been analyzed kinetically. The hapten **1** may be considered a multi-substrate analogue of the reaction, while its phosphonyl group also simulates the characteristics of a transition state. Antibodies may be useful in distinguishing these possible attributes of enzymatic inhibitors. In the first case, the antibody would be acting as an "entropy trap",[15] while the weak binding of substrates and substrate analogues suggests it can selectively stabilize transition states. The poor binding of amides to mAb 17G8, as determined by inhibition experiments, may indicate that substrates bind in a destabilized conformation, requiring torsion about the scissile bond. The amide "resonance" makes this distortion energetically more costly for amides than for esters.[16] While **1** is not ideal as an analogue for the reaction investigated, the data show that antibody combining sites can accommodate two molecules in a chemically reactive complex at concentrations typical of enzymatic catalysis.[17] The precise mechanism of this reaction and the exploration of antibody catalysis for other bimolecular processes continue to be of interest in our investigations.[18]

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Ovothiols as Biological Antioxidants. The Thiol Groups of Ovothiol and Glutathione Are Chemically Distinct†

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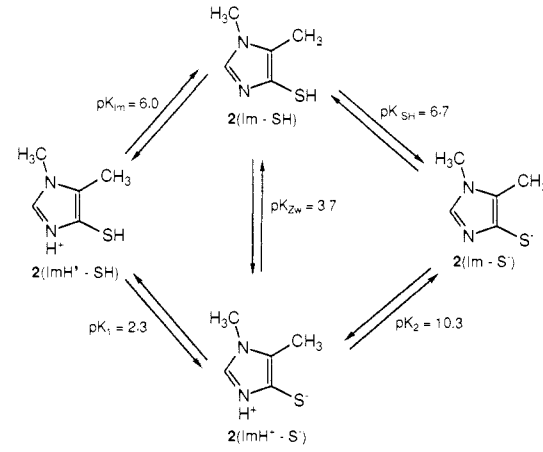
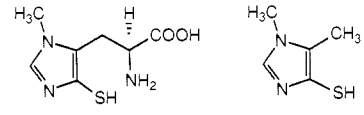
Mechanisms of action of biological antioxidants are of widespread interest because oxidative damage has been implicated in many disease states.[1] Shapiro et al. have suggested[2] that the ovothiols (e.g., **1**), a family of mercaptohistidines remarkably abundant (ca. 5 mM) in the eggs of marine invertebrates,[2,3] function as antioxidants. The presence in these eggs of both glutathione (ca. 2 mM)[4] and ovothiols suggests the possibility that these thiols may possess distinct antioxidant activities. Though

Scheme I



the antioxidant activity of aliphatic thiols such as glutathione has been widely discussed,[5] no comparison with aromatic thiols has been made.[6] The observation[7] that the 4-mercaptoimidazole **2**[3d] is at least 50-fold superior to glutathione in inhibiting the air oxidation of pyrogallol[8] led us to investigate differences between the thiol functions of glutathione and **2**; described herein are differences in protonation state, nucleophilicity, and one-electron donating ability that are relevant to the putative role of ovothiols as antioxidants.



1 **2**

Relevant pK_a data were measured for **2**, since thiol and thiolate functions differ in reactivity. Potentiometric titration of **2** in water afforded two macroscopic pK_a's of 2.3 and 10.3; the *S*-methyl derivative of **2** yielded a single pK_a of 6.0.[9] Assuming that the latter is identical with pK_{Im} (Scheme I), the pK_a's shown in Scheme I can be calculated.[10] In sharp contrast to the thiol group of glutathione, pK_a 8.65,[11] the pK_a of the thiol of **2** is 2.3, implying that marine invertebrates are in fact 5 mM in a thiolate anion! These data indicate that **2** exists predominantly (~99.9%) as the zwitterion **2** (ImH^+–S^-) at pH 7.[12]

The predominance of an aromatic thiolate function in **2** at pH 7 suggested that the 4-mercaptoimidazoles may be more nucleophilic than glutathione. The relative nucleophilicity of **2** and glutathione in phosphate buffered water (pH 7) at 23 °C was measured by competition for a deficiency of iodoacetamide. ^{1}H NMR analysis of the resulting mixture of thioethers indicated that the rate constants for thioether formation differ by a factor of 9 in favor of **2**.[13,14] These data are in qualitative agreement with the finding that at pH 7.2 ovothiol consumes hydrogen peroxide in a second-order process 5 times as quickly as does glutathione.[2]

The most striking difference between **2** and glutathione is in the kinetics of its reactions as a one-electron donor, as might be

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‡ Chevron Fellow, 1987.
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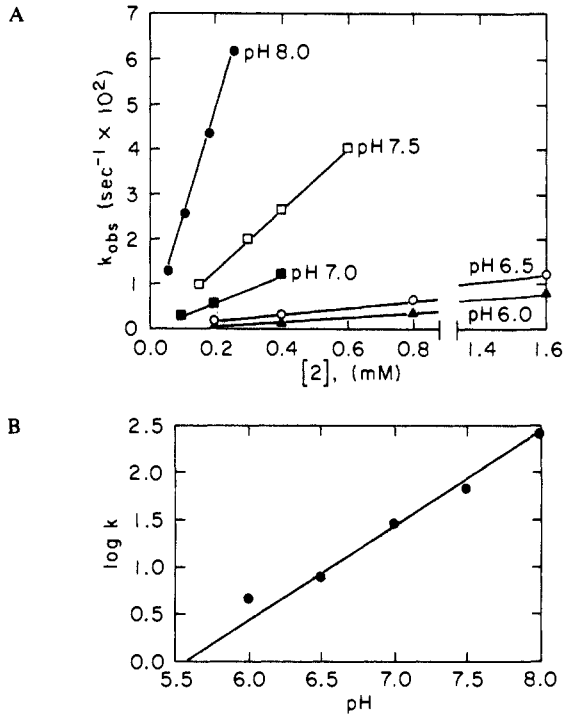

Figure 1. Reduction of ferricytochrome *c* with **2**. (A) Plot of pseudo-first-order rate constant (k_{obsd}) for the reduction of horse heart ferricytochrome *c* (Sigma, Type III, 8 μM at pH 6.0, 6.5, 7.5; 2 μM at pH 7.0, 8.0) by **2** vs [**2**] at 23 °C. Reactions were 0.10 mM in diethylenetriaminepentaacetic acid, buffered (ionic strength 0.05) with 2-*N*-morpholinoethanesulfonic acid (pH 6.0 and 6.5) or *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid (pH 7.0, 7.5, and 8.0), monitored at 550 nm for up to 2 half-lives. Rate constants were calculated from plots of ln ($A_\infty - A_t$) vs time; A_∞ was obtained by reduction with $Na_2S_2O_4$. (B) Plot of log second-order rate constant for reduction of ferricytochrome *c* by **2** vs pH. Data taken from (A). Indicated line is best line of slope 1 through the data points.

required of an antioxidant in the repair/destruction of free radicals. A preliminary survey indicated that **2** reacts at least one order of magnitude more rapidly than glutathione with several one-electron acceptors, including Fremy's salt,[15] Banfield's radical,[16] galvinoxyl radical,[17] and horse heart ferricytochrome *c*. The latter system was investigated in some detail.

The pseudo-first-order reaction of ferricytochrome *c* with excess **2** was studied as a function of pH and [**2**]. Unlike glutathione, whose reactivity with ferricytochrome *c* is negligible,[18] **2** reacts with ferricytochrome *c* at an appreciable rate. **2** reacts in a process that is first order in each of the two starting materials (Figure 1A). The second-order rate constant is strongly pH dependent in the range 6 to 8, a range in which the reduction potential of the cytochrome is known to be invariant;[19] a plot of the log of the second-order rate constant vs pH (Figure 1B) is linear with a slope of 1. Gel filtration afforded a 95% yield of the disulfide of **2**, identified and quantified by UV.

The simplest interpretation of the data in Figure 1 is that in the concentration range studied the mercaptoimidazole **2** reduces ferricytochrome *c* by outer-sphere single-electron transfer. The linearity of log *k* vs pH from pH 6 to 8 further indicates that the predominant reduction initiating species is the thiolate anion **2** (Im-S$^-$), not the zwitterion **2** (ImH$^+$-S$^-$).[20,21] If all reduced cytochrome *c* is attributed to this pathway, a rate constant of 4 × 10^4 M^{-1} s^{-1} can be estimated for the reaction of **2** (Im-S$^-$) with ferrocytochrome *c*.[22] We speculate that the superiority of **2** (relative to glutathione) as a one-electron donor has its origin in the thermodynamic advantage of forming an aromatic rather than aliphatic thiyl radical.[23]

In summary, the thiol groups of **2** and glutathione are chemically distinct. The mercaptoimidazole in solution at physiological pH is both more nucleophilic and more reactive as a one-electron donor, the latter despite the fact that the oxidation of 2 mole of thiol to one of disulfide is less favorable for ovothiol by 4 kcal.[2a] While generic differences between aliphatic and aromatic thiols account in part for these differences, the unusual pK_a's of the thiol and imidazole functions are also important in fine tuning the chemical reactivity of the mercaptoimidazoles. The antioxidant activities of ovothiol and glutathione will likely be significantly different from one another; the ovothiols warrant further investigation as biological antioxidants.

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$$RS^- + \text{cyt } c_{(ox)} \rightarrow RS^\bullet + \text{cyt } c_{(red)} \quad (1)$$

$$RS^\bullet + RS^- \rightarrow RSSR^{\bullet-} \quad (2)$$

$$RSSR^{\bullet-} + \text{cyt } c_{(ox)} \rightarrow RSSR + \text{cyt } c_{(red)} \quad (3)$$

(23) The best currently available estimate of the thermochemical superiority of benzenethiolate over methanethiolate anion in aqueous solution as a one-electron donor is 6 ± 3 kcal/mol, based upon the known solution-phase acidities and gas-phase S-H bond dissociation energies of the corresponding thiols.[24] This estimate does not account for any differences in solvation energy of the thiols and thiyl radicals, a difference that has been previously suggested to be small.[25]
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A Versatile Route to Diastereomeric Tungstenocene Complexes Containing Chiral Metal Centers[1]

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The availability of two adjacent coordination sites in bent tungstenocene complexes has resulted in the observation in this system of examples of many of the fundamental reactions of organometallic chemistry,[4] and mechanistic studies of these reactions would be facilitated by access to derivatives in which differentially substituted cyclopentadienyl ligands resulted in a prochiral or chiral metal center;[5] "the most valuable single type

(1) Dedicated to Professor E. J. Corey on the occasion of his 60th birthday, with gratitude for his continuing inspiration, stimulation, and scientific leadership.
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