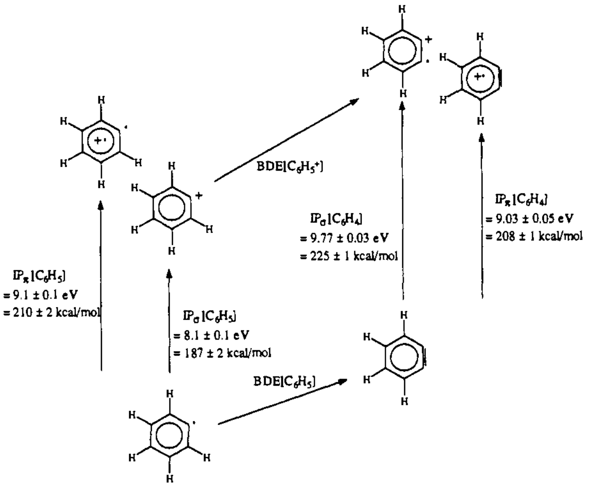
Scheme I. Thermochemical Cycle Relating Ionization Potentials to Bond Strengths and the Singlet-Triplet Gap (Phenyl Radical Data Taken from Reference 11)



previously determined by photodetachment of $C_6H_5^{\bullet -}$ and the carbocyclic skeleton is relatively rigid. This establishes that the hypothetical "noninteracting biradical" computed by additivity methods can be identified with triplet o-C_6H_4 and that the homolytic C-H bond dissociation energy (BDE) of phenyl radical (at the ortho position) is reduced from that in benzene by $\Delta_{ST}[o\text{-}C_6H_4]$.

While $\Delta_{ST}[o\text{-}C_6H_4]$ is known from the work of Leopold[17] et al., singlet-triplet gaps are usually difficult to infer. We therefore propose the relationship expressed in eq 1 as a measure of Δ_{ST} for geometrically-rigid singlet biradicals and check the proposition using o-C_6H_4 as the prototypical test case. Referring to Scheme I, we see that $IP_\sigma[o\text{-}C_6H_4] - IP_\sigma[C_6H_5] = BDE[C_6H_5^+] - BDE[C_6H_5]$ is an identity. While the VB promotion energy model that rationalized $\Delta H_f[o\text{-}C_6H_4]$ predicts a weakening of the ortho C-H bond in phenyl radical by $\Delta_{ST}[o\text{-}C_6H_4]$, the same picture finds no comparable weakening of the corresponding bond in phenyl cation. We therefore use the ortho C-H bond in $C_6H_5^+$ as the reference bond to which the bond in C_6H_5 is compared. It is this assumption that relates the difference in ionization potentials to the singlet-triplet gap of o-benzyne. The numerical test of the proposition is

$$BDE[C_6H_5^+] - BDE[C_6H_5] \approx \Delta_{ST}[o\text{-}C_6H_4] \approx IP_\sigma[o\text{-}C_6H_4] - IP_\sigma[C_6H_5]$$

This predicted relationship is tested with our spectroscopic measurements. We find

$$IP_\sigma[o\text{-}C_6H_4] - IP_\sigma[C_6H_5] = (9.77 \pm 0.03) - (8.1 \pm 0.1) = 1.67 \pm 0.1 \text{ eV} = 38.5 \pm 2.4 \text{ kcal/mol}$$

which is very close to the independently determined singlet-triplet gap of o-benzyne of $\Delta_{ST}[o\text{-}C_6H_4] = 38 \pm 0.7$ kcal/mol. *The remarkable agreement validates eq 1 and establishes ΔIP as a measure of a singlet biradical's singlet-triplet splitting.*

Because we identify triplet o-C_6H_4 with the "noninteracting biradical" whose ΔH_f is computed by additivity, and because a strict additivity estimate (which does not include the Δ_{ST} correction) does not distinguish[18] between ortho, meta, and para isomers, eqs 1 and 2, along with the recently determined[14] $\Delta H_f[m\text{-}C_6H_4] = 116 \pm 3$ kcal/mol and $\Delta H_f[p\text{-}C_6H_4] = 128 \pm 3$ kcal/mol, predict that $\Delta_{ST}[m\text{-}C_6H_4] \approx 26$ kcal/mol, $\Delta_{ST}[p\text{-}C_6H_4] \approx 14$ kcal/mol, $IP_\sigma[m\text{-}C_6H_4] \approx 9.2$ eV, and $IP_\sigma[p\text{-}C_6H_4] \approx 8.7$ eV. Experimental confirmation of these predictions is in progress.

We have observed a regularity in the thermochemistry of singlet biradicals that is confirmed by photoelectron spectroscopic measurements of biradical ionization potentials. Further work is underway to explore the generality of the relationship expressed in eq 1 as a way to determine biradical singlet-triplet gaps.

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[18] Ab initio calculations for **2**, **3**, and **4** predict correct relative energies among the singlet isomeric benzynes and also find that the triplet states of the three isomers are degenerate to within 3 kcal/mol. See: Noell, J. O.; Newton, M. D. *J. Am. Chem. Soc.* **1979**, *101*, 51.

Unexpected Frequency Effects on the Rate of Oxidative Processes Induced by Ultrasound

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Sonication of water generates radicals which oxidize compounds in solution.[1] In this work we give evidence that in the presence of argon or oxygen these oxidative processes occur in enhanced yields when a high frequency (514 kHz) is used in comparison with the more commonly used low frequency (20 kHz).

In water cavitation bubbles are filled with vapor and dissolved gas. They oscillate with pressure and then collapse.[2] At the end of the compression phase, the internal temperature can reach several thousand degrees (K), and the pressure several hundred bars.[1,3] Under these extreme conditions, the water molecule is cleaved to $H^\bullet$ and $OH^\bullet$ (eq 1).[4] Molecular oxygen if present in the bubble can also decompose (eq 2).[5]

$$H_2O \rightarrow H^\bullet + OH^\bullet \tag{1}$$

$$O_2 \rightarrow 2O \tag{2}$$

Transient radicals can recombine or escape from the collapsed bubble to react with dissolved molecules.[6] In several cases a dependence is suggested among the rates of the sonochemical oxidative process, the frequency of the wave, and the nature of the dissolved gas.[7] As no extensive study has reported on the

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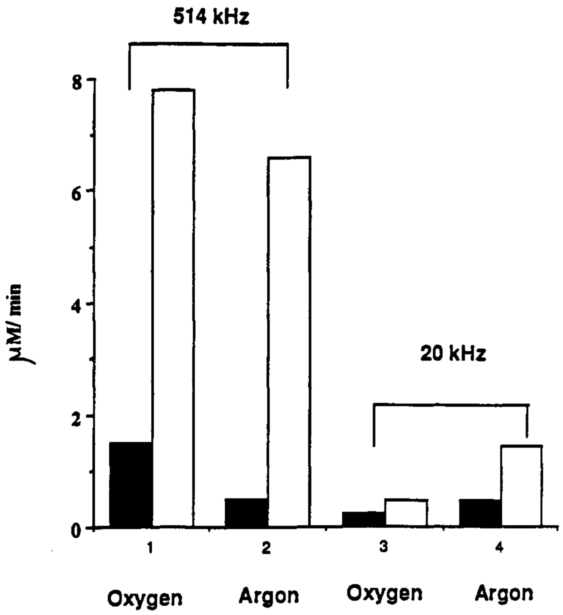

Figure 1. Comparative rates of formation for triiodide in KI, 10^{-2} M solution (■), and for hydrogen peroxide (□) in water at 514 and 20 kHz for solutions saturated with argon or oxygen. Power for each reactor was adjusted to make I_3^- rates in argon atmosphere equal to 0.45 µM/min.

nature of the phenomena, despite their considerable intrinsic interest, we recently started an investigation on the poorly understood frequency effect.[8]

Because of the differences in wavelengths, beaming effects, and multiple reflections on the walls of the cell, the correlation of the efficiency of reactions carried out at different frequencies is not possible by a direct physical measurement.[6b,9] Iodide ion oxidation (eq 3) and hydrogen peroxide production (eq 4), two dosimetric methods commonly used in sonochemistry, were then examined for calibration purposes.[10,11]

$$2OH^\bullet + 3I^- \rightarrow 2^-OH + I_3^- \qquad (3)$$

$$2OH^\bullet \rightarrow H_2O_2 \qquad (4)$$

The energy outputs of both generators were adjusted in order to obtain comparable rates for the triiodide ion formation from an argon-saturated 10^{-2} M solution of KI. Surprisingly, replacement of argon by oxygen leads to a lower rate at low frequency but a 3-fold increase at 514 kHz (Figure 1).

Hydrogen peroxide is one of the products resulting from recombination of the hydroxyl radicals (eq 4). Results (Figure 1) show that hydrogen peroxide is always formed in larger amounts at 514 kHz than at 20 kHz and, as previously seen, efficiency is better under oxygen at high frequency, but under argon at low frequency. The overall efficiency is obviously higher at 514 kHz than at 20 kHz. The difference cannot be attributed to a catalytic effect of the metal of the horn because similar results are obtained when the experiment is done in a quartz sealed tube. This experiment also eliminates any possibility of oxygen leakage into the reactor.

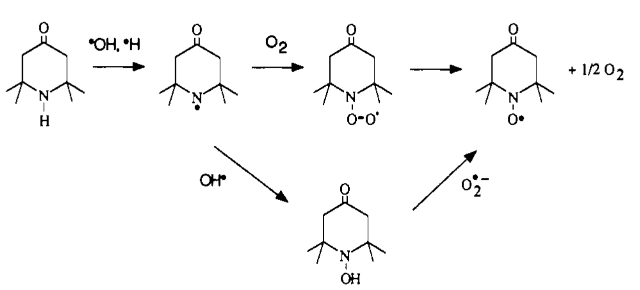

Figure 2.

With these results in hand, sono-oxidations were effected in the presence of 2,2,6,6-tetramethyl-4-piperidinone (TMPone, 10^{-2} M), to determine (ESR spectrometry) the amount of sonically produced stable nitroxide, 2,2,6,6-tetramethyl-4-piperidinone-*N*-oxyl (TMP). The reaction requires the presence of $OH^\bullet$ and either molecular oxygen or superoxide radical anion (Figure 2).[12] The higher rate for nitroxide formation (3.6×10^{-6} M.min^{-1}) is obtained at high frequency with the oxygen saturated solution, but under argon, no ESR signal is detected, in agreement with the proposed reaction scheme.[13] The same experiment run at 20 kHz proceeds at a low rate for an oxygen saturated solution (0.083×10^{-6} M.min^{-1}) but at a comparatively higher rate under argon (1.08×10^{-6} M.min^{-1}). As oxygen is necessary to achieve this oxidation, the latter result suggests that it must be formed in higher yield during low-frequency sonolysis under argon.[5]

Describing the behavior of radicals from water sonolysis, which has not yet received a comprehensive interpretation, is a formidable challenge.[14] Radicals produced in the superheated medium inside the bubble can migrate through the boundary layer around it and escape into the solution. At each of these steps, the primarily formed radicals can react or recombine in multiple ways according to the local conditions. Increasing the frequency has two effects: the collapse of the bubble releases less energy, and it occurs in a shorter period of time, 3×10^{-7} s at 514 kHz and 10^{-5} s at 20 kHz.[15] Then at high frequency, $OH^\bullet$ radicals can be ejected out of the bubble before undergoing any reaction. Their chemical evolution, including H_2O_2 formation, I^- oxidation, and reaction on TMPone, starts when they reach the limit layer and then the solution. At 20 kHz in the bubble, radicals have time enough to follow reaction pathways analogous to those found in flame chemistry, in particular those leading to oxygen generation (eqs 5 and 6).[5] Oxygen generation allows TMP formation in argon atmosphere but limits $OH^\bullet$ recombination and oxidative processes.

$$OH^\bullet + OH^\bullet \rightarrow H_2O + O \qquad (5)$$

$$2O \rightarrow O_2 \qquad (6)$$

The differences between the results obtained at high and low frequency under oxygen are explained if reactions 7 and 8 are

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(8) Reactions were carried out at 24 ± 1 °C in a circulating loop incorporating the high- or low-frequency reactor. The system has already been described: Petrier, C.; Reverdy, G.; Luche, J.-L. *Récents Progrès en Génie des Procédés*, **1991**, *5* (16), 241. The high-frequency reactor is constituted of a glazed titanate-lead zirconate disk (diameter 2 cm) fixed at the bottom of a thermostated cylindrical tube. The system is operated at 514 kHz with 20 W HF electrical power. The low-frequency reactor is built around the titanium horn (diameter 1.23 cm) of a 20-kHz Branson 450 sonifier with 30 W electrical power. The water or the investigated aqueous solution (50 mL) is circulated with a diaphragm pump at a flow rate of 20 mL/min. The irradiated volume is 30 mL. The solutions are saturated with gas in a separated thermostated flask through a fritted disk bubbler (50 mL/min flow rate). Acoustical power determined by calorimetry for argon saturated solutions was 13.4 W at 20 kHz and 14.6 W at 514 kHz.

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(13) ESR measurements were made on a Varian E 112 spectrometer operating in the 100-kHz X band. The circulating system was connected to a quartz flat cell fixed in the cavity of the spectrometer. 2,2,6,6-Tetramethylpiperidine-*N*-oxyl was used as a standard. (Similar results were obtained with a 10^{-3} M solution of TMPone.)

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considered. Molecular oxygen scavenges the hydrogen atoms, forming OOH radicals (eq 7) and lowering the recombination of OH• and H• radicals (eq 8).

$$\mathrm{H^\bullet + O_2 \rightarrow {}^\bullet OOH} \quad (7)$$

$$\mathrm{H^\bullet + OH^\bullet \rightarrow H_2O} \quad (8)$$

Consequently more hydroxyl radicals are available if they escape from the bubble (high frequency) in the medium. At low frequency, in the long-lived bubble, OH• can be scavenged by reaction with the hydroperoxyl radical (eq 9); consequently H_2O_2 and I_3^- formation are more effective under argon.[16]

$$\mathrm{OH^\bullet + {}^\bullet OOH \rightarrow H_2O + O_2} \quad (9)$$

Changing the frequency of ultrasonic waves then produces modifications in the behavior of the species first formed in water sonolysis. This finding constitutes the first example of a clear frequency effect and should have important consequences for optimizing sonochemical oxidation yields.

Registry No. TMP, 2896-70-0; TMPone, 826-36-8; H_2O, 7732-18-5; O_2, 7782-44-7; HO•, 3352-57-6; I^-, 20461-54-5; I_3^-, 14900-04-0; H_2O_2, 7722-84-1.

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Photoswitchable Binding of Substrates to Proteins: Photoregulated Binding of α-D-Mannopyranose to Concanavalin A Modified by a Thiophenefulgide Dye

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Macromolecules exhibiting photoswitchable physical or chemical properties are extensively examined as information storage and signal amplification materials. Photoregulated "on-off" biomaterials provide a novel means to design targeted therapeutic agents activated and deactivated by external light signals. Various means to photoregulate biotransformations by light-switchable enzymes have been described and include the modification of the enzyme active site[1–4] and protein backbone[5,6] by photochromic components and immobilization of enzymes in photochromic copolymers.[7] Here we wish to report on the photoregulation of the binding properties of a protein by its chemical modification with photochromic units. We describe the photoswitchable binding of saccharides to concanavalin A modified by thiophenefulgide dye.

Concanavalin A (Con A) is a globular lectin composed of four subunits (MW = 26K).[8] Each subunit includes binding sites for Mn^{2+} and Ca^{2+} and monosaccharide binding sites.[9,10] The metal ions act cooperatively in the association of the monosaccharides to Con A. The affinity of Con A toward monosaccharides is sensitive to the steric configuration of the pyranose hydroxyl groups at positions C-3, C-4, and C-6 of the saccharide.[11] Two pyranoses, α-D-mannopyranose and α-D-glucopyranose, are recognized for binding by Con A, and the affinity of Con A toward α-D-mannopyranose is ca. 3.5-fold higher than toward α-D-glucopyranose.[12] Thus, small steric alterations of the protein backbone

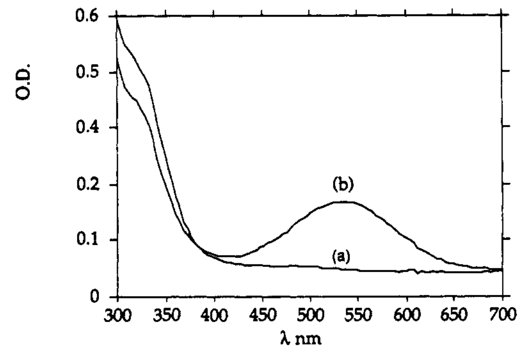

Figure 1. **2**-modified Con A: (a) **3**-*E* obtained after irradiation of **3**-*C* at λ > 475 nm; (b) **3**-*C* obtained after irradiation of **3**-*E* at 400 nm > λ > 300 nm.

Scheme I

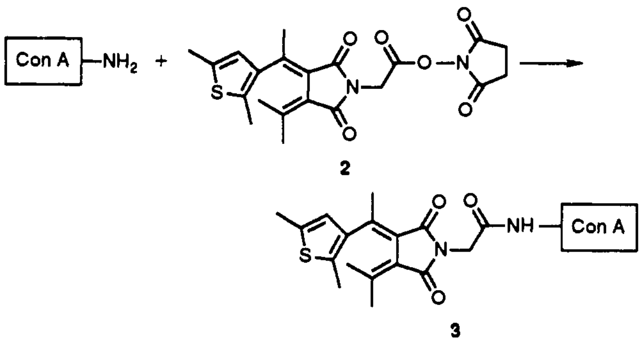

Table I. Association Constants of **4** to **2**-Modified Con A in Its Two Photochromic States (*E* and *C*) as a Function of Loading Degree by the Photochromic Material

loading degree[a]	dye configuration	$K_a \times 10^{-4}$ (M^{-1})[b]
0		2.2
6	*E*	1.64
6	*C*	2
9	*E*	0.78
9	*C*	1.21
12	*E* or *C*	0.64

[a] Loading degree is defined as the number of photochromic components anchored to each protein backbone. The total number of lysine residues in Con A corresponds to 12, and thus loading degree corresponds to the number of substituted lysine residues. [b] K_a = [(Con A)···(**4**)]/[Con A][**4**]. Association constants were determined by equilibrating Con A or **3**-*E* or **3**-*C* (30 μM) with 10 μM **4** in phosphate buffer solution (pH 7) of 0.0875 M, 0.1 mM $CaCl_2$, 0.1 mM $MnCl_2$, and 0.1 M NaCl at 20 °C. The protein was filtered off, and the concentration of free **4** was determined by HPLC (RP-18 column, eluent water/methanol, 50:50, at flow rate 1 mL/min, UV detection).

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