The Experimental Heats of Formation and Reaction of Singlet Carbenes via Time-Resolved Photoacoustic Calorimetry

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Abstract: The heats of formation and reaction of several singlet carbenes are determined by time-resolved photoacoustic calorimetry. The most selective and least reactive carbene examined, methoxychlorocarbene, is the most stable and reacts least exothermically with CH_3OH. The phenylhalocarbenes have similar stabilities and reaction exothermicities with CH_3OH. The experimental carbene heats of formation in solution are systematically lower and the stabilization energies higher than the values from MNDO calculations. This could potentially result from (i) solvation of the carbene, (ii) an unresolved reaction volume change, or (iii) incorrect MNDO calculated carbene and diazirine enthalpies.

Carbene chemistry has had a recent renaissance of theoretical[1] and experimental[2-4] interest. Carbene reactivity is highly dependent upon the substitution at the carbenic center and is reflected in the "philicity" of the carbene. Normal reactivity-selectivity relationships are often observed as measured by the "selectivity" index of carbene reactions with olefins. Although the reactivity order is usually rationalized in terms of the stabilities of the carbenes, little experimental thermochemical information about carbene reactions is currently available.[5] Consequently, no direct relationship has been established between carbene stability and selectivity or reactivity. In this regard, we wish to report the use of photoacoustic calorimetry (PAC)[6-8] to obtain the heats of reaction of several singlet carbenes with CH_3OH. This information can be used to determine their heats of formation in solution and their relative stabilization energies.

Experimental Section

The photoacoustic apparatus has been previously described.[6,7] Briefly, photolysis is initiated by a nitrogen laser (**1d**, 337 nm) or a pumped tunable dye laser (**1a–c**, 365 nm). The heat deposited is detected by a PZT transducer (Panametrics, Model A125S, 2.25 MHz or homebuilt ~0.5 MHz[9]). The signal is amplified (Panametrics preamp, Model 5676), digitized (LeCroy 9400), and transferred to a laboratory computer for data analysis. The waveforms are the average of 30–50 laser pulses (<20 μJ). The first 400 points of the acoustic waveform are analyzed by deconvolution methods.[6,7] The results are unaffected by sample concentration (0.2–0.8 OD), transducer employed (~0.5 or 2.25 MHz), or argon degassing of the sample.

The optical densities of the calibration and sample compounds are adjusted to be within 1% of each other. Sample absorbances did not change during the experiment. 2-Hydroxybenzophenone (Aldrich) is used as the calibration compound. The diazirines are prepared as previously described[10] and used immediately. Spectrograde CH_3CN, CH_3OH, and heptane are used as received.

Table I. The Experimental Heats of Reaction[a,b] and Formation[c] Determined by PAC[d]

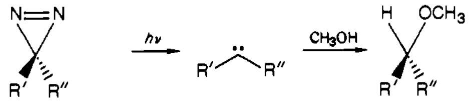
compd	$\Delta H_{(1\rightarrow 3)}$- ($CH_3OH$)	$\Delta H_{(1\rightarrow 2)}$- ($CH_3CN$)	$\Delta H_{(2\rightarrow 3)}$	$\Delta H_f(2)$	ΔE_{stab}[e]
1a	−57.3 ± 1.3[f]	−7.9 ± 2.1	−49.4 [−60.8][g]	27.3 [38.8]	62.2
1b	−62.0 ± 2.6	−11.1 ± 1.6	−50.9 [−78.2]	67.1 [94.4]	57.1
1c	−62.0 ± 2.0	−11.4 ± 2.0	−50.6 [−81.0]	79.8 [109.9]	55.9
1d	−71.06 ± 3.5[h]	−39.9 ± 2.9	−31.2 [−47.4]	−21.6 [−5.4]	76.2

[a] Excitation at 337 (**1d**) or 365 nm (**1a–c**) in CH_3OH or CH_3CN. [b] Using eq 1, the experimental α values and $\phi = 1$. [c] See text and ref 17. [d] Values are in kcal/mol. [e] Using eq 2 and ref 19. [f] Values are from at least four separate runs, the errors are 1σ. [g] MNDO values are in parentheses. [h] The α value used in eq 1 is the sum of the α values for the two heat depositions.

The quantum yields are determined by using phenylglyoxylic acid actinometry.[25] The disappearance of the diazirine in CH_3OH was followed by UV–vis spectroscopy (**1a–c**, 365 nm, ϵ_{365}:**1a** = 180, **1b** = 141, **1c** = 134, **1d**, 348 nm, ϵ = 70). A medium pressure Hg lamp with bandpass filters to isolate the appropriate wavelengths is used. The quantum yields for disappearance (**1a–d**) are 2.00 ± 0.09, 2.03 ± 0.14, 2.02 ± 0.08, and 1.05 ± 0.05, respectively.

Results and Discussion

Photolysis of the diazirines **1a–d** results in the loss of nitrogen to form the respective carbenes, **2a–d**.[2,3] In CH_3OH, the singlet ground state carbenes insert into the O–H bond, forming the methyl ethers, **3a–d**.[2,9c,11] In heptane or CH_3CN, the carbenes undergo bimolecular decay.[2,11]



1a, R′ = C_6H_5, R″ = F
b, R′ = C_6H_5, R″ = Cl
c, R′ = C_6H_5, R″ = Br
d, R′ = OCH_3, R″ = Cl

2a–d

3a–d

PAC permits the simultaneous determination of the energetics and dynamics of photoinitiated reactions. The deconvolution of the experimental acoustic waveform measures the amplitude and

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time evolution of heat deposition. The details of this method have been previously reported.[6,7] The enthalpic fitting parameter for the experimental waveform, α, is the fraction of the incident photon energy released in the given heat deposition. It can be converted to the corresponding heat of reaction via eq 1

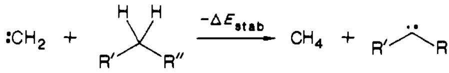
$$\Delta H = (1 - \alpha)E_{h\nu}/\Phi \tag{1}$$

where Φ is the reaction quantum yield and $E_{h\nu}$ is the incident laser power. The carbenes (**2a–d**) are generated with unit quantum efficiency from the diazirines (**1a–d**) in CH_3OH and CH_3CN.[12] The reaction enthalpies in CH_3OH and CH_3CN calculated from eq 1 are given in Table I. The experimental α values obtained from photodecomposition of diazirines (**1a–d**) in heptane are similar to those in CH_3CN.[13]

Irradiation of **1a–d** in CH_3CN results in only a single heat deposition, <10 ns, reflecting carbene formation. The experimental reaction enthalpy corresponds only to **1a–d** → **2a–d**.[13c] Irradiation of **1a–c** in CH_3OH also results in a single heat deposition, <10 ns. However, this decay includes both the initial carbene formation and the subsequent insertion into the O–H bond[11] so the reaction enthalpy corresponds to **1a–c** → **3a–c**. The difference between the experimental reaction enthalpies in CH_3OH, $\Delta H_{(1\rightarrow3)}(CH_3OH)$, and CH_3CN, $\Delta H_{(1\rightarrow2)}(CH_3CN)$, corresponds to the heat of reaction of the carbenes with CH_3OH to form adducts, $\Delta H_{(2\rightarrow3)}$, Table I.[13b] The two steps, **1a–c** → **2a–c** and **2a–c** → **3a–c**, can be time-resolved at low CH_3OH concentration in CH_3CN. The decay of **2b**, τ_{obsd}, in the presence of a large excess of CH_3OH (0.008–0.11 M) follows pseudo-first-order kinetics. The plot of $1/\tau_{obsd}$ vs $[CH_3OH]$ is linear ($r = 0.998$) and gives a slope corresponding to a second-order bimolecular quenching rate constant of 3.7×10^8 M^{-1} s^{-1}, which is similar to the literature value.[11,14a] The reaction enthalpy for the first decay, **1b**→**2b**, and for the sum of the two decays, **1b** → **3b**, is similar to those obtained in CH_3CN and CH_3OH, respectively.

Irradiation of **1d** in CH_3OH results in two heat depositions on different time scales. The first deposition, <10 ns, corresponds to a carbene formation.[14b] The reaction enthalpy associated with this heat deposition is equal to that determined in CH_3CN. The second decay, ~470 ns, presumably represents the O–H bond insertion of the carbene. The bimolecular quenching rate constant, $\sim 6.8 \times 10^4$ M^{-1} s^{-1}, is one of the first measured for a reaction of a non-aryl substituted or "invisible" carbene in solution.

The reaction enthalpies in CH_3OH and CH_3CN are calculated by MNDO, Table I.[15,16] The heats of formation of the carbenes **2a–d** in solution are determined using MNDO calculated heats of formation of the methyl ethers **3a–d** and the experimental heats of reaction in CH_3CN and CH_3OH.[17] The extent of stabilization of the various substituents can be evaluated by the comparison to the unsubstituted reference carbene, methylene. By using the following isodesmic equation,[1a] the stabilization energies are



(2)

calculated using the carbene heats of formation in solution and are given in Table I.[19] The difference in stabilization energy between **2a** and **2d**, 14 kcal/mol, is in good agreement with ab initio calculations, 14.6 kcal/mol.[1a]

The experimental carbene heats of formation in solution are systematically lower and the stabilization energies higher than the gas-phase values from MNDO calculations. This could potentially result from (i) solvation of the carbene,[13a] (ii) an unresolved reaction volume change,[22] or (iii) incorrect MNDO calculated carbene and diazirine enthalpies.

The relationship between carbene stability and selectivity can be examined by a comparison of the carbenes' empirical selectivity indexes, m_{cxy},[23] with either their stabilization energies or their heats of reaction with CH_3OH. The most stable carbene examined, **2d**, reacts least exothermically with CH_3OH and exhibits the greatest selectivity. Furthermore, its rate of O–H insertion in CH_3OH (~470 ns) is significantly slower than that of the phenylhalocarbenes **2a–c** (<10 ns). This decreased reactivity and increased selectivity presumably results from decreased reaction exothermicity and a "later" transition state. For comparison, the reaction of singlet methylene, the least stable, most indiscriminate carbene, with CH_3OH is exothermic by ~98 kcal/mol.[15,19]

The selectivity differences between the electrophilic phenylhalocarbenes, **2a** > **2b** > **2c**,[23] are not reflected, within experimental error, in their heats of reaction with CH_3OH nor in their stabilization energies. The differences in these values between the phenylhalocarbenes are also significantly smaller than those predicted from MNDO values. This may be a result of differential solvation of the carbenes; the heat of solvation is more exothermic

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(12) The initial photoproducts in CH_3OH, **3a–c**, are not stable under the reaction conditions. It is assumed that the subsequent reactions occur on a time scale beyond the detection limit of the PAC apparatus used, <5 μs. For **1a–c**, a secondary, nonphotolytic decomposition of the diazirine (see ref 11) occurs which results in $\phi \sim 2$. GC–MS analysis of the photolysate of **1a–c** in CH_3OH showed only the products reported in ref 11. The quantum yields for carbene formation in CH_3CN are assumed to be unity, i.e., no solvent dependence.

(13) (a) The PAC ΔH values include the differential heat of solvation energy between the reactants and products. In the reaction **1** → **2**, if the heat of solvation of the diazirine is not the same as that of the carbene in the solvents studied, the heat of reaction will include this difference. It may be significant for **1** → **2** because of the potential stabilization of the carbene by the solvent. The exothermic heat of solvation would result in a larger experimental $\Delta H_{(1\rightarrow2)}(CH_3CN)$ value than in the gas phase and a lower heat of formation of the carbene in solution and a higher stabilization energy. However, spectroscopic studies indicate that these electrophilic carbenes do not undergo appreciable solvation with the solvent CH_3CN.[2] In addition, the $\Delta H_{(1\rightarrow2)}$ for each carbene is the same in heptane or CH_3CN, which suggests that solvation may not be important. (b) The value $\Delta H_{(2\rightarrow3)}$ includes the heat due to the change in hydrogen bonding when an alcohol is converted to an ether. (c) The oscillation frequency of the transducer determines the time response of the experiment. The employed transducers (0.5–2.25 MHz) are insensitive to the slow heat depositions from the bimolecular decay of the carbenes.

(14) (a) The anticipated curvature in the plot due to different reactivities of **2b** with CH_3OH monomers and oligomers is apparently absent over the range employed. (b) It is possible that the first heat deposition is from a diazirine–diazoalkane isomerization, and the second is from the decomposition of the diazoalkane. Although this isomerization is observed with **1a** at low temperature, it does not occur with **1d**.[21] Furthermore, the isomerized **1d** should be more unstable than **1a** and decompose more rapidly. Decomposition of **1a** at 25 °C occurs in <10 ns.

(15) By MNDO, $\Delta H_f(\mathbf{1a}) = 48.95$, $\Delta H_f(\mathbf{1b}) = 88.59$, $\Delta H_f(\mathbf{1c}) = 98.75$, $\Delta H_f(\mathbf{1d}) = 24.02$, $\Delta H_f(\mathbf{2a}) = 38.77$, $\Delta H_f(\mathbf{2b}) = 94.39$, $\Delta H_f(\mathbf{2c}) = 109.87$, $\Delta H_f(\mathbf{2d}) = -5.44$, $\Delta H_f(\mathbf{3a}) = -70.27$, $\Delta H_f(\mathbf{3b}) = -31.97$, $\Delta H_f(\mathbf{3c}) = -19.31$, $\Delta H_f(\mathbf{3d}) = -101.01$, $\Delta H_f(\mathrm{MeOH}) = -48.18$ kcal/mol.[18]

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(17) (a) $\Delta H_f(2) = \Delta H_f(3) - \Delta H_f(CH_3OH) - \Delta H_{(2\rightarrow3)}$. Alternatively, the heats of formation of the carbenes **2a–d** can be calculated with the diazirine heats of formation and the experimental heats of reaction in CH_3CN.[15] The MNDO values for the diazirines **1a–d** appear too large, and so the methyl ether MNDO values are used. For example, the MNDO value for diazirine (CH_2N_2) is 73 kcal/mol, and the experimental value is 66 kcal/mol.[24] (b) This assumes the heat of solvation of the diazirines is small. The heats of formation in solution include the solvation of the carbenes.[13a]

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(22) (a) The PAC experiment measures the total volume change of the associated reaction. This includes volume changes from thermal, ΔV_{th}, and reaction volume, ΔV_{rx}, changes. The reaction volume contribution has previously been neglected,[6-8] but it can be significant. We are presently investigating the magnitude of such changes.[22b] In **1** → **2**, the reaction volume change should be positive which would give an erroneously large value for $\Delta H_{(1\rightarrow2)}(CH_3CN)$ and a lower heat of formation of the carbene. (b) Herman, M. S.; Goodman, J. L. *J. Am. Chem. Soc.*, in press.

(23) The carbene selectivity indices, m_{cxy}, for **2a–d** and $^1CH_2$ are 0.89, 0.83, 0.70, 1.59, and ≪0.5.[4]

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for the less stable carbene. In fact, the stabilization of carbenes by solvent can be significant. The heats of solvation of methylene with CH_3CN and acetone to form nitrile and carbonyl ylides, respectively, are estimated to be exothermic by 45–50 kcal/mol.[6c] In an attempt to examine potential differences in solvation energies, $\Delta H_{(1\rightarrow2)}$ was measured for the carbenes in solvents of different polarities. However, the difference in solvation enthalpies between heptane and CH_3CN for each carbene is small based on their similar $\Delta H_{(1\rightarrow2)}$ values.

In conclusion, we have used PAC to determine the heats of formation and reaction with CH_3OH of several singlet carbenes in solution. The stabilization of the carbenes by substitution can be related to their reaction rates and exothermicities. Further studies will examine substituent and temperature effects on the energetics and dynamics of carbene reactions.

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Registry No. **1a**, 87282-19-7; **1b**, 4460-46-2; **1c**, 4222-25-7; **1d**, 4222-27-9; CH_3OH, 67-56-1; CH_3CN, 75-05-8.

An Investigation of a Human Erythrocyte SOD Modified at Position 137

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Abstract: A human copper–zinc superoxide dismutase mutant in which the active site residue Thr-137 is substituted with Ile has been characterized by activity measurements, electronic, CD, and EPR spectroscopies, and ^1H NMR spectroscopy of its cobalt-substituted derivative. The mutant displays gross changes in the metal coordination sphere. The chromophore is considerably more tetragonal than in the WT and its coordination may be consistent with no apical water. The formation of a more regular square plane results from a more regular binding of the four histidines. Despite these sizable alterations, the mutant has essentially the same enzymatic activity as the WT at physiological pH. It follows that the detailed stereochemistry about copper is not relevant for activity. Consistent with this result, we find that the affinity of anions is similar to that of the WT. The mutant, however, shows a 0.8 unit lower pK_a for the high pH decrease of enzymatic activity. The possible candidates for the group responsible for such behavior are considered.

Site directed mutagenesis is increasingly proving its power as a tool in the investigation of enzyme mechanisms.[1,2] Since its successful cloning and expression in yeast[3] and *E. coli*,[4] we can now include erythrocyte Cu/Zn human superoxide dismutase (SOD) as well. The great advantage of site directed mutagenesis is the possibility of directing specific substitutions anywhere within a protein at enzyme active sites and, accordingly, permitting mechanistic hypotheses to be tested.

SOD catalyzes the dismutation of superoxide with very high efficiency;[5] human and bovine isoenzymes typically show k_{obs} of the order of 10^9 M^{-1} s^{-1}. Recent measurements, under saturating amounts of O_2^-, yielded k_{cat} and K_M values of $\approx 1 \times 10^6$ s^{-1} and $\approx 3 \times 10^{-3}$ M, respectively, for the bovine isoenzyme.[6]

SOD is a dimeric protein of MW 32 000, each monomer containing one copper and one zinc ion bridged by a histidinato residue. The copper ion is the catalytic center. The active site structure as it appears from X-ray studies[7] on the strictly similar bovine isoenzyme is shown in Figure 1. Besides the metal ligands, a particularly important residue present at the entrance of the active site cavity of all known natural isoenzymes is an arginine, which in the human isoenzyme is Arg-143.[8] This residue has been often discussed as playing a role in the catalysis.[9,10] It is possible that it attracts O_2^-,[11] as well as other anions,[12] into the active site. Modification of Arg with phenylglyoxal produces a strong decrease in the enzyme activity[13] and in anion affinity.[14] Recently, studies on derivatives substituted by mutagenesis with Ile, Lys, and Glu at position 143 have also shown a sharp decrease in both activity,[15,16] and anion affinity.[12] Yet, the metal geometry

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