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ENTHALPY OF DECOMPOSITION OF HYDROGEN PEROXIDE BY CATALASE AT 25°C (WITH MOLAR EXTINCTION COEFFICIENTS OF H_2O_2 SOLUTIONS IN THE UV)

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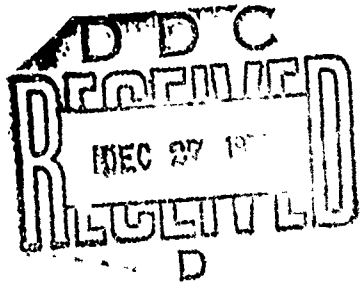

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13. ABSTRACT

The enthalpy of decomposition of hydrogen peroxide by catalase has been determined calorimetrically in isotonic saline solutions at 25° C. Extinction coefficients are also reported by hydrogen peroxide soluations in the ultraviolet.

I

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III

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Enthalpy of Decomposition of Hydrogen Peroxide by Catalase at 25°C (with Molar Extinction Coefficients of H_2O_2 Solutions in the UV)[1]

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Thermoanalytical 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 value for the decomposition of H_2O_2 solutions by catalase (EC 1.11.1.6) at 25°C and the molar extinction coefficients of H_2O_2 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 adsorb 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 H_2O_2 as substrates or produce H_2O_2 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 O_2 can be studied in a closed system calorimetrically by generating oxygen gas *in situ* through the decomposition of H_2O_2 with catalase. The enthalpy change associated with the decomposition of H_2O_2, 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%

[1] From the Bureau of Medicine and Surgery, Navy Department Research Subtask MR011.20.01.0361. The opinions and statements contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or of the Naval Service at Large.

sodium pyrophosphate as a preservative. Solutions were prepared in 0.154 *M* NaCl solution, Pioneer Chemical, ACS reagent, with EDTA-disodium salt (0.0001 *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 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 $KMnO_4$, Mallinckrodt, A.R., which was standardized against oxalic acid, Titrisol pre-standardized ampules, E. Merck & Co. Optical densities of the H_2O_2 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 H_2O_2 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 H_2O_2 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. 15 ml of catalase solution, prepared as above, were placed in the annular space of the reaction vessel and a precisely metered volume (0.2-0.4 ml) of H_2O_2 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 $NaOH + HCl \rightarrow NaCl + H_2O$ 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 0.0394 ± 0.002 cm^2/μmole was obtained for the extinction coefficient of H_2O_2 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 H_2O_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 $cm^2/\mu mole$ and 0.0436 $cm^2/\mu mole$) 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:

$$245 \text{ nm} = 0.0308 \pm 0.0003$$
$$240 \text{ nm} = 0.0304 \pm 0.0002$$
$$235 \text{ nm} = 0.0500 \pm 0.0006$$
$$230 \text{ nm} = 0.0824 \pm 0.0013$$

The standard deviations represent the error in 12 titrations and 16 manometric measurements. The optical absorbance was measured at the four wavelengths for each independent determination of concentration.

The value for the ΔH of decomposition was determined to be

$$24.0 \pm 0.3 \text{ kcal/mole}$$

for the reaction

$$H_2O_2\,(aq) \xrightarrow{\text{catalase}} H_2O + \tfrac{1}{2}\,O_2$$

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 ΔH of the reaction. A pressure change of 1.4 mm Hg, calculated from the millimoles of 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 ΔH of the reaction

DISCUSSION

The determination of heat change in enzymic 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 H_2O_2 and upon the heat of decomposition of H_2O_2 by catalase. Combination of measured heat values with the decomposition enthalpy reported here yields ΔH values for chosen enzymic reactions involving H_2O_2. Heat of reaction values can then be related to changes in H_2O_2 concentration using the ΔH value for that reaction

Examples of enzymic reactions amenable to such treatment include those utilizing H_2O_2 as substrate, e.g., catalase and peroxidase, those producing H_2O_2 as a product, e.g., glucose oxidase, xanthine oxidase, and amino acid oxidase, and those which can be coupled to reactions which involve H_2O_2 directly. Reactions which are directly concerned with the metabolism of oxygen can be studied in a single phase by producing O_2 partial pressures in the liquid phase through the decomposition of H_2O_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 deoxyhemoglobin with the O_2 produced by the H_2O_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 ΔH of decomposition of H_2O_2 in liquid solution has been reported in the literature by several investigators (7–19) to be −22.7 kcal/mole. It is reported to be rather insensitive to concentration and temperature changes. The decomposition of H_2O_2 by catalase at very low concentrations has not been studied, however. Reported values were determined at much higher concentrations and coarsely extrapolated to dilute solutions. Differences in the ΔH 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 enzymic 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 25°C. Extinction coefficients are also reported for hydrogen peroxide solutions in the ultraviolet.

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