indeed general base abstraction of a proton from the serine hydroxyl can be an efficient mechanism in acylation of the enzyme by its substrates and is in fact required in reactions of the latter compounds.

Transition-State Stabilization. The lack of pronounced pH dependence in the acylation reaction of α-chymotrypsin by N-acylimidazoles at pH >7 is in marked contrast to the bell-shaped plots of k_{cat}/K_m vs. pH obtained with specific amide substrates.[32,58] The pH optimum is generally close to 8, and pK_2 is usually close to 9. The acidic group of $pK_a = 9$ may be the α-ammonium group of N-terminal isoleucine,[59] which has been considered to be important in the binding process.[60-62] Thus, the acylimidazoles may bind to the enzyme in a manner that is different from specific amide substrates even though binding is productive.[63]

It should be noted that values of k_2/K_m for the N-acylimidazole substrates with long-chain acyl groups are very large even though K_m must be quite high, e.g., k_2/K_m for the N-hexanoyl derivative is 50 000 M^{-1} s^{-1} at pH 7.5, and k_2/K_m for N-(β-phenylpropionyl)imidazole is 1.2×10^6 M^{-1} s^{-1} at pH 7.5, making it one of the kinetically most reactive substrates known for the enzyme. In comparison, k_{cat}/K_m (which is equal to k_2/K_s) for the typical amide substrate N-acetyl-L-phenylalanineamide is 1.5 M^{-1} s^{-1} at pH 7.9 (25 °C).[30] The large values of k_2/K_m for the N-acyl-imidazoles must be a reflection of the magnitude of k_2. These rate constants are in part understandable in view of the much better leaving group of an N-acylimidazole than a conventional amide (the pK_a of imidazole is 14.5 for ionization to the anionic species.[64] However, the relatively favorable leaving group of N-acylimidazoles cannot be totally responsible for the high k_2/K_m ratios. In Figure 3, it can be seen that k_2/K_m for N-hexanoylimidazole is considerably greater than the limiting k_2/K_m for the N-(3,3-dimethylbutyryl)-N'-methylimidazolium ion even though the leaving group pK_a of the latter compound is ~7, i.e., 7 pK_a units more favorable. Likewise, the limiting k_2/K_m for acylation by p-nitrophenyl hexanoate (pK_a of the leaving group = 7) is only 14 000 M^{-1} s^{-1} (25 °C).[5] It is clear that the k_2 values are being profoundly influenced by the binding process. Certainly, the large variation in k_2/K_m within the series acetyl to hexanoyl ((2.5 $\times$ 10^3)-fold) would not be expected solely on the basis of changes in K_m considering the weak binding of all the compounds. Binding of the N-acylimidazoles must improve the steric fit of the substrate carbonyl and the participating groups in the active site. In fact, these substrates may represent cases in which binding in the transition state is greatly superior to binding of reactant. Thus the transition state will be stabilized while reactant binding is weak, thereby maximizing k_2 and values of k_2/K_m.

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Diffusive and Nondiffusive Time Scales in the Dissociation and Recombination of I2 in Linear Alkanes

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Abstract: By measuring both the initial yield of I atoms and the cage escape probability of two caged I atoms, via picosecond spectroscopy, it is possible to distinguish two distinct regimes in liquid behavior. The initial yield is not dependent on bulk parameters such as viscosity but on a measure of compressibility. The cage escape probability is dependent on the diffusion constant calculated from the Stokes–Einstein relation. Thus, at early times the discrete molecular nature of the liquid is important, whereas at longer times the average behavior (diffusion) is the determining factor.

The subject of iodine photodissociation and recombination has been of interest to chemists for some time.[1] The interest is due to the apparent simplicity of the system, its ease of experimental investigation, and its applicability to studying fundamental processes in liquids. The earlier studies of I2 recombination were done by scavenger techniques[1] where time resolution was not feasible. Recently, the first picosecond studies of this simple reaction were reported.[2,3] We have extended these results to a large number of solvents of the noninteractive type. A preliminary report of this work was presented last year.[4] We report here on one aspect of our more refined results. Future reports will consider further aspects of these studies.

The concern of this paper is with the initial dissociation process. We will offer evidence to suggest that the dissociation is controlled by the discrete, molecular nature of the solvent and not by macroscopic value measures of the solvent such as viscosity. On the other hand, the longtime yield of iodine atoms does seem to be bulk (viscosity) dependent. The distinction between these two limits seems to begin to define the limits of applicability of continuum theory to molecular motion in liquids.

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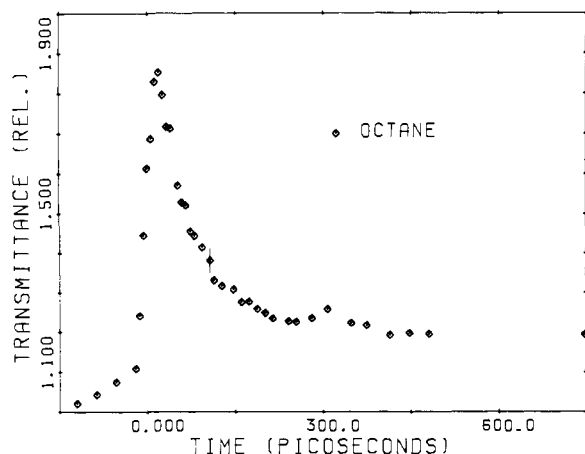
Figure 1. Bleaching curve of I_2, 1×10^{-2} M, in octane.

The basic description of the process is quite simple. The excited I_2 molecule dissociates with a given kinetic energy presumably fixed by the excitation wavelength.[5] Our experiments are conducted at one excitation wavelength, and presumably the initial kinetic energy is nearly the same for all solvents. The atoms slow down due to the dissipative effects of the liquid environment. In the continuum approximation, this dissipation is a viscous drag. In reality, of course, the dissipation is due to the large number of collisions with solvent molecules. Regardless of the origin of the damping, the I atoms quickly become thermalized and recombination can occur, if possible. The further the I atoms move apart initially, the less likely any geminate recombination (i.e., between the original partners) will occur. Noyes defines two different types of geminate recombination, primary and secondary.[6] Primary recombination occurs when the original partners slow down fast enough to not escape the first solvent shell cage. This recombination is presumed to occur very rapidly ($\lesssim 10^{-12}$ s) and would appear to our experiments as no dissociation at all, since our time resolution is ~ 25 ps at best. Those atoms that initially escape the first solvent shell can still recombine by diffusive-like motion. Recombination by these atoms is secondary, if they are the original partners or nongeminate if they were not originally bound. This paper is concerned with the initial yield of "secondary" I atoms, i.e., total dissociation yield minus the primary yield, and with the nongeminate yield.

It should be pointed out that many aspects of the above discussion are of necessity quite vague. Just where does one draw the line between caged and noncaged atoms? Or equivalently, is there really a sharp distinction between primary and secondary recombination as defined? The answer to this question is most likely no. What we present in the following discussion is an operating definition of these quantities and some discussion of what they reveal about I atom motions in liquid environments.

Experiments and Results

Basic Experiment. The experiment is the same as was done originally by Chuang et al.[2] It is a bleaching experiment in which the transmittance (or absorbance) of the sample is measured as a function of time relative to a strong excitation pulse. This bleaching curve is a measure of the concentration of I_2 molecules in relative time. The excitation and probing wavelengths are the same, the second harmonic of a mode locked Nd:YAG laser. The details of our experimental setup will be published elsewhere.[7] We have achieved a considerable improvement in data quality over the original work[2,3] by the use of higher repetition rates and computer-averaging techniques. In Figure 1 we show the results of bleaching I_2 in octane.

The characteristics of this curve are typical of those measured in all noninteracting solvents. A rapid increase in transmittance

Table I

alkane	peak trans, rel	non-geminate yield	viscosity, cP	compressibility, TPa⁻¹	mol vol var, 10³ cm³/EPa
pentane	1.166 (0.015)	0.60 (0.05)	0.219	2056	−253.1
hexane	1.276 (0.019)	0.65 (0.04)	0.329	1669	−219.6
heptane	1.351 (0.020)	0.42 (0.03)	0.403	1438	−212.1
octane	1.852 (0.067)	0.34 (0.04)	0.542	1282	−209.7
nonane	1.694 (0.036)	0.36 (0.03)	0.624	1175	−211.0
decane	1.358 (0.026)	0.26 (0.03)	0.861	1094	−214.3
undecane	1.318 (0.015)			1031	−218.8
dodecane	1.292 (0.022)	0.31 (0.05)	1.28	988	−225.8
tetradecane	1.258 (0.026)	0.27 (0.03)	1.90	910	−237.8
hexadecane	1.184 (0.009)	0.23 (0.05)	2.94	857	−252.08

to a peak value is followed by a slower decrease to a transmission above the original preexcitation level. This pseudo steady state also decays, but on a time scale greater than $\simeq 50$ ns. Measurements done by nanosecond flash photolysis indicate a microsecond time scale.[8] The curious aspect of these curves is that the initial decrease, which takes about 150–200 ps, is virtually independent of solvent for pure hydrocarbons, but the peak and long time transmittances are very solvent dependent. We will present a discussion of the implications of the time decay of the bleaching in a future publication.[9] The results and discussion here are based on the transmittances at the peak or the bleaching and in the long time tail and are independent of the mechanism of the fast decay.

The experiment consists of measuring three signals per laser shot. Two are the probe initial and transmitted intensities, which are ratioed in the computer to give a transmittance. The third is the excitation intensity. It was found that the transmittance is a linear function of excitation intensity. The data were analyzed by a linear least-squares fit of the log of the transmittance of the probe vs. excitation intensity. The least-squares line is evaluated at a particular intensity. This evaluated absorbance is then used to calculate the transmittance value (after dividing by the no-excitation transmittance) for that particular setting of the optical delay line. The delay line is moved and the procedure repeated. This procedure removes the effect of laser shot-to-shot intensity fluctuations, greatly improving data quality and reproducibility. Comparing solvents is then straightforward, since intensities can be accurately compared from day to day even if the laser changes characteristics. Great care was taken during the collection of these data to ensure compatability over the range of solvents. Many of the solvents were checked several times, over a period of several months, with good agreement. The error quoted is the statistical error of a single data-collection period. Other runs on different days did not significantly differ from these results.

The peak transmittance data were simply taken as the highest value transmittance. Some differences in the actual time of this peak from solvent to solvent were noticed. This jitter was less than $\simeq 10$ ps and was not considered significant because of the relatively long duration of the laser pulses ($\simeq 25$–30 ps). The longtime transmittances were calculated by averaging the data points from the trial at which the curves leveled off to the longest time measured. The validity of this procedure was demonstrated for several solvents by measuring the bleaching curves out to $\simeq 5$ ns. From $\simeq 150$ ps to 5 ns there was no significant decrease in transmission, so a linear least-squares fit reduces to a simple weighted average. The data for the normal alkanes are listed in Table I and are plotted in Figure 2. The values in the table for the initial yield are $-\log T_0$, where T_0 is the transmittance at the peak. This number is proportional to the concentration of I atoms at the peak (hereafter referred to as time zero).

It should also be noted that no net chemical reaction takes place with the pure hydrocarbon solvents used here. All I atoms recombine on at least a few millisecond time scale. For each new

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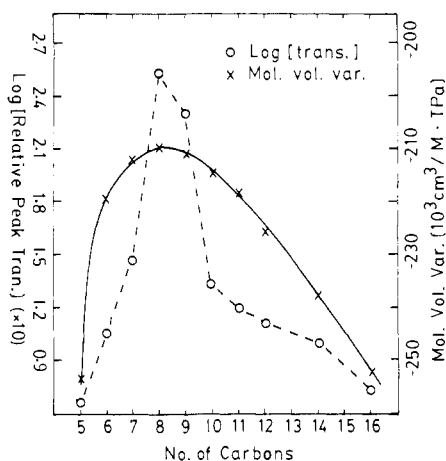(9) Langhoff, C. A.; Moore, B.; DeMeuse, M., in preparation.

Figure 2. -log peak transmittance for a given solvent vs. the number of carbons in solvent. Also, the molar variation vs. alkane number.

laser shot, the absorbance is always the same. This is not necessarily true for some chlorinated or brominated solvents we have used.

Initial Yield and Solvent Correlations. The simplest correlation expected comes from a continuum model.[10,11] The higher the viscosity, the faster the I atoms slow down and the lower the yield. This correlation obviously is wrong, since the viscosities monotonically increase with alkane number, but the initial yield peaks at octane and is lowest at both extremes. In fact, pentane and hexadecane have approximately the same yield but the largest viscosity difference. Another possible correlation is with the isothermal compressibility. Again, there is no correlation, as the compressibility decreases monotonically with the number of carbons.

The compressibility is related in an unspecified manner to the free volume in the liquid. It can be written as

$$\beta = -\frac{1}{\bar{v}} \left(\frac{\delta \bar{v}}{\delta P} \right)_T$$

where $\bar{v}$ is the molar volume. This is a measure of available free volume in the liquid expressed as a percentage of the total volume. A better measure of the room available to an I atom is the absolute volume change with respect to pressure, or $(\delta \bar{v}/\delta P)_T$. This is called the molar volume variation.[12] It is computed from the compressibility by

$$\left(\frac{\delta \bar{v}}{\delta P} \right)_T = \frac{MW}{\rho} \beta = \bar{v} \beta$$

where ρ is the density in g/cm^3, and MW is the molecular weight. This has been tabulated for the n-alkanes with $n \geq 6$ by Peña and Tardajos[12] and for pentane in ref 13. The actual values and a plot of $(\delta \bar{v}/\delta P)_T$ vs. alkane number is superimposed on the initial yields in Figure 2. It peaks at octane and decays away to higher and lower alkanes. The correlation between initial yield and $(\delta \bar{v}/\delta P)_T$, while not exact, is strikingly close qualitatively. Apparently, the room in which the I atom has to move is important for the initial dissociation.

A note of caution regarding the "free volume" interpretation should be issued. One should, as we do, regard this correlation as a loose suggestion. The correlation is obviously only qualitative and does not seem to apply to other nonlinear alkanes. Molar volume variation data for cyclohexane are available, but cyclohexane does not seem to fit on the same curve as the normal alkanes. A final point is that it is difficult to imagine how a bulk property such as $(\delta \bar{v}/\delta P)_T$ could really apply to the molecular
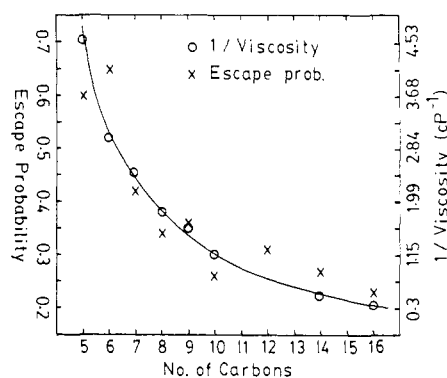

Figure 3. The escape probability (-log T_∞/-log T_0) vs. alkane number. Also plotted is 1/viscosity vs. alkane number.

motion behavior of I atoms. Also, care should be exercised as to the interpretation of "free volume". It is most likely the case that voids or vacancies do not exist in the liquid. Thus, free volume reflects an average measure of net binding in the bulk. Expanding into a free volume means not simply moving into unoccupied space, but also pushing solvent out of the way. Hence, the pressure derivative does have some relevance.

Two references prior to this work may be related to our observations. Muller[14] has noted a correlation of cohesive energy density with ^{19}F chemical shifts in some saturated hydrocarbons. The cohesive energy density is related to the volume of mixing changes. This is an indication of a similar effect, although it is not that close to our effect. Recently, in a study of vibrational relaxation in liquids, a correlation between inhomogeneous broadening and free volume, as well as with the product $\rho \beta^{1/2}$,[15] was suggested. Again, this effect may be different, but the point is that the discrete structure of the liquid seems to be important in understanding the motion and energetics of small solute particles in a solvent.

Escape Probability. The escape probabilities vs. alkane number are shown in Figure 3. The excape probability is defined as follows. Since -log transmittance is proportional to the I atom concentration at a given time, the ratio

$$E_P = -\log T_\infty / -\log T_0$$

is the proportion of initially dissociated I atoms that have recombined due to secondary geminate recombination. This is then the fraction (or probability) of an initially dissociated I atom to escape the cage. T_0 is the peak transmittance used to calculate the initial yield, and T_∞ is the long time transmittance.

Unlike the initial yields, they show a monotonic decrease with alkane number. Also plotted in Figure 3 is $1/\eta$, which is proportional to the diffusion constant of an I atom in a solvent by the Stokes–Einstein theory. The agreement is quite good, leading one to believe that diffusion theory or continuum mechanics is a valid description of the overall motion for this time regime. This is to be contrasted with the short time, initial-yield results, which do not appear to be related to diffusion.

Dicussion

There seem to be three distinct regimes of motion of I atoms that we are able to distinguish. In the dissociation process, the net initial yield of I atoms is apparently not diffusive in nature. The correlation with the molar volume variation indicates a dependence on free volume in the liquid rather than viscosity effects. This is the first time regime and lasts for ≤ 20 ps. After the I_2 molecules are initially dissociated, the probability of recombination does correlate with the diffusion coefficient as estimated from the Stokes–Einstein relationship. This latter motion is relatively slow (≥ 150 ps) compared to the motion governing initial yield (≤ 20 ps). The third regime is the very long-lived, nongeminate, pre-

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sumably diffusion-controlled recombination. We cannot determine this time with our apparatus, but it is most likely on the microsecond or longer time scales.

It should be pointed out that, although long time yields seem to be diffusion theory controlled, the actual approach to this long time limit is not describable by diffusion theory. Attempts at fitting these curves with solutions to the diffusion equation failed almost completely. We will report on these results in a future paper.[9] It also should be mentioned that there exist alternative interpretations to the simple motion description of I atoms. Hynes[16] and Wilson[17] have suggested, on the basis of theoretical calculations, that the decay of bleaching is actually a measure of vibrational relaxation of I_2 as it forms from I atoms. Our data can in no way directly distinguish between these two interpretations. Regardless of the interpretation of the time course, our arguments here of yields and escape probabilities should still be valid.

To understand this two-regime result, we present the following picture of the dissociation and recombination. Excited I_2 dissociates with I atoms flying apart at high (nonthermal) velocities. Some pairs slow down due to hard I–solvent collisions (presumably) and recombine immediately (primary recombination). Immediately is defined as ≤ 10–20 ps. The remaining pairs get far enough apart to be considered dissociated. This would be about at least 5–6 Å. The ratio of these two types of processes determines the initial yield.

The independence of this yield on diffusion parameters can be rationalized in at least two different ways. In the diffusive regime, the mean-square displacement is proportional to time. From molecular dynamics calculations[18] on Ar, for thermal velocities, it appears one reaches this regime in less than a picosecond. Perhaps then, for high, nonthermal velocities, this approach to the diffusion limit may be much longer, e.g., 10–20 ps. Thus, during this time regime when the initial dissociation and primary recombination is taking place, diffusion parameters make no sense.

A second and more plausible explanation is that the I–I potential, which swamps I–solvent interactions at close approach of two I atoms, drastically perturbs the diffusion parameters at early times (or equivalently at short I–I distances). Diffusion theory ignores explicit potentials completely assuming all interaction is described by the friction coefficient (or diffusion constant). Thus, the initial yield is concerned with I atom motion when it is close to a second I atom, which is not then describable by isolated I atom diffusion parameters. The concept of a local

or spacially dependent diffusion constant may help here.[19]

The description of the escape probability begins with the thermalized I atoms at separations where the I–I potential is not significant. These atoms then diffuse independently until they either recombine or separate. As mentioned previously, the time course of recombination is a subject of controversy, but the net amount of recombination is apparently determined, at least for the *n*-alkanes, by diffusive processes. The time course is most likely a convolution of diffusive motion and vibrational relaxation. Since neither is known with any confidence, the problem of an interpretation of the time course is doubly hard.

It should also be noted that the time scales quoted in this paper may be applicable only to I atom systems. This would certainly not be surprising. Indications from measurements of reorientational relaxation times of large dye molecules seem to indicate that diffusive descriptions do work quite well. One parameter that may be of importance is the relative size of the solute and solvent. When the solute is of the order of or smaller than the solvent, the onset of diffusive-like motion is delayed. When the solute is much bigger than the solvent, diffusive motion begins much earlier. These suggestions are based on simplistic physical models.

We cannot as yet suggest a molecular mechanism for the "octane effect" observed in the initial yields. The only reasonable speculation is that we are seeing a curious packing effect in the liquid. Perhaps this is related to a conformational preference of octane, but there is a almost no information to confirm or reject this hypothesis.[20] The yield is very sensitive to solvent. The commerical solvent hexanes, a mixture of *n*-hexane and methylcyclopentane, shows a significantly greater yield than pure *n*-hexane. Cyclohexane and methylcyclohexane also have large initial yields. These observations may be of relevance to many workers attempting to simulate liquid alkanes on a computer.[21-23] Although they have not looked for bulk free volume (or more accurately $(\delta \bar{v}/\delta P)_T$), these studies could potentially give us quite valuable information.

Registry No. Pentane, 109-66-0; hexane, 110-54-3; heptane, 142-82-5; octane, 111-65-9; nonane, 111-84-2; decane, 124-18-5; undecane, 1120-21-4; dodecane, 112-40-3; tetradecane, 629-59-4; hexadecane, 544-76-3; I₂, 7553-56-2.

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