is due to the difference in crystallite size only, then one could possibly calculate "crystallite boundary" tension, energy and entropy of graphite provided that certain essential information is also available. Further data necessary would be the difference in surface area of the crystallites in the two samples and the difference in the heats of combustion.[14] If the surface area measurements on the CS grade graphite and the average heats of combustion between Ceylon natural graphite and the CS material reported by Bupp and associates[15] are used, the crystallite boundary entropy of graphite is roughly 4.8 ergs cm.$^{-2}$ deg.$^{-1}$ g-atom.$^{-1}$. The value of heat content for the crystallite surface is 1950 ergs cm.$^{-2}$, while the crystallite boundary tension is 530 ergs cm.$^{-2}$.

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These values are probably reliable only as to order of magnitude.

(14) G. Jura and C. W. Garland, THIS JOURNAL, **74**, 6033 (1952).

(15) L. P. Bupp, private communication.

SCHENECTADY, N. Y.

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[CONTRIBUTION NO. 1296 FROM THE DEPARTMENT OF CHEMISTRY OF YALE UNIVERSITY]

# On the Detailed Mechanism of a New Type of Catalase-like Action

BY JUI H. WANG

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A model catalyst was constructed for the catalytic decomposition of hydrogen peroxide. The detailed mechanism of this new type of catalase-like action was studied with respect to ligand specificity, metal ion specificity, inhibition and activation by other ions and the isotope-effect. Comparison of the model catalysts with natural catalases was made and the similarities were briefly discussed.

The elucidation of the detailed mechanisms of enzyme action is a problem of common interest to physico- and bio-scientists. At the present time the major difficulty of this problem seems to be due to our lack of knowledge on the detailed structure of enzyme molecules. While the complexity of the detailed structures of these molecules shows little promise of their complete elucidation in the near future, this important problem of enzyme action may meanwhile be approached from a new angle, *i.e.*, by constructing and studying small model molecules with enzyme-like activity. This paper presents some first results obtained in a research project planned in this direction. The general method of approach in this project may be summarized as follows. Firstly, with the help of our knowledge on the structure of the substrate molecule, properties of the corresponding enzyme and the nature of the chemical bond, small model molecules with possible enzyme-like activity are designed, synthesized and tested for their catalytic activity. Secondly, detailed kinetic and structural studies will be carried out on those synthetic molecules with outstanding catalytic activity. Finally, attempts will be made to improve the catalytic activity of these model molecules by modifying their structure and by incorporating them, if necessary, into the structure of macromolecules. It is the hope of the present writer that the results obtained in this direction could accelerate our present pace of progress toward the understanding of enzyme action.

The decomposition of hydrogen peroxide is catalyzed by a large variety of substances.[1] But molecule for molecule, none can compete with catalases for catalytic efficiency. Even hematin and hemoglobin are about $10^7$ times less efficient as compared to catalases according to Lemberg and Legge.[2]

(1) For reference to the literature see a comprehensive review by J. H. Baxendale in "Advances in Catalysis" (Edited by W. G. Frankenburg, V. I. Komarewsky and E. K. Rideal), Vol. IV, Academic Press, Inc., New York, N. Y., 1952, p. 31.

(2) R. Lemberg and J. W. Legge, "Hematin Compounds and Bile Pigments," Interscience Publishers, Inc. New York, N. Y., 1949, p. 402.

It was reported in a recent communication that a very efficient model catalyst for the decomposition of hydrogen peroxide can be made by combining triethylenetetramine (TETA), $H_2NCH_2CH_2NHCH_2CH_2NHCH_2CH_2NH_2$, with ferric ion.[3] The results of a more extensive study on the catalytic action of this model catalyst, $(TETA)Fe(OH)_2^+$, and related compounds are given below.

**The Catalytic Mechanism.**—It was pointed out earlier[3] that steric considerations show that it is energetically improbable to have the four N atoms and the $Fe^{III}$ ion in $(TETA)Fe(OH)_2^+$ located in one plane, but that the structure with one primary amine N atom above and the other below the plane determined by the two secondary amine N atoms and the $Fe^{III}$ ion is stable. The detailed mechanism of the catalytic action of $(TETA)Fe(OH)_2^+$ for the decomposition of hydrogen peroxide is illustrated in Fig. 1. The configuration of $(TETA)Fe(OH)_2^+$ is depicted by structure I in Fig. 1. In the presence of hydrogen peroxide the two attached $OH^-$ ions in compound I may be replaced by an $OOH^-$ ion, yielding compound II. But since the O-O bond length in $OOH^-$ is only about 1.3 Å., compound II is unstable and tends to stretch the O–O bond until compound III is formed. Direct splitting of the O–O bond in an isolated $H_2O_2$ molecule requires about 35 kcal. of activation energy per mole. But in the above reaction mechanism the energy consumed in splitting the O–O bond is partially compensated by the energy gained through the formation of more stable Fe–O bonds, because the O atom and the $OH^-$ ion are now separate ligands and can orient themselves for maximum overlapping with the two vacant octahedral $d^2sp^3$ hybrid atomic orbitals of the $Fe^{III}$. The binding between the O atom and $Fe^{III}$ in compound III is probably not a single covalent bond. It probably involves a considerable amount of a one-electron bond (depicted by the dotted line in Fig. 1) in addition to an ordinary electron-pair bond. Compound III can then readily react with a second $OOH^-$ ion to yield $O_2$ and regenerate compound I. This cyclic mechanism was

(3) J. H. Wang, THIS JOURNAL, **77**, 822 (1955).

subsequently substantiated by experimental measurements on the rate of catalytic decomposition of $H_2O_2$ by $(TETA)Fe(OH)_2^+$.

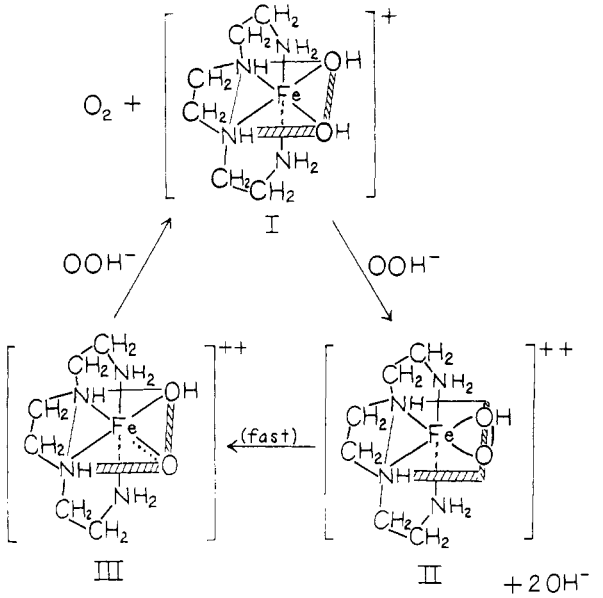


Fig. 1.—The detailed mechanism of the catalytic action of $(TETA)Fe(OH)_2^+$ for the decomposition of $H_2O_2$.

The rates of catalytic decomposition of $H_2O_2$ were measured both by direct titration and by manometric method. The titration procedure has already been described in the previous communication.[3] The procedure of manometric measurements is essentially the same as that used by George.[4] The turnover numbers of $(TETA)Fe(OH)_2^+$, expressed in moles of $H_2O_2$ decomposed per min. per mole of $(TETA)Fe(OH)_2^+$, were computed from the measured initial rates of the catalytic decomposition of $H_2O_2$. Values of the turnover number of $(TETA)Fe(OH)_2^+$ obtained by titration and by manometric methods showed very good agreement. The results are summarized in Table I.

TABLE I

CATALYTIC DECOMPOSITION OF $H_2O_2$ BY $(TETA)Fe(OH)_2^+$ AT 25°

(Concn. of total TETA = $4.8 \times 10^{-3}$ mole/l.; concn. of total $Fe^{III}$ = $6.3 \times 10^{-7}$ mole/l.)

| Initial concn. of $H_2O_2$ (mole/l.) | pH | Turnover no. of $(TETA)Fe(OH)_2^+$ (min.$^{-1}$) | Initial concn. of $H_2O_2$ (mole/l.) | pH | Turnover no. of $(TETA)Fe(OH)_2^+$ (min.$^{-1}$) |
|---|---|---|---|---|---|
| 0.149 | 6.0 | 270 | 0.149 | 10.0 | 11000 |
| .149 | 7.1 | 1300 | .314 | 10.0 | 20000 |
| .149 | 8.6 | 5900 | .418 | 10.0 | 25000 |
| .149 | 9.9 | 10300 | .616 | 10.0 | 34000 |
| .149 | 10.8 | 14400 | .628 | 10.0 | 35000 |
| .149 | 11.2 | 19000 | .86 | 10.0 | 49000 |
| .071 | 10.0 | 5200 | .94 | 10.0 | 52000 |

The results of earlier studies on the effect of varying temperature and catalyst concentration show that the activation energy for the catalytic decomposition of $H_2O_2$ by $(TETA)Fe(OH)_2^+$ is about 6.6 kcal., and that the decomposition rate is directly proportional to the catalyst concentration. Values in Table I show that the decomposition rate also continues to increase with $H_2O_2$ concentration up to 0.94 *M*, although the observed increase is slightly less than that predicted by a rate law first order with respect to $H_2O_2$ concentration. This shows that the transition of compound II to compound III as illustrated in Fig. 1 cannot be the rate-determining step in the catalytic cycle, for in that case a Michaelis–Menten type of saturation curve would be observed. Since Compound III is probably highly reactive, the rate-determining step in the above cycle is likely to be the reaction of compound I with $OOH^-$ to form compound II.

In order to correct for traces of $Fe^{III}$ present in the reaction system as impurity (from reagents and glassware), a blank measurement with TETA and $H_2O_2$ but without added $Fe^{III}$ were made for each $H_2O_2$ concentration and *p*H. These blank rates were subtracted from the corresponding measured rates before the computations for turnover numbers were made. Values of the turnover number of $(TETA)Fe(OH)_2^+$ in solutions with *p*H > 11 are less reliable, because in these solutions the blank rate becomes a substantial fraction of the measured rate. This is probably due to the increased rate of dissolution of glass, which presumably contains some iron, in alkaline solutions. The high efficiency of the present model system suggests that it could be used as basis of a new method for microanalysis of iron; *e.g.*, 0.000001% of iron in solution can be readily detected by the present reaction.

**Ligand Specificity.**—Ethylenediamine (EDA), $H_2NCH_2CH_2NH_2$ and diethylenetriamine (DETA), $H_2NCH_2CH_2NHCH_2CH_2NH_2$ also form chelate compounds with $Fe^{III}$ ion. The catalytic efficiency of these chelates for the decomposition of hydrogen peroxide are, however, much less than that of $(TETA)Fe(OH)_2^+$. The catalytic efficiencies of these $Fe^{III}$-chelates decrease in the order TETA > DETA > EDA. This is consistent with the considerations regarding the reaction mechanism illustrated in Fig. 1 as well as the relative stabilities of these chelate ions.

It may also be noticed from Fig. 1 that the splitting of the peroxide bond by $(TETA)Fe(OH)_2^+$ is due essentially to the availability of two adjacent octahedral hybrid atomic orbitals of $Fe^{III}$ for combination with $OOH^-$. If tetraethylenepentamine (TEPA), $H_2NCH_2CH_2NHCH_2CH_2NHCH_2CH_2NHCH_2CH_2NH_2$, is used as the ligand instead of TETA, the resulting chelate, $(TEPA)Fe(OH)^{++}$, will have only one octahedral hybrid atomic orbital of $Fe^{III}$ left for combination with $OOH^-$. In other words the fifth N atom in $(TEPA)Fe(OH)^{++}$ acts as an intramolecular inhibitor to prevent the splitting of the peroxide bond through the cyclic mechanism illustrated in Fig. 1. This reasoning was subsequently confirmed by the experimental finding that the catalytic efficiency of $(TEPA)Fe(OH)^{++}$ was even much less than the corresponding ethylenediamine chelate. Compared to $(TETA)Fe(OH)_2^+$, $(TEPA)Fe(OH)^{++}$ is practically inactive. This observation can be used as strong evidence in favor of the splitting mechanism illustrated in Fig. 1.

Of course, the fact that the catalytic decomposition of $H_2O_2$ by $(TETA)Fe(OH)_2^+$ takes place es-

(4) P. George, *Biochem. J.*, **43**, 287 (1948); *ibid.*, **44**, 197 (1949).

sentially through the above mentioned splitting mechanism does not exclude other catalytic decomposition processes from taking place simultaneously. For example, it is well-known that ferrous and ferric ions can catalyze the decomposition of $H_2O_2$ through a free radical mechanism.[1] However the fact that (TEPA)$Fe(OH)^{++}$, which may catalyze through the free radical mechanism but cannot catalyze through the above-mentioned splitting mechanism, is practically inert as compared to (TETA)$Fe(OH)_2^+$ shows that the splitting mechanism is very much more efficient than the free radical mechanism, even though the latter might also take place simultaneously.

Replacing the TETA in (TETA)$Fe(OH)_2^+$ by other negatively charged ligands of similar structure often results in a chelate of little catalytic activity. These ligands include nitrilotriacetate ion, $N(CH_2COO^-)_3$, ethylenediamine diacetate ion, $-OOCCH_2NHCH_2CH_2NHCH_2COO^-$, ethylenediaminetetracetate ion, $(-OOCCH_2)_2NCH_2CH_2N(CH_2COO^-)_2$, etc. Presumably this decrease in the catalytic activity is due to the increase in ionic character of the $Fe^{III}$–ligand bonds in these chelates. It is planned to give a more detailed discussion on this point in a later publication together with the results of some magnetic susceptibility studies on these systems.

**Metal Ion Specificity.**—A rather extensive study was carried out on the catalase-like activity of chelates formed by TETA and various metal ions. The metal ions selected for this study include $Mg^{II}$, $Al^{III}$, $Ca^{II}$, $Cr^{III}$, $Mn^{II}$, $Fe^{III}$, $Co^{III}$, $Ni^{II}$, $Cu^{II}$, $Zn^{II}$, $Sr^{II}$, $Ag^{I}$, $Cd^{II}$, $Ba^{II}$, $Tl^{I}$ and $Pb^{II}$. Of these only the TETA chelates of $Fe^{III}$ and $Mn^{II}$ showed remarkable catalytic activity for the decomposition of $H_2O_2$. The catalytic activities of the TETA chelates of all the other metal ions listed above are negligible as compared to (TETA)$Fe(OH)_2^+$ or (TETA)$Mn(OH)^+$. In dilute $H_2O_2$ solutions the catalytic efficiency of (TETA)$Fe(OH)_2^+$ is many times greater than that of (TETA)$Mn(OH)^+$. This shows that the present model catalyst has the highest degree of metal ion specificity, since $Fe^{III}$ and $Mn^{II}$ are isoelectronic. Obviously neither the size nor the charge of the metal ion can be the cause of this specificity, since many of the above ions have size and charge similar to $Fe^{III}$ or $Mn^{II}$. It seems likely that this specificity is due to some special requirement on the detailed electronic structure of the metal ion. In the present model catalyst the distribution of electrons around the metal nucleus must be such as to make compounds like I, II and III in Fig. 1 have appropriate amounts of stability. While highly unstable reaction intermediate (or activated complex) means high activation energy for the mechanism, extremely stable reaction intermediate will prevent rapid regeneration of the original catalyst.

Results of measurements on the catalytic decomposition of $H_2O_2$ by (TETA)$Mn^{II}(OH)^+$ at 25° are summarized in Table II.

Values in Table II show that at low $H_2O_2$ concentrations (TETA)$Mn(OH)^+$ is a much less efficient catalyst as compared to (TETA)$Fe(OH)_2^+$. This is not surprising, since a glance at the values of the successive ionization potentials of Fe and Mn shows that the $Fe^{III}$–O bonds in compounds II and III ought to be more stable than the corresponding $Mn$–$O^{II}$ bonds. However, there is considerable uncertainty in this interpretation, because the catalytic decomposition of $H_2O_2$ by (TETA)$Mn(OH)^+$ has several complications. For example, a plot of the measured turnover numbers of (TETA)$Mn(OH)^+$ at $p$H 10.0 in Table II *vs.* $H_2O_2$ concentration shows that at $[H_2O_2] > 0.2\ M$ the increase in turnover number with $[H_2O_2]$ is much faster than that predicted by a rate law first order with respect to $[H_2O_2]$. This is suggestive of the increased importance of another reaction mechanism at high $H_2O_2$ concentrations. Also, values in Table II show that the turnover number of (TETA)$Mn(OH)^+$ at $[H_2O_2] = 0.149\ M$ first increases with $p$H, reaches its maximum value at $p$H 10.8, and then decreases rapidly with increasing $p$H. This observed decrease in turnover number at high $p$H may be due to the simultaneous oxidation of $Mn^{II}$ to $MnO_2$ by $H_2O_2$ in alkaline solutions. Because of the greater affinity of $H^+$ for TETA, the catalyst (TETA)$Mn(OH)^+$ is unstable in dilute solutions at $p$H < 9. Consequently all measurements on (TETA)$Mn(OH)^+$ were carried out on solutions with $p$H > 9.5.

TABLE II

CATALYTIC DECOMPOSITION OF $H_2O_2$ BY (TETA)$Mn(OH)^+$ AT 25°

(Concn. of total TETA = $4.8 \times 10^{-3}$ mole/l.; concn. of total $Mn^{II}$ = $6.3 \times 10^{-7}$ mole/l.)

| Initial concn. of $H_2O_2$ (mole/l.) | $p$H | Turnover no. of (TETA)-$Mn(OH)^+$ (min.$^{-1}$) | Initial concn. of $H_2O_2$ (mole/l.) | $p$H | Turnover no. of (TETA)-$Mn(OH)^+$ (min.$^{-1}$) |
|---|---|---|---|---|---|
| 0.149 | 9.7 | 1900 | 0.149 | 11.4 | 1300 |
| .149 | 10.4 | 4800 | .149 | 10.0 | 2900 |
| .149 | 10.7 | 6300 | .314 | 10.0 | 7400 |
| .149 | 10.8 | 6700 | .628 | 10.0 | 25400 |
| .149 | 11.1 | 5100 | .942 | 10.0 | 48000 |

**Comparison of the Model Catalyst with Catalases.**—The present model catalyst and natural catalases have several striking similarities. Although the actual mechanism of catalase action may differ considerably from that of the present model catalyst, detailed studies on the latter may be helpful to investigations on catalase action itself. Both (TETA)$Fe(OH)_2^+$ and catalase contain $Fe^{III}$ iron, and both are inhibited by cyanide ions. Fluoride ion inhibits natural catalase but activates the model catalyst. The catalytic activity of $FeF_6^{---}$ and $F^-$ itself is negligible. The activation of (TETA)-$Fe(OH)_2^+$ by $F^-$ probably is due to the formation of (TETA)$FeF_2^+$ which can react with $OOH^-$ to form compound II at much faster rate than (TETA)-$Fe(OH)_2^+$ itself.

The important work of Chance[5] showed that catalase first reacts with one molecule of hydrogen peroxide to form an enzyme–substrate complex, this complex subsequently reacts with a second molecule of $H_2O_2$ to yield oxygen gas and regenerate the enzyme. The present model compound catalyzes by a similar mechanism as illustrated in Fig. 1. Moreover, Chance showed that the first cata-

(5) For references to the literature, see "A Symposium on the Mechanism of Enzyme Action," Edited by W. D. McElroy and B. Glass, The Johns Hopkins Press, Baltimore, Md., 1954, p. 398.

lase–$H_2O_2$ complex also reacts with excess of $H_2O_2$ to form a second enzyme–substrate complex which is relatively stable and has little tendency to decompose directly into $O_2$, $H_2O$ and the free enzyme. Although there is no experimental evidence to show the existence of such a second enzyme–substrate complex in our model system, it is interesting to notice that compound I in Fig. 1 is potentially capable of combining with two $OOH^-$ ions to form (TETA)-$Fe(OOH)_2^+$. The structure of this latter compound, which is formally obtained from compound I by replacing its two $OH^-$ groups by two $OOH^-$ ions, shows that it should be relatively stable and has little tendency to split the peroxide bond by the above proposed mechanism. Furthermore, Keilin and Hartree[6] showed that in the presence of other reducing substrates, catalase causes coupled oxidations and thus exhibits peroxidase-like activity. It may be noticed from Fig. 1 that the structure of compound III indicates it may oxidize molecules other than $H_2O_2$, and thus may also possess peroxidase-like activity. Qualitative experiments show that both formate ion and methanol inhibit the liberation of $O_2$ from $H_2O_2$ by $(TETA)Fe(OH)_2^+$. The observed inhibitory action of methanol may be due to its competition with $H_2O_2$ for compound III. But more experimental work in this direction is necessary before definite conclusions can be drawn.

Regarding the relative catalytic efficiencies, the present model catalyst is still much inferior as compared to natural catalases. Thus by plotting the turnover numbers in Table I *vs.* $[H_2O_2]$, the second-order rate constant $k_2$ (defined by the relationship $-d[H_2O_2]/dt = k_2[(TETA)Fe(OH)_2^+][H_2O_2]$) in dilute $H_2O_2$ solutions at *p*H 10.0 may be estimated to be about $1.2 \times 10^3$ sec.$^{-1}$ mole$^{-1}$. The value of $k_2$ for $(TETA)FeF_2^+$ is about twice this value. The corresponding value for catalases is about $5 \times 10^6$ sec.$^{-1}$ per mole of enzyme or $1.3 \times 10^6$ sec.$^{-1}$ per mole of hematin iron.[7] Thus the present model catalysts are inferior to catalases in efficiency by a factor of about $10^3$. On the other hand these model catalysts are superior to hemoglobin in efficiency by a factor of about $10^4$. They are, therefore, remarkable catalysts indeed as far as small molecules go. Attempts are now being made to improve the catalytic efficiency of these model catalysts by incorporating these and related compounds into the structure of macromolecules, and it is hoped to report the result of these studies in a later publication.

The question may be raised as to whether catalases may catalyze the decomposition of $H_2O_2$ by a similar mechanism but in a more efficient way. For example one may speculate that the initial stage of reaction between catalase and $H_2O_2$ involves the oxidation and rupture of the hematin ring of catalase at one of its four corner =CH— groups by $H_2O_2$. The resulting compound may then be locally refolded to give a configuration similar to that of compound I. In such a new hypothetical configuration the $Fe^{III}$ is coördinated with three of the four N atoms of the biliverdin (the ruptured hematin), the hybrid atomic orbital of $Fe^{III}$ directed toward the protein part of the enzyme molecule is still used to form a coördination link with an electron donor group on the apo-enzyme. The two remaining adjacent octahedral hybrid atomic orbitals of $Fe^{III}$ can now combine and split $OOH^-$ as illustrated in Fig. 1. Although the observed initial burst of oxygen evolution[4] and the ease with which catalase is destroyed during its reaction with $H_2O_2$ appear to be in favor to such a hypothetical mechanism, there is no direct evidence to support this kind of speculation.

In an attempt to get further information on the detailed mechanisms of catalase-like action, isotope-effect studies were made on the catalytic decomposition of $H_2O_2$ by various catalysts. The procedure involves mixing $\sim 3\%$ $H_2O_2$ solution with equal volume of catalyst solution in an evacuated vessel and subsequent mass-spectrometric analysis of the isotopic composition of the oxygen gas produced. All the oxygen samples were collected after complete decomposition of $H_2O_2$ at room temperature ($20 \pm 5°$). The atom per cent. of $O^{18}$ in the original $H_2O_2$ was determined by complete oxidation of the $H_2O_2$ by acid ceric sulfate solution and then analyzing the isotopic composition of the oxygen produced. Identical result was obtained when acid permanganate was used instead of ceric sulfate as the oxidizer. In most cases the oxygen gas obtained through complete catalytic decomposition of $H_2O_2$ was enriched in $O^{18}$ as compared to the original $H_2O_2$. The results are summarized in Table III, but the experimental details will not be given since most of the procedure consists of standard mass-spectrometric techniques.

TABLE III

ISOTOPE-EFFECT STUDIES ON THE CATALYTIC DECOMPOSITION OF HYDROGEN PEROXIDE

| Catalyst | Total no. of mass spectroscopic analyses | Enrichment factor |
|---|---|---|
| $Fe(OH)_3$, freshly ppt., colloidal | 6 | $1.028 \pm 0.0026$ |
| $Fe(OH)_3$, by Dole, *et al.* | | 1.024 |
| $Fe(OH)_3$, ppt., boiled and aged for 5 hr. | 7 | $1.022 \pm 0.005$ |
| $(TETA)Fe(OH)_2^+$ | 12 | $1.011 \pm 0.003$ |
| Catalase (crystalline) | 11 | $1.004 \pm 0.004$ |
| Catalase, by Dole, *et al.* | | 1.001 |
| Methemoglobin (crystalline) | 8 | $1.023 \pm 0.005$ |

The term "enrichment factor" in Table III is defined as the ratio of the atom per cent. of $O^{18}$ in the oxygen gas produced to that in the original $H_2O_2$. Two of the earlier values obtained by Dole and co-workers[8] are included in Table III for comparison and are shown to be consistent with the present results. The observed catalytic decomposition of $H_2O_2$ by $Fe(OH)_3$ has already been interpreted by Dole and co-workers through the free radical mechanism.[1,8] The observed high enrichment factor for methemoglobin indicates that the latter probably catalyzes through a similar mechanism. The low enrichment factor for $(TETA)Fe(OH)_2^+$ shows that the free radical mechanism is of minor importance in $(TETA)Fe(OH)_2^+$ catalysis. The exact interpretation of these observations appears to be rather complex, and will probably await more

(6) D. Keilin and E. F. Hartree, *Proc. Roy. Soc.* (*London*), **B119**, 141 (1936).

(7) See p. 403 of reference 5.

(8) M. Dole, R. DeForest, G. Muchow and C. Compte, *J. Chem. Phys.*, **20**, 961 (1952).

experimental results in this direction. There are several possible ways in which catalase can decompose $H_2O_2$ without causing a detectable $O^{18}$-enrichment in the oxygen produced. First, the oxygen may be produced through an action of the enzyme on $H_2O_2$ without breaking the peroxide bond, *e.g.*, by dehydrogenation process. This, however, seems most unlikely, because the peroxide bond is the weakest link in the $H_2O_2$ molecule. Since catalase is the most efficient of all enzymes, it is very unlikely that such an enzyme would skip the weak peroxide bond and try to break the much stronger O–H bonds. Secondly, one may suggest that the reaction is diffusion-controlled, so that no isotope effect is not detected. But this suggestion contradicts experimental evidence. Chance estimated the activation energy for the catalytic decomposition of $H_2O_2$ by catalase to be 1400 cal./mole or less,[5] whereas for a diffusion-controlled reaction in aqueous solution at room temperatures the activation energy should be close to 4.6 kcal./mole. The third possibility is that the splitting of the peroxide bond by catalase is preceded by a slower and hence rate-determining step in which the substrate combines with the enzyme; *i.e.*, once the substrate succeeded in combining with catalase, the peroxide bond is immediately split irrespective of whether it is $O^{16}$–$O^{16}$ bond or $O^{16}$–$O^{18}$ bond. The transition of compound I to compound III in Fig. 1 has some resemblance to this possibility. Finally, the peroxide bond may not be completely broken until fairly stable bonds have been formed between the two O atoms and catalase. The energy consumed in breaking the stronger $O^{16}$–$O^{18}$ bond is mostly compensated by the energy gained in forming the stronger $Fe^{III}$–$O^{18}$ bonds, etc.; and if the splitting of the peroxide bond and the formation of the new enzyme–substrate bonds are not isolated events but occur in a more or less continuous transition, this would minimize the activation energy as well as the isotope effect. The mechanisms of most catalytic processes, including the one illustrated in Fig. 1, probably have some feature of this continuous transition from old to new bonds. In general, the efficiency of the catalyst depends largely on the nature of this kind of transition.

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NEW HAVEN, CONNECTICUT

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[CONTRIBUTION FROM THE COMBUSTION BRANCH, U. S. NAVAL ORDNANCE TEST STATION]

# The Photolysis and Pyrolysis of Acetone-$d_6$ in the Presence of Ethane and of Acetone in the Presence of Ethane-$d_6$

BY JAMES R. MCNESBY AND ALVIN S. GORDON

RECEIVED MARCH 1, 1955

The photolysis and pyrolysis of mixtures of acetone-$d_6$ and ethane, and of acetone and ethane-$d_6$ have been studied with particular emphasis on abstraction of H and D and by $CD_3$ and $CH_3$, respectively (reaction 1 and 2). The activation energies found are $E_1 = 11.5$ kcal. and $E_2 = 14.8$ kcal. Changing light intensity and introducing additional surface has no measurable effect on the relative rates of (1) and (3). Small amounts of ethylene-$d_4$, HD and $D_2$, but no $H_2$ within the accuracy of the mass spectrometer are found in the products of the photolysis and pyrolysis of the ethane-$d_6$–acetone mixture. HD, $H_2$ but no $D_2$ were found in the acetone-$d_6$–ethane reaction, A fifty-fold increase in surface had no measurable effect upon the HD/$D_2$ ratio. Similarly, argon does not appear to affect the ratio measurably. Both the HD/$H_2$ and HD/$D_2$ ratios increased with temperature. The most reasonable interpretation of the hydrogen analyses appears to be that a H (or D) atom is formed by decomposition of the ethyl radical and the H (or D) atom subsequently either abstracts H or D from acetone or ethane, or recombines with a similar atom.

## Introduction

The measurement of activation energies of metathetical reactions of the type $CH_3 + HR \rightarrow CH_4 + R$ has been greatly facilitated in recent years by a technique first introduced by Steacie[1] which involves the use of the $CD_3$ radical. The rate of formation of $CD_3H$ is compared with the rate of formation of $CD_4$ in the reaction of $CD_3$ with acetone-$d_6$ from which the $CD_3$ radicals are generated. This comparison is made over a temperature range and the pre-exponential factors and activation energies are obtained relative to those for the reaction of $CD_3$ with acetone-$d_6$. To obtain a meaningful measurement, the amount of $CD_3H$ arising from the incompletely deuterated acetone must represent a very small proportion of the $CD_3H$ produced by the abstraction of hydrogen from the compound under investigation. This requirement demands a very highly deuterated acetone-$d_6$. The acetone-$d_6$ used by Trotman-Dickenson, Birchard and Steacie[1] in their work on abstractions by $CD_3$ of hydrogen from hydrocarbons contained some 30% acetone-$d_5$. This results in large percentages of the total $CD_3H$ arising from the partially deuterated acetone. We calculate about 80% in the case of $C_2H_6$. Recently the reaction between $CD_3$ and $CH_4$[2] was studied in this Laboratory. Since methane has only one kind of hydrogen, the significance of these experiments is clear.

In the present investigation the reactions

$$CD_3 + C_2H_6 \longrightarrow CD_3H + C_2H_5 \quad (1)$$
$$CH_3 + C_2D_5 \longrightarrow CH_3D + C_2D_5 \quad (2)$$

were studied since they can be interpreted unambiguously by the observation of the $CD_3H/CD_4$ and $CH_4/CH_3D$ ratios, respectively. Previous work[3]

(1) A. F. Trotman-Dickenson, J. R. Birchard and E. W. R. Steacie, *J. Chem. Phys.*, **19**, 163 (1951).

(2) J. R. McNesby and A. S. Gordon, THIS JOURNAL, **76**, 4196 (1954).

(3) J. R. McNesby and A. S. Gordon, *ibid.*, **76**, 1416 (1954).