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APPLICATION OF SEMIEMPIRICAL MOLECULAR ORBITAL TECHNIQUES TO THE STUDY OF PEROXIDASE-MEDIATED OXIDATION OF PHENOLS, ANILINES, SULFIDES AND THIOBENZAMIDES

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Abstract Reaction rates for a variety of enzymatically mediated oxidations were obtained and related to semiempirical (AM1) ionization potentials and molecular orbital structures. Highly significant correlations were generated for both vertical and adiabatic ionization potentials and horseradish peroxidase-mediated oxidation of substituted phenols, anilines and thioanisoles The highest occupied molecular orbital for all substrates were clearly π in nature and associated with significant contributions by component heteroatoms The results suggest that the oxidations considered proceed via an initial electron abstraction giving rise to a substrate radical or radical cation Finally, theoretical approaches were used in concert with experimental paradigms to define the mechanism of lactoperoxidase-catalyzed oxidation of thiobenzamides The sulfoxidative process was shown to proceed via a tightly coupled oxygen transfer from the enzyme to the substrate in which the initial step involved electron loss from the thiobenzamide In addition, the thiobenzamide S-oxide oxygen was found to be exclusively derived from the hydrogen peroxide and not from other sources

INTRODUCTION

Theoretical approaches including a variety of computational chemistry methods have been shown to be useful in examining enzymatically catalyzed reactions Specifically, semiempirical molecular orbital approximations have been used to assess substrate binding, product dissociation and the chemical transmutation inherent in enzymatic processes Thus, suggestions on the structure of the active site of an enzyme and the importance of various prosthetic groups

can be analyzed, evaluated and optimized. Kuthan, for example, investigated the structural requirements for NAD-associated oxidation of ethanol and reduction of acetaldehyde using semiempirical methods.[2] Other investigators have delved into the workings of charge transfer systems[3] and transition state structure.[4]

Another application of molecular orbital theory and one which has not been extensively applied is evaluation of the electronic properties of a substrate or series of substrates relative to the rate of their enzymatic conversions.[5,6] The relationships drawn from such comparisons can be informative in a number of circumstances including suggesting mechanisms of reaction, allowing one to narrow down a number of possible mechanisms and providing information on mechanism uniformity over a range of structural manipulations and reactivities. In the present communication, the all-valence electron AM1 semiempirical molecular orbital technique[7,8] was applied to the examination of several enzymatic oxidations including horseradish peroxidase- and lactoperoxidase-mediated transformations of various aromatic substrates The results were then used to suggest or support metabolic routes of oxidation

MATERIALS AND METHODS

AM1 calculations[7,8] were performed using either an IBM 3084 Model K dual processor computer (15 MIPS) or a Tektronix CAChe® computer-aided chemistry workstation. The Tektronix system contains an Apple® MacIntosh® IIx 68030 processor with a 68882 floating point coprocessor In addition, a Motorola 20 MHz 88000 RISC processor (17 MIPS) is incorporated into the Apple® platform to speed molecular orbital calculations The output is displayed on a 640 x 480 double-buffered 3-D stereo display For the IBM system, structural inputs were generated using a SYBYL/MOPAC interface Molecular geometries were obtained by minimizing the total energy of a system with respect to all structural variables using the standard Davidon-Fletcher-Powell optimization procedure in the case of the IBM system and the Broyden-Fletcher-Goldfarb-Shanno method in the case of the Tektronix® system For calculations on sulfur-containing molecules, the MNDO parameters for sulfur were imported into the AM1 framework Vertical ionization potentials were calculated using Koopman's theorem and adiabatic ionization potentials were obtained as the difference in energy (heat of formation, ΔH_f) between the generated radical cations and ground states Calculations of the radical cation energies were performed using the half-electron paradigm. Experimental data for the rate of horseradish peroxidase-medicated oxidation of phenols, anilines[6] and thioanisoles[9,10] were obtained from the literature Lactoperoxidase-mediated sulfoxidation of thiobenzamides were evaluated as part of the present study 4-Substituted thiobenzamides and the corresponding sulfoxides were

synthesized according to literature precedent[11,12] and extinction coefficients determined for the thiobenzamide sulfoxides (0.1 M phosphate buffer, pH 7.0). Lactoperoxidase was obtained from Sigma Chemical Co. and was analyzed spectrophotometrically at 412 nm as previously described [13] Initial rates of enzyme-catalyzed sulfoxidation were determined in duplicate by monitoring the absorbance at 340 nm using a Hewlett Packard HP8452A diode array spectrophotometer Studies utilized an initial concentration of 62 μm lactoperoxidase, 200 μm hydrogen peroxide and thiobenzamide concentrations ranging from 50-500 μm in 0.1 M (pH 7 0) phosphate buffer All determinations were conducted at 22°C. Second order rate constants were obtained by a linear least squares regression fit of pseudo first order rate constants and thiobenzamide concentrations.

RESULTS AND DISCUSSION

Peroxidases are heme-containing enzymes which catalyze the oxidation of various organic substrates at the expense of hydroperoxides.[14] Depending on the substrates involved and the conditions under which the reactions are carried out, a variety of mechanisms have been suggested to explain the accumulated experimental data. In the case of horseradish peroxidase-catalyzed oxidation of phenols and anilines, there is general agreement that the catalytic process begins with interaction of the native enzyme (HRP) with hydrogen peroxide to give an activated form of the enzyme known as compound I (HRP-1).[6,15,16] Compound I is associated with two oxidizing equivalents over that of HRP with electrons lost from iron (the valence state increase from +3 to +4) and the porphyrin ring system [17] In the latter case, a radical cation is produced Compound I then reacts with the phenol or aniline substrate (AH_2) resulting in the formation of a neutral radical (AH ·) and compound II (HRP-II) which is associated with one oxidizing equivalent over that of HRP which is localized on the iron atom This process appears to involve more or less simultaneous transfer of an electron and proton as Hammett sigma-rho studies do not indicate the build-up of significant positive charge in the transition state Compound II can subsequently interact with a second molecule of substrate and in the process regenerate the native HRP enzyme The two moles of $AH^{\cdot}$ produced can then dimerize to give A_2H_2 or react to give $A + AH_2$ These conversions are summarized in Scheme I below

Scheme I

$$HRP + H_2O_2 \xrightarrow{k_1} HRP\text{-}I$$

$$HRP\text{-}I + AH_2 \xrightarrow{k_2} HRP\text{-}II + AH\cdot$$

$$HRP\text{-}II + AH_2 \xrightarrow{k_3} HRP + AH\cdot$$

$$2AH \rightarrow A_2H_2 \text{ or } A + AH_2$$

As a number of studies with phenols and anilines have indicated that step 3 is rate-limiting, the interaction of HRP-II with various substrates has been examined to give some insight to the kinetics of the overall process [6,15,16] The above proposed mechanism suggests that reaction rate is dependent on a one-electron oxidation involving electron transfer from the phenol or aniline substrate to HRP-II The energy associated with electron removal from the highest occupied molecular orbital (HOMO) of the substrate should, therefore, correlate with the log of the reaction rate Two different types of ionization potentials were calculated using the AM1 semiempirical molecular orbital method Vertical ionization potentials were estimated based on Koopman's theorem which holds that the energy required to remove an electron from the HOMO is simply the negative of the HOMO energy Adiabatic ionization potentials were obtained as the difference in energy between the radical cation for a particular substrate and its closed shell ground state This latter parameter includes in its magnitude molecular relaxation subsequent to electronic ionization Other values which were estimated in this study included lowest unoccupied molecular orbital energies (LUMO), partial atomic charge distribution and HOMO/LUMO structure based on individual atomic orbital coefficient contributions

Correlation of AM1-derived vertical ionization potentials with the log of the reaction rate for HRP-II mediated oxidation (k_3) with a set of 18 substituted phenols[6] gave a highly significant linear correlation ($p < 0.001$) as shown in Figure 1 and Table I ($r = 0.871$) Use of adiabatic ionization potentials gave similar results ($r = 0.831$) In addition, a plot of LUMO virtual orbital energies and log (k_3) was linear ($r = 0.86$) Correlation coefficients were lower when the energies associated with either proton loss ($r = 0.732$) or hydrogen atom abstraction ($r = 0.647$) were related to reaction rate (Table I) Analysis of a set of 10 substituted anilines gave similar results (Table II) with significant relationships generated between log (k_3) and vertical ($r = 0.934$) (Fig 1) and adiabatic ($r = 0.935$) ionization potentials Attempts to link reactivity to atomic partial charge distribution (Table I) were interesting in that a plot of reaction rates and nitrogen partial charge in the aniline series produced a significant ($r = 0.84$) relationship characterized by a positive slope By contrast, phenolic oxidation rates were negatively correlated with oxygen partial charge ($r = 0.68$) It appears, therefore, that there is no consistent effect of atomic partial charges on the process of the enzymatic reactions considered Finally, the AM1 approach suggested that the anilines and phenols examined had π-type HOMO's with significant atomic orbital contributions by the $2p_z$-orbitals centered on both the aromatic carbon atoms as well as the heteratomic components as indicated by the equations below

$$\Psi^{HOMO}_{Phenol} = -0.476\ C\text{-}1\ 2p_z - 0.326\ C\text{-}2\ 2p_z + 0.254\ C\text{-}3\ 2p_z + 0.532\ C\text{-}4\ 2p_z + 0.178\ C\text{-}5p_z - 0.374\ C\text{-}6\ 2p_z + 0.384\ O\text{-}7\ 2p_z$$

$$\Psi^{HOMO}_{Aniline} = +0.398\ C\text{-}1\ 2p_z + 0.362\ C\text{-}2\ 2p_z - 0.170\ C\text{-}3\ 2p_z - 0.485\ C\text{-}4\ 2p_z - 0.170\ C\text{-}5\ 2p_z + 0.362\ C\text{-}6\ 2p_z - 0.492\ N\text{-}7\ 2p_z$$

The data generated are wholly consistent with the proposed mechanism in Scheme I and indicate that electron loss is the rate-limiting or predominantly rate-limiting step in the oxidation These results are also in general agreement with those obtained using other theoretical techniques Hosoya, for example, found good correlation between enzymatically catalyzed reaction rates and CNDO/2 HOMO energies although the relationships generated were characterized by a lower correlation coefficient than those observed in the present investigation [5] Thus, a set of 33 substituted anilines gave an r value of 0 56 when similar rate-energy relationships were examined The same group later used *ab initio* methods at the STO-3G level of sophistication and improved the correlation for anilines to r = 0 989 even though phenolic derivatives were somewhat less well-behaved (r = 0 641) [6] The higher correlation obtained using the AM1 technique compared with minimal basis set *ab initio* methods is not surprising based on reports that the AM1 model compares favorably with the 6-31G* method in terms of its accuracy but requires a fraction of the computational effort [8]

Early reports of Bordeleau and Bartha indicated that charge density may be an important factor in affecting rates associated with fungal enzyme-catalyzed oxidation of anilines [18] The studies presented here, as well as those of Hosoya, show no consistent effect of reaction rate on charge distribution and tend not to support this speculation at least in terms of horseradish peroxidase Finally, the semiempirical tools employed here suggest that the anilines and phenols examined contained a π-type HOMO This observation is consistent with possible electron transfer from the substrate to the heme prosthetic group edge, but does not rule out direct substrate interaction with the d_{xz}-p_x hydridized iron-oxygen orbitals [5,17] These data corroborate the assigned mechanism for oxidation

of anilines and phenols in that reaction rates appear to be dependent on one-electron oxidation energies Furthermore, the changes in rates over a series of derivatives could not be correlated with changes in charge distribution

Table I Vertical and Adiabatic Ionization Potentials, Lowest Unoccupied Molecular Orbital (LUMO) Energies, Atomic Partial Charge on the Phenolic Oxygen, Heats of Formation Differences of the Phenoxide Anion and the Phenoxy Free Radical and the Log of the Rate Constant for Horseradish Peroxidase-mediated Oxidation[a] of a Series of Substituted Phenols

Substituent (R)	Vertical Ionization Potential (eV)	Adiabatic Ionization Potential (eV)	LUMO Energies (eV)	Atomic Partial Charge	$\Delta\Delta H_f$ $(ArO^-)^b$ (Kcal/mol)	$\Delta\Delta H_f$ $(ArO^-)^c$ (Kcal/mol)	Log k_o
H	9.115	8 651	0.397	-0.253	-18.73	37.66	5 498
p-CH_3	8 881	8.394	0.426	-0.253	-19.41	36.17	6.025
p-Cl	9 124	8.634	0 095	-0.248	-26.00	36.85	6.041
p-OCH_3	8 648	8.074	0.313	-0.253	-21.86	33.44	6.775
p-CHO	9 493	8.994	-0.446	-0.245	-34.02	39.60	3.722
p-CN	9 510	9.041	-0.413	-0.244	-34.45	39.25	3.966
m-CH_3	9 052	8.555	0 373	-0 253	-18.48	37.63	5.598
m-Cl	9.335	8.843	0.019	-0.248	-25.67	38.45	4.833
m-OCH_3	9 040	8.534	0.433	-0.249	-21.26	39.04	5.004
m-CHO	9 437	8.894	-0.536	-0.246	-26.04	38.63	3.659
m-CN	9 576	9.049	-0.514	-0 246	-29.51	39.02	2.167
m-OH	9.125	8.678	0.366	-0.248	-22.12	39.22	5.547
o-CH_3	8.996	8.510	0.370	-0 254	-19.00	36.53	4.910
o-Cl	9 259	8.778	0 030	-0.245	-24.43	37.91	5.645
o-OCH_3	8 789	8.211	0.387	-0 250	-20.53	35.65	5.446
o-CHO	9 495	8 965	-0.571	-0.246	-35 53	36.66	3.436
o-OH	8.885	8 374	0 297	-0 249	-23.98	35.99	5.639
o-CN	9 556	9 050	-0.509	-0 241	-32.75	39.69	2 940

[a]Ref. 6
[b]$\Delta\Delta H_f$ (ArO^-) = ΔH_f (phenoxide) - ΔH_f (phenol)
[c]$\Delta\Delta H_f$ $(ArO^\cdot)$ = ΔH_f (phenoxy free radical) - ΔH_f (phenol)

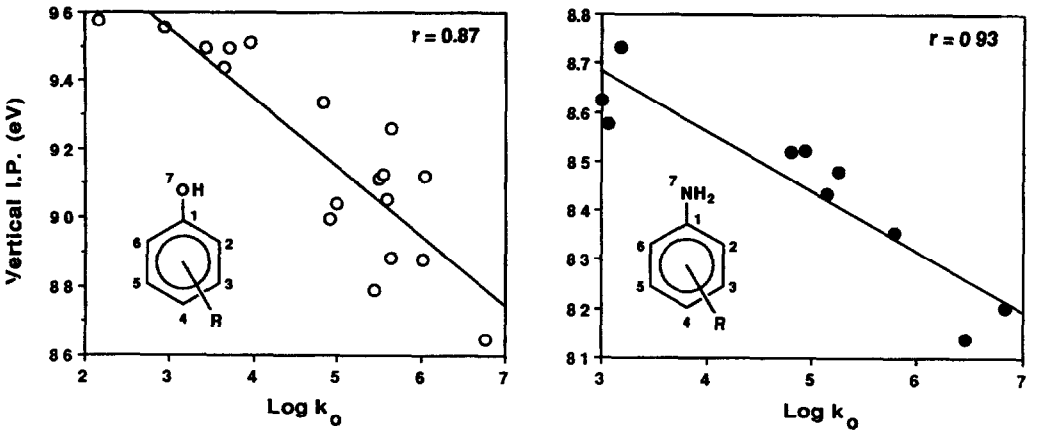

Figure 1 Correlation between horseradish and peroxidase-mediated oxidation of phenols (left panel) and aniline (right panel) and AM1-derived vertical ionization potentials.

Table II Vertical and Adiabatic Ionization Potentials, Lowest Unoccupied Molecular Orbital (LUMO) Energies, Atomic Partial Charge on the Aniline Nitrogen and the Log of the Rate Constant for Horseradish Peroxidase-Mediated Oxidation [a] of a Series of Substituted Anilines.

Substituent (R)	Vertical Ionization Potential (eV)	Adiabatic Ionization Potential (eV)	LUMO Energies (eV)	Atomic Partial Charge	Log k_o
H	8 522	7 866	0 639	-0.327	4 934
p-CH_3	8 356	7 676	0.615	-0 325	5 785
p-Cl	8 577	7 919	0 292	-0 330	3 064
p-OCH_3	8 203	7 434	0.517	-0.317	6 824
m-CH_3	8 479	7 769	0 605	-0 326	5.253
m-Cl	8 732	8 061	0 263	-0 330	3.182
m-OCH_3	8 520	7 854	0 613	-0 331	4 797
o-CH_3	8 435	7.748	0 600	-0 325	5 140
o-Cl	8 625	7 985	0 285	-0 331	3 004
o-OCH_3	8 319	7 485	0 546	-0 313	6 462

[a]Ref 6

While the mechanism for the oxidation of substrates bearing a hydrogen at the reaction center is fairly straightforward, the possible routes by which horseradish peroxidase and other peroxidases catalyze the oxidation of organic sulfides are more diverse [19,20] These species lack a source of hydrogen which can be transferred to the enzyme and the necessary proton must be derived from water or another source. Perez and Dunford summarized the three possible mechanisms for horseradish peroxidase-catalyzed oxidation of sulfides[19] which included, first, reaction of compound

I (HRP-I) with the sulfur-containing substrate to result in transfer of the peroxide-derived iron-bound oxene species in a single step. This concerted two electron process results in reduction of HRP-I to the native enzyme and production of the sulfoxide. A second possibility involves interaction of compound I with the substrate to give rise to the sulfur radical cation and the HRP-I one-electron reduction product, i e , compound II (HRP-II) HRP-II can then transfer the iron-bound oxygen to the radical cation either in the form of an anion or as the hydroxy radical This two-step process results in regeneration of HRP The third mechanism is a variation of the second in that HRP-I interacts with the sulfide to produce HRP-II and a radical cation. In this scenario, however, HRP-II interacts with a second molecule of substrate to give a second radical cation intermediate and the native enzyme The two moles of radical cation then disproportionate giving one mole of the regenerated sulfide and one mole of a sulfur dication which can react with environmental water to produce the sulfoxide and two protons These three processes are summarized below (Scheme II) and are designated as an (1) concerted oxygen transfer (involving an oxene intermediate), (2) sequential electron/oxygen transfer and (3) disproportionation mechanisms

Scheme II

Concerted Oxygen Transfer

$$HRP + H_2O_2 \xrightarrow{k_1} HRP\text{-}1 + H_2O$$

$$HRP\text{-}I + SR_2 \xrightarrow{k_2} HRP + S(O)R_2$$

Sequential Electron/Oxygen Transfer

$$HRP + H_2O_2 \xrightarrow{k_1} HRP\text{-}I + H_2O$$

$$HRP\text{-}I + SR_2 \xrightarrow{k_2} HRP\text{-}II + {}^{+}SR_2$$

$$HRP\text{-}II + {}^{+}SR_2 \xrightarrow{k_3} HRP + S(O)R_2$$

Disproportionation mechanism

$$HRP + H_2O_2 \xrightarrow{k_1} HRP\text{-}I + H_2O$$

$$HRP\text{-}I + SR_2 \xrightarrow{k_2} HRP\text{-}II + {}^{+}SR_2$$

$$HRP\text{-}II + SR_2 \xrightarrow{k_3} HRP + {}^{+}SR_2$$

$$2\ {}^{+}SR_2 \xrightarrow{k_4} SR_2 + {}^{++}SR_2$$

$${}^{++}SR_2 + H_2O \rightarrow S(O)R_2 + 2H^{+}$$

In applying semiempirical approaches to examine and possibly differentiate between these mechanisms, correlation of rate data with ionization potentials was again considered Analysis of reaction rate for the oxidation of 4-substituted thioanisoles by horseradish peroxidase and calculated vertical and adiabatic ionization potentials was completed[9,10] The results found significant

correlation in both cases with r = 0.71 when vertical ionization potentials were applied and r = 0.80 in the case of adiabatic ionization potentials. Importantly, when the energy associated with direct oxygen transfer was correlated to reaction rate, no significant relationship emerged (r = 0 4) (Table III) These electronic studies tend to support the mechanisms involving sequential single electron transfer, i e , those processes which result in sulfur radical cation formation, and not a single step concerted two-electron reaction The theoretical predictions appear to be borne out experimentally since HRP-II formation in the course of the reaction has been observed by spectrophotometric analysis [19] Furthermore, formation of sulfur radical cations as indicated by ESR spectroscopic studies in the oxidation of sulfides by cytochrome P-450 has been documented [21]

Table III Vertical and Adiabatic Ionization Potentials, Heats of Formation Differences of the Sulfoxides and the Log of the Horseradish Peroxidase-mediated Oxidation Rate Constants[a] for Sulfoxidation of *p*-Substituted Thioanisoles

Substituent (R)	Adiabatic Ionization Potential (eV)	Vertical Ionization Potential (eV)	$\Delta\Delta H_f$ ($ArS(O)CH_3$)[b] (kcal/mol)	Log k_o
Isopropoxy	8 11	8 33	10 99	-3.45
Methoxy	8 23	8.39	14 34	-3 60
Methyl	8 49	8 52	15 17	-3 87
H	8 65	8 65	15 36	-4 42
Chloro	8 73	8 75	15 79	-3 75
Cyano	9 03	9 02	16 69	-4 36
Nitro	9 41	9 41	17 84	-4 35

[a]Ref 9 and 10
[b]$\Delta\Delta H_f$ ($ArS(O)CH_3$) = ΔH_f(Sulfoxide) - ΔH_f (Sulfide)

Unfortunately, distinguishing between a sequential electron/oxygen transfer mechanism and disproportionation reactions using only electronic parameters is difficult and further experimental approaches are required In this context, and to demonstrate the power of combined use of theoretical and experimental techniques, the oxidation of thiobenzamides by lactoperoxidase was considered Lactoperoxidase is a useful model enzyme for the study of thyroid peroxidase, the catalyst responsible for synthesizing thyroid hormones [22-24] Thyroid peroxidase is an important biochemical target in the therapy of hyperthyroidism

Lactoperoxidase (LPX) is similar in many respects to HRP discussed herein but also has clear mechanistic differences For example, reaction of lactoperoxidase with hydrogen peroxide results in two forms of compound I (LPX-I) in which the oxidizing equivalent associated with LPX-I is centered either on the porphyrin system or on an amino acid present in the active site [17,25] In any

case, LPX catalyzed the sulfoxidation of organosulfur substrates in a manner similar to that induced by HRP.[13] Thus, three mechanisms. concerted oxygen transfer, sequential electron/oxygen transfer or disproportionation are possible.

The oxidation of a series of *p*-substituted thiobenzamides by lactoperoxidase was evaluated and obtained rate constants correlated with calculated ionization potentials as summarized in Figure 2 A highly significant relationship between vertical ionization potentials and the log of the oxidation rate constants was obtained with $r = 0.94$ Again, this finding is consistent with a rate-determining or partially rate-determining electron loss from sulfur to LPX giving rise to a sulfur-based radical cation Interestingly, vertical ionization potentials were found to correlate with experimentally derived electrochemical oxidation potentials [13]

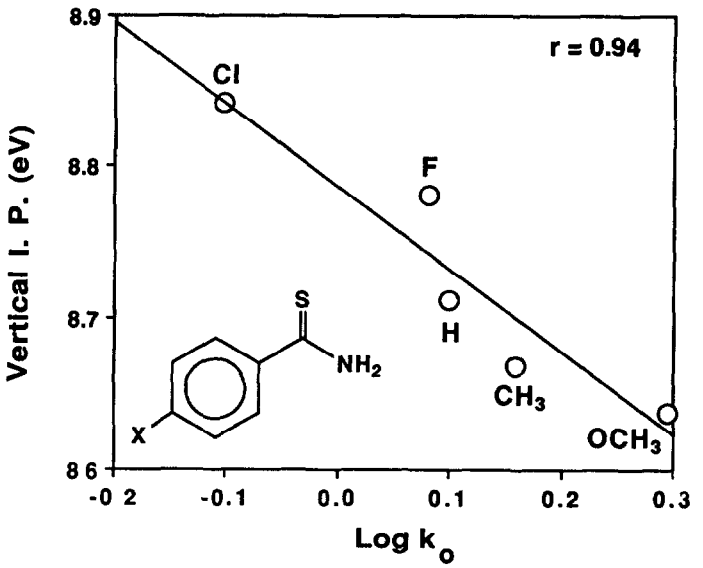

Figure 2 Correlation between lactoperoxidase-mediated sulfoxidation of *p*-substituted thiobenzamides and AM1-derived vertical ionization potentials.

These data suggest sequential electron transfer but other studies were required to specifically define the enzymatic process By using labelled hydrogen peroxide or water, it was possible to identify the source of oxygen in the sulfoxide.[26] Thus, significant label derived from hydrogen peroxide would indicate a tightly coupled rebound mechanism, i.e , a sequential electron/oxygen transfer scenario and oxygen obtained from water could be indicative of a disproportionation in which the sulfur dication forms the sulfoxide. Recent findings indicate that while little, if any, labelled water was incorporated in the thiobenzamide *S*-oxides subsequent to LPX-catalyzed oxidation, 98 1±2 0% incorporation was observed when labeled peroxide was used. The theoretical data suggest,

therefore, a multi-step mechanism involving a rate-determining electron transfer and labelling data suggests a tightly coupled oxygen rebound mechanism as the most likely possibility. Importantly, the isotope study, in and of itself, cannot differentiate between a single step oxene or a tightly coupled radical cage process In this case, experimental and theoretical approaches complement each other to provide mechanistic definition.

In conclusion, use of semiempirical molecular orbital methods can provide supportive information in studies of enzyme mechanism. Such assistance includes, in some cases, differentiation between single and multiple step reaction and whether component processes result in the formation of charged intermediates A caution, however, should be applied. Bruice found that in attempting to distinguish between radical or hydride-mediated oxidation of dihydropyridines, substituent and various electronic effects were not useful.[27] In this case, the product of oxidation is ultimately a pyridinium salt Hydride-mediated oxidation provides the quaternary species in one step while radical-induced oxidation proceeds through an electron-proton-electron transfer sequence in which the first electron loss is rate-determining or at least partially rate-determining Thus, the rate-limiting step in both processes involves the formation of a positively charged species, either a radical cation or a pyridinium salt, and substituents were found to have exactly the same effect on both systems In the examples presently under consideration, electronic semiempirical parameters are called upon to distinguish between a single step (oxene) process which results in the formation of a neutral sulfoxide or a multiple step mechanism in which sulfur radical cations are produced in the rate-limiting step

Finally, several studies have been published using Hammett sigma-rho techniques to evaluate enzymatic mechanisms of conversion with similar results of those reported herein [19] Henri-Rousseau and Texier have argued that when a series of substituted derivatives reacts with a common species, the difference in energy in the process depends on the frontier molecular orbital energy of the substituent [28] This suggests that Hammett sigma substituents are, to a first approximation, an indication of the difference in orbital energy imparted by a substituent and is consistent with the high degree of correlation between sigma values and HOMO energies The question then arises as to the advantage of using semiempirical methods over linear free energy relationships of the Hammett type Two points should be forwarded First, HOMO energies are specific, mensurate molecular characteristics correlation which have physical interpretations Second, Hammett analysis is limited to reaction schemes specifically designed to separate the substituent from the reaction center and therefore can be limited in its applicability In the ferricyanide-mediated oxidation of 3-substituted-1-methyl-1,4-dihydropyridines, for example, Hammett analysis was not possible but excellent correlation was observed between reaction rates and adiabatic ionization potentials [29]

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