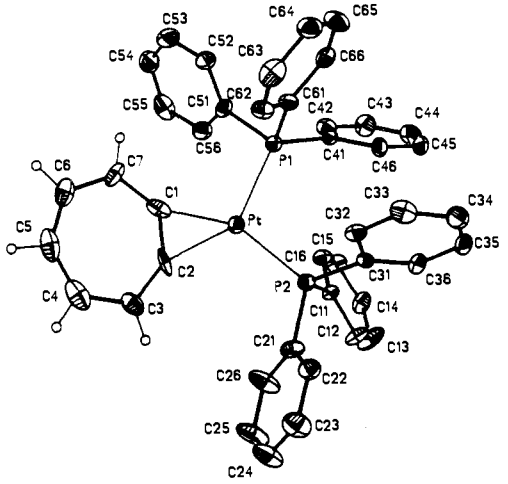


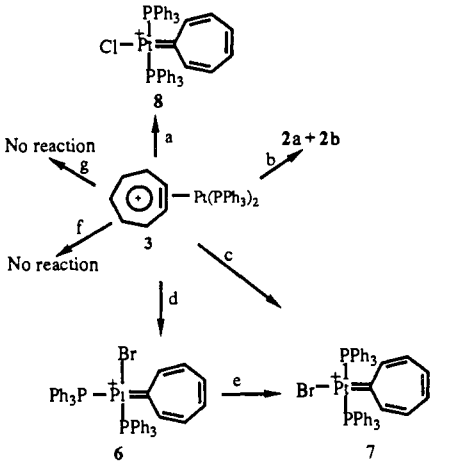
Figure 1. Thermal ellipsoid drawing of **3**, with 50% probability, showing the atom numbering scheme.

downfield than otherwise expected[14] [e.g., the Pt(II) complex **7** shows its Pt resonance at −4095 ppm],[13] and a reduction potential (−0.51 V vs Hg/HgCl) which is more negative than that of the tropylium ion (−0.29 V vs Hg/HgCl).[16]

Finally, the UV/vis spectrum of **3** shows a long wavelength absorption at 404 nm (log η = 3.44), 20 nm longer than the dienyne complex, suggesting communication between the platinum and the tropylium π-system. The crystal structure of **3** is shown in Figure 1. The seven-membered ring is planar [average atomic deviation from the least-squares plane is 0.02 (2) Å], and the platinum atom is at a distance of 0.274 (5) Å from this plane. It forms an angle of 7.8 (5)° with the plane containing Pt, C1, and C2. All C–C bonds in the seven-membered ring are equivalent within experimental error [bond average is 1.38 (2) Å]. This feature is also apparent in a Ni–benzyne complex[17] [bond averages: 1.367 (7) and 1.407 (7) Å] and a Zr–benzyne complex[18] [bond averages: 1.383 (9) Å]. However, it is in contrast to the short–long alternating C–C bonds observed in two Ta–benzyne complexes[19,20] and a Nb–benzyne complex.[20] The C–C triple bond [1.37 (2) Å] is longer than its counterparts in Pt–hexyne [1.297 (8) Å] and Pt–heptyne [1.283 (5) Å] complexes.[21] It is equivalent to the C–C triple bonds reported in the metal–benzyne complexes mentioned above. The Pt–C distances [2.00 (2) and 2.044 (13) Å] are similar to those in other metal–benzyne, metal–cyclohexyne, and metal–cycloheptyne complexes.[17-21]

The tropyne complex is relatively inert (Scheme II), showing no reaction with acetone or methanol at 70 °C for 12 h. It is also inert to acetonitrile at room temperature but, upon heating to 70 °C, is completely decomposed to a multitude of products in less than 2 h. It reacts cleanly and instantaneously with either HCl or HBr in THF to give Pt(II) complexes of cycloheptatrienylidene **7** and **8**;[13] in both cases only the trans isomer was detected. However, addition of HBr in CH_2Cl_2 gave first the cis-insertion product **6** (identified by ^1H and ^{31}P NMR), which slowly (ca. 4.8 h) isomerized to the trans isomer. The tropyne complex is also cleanly reduced to a mixture of **2a** and **2b**. Reductive coupling

Scheme II[a]



[a] (a) HCl in THF. (b) $KBEt_3H$ in THF. (c) HBr in THF. (d) HBr in CH_2Cl_2. (e) In CH_2Cl_2. (f) CH_3COCH_3, 70 °C, 12 h. (g) CH_3OH, 70 °C, 12 h.

reactions with alkenes and alkynes are under active investigation.

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Supplementary Material Available: Listings of details of the experimental procedures for the preparation of **2a**, **2b**, and **3**, ^1H, ^{13}C, 2D COSY, and C–H 2D NMR spectra of **3**, ^1H, 2D COSY, C–H 2D, and Pt–H 2D NMR spectra of **2a** and **2b**, and X-ray data for **3** (23 pages); table of observed and calculated structure factors for **3** (20 pages). Ordering information is given on any current masthead page.

EPR Evidence for Binuclear Mn(II) Centers in Rat Liver Arginase

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Arginase (L-arginine amidinohydrolase) catalyzes the hydrolysis of L-arginine to form L-ornithine and urea. A common feature of all arginases studied thus far is the requirement of divalent cations for activity. Mn^{2+} is the physiological cofactor, although activation of the enzyme by Co^{2+}, Ni^{2+}, Fe^{2+}, VO^{2+}, and Cd^{2+} has been reported.[1] The arginases from the *Agrobacterium* TiC58 plasmid,[2] *Neurospora crassa*,[3] and *Rhodobacter capsulatus* E1F1 cells[4] are specifically activated by Mn^{2+}, while the enzyme isolated from the thermophilic *Bacillus caldovelox* contains ≥1 Mn/subunit.[5] The function of the metal ion in the catalytic mechanism and/or structure of the protein is unknown, although recent work

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with the *Saccharomyces cerevisiae* arginase has implicated one catalytic Mn^{2+} and one structural metal, either Zn^{2+} or Mn^{2+}, per subunit.[6] Rat liver arginase is a manganoprotein which, as isolated, contains substoichiometric amounts of Mn(II) and displays catalytic activity that is proportional to the bound Mn(II) content.[7] Rat liver arginase with a full complement of Mn(II) and maximal catalytic activity is prepared by incubating the enzyme with millimolar concentrations of $MnCl_2$ at elevated temperatures.[7] In the present communication, we report that fully Mn-activated rat liver arginase contains spin-coupled Mn(II)–Mn(II) centers.

Arginase was purified from fresh rat livers using a modification of published procedures[8] that utilizes chromatography on columns of Amicon Green dye ligand media and carboxymethyl cellulose. Activity was measured with a radioactive assay[9] and protein concentration was determined spectrophotometrically from the absorbance at 280 nm.[10] The fully Mn-activated form of arginase was prepared by incubating the enzyme at 2 mg/mL in 50 mM Hepes[11]–KOH, pH 7.5, with 10 mM $MnCl_2$ at 60 °C for 10 min. Samples for EPR spectroscopy were prepared by concentration of the activated enzyme with an Amicon Centricon-30 concentrator. Free and loosely bound Mn(II) was removed from the samples by repeated dilutions of the concentrated protein with 50 mM Hepes–KOH, pH 7.5, followed by reconcentration using the Centricon. The stoichiometry of Mn(II) binding to the protein was determined by EPR analysis of perchloric acid extracts.[12]

At X-band frequencies (9.4 GHz) and a temperature of 0 °C, concentrated solutions of fully Mn-activated rat liver arginase do not give rise to discernible Mn(II) EPR signals, even at concentrations approaching 4 mM bound Mn(II). However, at 20 K the fully Mn-activated protein gives rise to the multiline spectrum shown in Figure 1A. Of particular interest in this spectrum are the regions centered around 575 G and 2000–3000 G. In contrast to mononuclear Mn(II) sites, which typically exhibit a hyperfine coupling constant of ~90 G,[13] the splitting between lines in these regions of the spectrum is ~45 G (inset to Figure 1A). Furthermore, these hyperfine structures consist of 11 lines, with relative intensities of approximately 1:2:3:4:5:6:5:4:3:2:1. These spectral features are characteristic of exchange-coupled Mn(II)–Mn(II) centers.[13,14] The weak narrow signal at 1565 G ($g \approx 4.3$) varies from sample to sample and is attributed to a high-spin ferric ion contaminant. Upon treatment of the arginase sample with perchloric acid, the characteristic six-line signal for the hexaaquo Mn(II) species is observed. Quantitation of Mn(II) in this sample provides a stoichiometry of 5.9 mol of Mn(II) bound per mole of enzyme trimer.[15] Thus, in the fully Mn-activated state, arginase contains two Mn(II) bound per subunit.

Further confirmation of the binuclear nature of the Mn(II) site of arginase is provided by the Q-band (33.8 GHz) EPR spectrum for the fully Mn-activated enzyme (Figure 1B). Measurements on multiple samples indicate that this spectrum is the superposition of spectra for two species: (i) a mononuclear Mn(II) site of low concentration, with a characteristic hyperfine coupling constant of ~90 G for the signal centered at $g = 2$, and (ii) an abundant binuclear Mn(II) center, with a hyperfine coupling constant of

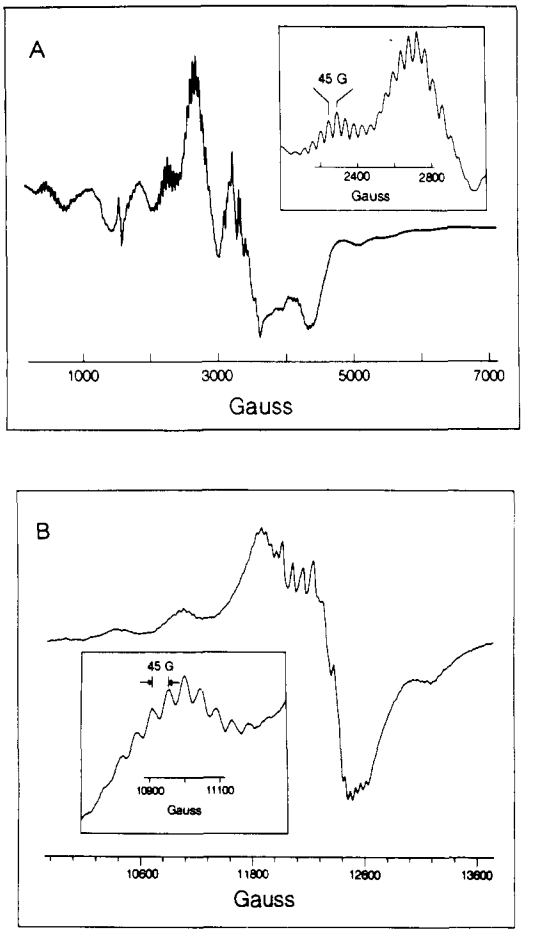

Figure 1. EPR spectra for Mn-activated arginase. (A) X-band spectrum at 20 K for a 51 mg/mL solution of arginase. EPR parameters: 9.45 GHz spectrometer frequency, 12.8 mW microwave power, 100 kHz modulation frequency, 4.9 G modulation amplitude, 3 accumulated scans. (B) Q-band spectrum at 155 K for a 54 mg/mL solution of arginase. EPR parameters: 33.8 GHz spectrometer frequency, 200 mW microwave power, 100 kHz modulation frequency, 12.6 G modulation amplitude, 4 accumulated scans.

~45 G. The spectral features due to the binuclear center are most apparent in the signals centered at 11 000, 11 600, and 12 500 G. The mononuclear signal likely results from the loss of Mn(II) from the binuclear site during preparation of the sample or a contaminant of free Mn(II).

The observed hyperfine interaction requires either that the two Mn(II) are bound sufficiently close for direct orbital overlap or that the metal ions are bridged by a common ligand. The coupling of two $S = {}^5/_2$ Mn(II) ions results in a manifold of new states with total spin S of 0, 1, 2, 3, 4, or 5. For antiferromagnetic coupling, the diamagnetic $S = 0$ spin state lies lowest in energy; in the case of ferromagnetic coupling the $S = 5$ spin state is lowest in energy. The presence of fine structure transitions displaying 11 lines separated by 45 G suggests that the exchange integral, J, is much greater than the hyperfine coupling constant. Analysis of the temperature dependence of the EPR signals can provide a value of J and establish the contributions of the various spin states to the observed spectra.[14] Ultimately, these analyses can provide an estimate of the distance separating the Mn(II) ions. These experiments are currently in progress.

The presence of a binuclear Mn(II) center in arginase raises intriguing questions concerning the function of this metal center in the catalytic cycle, particularly since catalysis by the related creatine amidinohydrolase has no apparent metal ion requirement.[17] Bimetallic centers have been documented for other

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enzymes that catalyze hydrolytic or reversible hydration–dehydration reactions, such as enolase[18] and leucine aminopeptidase.[19] For each of these enzymes the multinuclear metal center functions in substrate binding and in catalysis, suggesting that the binuclear Mn(II) center of arginase may have an analogous function. In addition, it is noteworthy that the X-band EPR spectrum for arginase shown in Figure 1A is remarkably similar to that reported for the Mn(II)–Mn(II) oxidation state of the Mn-catalase from *Thermus thermophilus*.[20] X-ray diffraction studies of the Mn-catalase indicate that the two metal ions are separated by 3.6 Å; however, protein ligands to the metal ions have not been identified.[21] Structure–function analysis of the binuclear Mn(II) center of arginase is the focus of our ongoing spectroscopic and crystallographic studies.

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Self-Assembled Multifunctional Receptors for Nucleotides at the Air–Water Interface

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We report here that multifunctional receptors specific for mononucleotides are spontaneously formed from guanidinium-functionalized monolayers and their combination with nucleobase-containing monolayers.

The recognition process of the artificial receptors for nucleotides and oligonucleotides is facilitated through multifunctional interactions which combine ionic pairing, aromatic stacking, and complementary hydrogen bonding.[1-4] Such multifunctional receptors may be realized more readily by utilizing self-assembly of amphiphilic molecules at the air–water interface. We established that Langmuir monolayers at the air–water interface served as powerful, selective hosts for a variety of biorelated water-soluble compounds via hydrogen bonding[5,6] or ionic pairing.[7] In particular, a guanidinium-functionalized monolayer of **1** specifically recognized phosphate units of AMP and ATP, with formation of the guanidinium/phosphate pair through ionic and hydrogen-bonding interactions.[7] We intended to achieve discrimination of different nucleotides by the addition of nucleobase monolayer components **2** and **3**.[8]

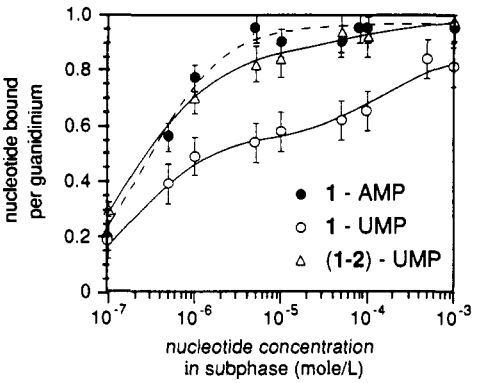

Figure 1. Nucleotide binding to monolayers of **1** and **1–2**. Solid lines are theoretical adsorption curves obtained by using K and α values from Table I.

Table I. Binding of Mononucleotides to Guanidinium-Functionalized Monolayers

amphiphile	substrate[a]	$10^6 \times K$,[b] M^{-1}	α[b]
1	AMP	3 ± 0.5	1.0
1–2	AMP	nonspecific[c]	
1–3	AMP	8 ± 2	0.7
4	AMP	3 ± 1	1.0
1	UMP	5 ± 1	0.6
1–2	UMP	5 ± 2	0.9
1–3	UMP	6 ± 2	0.6
4	UMP	2 ± 1	1.0

[a] AMP (Oriental yeast, 99%) and UMP (Sigma, 98%) were used as received. [b] The adsorption equation is applied to the concentration range where simple substrate saturation is observed. [c] Binding saturation is not observed within the concentration range of this study.

Monolayer characteristics of **1** have been described briefly.[7] Monolayers consisting of equimolar mixtures of **1** and **2** and of **1** and **3** showed good mixing behavior as inferred from nonlinear changes in surface pressure with component ratios.

Monolayer-bound nucleotides were determined by XPS analyses of LB films transferred from the aqueous nucleotide-laden subphase (10^{-7}–10^{-3} M^{-1}).[5-7] A saturation phenomenon in the binding curve (Figure 1) indicates the presence of a specific binding site. Binding constants, K, and the fraction of the occupied guanidinium sites at saturation, α, were determined by fitting these binding data to a general adsorption isotherm via iteration[10] and are given in Table I.

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$$n = \alpha[S]/(1/K + [S])$$

where n is the number of substrate bound per guanidinium group, α is the number of substrate bound per guanidinium group at saturation binding, [S] is the substrate concentration in the subphase, and K is the binding constant. Marshall, A. G. *Biophysical Chemistry: Principles, Techniques, and Applications*; John Wiley & Sons, Inc.: New York, 1978.

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