kcal./mole). However, reaction 2 is rather more complicated than (7), since it involves both carbonate replacement and H^+ transfer, precluding direct comparisons between them. $E'' = E_6 + \Delta H_5$ for both N_4 and N_5, making $E_6 \sim 30$ kcal./mole for both ammines, in acceptable agreement with Brönsted's data for the corresponding reaction of the nitrato salt.[25] For tn_2, E' lies between E_2 and $E_2 + \Delta H_1$, depending on how small K_1 is. Similarly, E'' lies between E_3 and $E_3 + \Delta H_1$ for tn_2. ΔH_1 is not known but is probably quite small, so that $E_2 \cong E_3 \cong 25$ kcal./mole in this case.

Support of this research by the U. S. Atomic Energy Commission through Contract AT(30-1)-1578 is gratefully acknowledged.

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[CONTRIBUTION NO. 1463 FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

Investigation of the Catalytic Mechanism of Catalase and Other Ferric Compounds with Doubly O^{18}-labeled Hydrogen Peroxide

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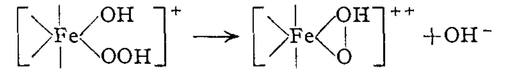
RECEIVED AUGUST 19, 1957

A method for determining the origin of molecular oxygen in the catalytic decomposition of hydrogen peroxide is developed and applied to the catalytic decomposition of hydrogen peroxide by catalase, polyamine–Fe(III) chelates and ferric hydroxide. In all cases studied, the oxygen molecule originates from the intact O–O bond in hydrogen peroxide.

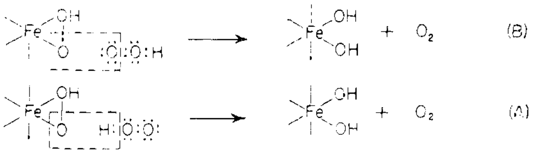
One method of approach to the study of catalysis is *via* the synthesis and investigation of relatively simple model molecules of known structure. By this means Wang[2] discovered the remarkably high catalytic efficiency of triethylenetetramine-Fe(III) chelate for the decomposition of hydrogen peroxide. The postulated mechanism of catalysis involves the metathetical displacement of a bound OH^- by OOH^-, followed by the displacement of another bound OH^- by the second oxygen atom of the same OOH^- as illustrated below.



I II

Since the O–O bond in compound II is under strain, it becomes more reactive and reacts subsequently with a second OOH^- to produce O_2 and regenerate the catalyst.

There are two alternative mechanisms for this last reaction step to take place. These are depicted below as mechanisms (A) and (B), respectively.



It is of particular interest to find out whether the catalytic decomposition of hydrogen peroxide by triethylenetetramine–Fe(III) chelate takes place through mechanism (A) or (B).

In general even for cases in which the detailed mechanism is unknown, it is still of interest to know whether the two O-atoms in each O_2 liberated came from the same H_2O_2 molecule or from two separate H_2O_2 molecules.

Principle of the Method

Suppose O^{18}-enriched hydrogen peroxide is made directly by an electric discharge through O^{18}-enriched water vapor. If the isotopic atom-fraction of O^{18} in the resulting H_2O_2 is X, the mole-fraction of $HO^{18}O^{18}H$ should be approximately equal to X^2. (Here it is assumed that the isotope-effect due to the difference in zero point energies of light and heavy O-atoms in H_2O_2 is negligible.) When this H_2O_2 is catalytically decomposed, the mole-fraction of $O^{18}O^{18}$ in the oxygen gas liberated should also be X^2 no matter whether the decomposition takes place through mechanism (A) or mechanism (B), because the isotopic distribution in this H_2O_2 is in accordance with natural probabilities. However, when each mole of this O^{18}-enriched hydrogen peroxide is mixed with q moles of ordinary hydrogen peroxide, the isotopic atom-fraction of O^{18} and the mole-fraction of $HO^{18}O^{18}H$ in the mixed H_2O_2 become $[X + q(0.0020)]/(1 + q)$ and $[X^2 + q(0.0020)^2]/(1 + q)$, respectively, where 0.0020 is the isotopic atom-fraction of O^{18} in ordinary H_2O_2. This last isotopic distribution is unnatural, for had this H_2O_2 been made directly from enriched water vapor with atom-fraction of O^{18} equal to $[X + q(0.0020)]/(1 + q)$, then the mole-fraction of $HO^{18}O^{18}H$ in the resulting H_2O_2 would be $[X + q(0.0020)]^2/(1 + q)^2$. Now if this O^{18}-enriched H_2O_2 with unnatural isotopic distribution is catalytically decomposed by, say, triethylenetetramine–Fe(III), the mole-fraction of $O^{18}O^{18}$ in the liberated O_2 will be different for mechanisms (A) and (B), respectively. Mechanism (B) involves a reshuffling of the O-atoms, *i.e.*, restoration to the natural distribution of the isotopic species, and hence predicts a mole-fraction of $[X + q(0.0020)]^2/(1 + q)^2$ for $O^{18}O^{18}$ in the liberated O_2 gas. But a mechanism such as (A), which produces all the O_2 from the intact O–O bonds, leaves the original isotopic distribution undisturbed, and hence predicts the mole-fraction of $O^{18}O^{18}$ to be $[X^2 + q(0.0020)^2]/(1 + q)$.

If $X = 0.10$ and $q = 1.0$, we have $[X^2 + q(0.0020)^2]/(1 + q) \approx 0.0050$ and $[X + q(0.0020)]^2/(1 + q)^2 \approx 0.0026$. Consequently a mass-spectrometric analysis of the O_2 produced by the catalytic decomposition of hydrogen peroxide could

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[1] National Science Foundation Predoctoral Fellow, 1957–1958.

[2] J. H. Wang, THIS JOURNAL, **77**, 4715 (1955).

distinguish between these two alternative mechanisms.

Results and Discussion

In the present study, water which is about 10 atom % in O^{18} was converted to hydrogen peroxide in an electric discharge. The hydrogen peroxide so obtained contained about 1 mole % of $HO^{18}O^{18}H$. This was diluted with an approximately equal amount of ordinary hydrogen peroxide. The H_2O_2 in the resulting mixture contained about 0.5 mole % of $HO^{18}O^{18}H$ and about 5 atom % of O^{18}. This hydrogen peroxide with unnatural isotopic distribution was catalytically decomposed to completion by triethylenetetramine-Fe(III) chelate, catalase, tris-(β-aminoethyl)-amine–Fe(III) chelate, freshly prepared $Fe(OH)_3$, respectively, and by thermal means. The liberated oxygen gas was analyzed mass-spectrometrically. The isotopic atom-fraction of O^{18} and the mole-fraction of $HO^{18}O^{18}H$ in the H_2O_2 were determined by complete oxidation with Ce(IV) solution,[3] followed by mass-spectrometric analysis. The results are summarized in Table I.

TABLE I

$[H_2O_2]$, 0.2 to 0.4 *F*; temp. 25 ± 5°

Catalyst	Atom % of O^{18} in H_2O_2	Mole % of HO^{18}-$O^{18}H$ in H_2O_2	Mole % of $O^{18}O^{18}$ in the liberated O_2		
			Expected from intact O–O bonds	Expected from reshuffling of O-atoms	Found
Triethylenetetramine–Fe(III)[a]	10.78[b]	1.18	1.18	1.18	1.19 1.19
Triethylenetetramine–Fe(III)[a]	1.071	0.098	0.098	0.012	0.098 .098
Triethylenetetramine–Fe(III)[a]	5.20	.559	.559	.27	.556 .547
Catalase[c]	5.22	.564	.564	.27	.556 .547
Tris-(β-aminoethyl)-amine–Fe(III)[d]	6.58	.714	.714	.43	.730 .728
$Fe(OH)_3$[e]	7.22	.778	.778	.52	.816 .818
Thermal decomposition (in Pyrex tube)[f]	7.22	.778	.778	.52	.827 .806

[a] All triethylenetetramine–Fe(III) decompositions were carried out at *p*H 10.0 with [TETA] = $4.5 \times 10^{-2}F$ and [Fe(III)] = $4.9 \times 10^{-6}F$. [b] No dilution of doubly labeled H_2O_2 to give unnatural isotopic distribution. [c] Catalase in Sörensen's phosphate buffer, *p*H 7.0, with [catalase] = 0.42 μM estimated spectroscopically. [d] [polyamine] = $8.5 \times 10^{-3}F$, [Fe(III)] = $4.9 \times 10^{-6}F$, *p*H 10.0. [e] $Fe(OH)_3$, freshly prepared at *p*H 10.0. [f] Temperature = 84.5 ± 0.2°.

The results in all cases investigated unambiguously show that the O_2 evolved originates from intact O–O bonds in hydrogen peroxide. This shows that the O_2 is liberated through a hydrogen atom or hydride ion-removal process in all of the above cases. It does not mean, however, that the complete reaction mechanism is the same in all these cases. In fact the complete mechanism for the catalytic decomposition of H_2O_2 by ferric ion,[5,3] ferric hydroxide[4,2] and methemoglobin,[2] and by triethylenetetramine-Fe(III) chelate[2] and catalase,[3,4,2] respectively, have been shown to be quite different. Further studies on the oxygen and hydrogen isotope-effects in the decomposition of hydrogen peroxide by various catalysts will be reported in a later communication.

Experimental

Doubly O^{18}-Labeled H_2O_2.—Water containing 10 atom % of O^{18} (Dajac Laboratories, Leominster, Mass.) was passed through an electric discharge, and the product was trapped on a liquid nitrogen-cooled glass surface.[6] Aluminum electrodes spaced 2 meters apart were used in a Pyrex glass tube, 28 mm. i.d. The electric field strength was 10 v./cm., maintained by a neon-sign transformer. Two U-tubes, 13 mm. i.d., cooled by liquid nitrogen, were used as traps. The first U-tube was sealed directly to the discharge tube about 10 cm. in front of the downstream electrode. The second U-tube was 50 cm. downstream from the first and served to prevent the diffusion of pump vapors into the discharge system. The system was evacuated by a Welsh Duo Seal Pump with a capacity of about 0.4 l./sec. at 0.2–0.4 mm.

Water vapor was admitted through a capillary about 1/3 mm. in diameter and 5 cm. long. This size of capillary permitted convenient control of the flow rate of water vapor by regulating the temperature (25–45°) of the water introduction tube.

The product H_2O_2 was pumped out of the first trap and collected in a small U-trap connected to the system by a 10/30 joint and stopcock situated between the two larger traps.

The amount of water admitted was often between 80 and 200 mg. A typical charge is as follows: total water admitted, 96 mg.; time required, 25 minutes; H_2O_2 collected, 33 mg.; conversion, 35%.

Mass-spectrometric Analysis.—All mass-spectrometric analyses were made on a Consolidated Engineering Mass-spectrometer Model 21-401. Standard techniques and procedures were used.

Reagents.—Ordinary H_2O_2, either Fisher or Merck, 30%, C.P.; catalase, crystalline, purchased through Delta Chemical Works; triethylenetetramine, Matheson, Coleman and Bell, practical grade; tris-(β-aminoethyl)-amine, synthesized according to the method of Ristenpart.[7]

Acknowledgments.—The authors wish to thank Dr. Martin Saunders who first suggested the doubly O^{18}-labeled tracer experiment. This work was supported in part by a research grant (US-PHS-RG-4483) from the Division of Research Grants, Public Health Service.

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