formed by electron impact on $Mn_2(CO)_{10}$ and the equivocal nature of data available on $D(Mn^+-Mn)$. Nonetheless it is possible to make tentative assignments of absolute Mn^+ affinities which, though not very precise, reveal important features of bonding between Mn^+ and alcohols, in particular.

Mn^+ may bond the alcohols electrostatically or by oxidative addition. If oxidative addition occurs, however, the results suggest that the methyl group in CH_3MnOH^+ decreases the bond strength between the metal ion and OH by 20–30 kcal/mol and the OH group weakens the metal–methyl bond by ~23 kcal/mol.

The affinity of methanol for Mn^+ is substantially less than that of methanol for $NiC_5H_5^+$ (~29 vs. 44.5 kcal/mol). This suggests either that $NiC_5H_5^+$ has a small metal ion center with a high concentration of charge or that $NiC_5H_5^+$ adds CH_3OH (and perhaps other bases) oxidatively and forms σ bonds in CH_3 and OH that are not weakened by the presence of more than one ligand.

Acknowledgment. The NSF partially supported this research (CHE 8110516).

Registry No. Mn_2^+, 73145-85-4; Mn^+, 14127-69-6; CH_3OH, 67-56-1; C_2H_5OH, 64-17-5; t-C_4H_9OH, 75-65-0; n-C_3H_7OH, 71-23-8; CH_2O, 50-00-0; CH_3SH, 74-93-1; H_2O, 7732-18-5; H_2S, 7783-06-4; n-C_4H_{10}, 106-97-8; C_3H_8, 74-98-6; CH_4, 74-82-8; $(CH_3)_2CO$, 67-64-1.

Low-Pressure Study of the HO_2 + HO_2 Reaction at 298 K

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The rate constant for the reaction $HO_2 + HO_2 \rightarrow H_2O_2 + O_2$ was measured at 298 K and 1 torr total pressure of helium by using the discharge flow technique. A quadrupole mass spectrometer was used to detect HO_2 in conjunction with in situ long-path ultraviolet absorption which was used for calibration. A value of $(1.5 \pm 0.4) \times 10^{-12}$ cm^3 molecule^{-1} s^{-1} was obtained for k_1 where the rate constant is defined by the relation $-d[HO_2]/dt = 2k_1[HO_2]^2$.

Introduction

Recent work in our laboratory and elsewhere has established that the observed rate constant for the gas-phase reaction

$$HO_2 + HO_2 \rightarrow H_2O_2 + O_2 \qquad (1)$$

is pressure dependent.[1-5] Using the flash photolysis/ultraviolet absorption technique over the pressure range 100–700 torr of Ar and N_2 and temperature range 230–420 K, it was found[1,2] that the observed rate constant could be expressed as a sum of pressure-dependent and pressure-independent terms:

$$k_1([M],T) = k_{II}(T) + k_{III}(T)[M]$$

where k_{II} and k_{III} are the biomolecular and termolecular rate constants, respectively. Both k_{II} and k_{III} exhibited negative temperature dependences as observed in many bimolecular and termolecular radical–radical reactions.[2]

While there have been several previous measurements of k_1 over a wide range of conditions, there are discrepancies in the values reported in the low-pressure limiting region below 10 torr. Using the discharge flow/laser magnetic resonance (DF/LMR) technique at 298 K, Thrush and Wilkinson[3] observed a proportionality between k_1 and helium pressure with k_1 varying between 2.9×10^{-13} and 5.5×10^{-13} cm^3 molecule^{-1} s^{-1} in the 2–4-torr range. These measurements seemed to suggest a very large value for k_{III}, about 4×10^{-30} cm^6 molecule^{-2} s^{-1}, and a very small or zero value for k_{II}. Cox and Burrows[4] studied reaction 1 over a wide range of pressures using molecular modulation with UV absorption and found a pressure dependence in the 3–10-torr range which was consistent with the Thrush and Wilkinson observations. In contrast, flash photolysis studies by ourselves[1,2] and Simonaitis and Heicklen[5] implied a much smaller value for k_{III}, 2.3×10^{-32} cm^6 molecule^{-2} s^{-1} for M = He at 298 K, and a significant zero-pressure intercept, $(1.6 \pm 0.2) \times 10^{-12}$ cm^3 molecule^{-1} s^{-1}. Two other recent studies in the low-pressure regime support the findings of a finite bimolecular intercept. Thrush and Tyndall[6,7] using flash photolysis coupled with infrared laser diode absorption obtained a value of $(1.6 \pm 0.1) \times 10^{-12}$ cm^3 molecule^{-1} s^{-1} at 298 K between 7 and 20 torr of O_2. Takacs and Howard[8] measured the rate constant between 1 and 7 torr of He and obtained $(1.5 \pm 0.3) \times 10^{-12}$ cm^3 molecule^{-1} s^{-1} at 298 K, independent of pressure.

In view of the importance of the HO_2 + HO_2 reaction to atmospheric and combustion chemistry and the difficulty of measuring the rate constant due to problems encountered in the absolute calibration of HO_2, we have repeated the rate constant measurement at low pressure. In this study, the discharge flow/mass spectroscopy (DF/MS) technique was used at about 1 torr total pressure of He to measure k_1 at 298 K. Ultraviolet absorption of HO_2 at 220 nm was used to determine the absolute mass spectrometer sensitivity for HO_2 radicals, thus providing a direct link between the calibration factors in this experiment and our previous flash photolysis work.[1,2]

Experimental Section

The experimental apparatus is shown in Figure 1. The main components are the flow tube with movable HO_2 source, the UV absorption system for HO_2 and H_2O_2 calibrations, and the molecular beam quadrupole mass spectrometer which has been described previously.[9,10]

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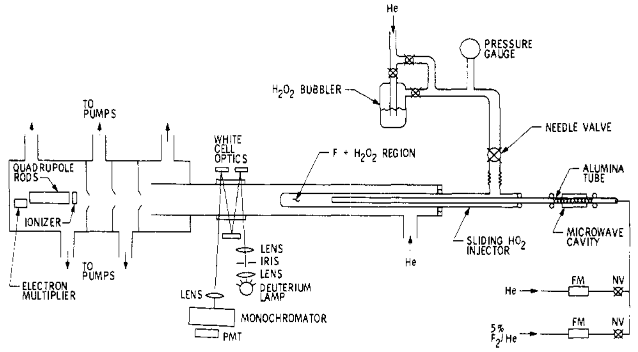

Figure 1. Schematic diagram of the discharge flow/mass spectrometer–ultraviolet absorption system showing the flow tube, HO_2 injector system, UV calibration cell, and four-stage quadrupole mass spectrometer. FM, flowmeter; NV, needle valve.

Flow Tube. The flow reactor used in these studies was a 4.86-cm-i.d. Pyrex tube which was operated at room temperature. Two 2-in.-diameter Suprasil windows were epoxied into place at opposite points about 22 cm from the end of the flow tube. Because wall coatings such as halocarbon wax had a tendency to migrate onto the inside window surfaces and affect the window transmissivity, the flow tube was uncoated. The maximum length of the reaction zone was about 75 cm.

HO_2 Injector. HO_2 radicals were produced by a movable double injector similar to that used in previous studies.[8,9,11,12] The injector consisted of two concentric tubes having i.d.'s of 8 and 12.4 mm, respectively. Fluorine atoms were produced in the inner tube by flowing a dilute F_2/He mixture through a low-power microwave discharge contained in an alumina tube attached to the upstream end of the injector. A flow of H_2O_2 in He was added to the annular region between the two injector tubes through a thermostated bubbler containing concentrated H_2O_2. To minimize the surface decomposition of H_2O_2, all surfaces downstream of the bubbler, including the needle valve, were either glass or Teflon. The inside surface of the 8-mm-i.d. tube was coated with halocarbon wax while the 12.4-mm tube was uncoated. The length of the F + H_2O_2 reaction region was adjustable but was fixed at 20 cm for most of the experiments.

Several different methods of HO_2 formation were tried. The most successful, and the one employed in all of the kinetic measurements, employed the F + H_2O_2 reaction

$$F + H_2O_2 \rightarrow HF + HO_2 \qquad (2)$$

$$k_2 = 8 \times 10^{-12}\ \mathrm{cm^3\ molecule^{-1}\ s^{-1}}\ ^{13}$$

as the HO_2 source with H_2O_2 in excess. In this system, a small (5–20 cm^3 atm min^{-1}) flow from a premixed 5% F_2/He cylinder was mixed with a larger (250 cm^3 atm min^{-1}) helium flow which passed through the discharge. The dissociation of F_2 was very high, typically greater than 95%, as indicated by monitoring the F_2^+ ion signal on the mass spectrometer. The initial F atom concentration, and thus the HO_2 concentration, was adjusted by varying the F_2 flow. F atoms reacted with H_2O_2 in the last 20 cm of the 12.4-mm outer tube of the injector. In this section, H_2O_2/He flow rates varied from 300 to 700 cm^3 atm min^{-1}, resulting in H_2O_2 concentrations of $(0.6–3.8) \times 10^{14}$ molecules cm^{-3}. These flow rates yielded reaction times in the outer injector tube of 1.4–3.8 ms. Additional helium could be added in the main flow tube section to vary the flow velocity and/or pressure without affecting the conditions in the injector. Initial HO_2 concentrations in the flow tube varied from 4.2×10^{12} to 15×10^{12} molecules cm^{-3}.

Because H_2O is present as a substantial impurity in the H_2O_2 stream, there is a possibility of secondary reactions involving the OH radical in the injector. With H_2O present, OH will be produced by the reaction

$$F + H_2O \rightarrow HF + OH \qquad (3)$$

$$k_3 = 1.1 \times 10^{-11}\ \mathrm{cm^3\ molecule^{-1}\ s^{-1}}\ ^{14}$$

and lost primarily by the reactions

$$OH + H_2O_2 \rightarrow HO_2 + H_2O \qquad (4)$$

$$k_4 = 1.7 \times 10^{-12}\ \mathrm{cm^3\ molecule^{-1}\ s^{-1}}\ ^{14}$$

$$OH + HO_2 \rightarrow H_2O + O_2 \qquad (5)$$

$$k_5 = 7 \times 10^{-11}\ \mathrm{cm^3\ molecule^{-1}\ s^{-1}}\ ^{14}$$

The presence of significant OH concentrations exiting the injector could interfere with the determination of k_1 by providing a secondary loss path for HO_2 via reaction 5. With the present system it is difficult to monitor OH directly due to the presence of an interfering background peak. It can be shown, however, that under conditions typical of those used in the kinetic runs the characteristic time for OH removal in the injector due to reactions 4 and 5 is less than 1 ms and is always less than the injector residence time. This was verified by detailed numerical simulations which indicated that, in the flow tube, $[OH]_0/[HO_2]_0$ was less than 0.01 in all cases. This result was verified experimentally by Takacs and Howard[8] who found $[OH]_0/[HO_2]_0$ to be about 5×10^{-5} using a similar injector configuration and somewhat higher H_2O_2 concentrations ($(0.4–1.5) \times 10^{15}$ molecules cm^{-3}).

Several other HO_2 production mechanisms were tried including

$$Cl + H_2O_2 \rightarrow HCl + HO_2 \qquad (I)$$

$$H_2 \xrightarrow{\text{microwave}} 2H \qquad (II)$$

$$H + F_2 \rightarrow HF + F$$

$$F + H_2O_2 \rightarrow HF + HO_2$$

$$H_2O_2 \xrightarrow{\text{microwave}} 2OH \qquad (III)$$

$$OH + H_2O_2 \rightarrow HO_2 + H_2O$$

All those systems produced HO_2 radicals to some extent but were unsuitable for the $HO_2 + HO_2$ study either because the HO_2 production rate was too low or because of the presence of substantial impurities.

Optical Absorption System. Measurement of the $HO_2 + HO_2$ rate constant required that an absolute calibration of the mass spectrometer HO_2^+ ion signal be obtained. This was accomplished by simultaneous detection of the HO_2 by MS and by long-path UV absorption in the flow tube at 220.0 nm. The absorption system consisted of a D_2 lamp, 12-pass White Cell optics, $^1/_4$-m monochromator, and a Hamamatsu R166 UH photomultiplier. Monochromator entrance and exit slit widths were 500 μm, resulting in a resolution of 0.7-nm fwhm. The effective path length of the optical system was calibratd at 200 nm by using known flow rates of CH_3Br which had a measured cross section of $(3.91 \pm 0.08) \times 10^{-19}$ cm^2 $molecule^{-1}$. From a Beer's law plot, the path length was determined to be 72.5 ± 1.5 cm. This is less than the geometric value, 85.9 cm, because of scattered light from the window surfaces which affects the linearity of photometric measurements only at absorbances much larger than those encountered here. The output of the photomultiplier was recorded with a Keithley 417 picoammeter using the current suppression feature.

Mass Spectrometer. The quadrupole mass spectrometer has been described in detail previously.[9,10] Conventional electron-impact ionization at 15-eV electron energy is used coupled with analog detection of the electron multiplier output current. The

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detection of HO_2 at *m/e* 33 is complicated somewhat by the presence of the enormous neighboring *m/e* 34 mass peak from H_2O_2 which is present in excess. Not only can the wings of this line contribute to the *m/e* 33 signal due to the finite quadrupole resolution, but the dissociative ionization of H_2O_2 can also lead to the formation of HO_2^+. Both these factors can contribute to the *m/e* 33 background although they can be reduced by operating at the highest resolution and lowest electron ionization energy consistent with ion throughput. Under typical conditions, the *m/e* 33 signal equalled the background current for $[HO_2] \sim 5 \times 10^{10}$ molecules cm^{-3}. Several points should be noted in connection with this sensitivity figure. First, the background originates from the molecular beam itself rather than the intrinsic chamber background; reducing $[H_2O_2]$ in the flow tube reduced the background proportionately. Second, because the $HO_2 + HO_2$ reaction is second order, the reaction rate drops quickly with decreasing $[HO_2]$. Even at the longest reaction times the observed HO_2 concentration is always greater than about 1×10^{12} molecules cm^{-3}. Thus, for all the kinetic runs the minimum signal/background ratio was greater than 20.

Calibration. Calibration of the mass spectrometer was performed prior to every run. In a typical experiment, the injector was first positioned 1–2 cm upstream of the White Cell and an absorption measurement taken. The injector was then positioned a similar distance from the MS pinhole and an ion current measurement made. The calibrations were performed at HO_2 concentrations which were low enough that the exact position of the injector relative to the UV and MS detection points was not critical. Measurement of the $[HO_2]$ by UV absorption was accomplished by recording the transmitted light intensity with the discharge fluorine flow on and off to establish I and I_0 values. Because H_2O_2 was present as a background absorber ($\sigma^{220}_{H_2O_2} = 2.54 \times 10^{-19}$ cm^2 $molecule^{-1}$), the HO_2 measurement was sensitive to the change in $[H_2O_2]$ caused by the $F + H_2O_2$ reaction. A correction for this background change was made by recording the *m/e* 34 ion current with the fluorine atoms on and off. This measurement, combined with the H_2O_2 cross section, could be used to correct the observed HO_2 absorption. These corrections were generally in the range of 10–30%. The HO_2 concentration was then given by

$$[HO_2] = \frac{A_{TOT} - A_{H_2O_2}(S_{34}/S_{34}^{\circ} - 1)}{\sigma_{HO_2} l}$$

where A_{TOT} = absorbance with H_2O_2 flow on and F_2 flow on/off, $A_{H_2O_2}$ = absorbance with H_2O_2 flow on/off and F_2 flow off, S_{34}, S_{34}° = *m/e* 34 signal with H_2O_2 flow on and F_2 flow on/off, l = absorption path length, and σ_{HO_2} = HO_2 cross section. Another correction was made for the decrease in $[HO_2]$ due to HO_2 disproportionation through the 5-cm viewing zone along the flow tube axis. This correction was made by assuming a k_1 a value of 1.6×10^{-12} cm^3 $molecule^{-1}$ s^{-1} and averaged about 10%.

In each kinetic run HO_2 measurements by the UV absorption system were made by manually switching the discharge fluorine flow on and off for a period of about 15 s. The resulting modulated HO_2 absorptional signal was integrated over a 10-min period to achieve a minimum detectable absorbance of 1×10^{-4}, corresponding to an HO_2 detection limit of about 5×10^{11} molecules cm^{-3}. The value used for the HO_2 cross section at 220 nm, 3.4×10^{-18} cm^2 $molecule^{-1}$, is based on the spectrum of Cox and Burrows,[4] which is in good agreement with our previous cross-section measurement at 227.5 nm[1] on which the determination of k_1 by flash photolysis is based. This calibration method thus establishes a correspondence between rate measurements of reaction 1 by two totally different methods.

Reagents. Hydrogen peroxide was obtained from the FMC Corp. in concentrations of 90% by weight and then evaporated under vacuum to half its original volume to increase the concentration to 95%.[11] Helium was UHP grade (99.999%).

Results and Discussion

As indicated above, all calibrations and kinetic runs were carried out at about 1 torr total pressure of helium at 298 K over a range of $[HO_2]_0$, $[H_2O_2]$, and flow velocity conditions (Table I). Plots of 1/(*m/e* 33 signal) vs. reaction time were linear as indicated in Figure 2. The results from all 13 runs are listed in Table I. An average value of $(1.47 \pm 0.20) \times 10^{-12}$ cm^3 $molecule^{-1}$ s^{-1} is obtained for k_1. When the additional uncertainties associated with HO_2 calibration and other systematic errors are factored in, an overall uncertainty of ±25% (1σ) is recommended, giving $k_1 = (1.5 \pm 0.4) \times 10^{-12}$ cm^3 $molecule^{-1}$ s^{-1}.

TABLE I: Experimental Results for the $HO_2 + HO_2$ Reaction at 1 torr and 298 K (M = He)

$10^{-12}[HO_2]_0$, molecules cm^{-3}	$10^{-14}[H_2O_2]$, molecules cm^{-3}	flow velocity, cm s^{-1}	$10^{12}k_1$,[a] $molecule^{-1}$ s^{-1}
4.2	2.8	434	1.62
6.6	2.9	680	1.53
6.8	2.9	398	1.29
12	2.2	994	1.71
8.2	2.2	994	1.47
4.1	1.8	417	1.62
7.5	1.8	424	1.53
13	3.8	980	1.37
15	3.8	988	1.26
10	3.8	973	1.22
10	3.5	437	1.22
5.8	3.5	435	1.84
4.7	3.5	434	1.47
			av = 1.47 ± 0.20

[a] k_1 is defined by the expression $-d[HO_2]/dt = 2k_1[HO_2]^2$.

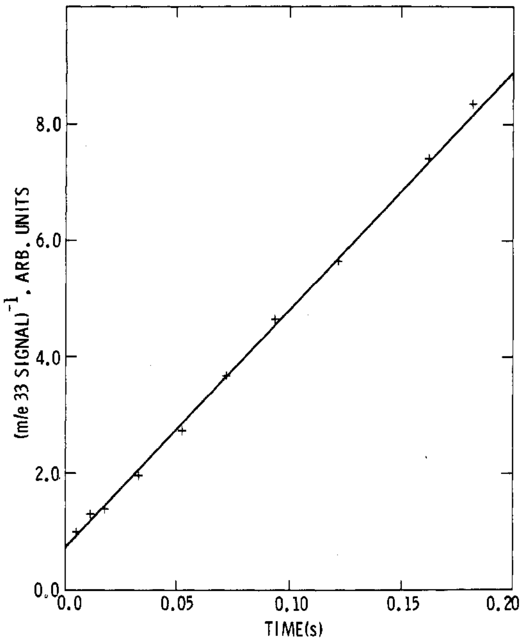

Figure 2. Second-order decay plot for the $HO_2 + HO_2$ reaction.

The first-order wall loss of HO_2 in the uncoated Pyrex flow tube was measured at HO_2 concentrations less than 1×10^{12} molecules cm^{-3} and found to be less than 1–2 s^{-1}. This is small relative to the HO_2 loss rate by disproportionation and can be neglected.

A number of runs were also carried out using NO as a titrant for HO_2 and the mass spectrometer to detect the NO_2 product. Regeneration of HO_2 from the reaction of H_2O_2 with the OH produced in the titration was prevented by adding a large excess ($\sim 1 \times 10^{15}$ molecules cm^{-3}) of C_2F_3Cl which reacts rapidly with OH to form a stable adduct.[16,17] Calibrations performed using this method showed considerable scatter with poor reproducibility due to both the effect of C_2F_3Cl on the efficiency of the mass

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TABLE II: Previous Results for the $HO_2 + HO_2$ Reaction in the Low-Pressure Limit at 298 K

ref	technique[a]	pressure, torr	$10^{12}k_1$, cm^3 $molecule^{-1}$ s^{-1}
15	DF/MS	0.6	~3
3	DF/LMR	2.0	0.29 ± 0.12
		3.0	0.43 ± 0.18
		4.0	0.55 ± 0.14
6, 7	FP/IR	7–20	1.6 ± 0.1
4	MM/UV	3.0	1.1
		5.3	1.5
		10	1.8
1	FP/UV	*b*	1.6 ± 0.2
5	FP/UV	*b*	1.4 ± 0.2
8	DF/LMR	1–7	1.5 ± 0.3
this work	DF/MS–UV	1	1.5 ± 0.4

[a] DF, discharge flow; FP, flash photolysis; MM, molecular modulation; MS, mass spectrometry; UV, ultraviolet absorption; LMR, laser magnetic resonance; IR, infrared absorption. [b] Experiments were carried out at pressures up to 1 atm. Quoted zero-pressure limiting rate constant is obtained from extrapolation of these data.

spectrometer ionizer and the additional steps involved in the calibration. It was therefore concluded that UV absorption was the more reliable of the two calibration methods, at least when using mass spectrometric detection of HO_2.

Previous work on the low-pressure room-temperature behavior of the $HO_2 + HO_2$ reaction is summarized in Table II. We have recently found[2] that over the pressure range 50–700 torr (Ar) and temperature range 240–417 K the rate constant fits the expresion

$$k_1 = 2.3 \times 10^{-13} \exp((600 \pm 130)/T) + 8.4 \times 10^{-34}[\mathrm{Ar}] \exp((1100 \pm 300)/T)$$

Our earlier study[1] indicating that the observed rate constant extrapolates to a zero-pressure limiting value of (1.6 ± 0.2) × 10^{-12} cm^3 $molecule^{-1}$ s^{-1} agrees with the low-pressure results of Thrush and Tyndall,[6,7] Simonaitis and Heicklen,[5] Takacs and Howard,[8] and this work. The results of Thrush and Wilkinson[3] which extrapolate to a zero bimolecular intercept and suggest a large termolecular component appear to conflict with the later flash photolysis work of Thrush and Tyndall.[6,7] The significant pressure dependence observed by Cox and Burrows[4] between 3 and 10 torr also disagrees with the recent group of studies[1–3,5–8] which report a much smaller termolecular component.

In the only other DF/MS study of this reaction, Foner and Hudson[15] obtained $k_1 \sim 3 \times 10^{-12}$ cm^3 $molecule^{-1}$ s^{-1} in the low-pressure regime using a low-power electrical discharge in H_2O_2 as the HO_2 source. This value must be considered somewhat uncertain due to a number of experimental problems including heterogeneous reactions and possible secondary chemistry involving the OH radical. In addition, no details were provided concerning the HO_2 calibration method.

Acknowledgment. We thank J. J. Margitan for several useful discussions and C. J. Howard for communicating his results prior to publication. The research described in this paper was carried out by the Jet Propulsion Laboratory, California Institute of Technology, under contract with the National Aeronautics and Space Administration.

Registry No. HO_2, 3170-83-0.

Pressure Dependence of the α-Chymotrypsin-Catalyzed Hydrolysis of Amide and Anilides. Evidence for the Single-Proton-Transfer Mechanism

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The rates of hydrolysis of *N*-acetyl-L-tryptophanamide (ATA), *N*-acetyl-L-tryptophan *p*-chloroanilide (ATpCA), and succinyl-L-alanyl-L-alanyl-L-alanine *p*-nitroanilide (SApNA), catalyzed by α-chymotrypsin (α-CHT), were measured at pressures up to 2 kbar at 25 °C in Tris buffer solutions. Three substrates follow the kinetics of the Michaelis–Menten type. From the pressure dependence of the Michaelis constants K_M, the volume changes ΔV_{K_M} were −3 ± 2 cm^3/mol for ATA, 12 ± 2 cm^3/mol for ATpCA, and 5 ± 2 cm^3/mol for SApNA. From the pressure dependence of the rate constant k_{cat} of product formation, the activation volumes ΔV^*_{cat} were −3 ± 2 cm^3/mol for ATA, 10 ± 2 cm^3/mol for ATpCA, and 15 ± 2 cm^3/mol for SApNA. From the volume changes it is concluded that the reaction mechanism of α-CHT follows single-proton transfer.

Introduction

A number of investigations[1] of the reaction mechanism of α-chymotrypsin have led to the conclusion that Ser-195, His-57, and Asp-102 in the active site contribute to the catalytic reaction. X-ray diffraction studies have suggested a "charge relay system"[2] which forms the hydrogen-bonding (H-bonding) bridges among the carbonyl oxygen of Asp-102, the nitrogen atoms of the imidazole ring of His-57, and the hydrogen atom of Ser-195. The system permits the transfer of negative charge from Asp-102 to Ser-195 which then acts as a powerful nucleophile in acylation. On the other hand, kinetic investigations have indicated that there cannot be a normal H bond between the serine and the histidine residues and that simultaneously no H bond between the carboxylate ion and the NH group of the imidazole ring is formed.[3] This H-bonding system (single-proton transfer) results in stabilization[3] of the imidazole against a possible release of its proton. Additional kinetic studies[4–6] of the pH dependence of the hydrolysis of amides have implied that there is an additional intermediate step between the Michaelis complex and the acyl enzyme. In spite of these detailed studies of the mechanism of action of α-CHT, the question still exists as to whether the mechanism is a sin-

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