absorbance of the solution at a wavelength of 240 nm with a 1 cm optical path length and the extinction coefficient reported here. Heat of dilution measurements were made in the absence of catalase and subtracted from the heat values observed in the decomposition runs. The calorimeter was calibrated using the acid-base neutralization reaction:

\[ \text{NaOH} + \text{HCl} \rightarrow \text{NaCl} + \text{H}_2\text{O} \] at 25°C. The enthalpy value 13.37 kcal/mole was assigned to this reaction at this temperature. Calibration reactions involving the protonation of tris(hydroxymethyl)aminomethane also yielded the same calibration factor for the calorimeter within the standard deviation of the standardization runs.

RESULTS

A value of 0.0304 ± 0.002 cm²/μmole was obtained for the extinction coefficient of H₂O₂ at 240 nm. This value is valid at concentrations 0.02 M
and below and is within the region of linearity as indicated by dilution experiments. The isotonic saline-EDTA solution was used as a spectrophotometric blank and therefore did not contribute to the observed absorbance. The possibility that the sodium pyrophosphate contained in the original \( \text{H}_2\text{O}_2 \) solution might contribute to the absorbance at 240 nm was investigated and found to be insignificant in the concentration ranges studied. Values for the absorption coefficient taken from the literature (0.030 cm\(^2\)/µmole and 0.0430 cm\(^2\)/µmole) exhibit a wide variance (5,6). The value reported here is roughly the mean of these two values. The values for the extinction coefficients at the four wavelengths are:

\[
\begin{align*}
245 \text{ nm} & = 0.0305 \pm 0.0003 \\
240 \text{ nm} & = 0.0301 \pm 0.0002 \\
235 \text{ nm} & = 0.0500 \pm 0.0000 \\
230 \text{ nm} & = 0.0424 \pm 0.0013
\end{align*}
\]

The standard deviations represent the error in 12 titrations and 15 manometric measurements. The optical absorbance was measured at the four wavelengths for each independent determination of concentration.

The value for the \( \Delta H \) of decomposition was determined to be

\[24.0 \pm 0.3 \text{ kcal/mole}\]

for the reaction

\[
\text{catalase} \quad \text{H}_2\text{O}_2 (aq) \rightleftharpoons \text{H}_2\text{O} + \frac{1}{2} \text{O}_2
\]

at 25°C. Since the reaction vessel in which the decomposition takes place is a closed vessel, the oxygen gas produced in the reaction can cause a pressure change in the vessel. The heat change in the reaction does not then reflect identically the \( \Delta H \) of the reaction. A pressure change of 1.4 mm Hg, calculated from the millimoles of \( \text{O}_2 \) produced in a typical decomposition reaction is so small, however, compared with atmospheric pressure, that the heat change observed is equal within the standard error of the calorimetric procedure to the \( \Delta H \) of the reaction.

**DISCUSSION**

The determination of the change in enthalpy reactions involving hydrogen peroxide is often the most convenient method for observing these reactions. This measurement is dependent only upon the heat of the reaction which produces or utilizes \( \text{H}_2\text{O}_2 \) and upon the heat of decomposition of \( \text{H}_2\text{O}_2 \) by catalase. Combination of measured heat values with the decomposition enthalpy reported here yields \( \Delta H \) values for chosen enzymatic reactions involving \( \text{H}_2\text{O}_2 \). Heat of reaction values can then be related to changes in \( \text{H}_2\text{O}_2 \) concentration using the \( \Delta H \) value for that reaction.
Examples of enzymatic reactions amenable to such treatment include those utilizing \( \text{H}_2\text{O}_2 \) as substrate, e.g., catalase and peroxidase, those producing \( \text{H}_2\text{O}_2 \) as a product, e.g., glucose oxidase, xanthine oxidase, and amino acid oxidase, and those which can be coupled to reactions which involve \( \text{H}_2\text{O}_2 \) directly. Reactions which are directly concerned with the metabolism of oxygen can be studied in a single phase by producing \( \text{O}_2 \) partial pressures in the liquid phase through the decomposition of \( \text{H}_2\text{O}_2 \), and by then monitoring heat effects associated with the consumption of this oxygen. A particular example of the latter is the oxygenation of hemoglobin by the association of deox-hemoglobin with the \( \text{O}_2 \) produced by the \( \text{H}_2\text{O}_2 \) decomposition reaction. Studies of the influence of various metabolites on the enthalpy of oxygenation of deoxyhemoglobin are underway in this laboratory.

The value for the \( \Delta H \) of decomposition of \( \text{H}_2\text{O}_2 \) in liquid solution has been reported in the literature by several investigators \( 17-19 \) to be \( \approx 22.7 \text{ kcal mole} \). It is reported to be rather insensitive to concentration and temperature change. The decomposition of \( \text{H}_2\text{O}_2 \) by catalase at very low concentrations has not been studied, however. Reported values were determined at much higher concentrations and hence extrapolated to dilute solutions. Differences in the \( \Delta H \) values reported in the literature and that reported here are not particularly surprising since the values obtained in the literature were determined by somewhat indirect methods, and the choice of catalyst and ionic strength of the solution are quite different. The value reported here is, of course, more applicable to biochemical investigations since it involves an enzymatic decomposition catalyst and a physiological ionic strength.

**SUMMARY**

The enthalpy of decomposition of hydrogen peroxide by catalase has been determined calorimetrically in isotonic saline solutions at 23°C. Extinction coefficients are also reported for hydrogen peroxide solutions in the ultraviolet.

**REFERENCES**