cellent selectivity and compatibility with other potentially interfering functionalities in complex substrates.

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Supplementary Material Available: ^{1}H NMR spectra for compounds **3–6**, **11** (natural and synthetic), and **12–22**, combustion analytical data for compounds **6**, **14**, **16–18**, and **21**, and HRMS data for compounds **3**, **4**, **8–10**, **12**, **19**, **20**, and **22** (18 pages). Ordering information is given on any current masthead page.

Time-Resolved IR Spectroscopy in Liquid Rare Gases: Direct Rate Measurement of an Intermolecular Alkane C–H Oxidative Addition Reaction

B. H. Weiller, E. P. Wasserman, R. G. Bergman,*
C. B. Moore,* and G. C. Pimentel[†]

Department of Chemistry, University of California
and Materials and Chemical Sciences Division
Lawrence Berkeley Laboratory
Berkeley, California 94720

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Since the first demonstration of the intermolecular oxidative addition of alkane C–H bonds to transition-metal centers[1,2] illustrated in eq 1, there have been many studies of the mechanism of this reaction.[3] While these studies have illuminated many

$$\eta^5\text{-}C_5R_5M(L)X + RH \xrightarrow{h\nu} \eta^5\text{-}C_5R_5M(L)(R)(H) + X \qquad (1)$$

$$R = H, CH_3;\ M = Rh \text{ or } Ir,\ L = CO \text{ or } PMe_3,\ X = CO \text{ or } H_2$$

aspects of the C–H activation process, they do not provide direct information about the reactive intermediates or the potential energy surface for the elementary insertion reaction. Flash photolysis studies have been thwarted by extremely fast insertion rates in neat alkane solution[4] and by the lack of a suitable inert and transparent solvent for dilution of the alkane. We have overcome these difficulties with the use of liquid rare gases as solvents. Using a novel combination of low-temperature and IR laser flash kinetic techniques, we are able to detect the C–H activating transient intermediate formed from Cp*Rh(CO)$_2$ (Cp* = (η^5-C$_5$Me$_5$)) and measure its rate of reaction with cyclohexane over a wide range of concentrations and temperatures.[5]

The experimental apparatus is an IR laser flash kinetic spectrometer that incorporates a pulsed UV laser (XeCl, 308 nm) for excitation and a continuous-wave IR laser (CO, 2100–1800 cm^{-1}) for monitoring the CO stretching frequencies of transient species.[6]

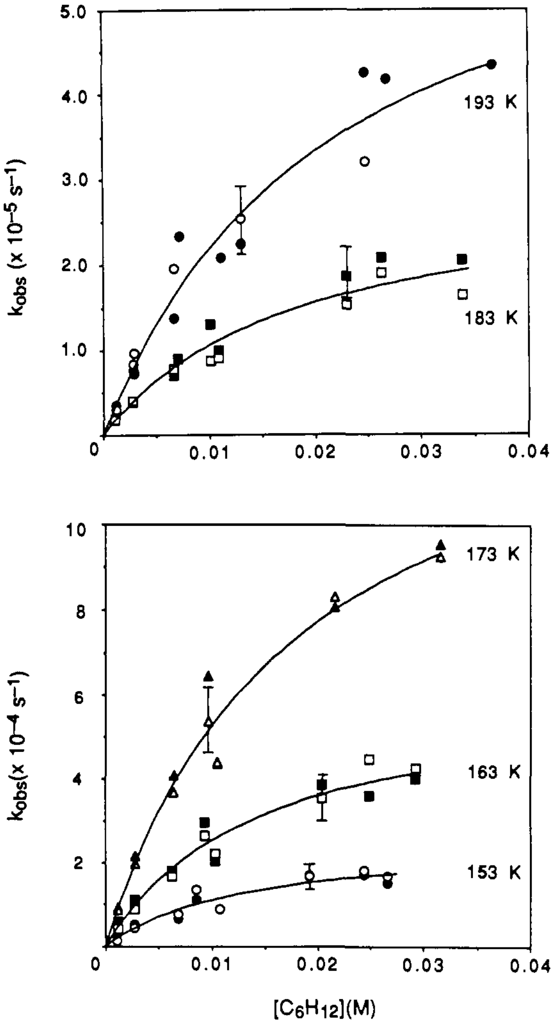

Figure 1. The observed rate constants (k_{obsd}) for the decay of the transient at 1947 cm^{-1} (filled symbols) and for the formation of the product at 2003 cm^{-1} (open symbols) as a function of cyclohexane concentration and temperature. For clarity, only representative error bars are indicated explicitly.

In this study we use a high-pressure, low-temperature cell similar to those described by others[7a,b] with some improvements.[7c] The UV and IR beams pass colinearly through a long path (5 cm) while a perpendicular short path (1.4 cm) is used to monitor the overall changes in the sample with an FTIR spectrometer. The initial concentration of Cp*Rh(CO)$_2$ is held constant at $\approx 5 \times 10^{-6}$ M, and the concentration of cyclohexane[8] or CO[9] is determined from the FTIR spectrum.[10]

Upon UV irradiation of Cp*Rh(CO)$_2$ in liquid xenon at 242 K, a new monocarbonyl species is detected that exhibits an absorption at 1943 cm^{-1}.[11] In the presence of 0.017 M CO, this species decays surprisingly slowly at this temperature ($k = 4 \times 10^4$ s^{-1}) to reform starting material. In liquid Kr at lower temperatures (193–153 K), irradiation once again produces a single transient absorption with a similar band at 1947 cm^{-1}. However, this species appears to be substantially more reactive. It decays slowly ($k = 5 \times 10^3$ s^{-1}) in the absence of added reagents[12] and

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[†] Deceased June 18, 1989. This paper is dedicated to the memory of Professor G. C. Pimentel.

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at a much faster rate in the presence of 0.006 M CO, $k = 7 \times 10^5$ s^{-1} at 193 K, again regenerating starting material. The fact that we observe only one ν_{CO} IR band, coupled with the CO reactivity and matrix isolation data,[13a] leads us to assign this transient species as a monocarbonyl resulting from photodissociation of CO from Cp*Rh(CO)$_2$. Due to the ability of noble gases to bind to Cr(CO)$_5$,[14-16] we believe that the unusually stable transient in liquid xenon is Cp*Rh(CO)(Xe). Analogously, we infer that the more reactive transient detected in krypton is Cp*Rh(CO)(Kr).

When cyclohexane is added to the sample, Cp*Rh(CO)(Kr) decays exponentially with a rate constant k_{obsd} that increases monotonically with increasing cyclohexane concentration. Furthermore, we observe a new species at 2003 cm^{-1} grow in with an exponential constant (k_{obsd}) that matches the decay at 1947 cm^{-1}. The absorption of this product at 2003 cm^{-1} is identical with the only photoproduct band observed at longer times in the FTIR spectrum and is consistent with the matrix assignment of Cp*Rh(CO)(CH$_3$)(H) at 2009 cm^{-1}.[13b] Therefore we assign this species[17] as Cp*Rh(CO)(C$_6$H$_{11}$)(H) and k_{obsd} as the phenomenological rate constant for C–H oxidative addition. It should be noted that the observation of Cp*Rh(CO)(Kr) as the intermediate for this C–H insertion reaction is not consistent with an associative mechanism (proceeding via cyclopentadienyl "ring slip") as proposed by others.[18]

The concentration dependence of k_{obsd} is shown in Figure 1 for the temperature range 193–153 K. The data at each temperature show distinct curvature and can be fit to a function of the form

$$k_{obsd} = \frac{\alpha[C_6H_{12}]}{[C_6H_{12}] + \beta} \qquad (2)$$

as shown by the solid lines. The asymptotic rate constant at high [C$_6$H$_{12}$] is given by α, and for 193, 183, 173, 163, and 153 K, these values are 690 ± 75, 290 ± 30, 150 ± 13, 62 ± 5, and 26 ± 3 × 10^3 s^{-1}, respectively.

One possible interpretation of the data in Figure 1 involves a dissociative substitution mechanism:

$$\mathrm{Cp^*Rh(CO)(Kr)} \underset{k_{-1}[\mathrm{Kr}]}{\overset{k_1}{\rightleftharpoons}} \mathrm{Cp^*Rh(CO)} \xrightarrow{k_2[\mathrm{C_6H_{12}}]} \mathrm{Cp^*Rh(CO)(C_6H_{11})(H)} \qquad (3)$$

Using the steady-state assumption for Cp*Rh(CO), the derived rate law for this mechanism is of the form given in eq 2 where $\alpha = k_1$ and $\beta = \{k_{-1}[\mathrm{Kr}]\}/k_2$. Here the asymptotic rate constant is k_1, for the dissociation of Kr from Cp*Rh(CO)(Kr). Importantly, because this asymptotic k_1 involves only dissociation of Kr from the rhodium center, it should be independent of the nature of the alkane. However, the observed rate constants (k_{obsd}) are dramatically different when C$_6$D$_{12}$ is used instead of C$_6$H$_{12}$. Using the deuterated alkane, a plateau is reached at only ≈0.01 M concentration of hydrocarbon, whereas for C$_6$H$_{12}$ k_{obsd} continues to increase even at ≈0.025 M. Furthermore, the asymptotic rate constant for C$_6$D$_{12}$ is much smaller than that for C$_6$H$_{12}$; for 193, 183, 173, and 163 K, $\alpha_H/\alpha_D = 19 \pm 3$, 20 ± 3, 24 ± 5, and 29 ± 6, respectively.[19]

These difficulties with the dissociative mechanism lead us to postulate the following as an alternative:

$$\mathrm{Cp^*Rh(CO)(Kr)} + \mathrm{C_6H_{12}} \overset{K_{eq}}{\rightleftharpoons} \mathrm{Cp^*Rh(CO)(C_6H_{12})} + \mathrm{Kr} \xrightarrow{k_i} \mathrm{Cp^*Rh(CO)(C_6H_{11})(H)} \qquad (4)$$

Here we propose a rapid exchange equilibrium (K_{eq}) between the Kr complex and an uninserted alkane complex, followed by a unimolecular insertion (k_i) to form the alkyl hydride. There is much evidence for the existence of complexes with significant intra- and intermolecular alkane–metal interactions,[14,20] and such a complex is predicted to be an intermediate in some C–H oxidative addition reactions.[3e,f,20f] We would not expect to resolve the Kr and alkane complexes spectroscopically, given our limited resolution; this is consistent with earlier observations of only small differences in the CO stretching frequencies of Cr(CO)$_5$ in Kr and CH$_4$ matrices.[14]

The rate law for this mechanism can be derived by using mass balance and equilibrium constant expressions and is of the same form as eq 1 with $\alpha = k_i$ and $\beta = [\mathrm{Kr}]/K_{eq}$. Here the asymptotic rate constant (k_i) is for C–H insertion in the alkane complex, and a significant isotope effect is expected.[21] The temperature dependence of k_i gives $E_a = 4.8 \pm 0.2$ kcal/mol and log $A = 11.2 \pm 0.2$ for C$_6$H$_{12}$ for the insertion step. The value for E_a is in good agreement with estimates based on comparisons of kinetic and bond energy data for Cp*Ir(PMe$_3$) + C$_6$H$_{12}$.[3e,h,22] The value of A is low for a unimolecular reaction ($\Delta S^\ddagger = -8.2 \pm 0.9$ cal/(mol K)), but this is not inconsistent with a three-center transition state. The temperature dependence of K_{eq}[23] can be fit with $\Delta H = -1.0 \pm 0.1$ kcal/mol and $\Delta S = +8 \pm 1$ cal/(mol K) for the equilibrium step. There are no bond energy data available for Kr–ML$_x$ complexes, but this value of ΔH is consistent with spectroscopic data indicating that Cr(CO)$_5$ may be stabilized more by CH$_4$ than by Kr.[14] Although we would expect a negative ΔS for this reaction in the gas phase due to the loss of rotational degrees of freedom of cyclohexane, it is possible that solvation effects could account for the positive value.

Experiments are continuing aimed at carrying out analogous kinetic measurements with other alkanes and complexes, spectroscopically resolving the IR bands of the krypton solvate from the alkane complex, and confirming our derived activation parameters for the C–H oxidative addition reaction.

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(12) The origin of this background decay rate is not fully understood at this time. Some likely causes are the following: reaction with starting material to form Cp*$_2$Rh$_2$(CO)$_3$ or [Cp*RhCO]$_2$, reaction with photogenerated CO to reform starting material, reaction with impurities, or diffusion out of the IR probe beam. Some regeneration of starting material was observed to occur at the same rate as the decay of Cp*Rh(CO)(Kr). We could not observe the formation of Cp*$_2$Rh$_2$(CO)$_3$ with the CO laser, nor could we see any bridging CO bands in the FTIR spectrum. In any case, the background decay rate is negligible relative to the C–H oxidative addition rate under the conditions of this study.

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Registry No. Cp*Rh(CO)$_2$, 32627-01-3; Cp*Rh(CO)(C$_6$H$_{11}$)(H), 122699-84-7; Cp*$_2$Rh$_2$(CO)$_3$, 75713-08-5; [Cp*Rh(CO)]$_2$, 69728-34-3; Cp*Rh(CO), 92586-25-9; C$_6$H$_{12}$, 110-82-7; Xe, 7440-63-3; Kr, 7439-90-9.

Demonstration of Structural Integrity of an Enzyme in Organic Solvents by Solid-State NMR

Paul A. Burke

Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Steven O. Smith

Department of Molecular Biophysics and Biochemistry
Yale University, New Haven, Connecticut 06511

William W. Bachovchin

Department of Biochemistry
Tufts University School of Medicine
Boston, Massachusetts 02111

Alexander M. Klibanov*

Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Received June 13, 1989

Recently, enzymes have been shown to function as catalysts in anhydrous organic solvents,[1] where they exhibit such novel properties as enhanced thermostability[2] and altered specificity,[3] and can catalyze reactions impossible in water.[4] Despite the immense potential of nonaqueous enzymology,[1,4] our understanding of this phenomenon remains in its infancy largely because of the insolubility of proteins in nearly all organic solvents, which precludes the use of most classical biophysical techniques.

High-resolution solid-state NMR spectroscopy[5] with magic angle spinning (MAS) permits the measurement of isotropic chemical shifts in samples exhibiting anisotropic spin interactions, making it attractive for study of heterogeneous systems, e.g., membrane and crystalline proteins.[6] We now report a solid-state MAS ^{15}N NMR investigation of an enzyme suspended in organic solvents, which reveals that the catalytic site remains intact under these unnatural conditions.

In order to assess enzyme structural integrity, we examined the tautomeric structure and hydrogen bonding of His 36 in a serine protease, α-lytic protease (α-LP), as reflected by the isotropic ^{15}N chemical shifts of the imidazole nitrogens.[7] In aqueous solution, His 36 is a part of the catalytic triad of the active center and, at high pH, exists solely in the N$^{\delta1}$–H tautomeric form (while the N$^{\epsilon2}$–H tautomer predominates in peptides and proteins[8]), due to a hydrogen bond with the carboxylate of Asp 63.[9] This hydrogen bond, also present at low pH, results in a characteristic 8–12-ppm separation of the two ^{15}N peaks of the protonated imidazole.[9] This interaction is crucial for enzymatic activity in serine proteases; replacement of the participating Asp with Asn[10] drastically lowers k_{cat}, whereas K_{m} is affected only slightly.

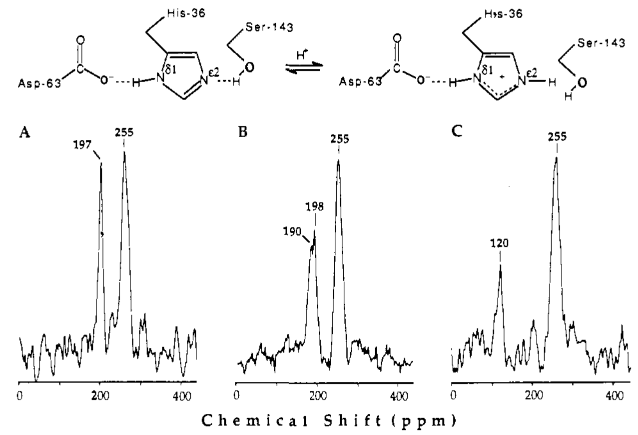

Figure 1. MAS ^{15}N NMR spectra of ^{15}N$^{\delta1}$-His α-lytic protease, "pH 8.6",[11] (A) and ^{15}N$^{\delta1}$,^{15}N$^{\epsilon2}$-His α-lytic protease, "pH 5.4",[11] (B) in acetone and ^{15}N$^{\delta1}$-His α-lytic protease, "pH 8.6",[11] reclaimed from dimethyl sulfoxide[17] (C). Chemical shifts (±2 ppm) are referenced to the peptide backbone and reported relative to 1 M H^{15}NO$_3$ in D$_2$O with positive shifts being upfield. Spectra were recorded on a Bruker MSL spectrometer with ^{1}H and ^{15}N frequencies of 200.1 and 20.27 MHz, respectively. The ^{1}H $\pi/2$ pulse was 6 μs, and the cross polarization mixing time was 2 ms. All spectra were recorded at room temperature. All samples consisted of 40–60 mg of protein and, in A and B, contained at least 65% w/w of the solvent. Each spectrum represents approximately 50000 scans, with 100 Hz line broadening applied.

First, we examined (singly labeled) ^{15}N$^{\delta1}$-His α-LP, "pH 8.6",[11] in anhydrous acetone. The spectrum consists of two peaks (Figure 1A). The peak at 255 ppm arises from the 197 amide nitrogens of the peptide backbone containing ^{15}N at its natural abundance. The peak at 197 ppm arises from N$^{\delta1}$ of His 36. It indicates that this nitrogen is exclusively in the pyrrole (>NH) form and that the N$^{\delta1}$ proton is engaged in a hydrogen bond.[9] (In both solids[12] and liquids,[9] a pyrrole-type nitrogen not serving as a hydrogen bond donor resonates at 210 ppm.[13]) The same result was obtained for α-LP in anhydrous octane.

Next, we examined (doubly labeled) ^{15}N$^{\delta1}$,^{15}N$^{\epsilon2}$-His α-LP at "pH 5.4"[11] in acetone. Since at this "pH" the imidazole ring is fully protonated, both nitrogens exist in the same form ($\geq$NH$^+$). In addition to the 255 ppm peptide backbone peak, there are peaks at 190 and 198 ppm (Figure 1B). ^{15}N$^{\delta1}$-His α-LP prepared and examined in the same fashion revealed that the low field peak arises from N$^{\delta1}$; its position indicates that the N$^{\delta1}$ proton is engaged in a hydrogen bond.[9] In contrast, the shift of 198 ppm for N$^{\epsilon2}$ shows that its proton is not involved in hydrogen bonding.[9]

The ^{15}N spectra of the α-LP His 36 demonstrate that the unique tautomeric structure and hydrogen bonding interactions in the active center of this enzyme are identical in acetone, octane, and water. Because the catalytic triad comprises residues remote from

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