bpy, and then, the spectrum converged to that of **1** at ~1.0 equiv of bpy. Furthermore, the spectrum of Figure 1c (0.6 equiv of bpy) was completely identical with the spectrum obtained when pure **1** and **2** were mixed so that the ratio Pd:bpy became the same. These observations support rapid equilibrium which mainly lies on the stable cyclic tetramer **1** as shown in Scheme I. It is noteworthy that *the thermodynamic cyclization realized quantitative formation of 1* without employing any special conditions such as high dilution.

A significant feature of this complex is its ability for molecular recognition in aqueous media.[12,13] When **1** was added to a D_2O solution of 1,3,5-trimethoxybenzene (**6**), high field shifts in ^1H NMR were observed for the signals of **6** ($\Delta\delta$ = 1.56 ppm for Ar *H*, 0.59 ppm for CH_3; [**1**] = [**2**] = 0.005 M, D_2O, 25 °C). The CPK model showed that **1** has an inner cavity (7.8 Å × 7.8 Å × 6.5 Å)[14] surrounded by π electrons of eight pyridine nuclei. The high field shift is most likely attributed to complexation in the cavity. Analysis of the chemical shift change ($\Delta\delta$ vs [**1**]/[**6**]) by the Benesi–Hildebrand[15] and nonlinear least-squares methods[12a] predicted that the complexation of **1** and **6** is 1:1 and the association constant (K_a) at 25 °C is 7.5×10^2 L mol^{-1}.[16]

(11) Fully characterized by ^1H NMR (270 MHz, D_2O).[7] Complex **3**: δ 2.59 (s, 8 H), 7.78 (d-like, *J* = 7.0 Hz, 4 H), 8.63 (d-like, *J* = 7.0 Hz, 4 H). Complex **4**: δ 2.57 (s, 8 H), 2.72 (s, 4 H), 7.70–7.75 (m, 8 H), 8.60 (d-like, *J* = 7.0 Hz, 4 H), 8.68 (d-like, *J* = 7.0 Hz, 4 H).

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(14) For the Pd–N bond length, 2.1 Å is adopted: Maitlis, P. M.; Espinet, P.; Russell, M. J. H. In *Comprehensive Organometallic Chemistry*; Wilkinson, G., Ed.; Pergamon Press: New York, 1982; Vol. 6, Chapter 38-1.

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(16) The K_a value roughly estimated by the Benesi–Hildebrand method[15b] was defined by the nonlinear least-squares method.[12a] Calculation was done according to the following equation: $\delta_{calcd} = ([\mathbf{1}]_0 + [\mathbf{6}]_0 + K_a^{-1} - (([\mathbf{1}]_0 + [\mathbf{6}]_0 + K_a^{-1})^2 - 4[\mathbf{1}]_0[\mathbf{6}]_0)^{1/2})(2[\mathbf{6}]_0)^{-1}(\delta_{complex} - \delta_{free}) + \delta_{free}$, where $[\mathbf{1}]_0$ and $[\mathbf{6}]_0$ are initial concentrations of **1** and **6**, respectively.

Selenosubtilisin as a Glutathione Peroxidase Mimic[†]

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There is considerable interest in the biosynthesis and mechanism of action of naturally occurring selenoenzymes, like glycine reductase and glutathione peroxidase.[1] The latter enzyme catalyzes the reduction of hydroperoxides by glutathione, thereby protecting mammalian cells against oxidative damage.[2] An active-site selenocysteine residue is essential for catalytic activity, but its mechanistic role is still debated.[2] We recently reported the preparation of an artificial selenoenzyme, selenolsubtilisin, which also contains a selenocysteine residue in its active site.[3] Here we report that this semisynthetic enzyme mimics key aspects of the chemistry of glutathione peroxidase.

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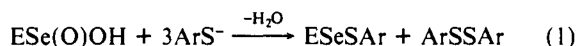
Selenosubtilisin was prepared by chemically converting the active-site serine (Ser 221) of the protease subtilisin into a selenocysteine.[3] Treatment of the freshly made selenoenzyme with hydrogen peroxide[4] leads to an oxidized form with the prosthetic group most likely in the seleninic acid oxidation state (ESe(O)-OH). The p*I* value of the oxidized protein is 5.7, considerably lower than that of the wild-type enzyme (7.8).[5] Moreover, the oxidized enzyme reacts with 3 equiv of 3-carboxy-4-nitrobenzenethiol (**1**) according to eq 1, in analogy to the reduction of benzeneseleninic acid by thiols.[6] In contrast to other alkaneseleninic acids with β-hydrogens which can syn eliminate,[2d] oxidized selenolsubtilisin is stable for months at 4 °C. Ongoing structural studies[7] are likely to show whether the protein provides specific stabilizing interactions for the seleninic acid moiety or sterically blocks the elimination pathway.



$$\mathrm{ESe(O)OH + 3ArS^- \xrightarrow{-H_2O} ESeSAr + ArSSAr} \quad (1)$$

Like glutathione peroxidase, selenosubtilisin catalyzes the reduction of alkyl hydroperoxides by thiols. The reduction of *tert*-butyl hydroperoxide (*t*-BuOOH) by 3-carboxy-4-nitrobenzenethiol (**1**) was studied in detail since it can be conveniently followed spectroscopically. Both the seleninic acid (ESe(O)OH)[4] and the selenenyl sulfide (ESeSAr)[6] forms of the enzyme substantially accelerate the rate of this reaction with multiple (>100) turnovers in processes that are first order in protein concentration. In both cases, enzymatic activity was observed to increase with decreasing pH, with the maximal rate occurring below pH 5.5.

The initial rates for the reduction of *t*-BuOOH by thiol **1** were determined as a function of substrate concentration at 25.0 °C and pH 5.5 by stopped-flow spectroscopy. These experiments were carried out by varying one substrate's concentration while keeping the other constant. Although the enzyme can be saturated by thiol, the kinetic behavior is complicated. On the other hand, typical Michaelis–Menten kinetics were observed with respect to the hydroperoxide. The apparent k_{cat} and $(K_m)_{t\text{-BuOOH}}$ values at 60 μM of thiol, for example, were determined to be 430 ± 10 min^{-1} and 160 ± 10 mM, respectively. As shown in Figure 1, the kinetic data at several thiol concentrations give characteristic parallel Lineweaver–Burk plots, indicating a ping-pong mechanism with at least one covalent intermediate.[8] Glutathione peroxidase behaves analogously,[9] and our observation that the oxidized enzyme reacts stoichiometrically with thiol to give an isolable selenenyl sulfide derivative is consistent with this kinetic pattern. Although further experiments are needed to characterize each of the intermediates in the catalytic cycle, the turnover reaction may proceed via the mechanism shown in Scheme I, which was

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(4) Freshly prepared selenosubtilisin was dialyzed exhaustively against 10 mM DTT, then against 20 mM H_2O_2, and finally against PIPES buffer (10 mM, pH 7.0). The resulting protein was homogeneous as judged by polyacrylamide gel electrophoresis.

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(6) See: Kice, J. L.; Lee, T. W. S. *J. Am. Chem. Soc.* **1978**, *100*, 5094. The stoichiometry of the enzymatic reaction was determined by monitoring the disappearance of thiol **1** spectroscopically at 412 nm in aqueous buffer (100 mM Mes, pH 5.0, 1.0 mM EDTA). Formation of 1 equiv of disulfide was verified by analytical reversed-phase HPLC and comparison with authentic samples. Moreover, as judged by the release of free **1** upon addition of DTT, ESeSAr was isolable in greater than 90% yield following treatment of the oxidized enzyme for 30 min at pH 5.0 with 3.1 equiv of thiol and gel filtration on G-25 Sephadex.

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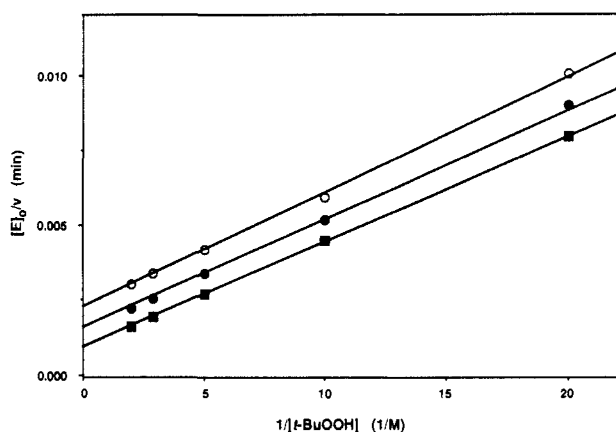
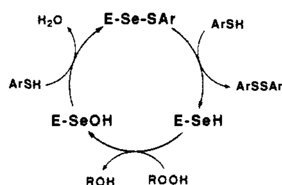


Figure 1. Lineweaver-Burk plots for reduction of *tert*-butyl hydroperoxide by 3-carboxy-4-nitrobenzenethiol catalyzed by selenosubtilisin (ESeSAr form) at 25.0 °C and pH 5.5 (100 mM MES buffer, 1 mM EDTA). The initial rates of the reaction were measured at 60 μM (○), 120 μM (●), and 400 μM (■) thiol **1**, respectively, by monitoring the disappearance of thiol at 412 nm. Enzyme concentration was determined by BCA titration using native subtilisin as a standard.[12]

Scheme I



originally proposed for glutathione peroxidase.[2] If this mechanism is correct, it should be possible to trap free selenol. Indeed, we found that treating the enzyme with excess iodoacetate in the presence of thiol results in complete loss of catalytic activity.[10]

To gauge the catalytic efficiency of selenosubtilisin, we compared the enzyme with diphenyl diselenide, a well-studied antioxidant.[11] At 25.0 °C and pH 5.5, the initial rate for the reduction of *t*-BuOOH (250 μM) by thiol **1** (100 μM) in the presence of 1 μM enzyme (ESeSAr form) is 1.5×10^{-6} M/min. Under similar conditions, but with 7.5 mM PhSeSePh as the catalyst, the initial rate is only 1.6×10^{-7} M/min. Thus, the protein binding site confers a rate advantage of at least 70000-fold to the artificial enzyme over diphenyl diselenide.

In short, we have shown that a semisynthetic selenium-containing enzyme catalyzes the reduction of *tert*-butyl hydroperoxide by an aryl thiol with much higher chemical efficiency than does a model selenium compound. Since the enzymatic reaction is mechanistically similar to the redox chemistry of glutathione peroxidase, further characterization of selenosubtilisin may lead to a better understanding of the natural system. Such information will be invaluable for the rational design of selenium-based antioxidants and practical redox catalysts.

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(10) Selenosubtilisin (ESeSAr form, 500 μM) was incubated with iodoacetic acid (50 mM) and treated successively with three portions of thiol **1** (5 mM) for 10 min each at room temperature and pH 5.0. Enzyme that was recovered after gel filtration on G-25 had no detectable redox activity. Enzyme similarly treated with iodoacetic acid but in the absence of thiol was recovered with full activity. For an analogous experiment with glutathione peroxidase, see: Forstrom, J. W.; Zakowski, J. J.; Tappel, A. L. *Biochemistry* **1978**, *17*, 2639.

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Metal-Promoted Binuclear C–H Activation of Ethylene and Formation of a Novel Heterobimetallic Ir–Pt Complex. X-ray Crystal Structure of $[(Ph_3P)_2(CO)Ir(\mu\text{-}H)(\mu\text{-}\eta^2{:}\eta^1\text{-}CH{=}CH_2)Pt(PPh_3)_2]^+CF_3SO_3^-$

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Heterobimetallic complexes containing asymmetric metal–metal bonds[1] as well as homogeneous C–H bond activation[2] by organometallic compounds are of considerable current interest largely because of their relevance to catalysis.[3] Although coordination of an alkene to transition metal systems is generally considered a necessary activation step in many catalytic and stoichiometric organometallic reactions,[4] little is known about alkene C–H bond activation[2] of precomplexed olefin substrates. In this paper we report the first intermolecular example of olefin C–H activation by a second, different metal system of a precomplexed π-ethylene transition-metal complex and the concomitant formation of a novel alkene-bridged heterobimetallic Ir–Pt complex.

Reaction of $(H_2C{=}CH_2)Pt(PPh_3)_2$ with 1 equiv of *trans*-$Ir(PPh_3)_2(CO)(CF_3SO_3)$[5] in CH_3NO_2 at room temperature for 2 h gave $[(PPh_3)_2(CO)Ir(\mu\text{-}H)(\mu\text{-}\eta^2{:}\eta^1\text{-}CH{=}CH_2)Pt(PPh_3)_2]^+CF_3SO_3^-$ (**1**) in 58% isolated yield (eq 1), as air-stable, bright-yellow crystals.[6] Compound **1** is very soluble in nitromethane, methylene chloride, and chloroform, but insoluble in hexane, benzene, and toluene. The structure of complex **1** was established

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(6) Data on **1**; IR (cm^{-1}, KBr) ν_{CO} 1981 s; ^1H NMR (δ, $CDCl_3$) 7.45–7.10 (m, 12 C_6H_5), 5.60 (m, μ-CH), 2.32 (m, μ-C*H*H′), 1.35 (m, μ-CH*H*′), −11.54 (m, $^1J_{Pt\text{-}H}$ = 515 Hz, Ir–H–Pt); $^{13}C\{^1H\}$ NMR (ppm, $CDCl_3$) 176.3 (d, $^2J_{C\text{-}P}$ = 12 Hz, CO), 141.8 (dd, $^2J_{C\text{-}P}$ = 85, 13 Hz, μ-CH), 135.6–127.8 (m, 12 C_6H_5), 121 (q, $^1J_{C\text{-}F}$ = 321 Hz, CF_3SO_3), 37.6 (br s, μ-CH_2); $^{31}P\{^1H\}$ NMR (ppm, $CDCl_3$, H_3PO_4) 21.3 (m, $^1J_{Pt\text{-}P}$ = 2383 Hz, P3 or P4), 13.6 (m, $^1J_{Pt\text{-}P}$ = 4633 Hz, P3 or P4), 9.4 (m, $^2J_{Pt\text{-}P}$ < 90 Hz, P1 or P2), 5.1 (m, $^2J_{Pt\text{-}P}$ = 160 Hz, P1 or P2); ^{19}F NMR (ppm, $CDCl_3$, $CFCl_3$) −77.9 (s); mp (gradual darkening) 179–182 °C dec. Anal. Calcd for $C_{76}H_{64}F_3IrO_4P_4PtS$: C, 55.61; H, 3.93. Found: C, 55.35; H, 3.95.

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