CUP2 spectrum also closely resembles that of the Cu(I) cluster in *N. crassa* metallothionein as well as those of several other metallothioneins,[22-24] most notably the *Saccharomyces cerevisiae* yeast metallothionein itself.[4] The edge energy position, along with the absence of a 1s → 3d transition, further establishes that it is indeed a Cu(I), and not a Cu(II), cluster.[20]

Taken together, the results thus suggest that CUP2 contains Cu atoms arranged in a cluster bridged by S atoms, presumably donated by protein cysteines. The Cu-S distance (2.26 Å) and coordination numbers determined from EXAFS are consistent with the electronic structure indicated by the edge transition. It is furthermore consistent with findings from Cu-S model clusters, where two-coordinate Cu-S distances average 2.16-2.17 Å, trigonal coordination 2.25-2.28 Å, and tetragonal coordination 2.3-2.42 Å.[17,25] CUP2 thus joins the class of Cu-S cluster-containing proteins, established through EXAFS in the metallothioneins of *S. cerevisiae* yeast,[4] *N. crassa* fungus,[16] β-domain of rat liver,[22] a mixed Cu:Zn metallothionein from pig liver,[23] and possibly also canine liver[24] (although in this study, the Cu-S distance was 2.27 Å but the coordination number four).

It is thus remarkable that the CUP2 copper cluster seems to resemble that of the very yeast metallothionein protein[4] that it regulates. What is the functional advantage for CUP2 to be activated by formation of a copper cluster, instead of a simple mononuclear copper center? There are several possibilities. A small amount of copper is necessary for viability of yeast, but high concentrations are deleterious. Induction of metallothionein synthesis could be controlled to a fine degree of cooperative construction of a copper cluster in CUP2 . Thiolate-bridged clusters are characteristic of copper(I) chemistry and offer a way to enforce specificity for copper in metallothionein activation. Effective DNA binding might require more than one structural domain to be formed in a protein. Although the zinc finger is a DNA-binding domain formed around one metal ion, all zinc finger proteins that have been demonstrated to bind to DNA contain more than one finger, which likely act cooperatively. Support for this hypothesis is provided by the analysis of a variant CUP2 protein in which a single cysteine residue was substituted by a tyrosine.[26] This protein, which binds less Cu than the wild-type protein, is capable of interacting with only a part of the DNA sequence recognized by the wild-type protein. These findings were interpreted to suggest that the DNA-binding domain of CUP2 contains at least two functional units involved in sequence recognition.[26] Another possibility is that the metalloregulatory protein CUP2 is evolutionarily related to the protein whose synthesis it controls. The structural similarity of the copper(I) clusters in yeast metallothionein[4] and CUP2, as we have demonstrated in this paper, makes this an attractive prospect.

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"Cation-like" Homogeneous Olefin Polymerization Catalysts Based upon Zirconocene Alkyls and Tris(pentafluorophenyl)borane

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Lewis acid cocatalysts such as aluminum alkyls and methylalumoxane are ubiquitous components of several important classes of highly active group 4 alkyl-based (e.g., titanocene, zirconocene) olefin polymerization catalysts.[1,2] Although electrodialysis,[3] chemical trapping,[4] model synthetic,[5-7] XPS,[8] surface chemical,[9] NMR spectroscopic,[10] and theoretical studies[11] argue indirectly that the role of the Lewis acid is to promote (e.g., by alkide abstraction) the formation of unsaturated "cation-like" active centers (e.g., Cp_2MR^+), the exact structural nature of the catalyst–cocatalyst interaction has remained elusive. We report here the use of the strong Lewis acid tris(pentafluorophenyl)borane[12] for the first synthesis of stoichiometrically precise, isolable/crystallographically characterizable, highly active "cation-like" zirconocene polymerization catalysts.

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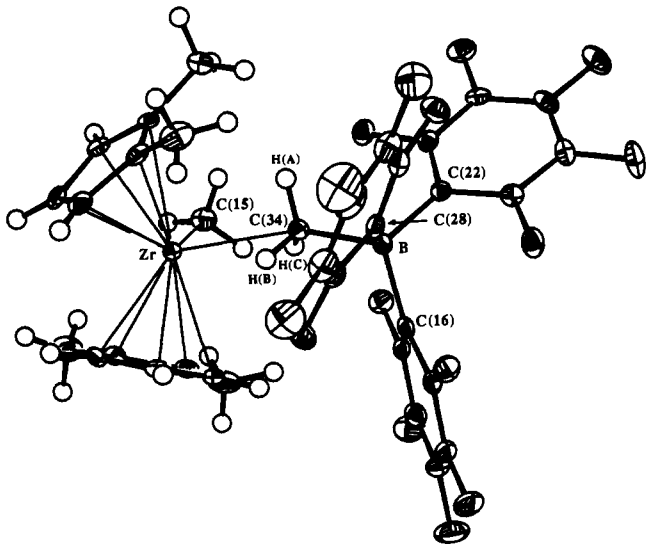

Figure 1. Molecular structure of $[1,2\text{-}(CH_3)_2C_5H_3]_2ZrCH_3^+$ $CH_3B(C_6F_5)_3^-$ (**2**). Important bond distances (Å) and angles (deg) are as follows: Zr–C(15) = 2.252 (4), Zr–C(34) = 2.549 (3), B–C(34) = 1.663 (5), Zr–H(34A) = 2.71 (3), Zr–H(34B) = 2.25 (3), Z–H(34C) = 2.30 (3), Zr–C_{ring} = 2.500 (1) (av), C(15)–Zr–C(34) = 92.0 (1), Zr–C(34)–B = 161.8 (2), ring centroid–Zr–ring centroid = 131.3 (1), C(16)–B–C(34) = 108.7 (3), C(22)–B–C(34) = 112.7 (2), C(28)–B–C(34) = 102.8 (3), C(16)–B–C(22) = 106.5 (3), C(16)–B–C(28) = 114.3 (3), C(22)–B–C(28) = 112.0 (3). Thermal ellipsoids are drawn at the 35% probability level.

The reaction of tris(pentafluorophenyl)borane with a variety of zirconocene dimethyl complexes proceeds rapidly and quantitatively (by NMR) to yield, after recrystallization from hydrocarbon solvents, methyltriarylborate complexes (eq 1). The

$$L_2Zr(CH_3)_2 + B(C_6F_5)_3 \xrightarrow[\text{or pentane}]{C_6H_6} L_2ZrCH_3^+CH_3B(C_6F_5)_3^- \quad (1)$$

1: L = η^5-C_5H_5
2: L = η^5-1,2-$(CH_3)_2C_5H_3$
3: L = η^5-$(CH_3)_5C_5$

new compounds have been characterized by standard analytic/spectroscopic techniques.[13] Particularly telling in the latter are quadrupolar-broadened $^1H/^{13}C$ NMR spectral features assignable to a $CH_3BR_3^-$ group[14] and low-field $Zr^{13}CH_3$ signals previously associated with "cation-like" species.[5,9] The diastereotopic ring CH and CH_3 signals in **2**[13] indicate loss of the time-averaged ring centroid–Zr–ring centroid mirror plane in eq 1. Regarding the lability of B–CH_3 complexation, NMR line broadening indicates $\Delta G^\ddagger$ = 18.7 (2) and 19.7 (2) kcal/mol (80 °C) for intramolecular Zr–CH_3/B–CH_3 interchange in **1** and **2**, respectively. However, as evidenced by the high-temperature interchange of the diastereotopic ring signals, inversion of the dissymmetric ion pair structure occurs at a slightly greater rate ($\Delta G^\ddagger$ = 18.3 (2) kcal/mol (80 °C)) in **2**.

The crystal structure of **2**[15] (Figure 1) consists of a "bent-sandwich" $[1,2\text{-}(CH_3)_2C_5H_3]_2ZrCH_3^+$ cation weakly coordinated to a $CH_3B(C_6F_5)_3^-$ anion via a nonlinear (161.8 (2)°), highly unsymmetrical Zr(μ-CH_3)B bridge. With the exception of a shortened Zr–C(15) bond (cf., 2.273 (5), 2.280 (5) Å in $Cp_2Zr(CH_3)_2$),[16,17] key aspects of the Zr coordination sphere such as the angle ring centroid–Zr–ring centroid and Zr–C_{ring}(av) are unexceptional (132.5° and 2.525 (12) Å, respectively, in $Cp_2Zr(CH_3)_2$[16,17]). The Zr–CH_3(bridge) distance is elongated by ca. 0.3 Å,[18] while the B–CH_3 distance appears to be normal,[14b] and the valence angles about B deviate only slightly from tetrahedral. The C(34) hydrogen atoms are bent away from B and toward Zr, with the closest Zr···H contact (2.25 (3) Å) exceeding typical terminal and bridging Zr–H bond distances (1.78 (2) and 1.94 (2), 2.05 (3) Å)[19] as well as a short Zr···H(C_{alkyl}) "agostic" distance (2.16 Å).[20]

Complexes **1–3** are active homogeneous catalysts for olefin polymerization. Using procedures described previously,[21] ethylene polymerization proceeds rapidly at 25 °C, 1 atm pressure, with N_t(**1**) ≈ 45 s^{-1} (~4.5 × 10^6 g of polyethylene (mol of Zr)$^{-1}$ h^{-1} atm^{-1}), roughly comparable in activity to typical zirconocene/alumoxane catalysts.[1f,2] The polyethylene produced is highly linear by NMR[22] with relatively high molecular weight ($\bar{M}_w$ = 124 000, $\bar{M}_n$ = 61 200).[23] With propylene[21] at 25 °C, **1** yields atactic (by NMR)[24] polypropylene with N_t(**1**) ≈ 4.2 s^{-1} ($\bar{M}_w$ = 15 600, $\bar{M}_n$ = 3000).[23] NMR experiments in which a toluene-d_8 solution of **1** was exposed to 10 equiv of propylene at −25 °C indicated that >70% of **1** undergoes olefin insertion under these conditions. This argues that the observed catalytic activity is not due to a minor component or impurity.

These results demonstrate the direct abstractive role of an organo-Lewis acid in the stoichiometric conversion of a zirconocene dialkyl to the corresponding "cation-like" zirconocene monoalkyl having high activity for homogeneous α-olefin polymerization.

(13) **1**: NMR (C_6D_6, 25 °C), ^1H, δ 5.37 (s, 10 H, Cp), 0.26 (s, 3 H, $ZrCH_3$), 0.10 (s, br, 3 H, CH_3B); ^{13}C, δ 148.63 (d, $^1J_{C-F}$ = 241.5 Hz, 6 C), 139.62 (d, $^1J_{C-F}$ = 247.9 Hz, 3 C), 137.57 (d, $^1J_{C-F}$ = 245.2 Hz, 6 C), 123.16 (br, 3 C), 114.01 (d, $^1J_{C-H}$ = 176.5 Hz, Cp_{ring}), 40.93 (q, $^1J_{C-H}$ = 122.6 Hz, $ZrCH_3$), 26.10 (s, br, BCH_3); ^{19}F, δ −135.54 (d, $^3J_{F-F}$ = 21.7 Hz, 6 F, *o*-F), −160.38 (t, $^3J_{F-F}$ = 21.0, 3 F, *p*-F), −165.89 (t, $^3J_{F-F}$ = 18.3 Hz, 6 F, *m*-F). Anal. Calcd for $C_{30}H_{16}B_1F_{15}Zr$: C, 47.20; H, 1.90; Found: C, 46.98; H, 1.90. **2**: NMR (C_6D_6, 25 °C), ^1H, δ 5.49 (t, $^3J_{H-H}$ = 2.8 Hz, 2 H), 5.23 (t, $^3J_{H-H}$ = 3.2 Hz, 2 H), 4.78 (t, $^3J_{H-H}$ = 2.8 Hz, 2 H), 1.51 (s, 6 H, CH_3), 1.20 (s, 6 H, CH_3), 0.08 (s, 3 H, $ZrCH_3$), 0.02 (s, br, 3 H, BCH_3); ^{19}F, δ −135.32 (d, $^3J_{F-F}$ = 23.2 Hz, 6 F, *o*-F), −160.78 (t, $^3J_{F-F}$ = 20.7 Hz, 3 F, *p*-F), −166.06 (t, $^3J_{F-F}$ = 18.7 Hz, 6 F, *m*-F). Anal. Calcd for $C_{34}H_{24}B_1F_{15}Zr$: C, 49.83; H, 2.95. Found: C, 49.69; H, 2.83. **3**: NMR (C_6D_6, 25 °C), ^1H, δ 1.37 (s, 30 H), 0.29 (s, 3 H, $ZrCH_3$), −0.30 (s, br, 3 H, BCH_3); ^{13}C, δ 124.01 (s, Cp), 50.36 (q, $^1J_{C-H}$ = 121.5 Hz, $ZrCH_3$), 14.34 (s, br, BCH_3), 11.02 (q, $^1J_{C-H}$ = 127.4 Hz, Cp-CH_3); ^{11}B (C_6D_6 + THF-d_8, 25 °C) δ −14.01. Anal. Calcd for $C_{40}H_{36}B_1F_{15}Zr$: C, 53.16; H, 4.01. Found: C, 53.07; H, 3.87.

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(23) We thank Dr. J. C. Stevens of Dow Chemical Co. for GPC measurements.

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Furthermore, they provide the first structural and molecular dynamics information on such a catalyst–cocatalyst ion pair and suggest that the scope of effective Lewis acid cocatalysts may be considerably broader than heretofore appreciated.

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Supplementary Material Available: Tables of atomic coordinates, anisotropic thermal parameters, and bond distances and angles for **2** (13 pages); listing of observed and calculated structure factors for **2** (29 pages). Ordering information is given on any current masthead page.

Electrically Wired Glutathione Reductase: A Biocatalyst for the Photochemical Reduction of Glutathione

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Electron transfer to (and from) redox sites of enzymes is inhibited by the insulating shell of the protein. Protein modification by electron relay components[1] and protein interaction with redox polymers[2] facilitate electron transfer to the active site by forming a "wire" that penetrates the insulating shell. Electrically wired biocatalysts (glucose oxidase and D-amino acid oxidase) were coupled to an electrode, and electron transfer was established.[1] Photosensitized electron transfer reactions in which the electron transfer between the photosystem and the active site of the enzyme is mediated by a redox polymer has recently been achieved with nitrate reductase by immobilizing the enzyme in a bipyridinium copolymer.[3] Unlike nitrate reductase, many other redox enzymes fail to establish electron transfer when immobilized in such a polymer, since the active site is shielded by a thick protein shell. This is the case with glutathione reductase. Here we report on a relay-modified glutathione reductase exhibiting electron-transfer properties. We show that the relay-modified enzyme interacts directly with excited species. We also demonstrate the improved biocatalytic performance of the relay-modified enzyme when it is immobilized in a redox polymer matrix. It should be noted that native glutathione reductase requires NAD(P)H as a cofactor for its catalytic activity. With the relay-modified enzyme, however, the cofactor is excluded and electrical wiring is maintained in the assembly.

Glutathione reductase (EC 1.6.4.2, Sigma type III from bakers' yeast) is modified by anchoring 4,4′-bipyridinium-1,1′-dipropionate,[4] PAV, to its lysine residues using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide, EDC, as the coupling agent.[5] Illumination ($\lambda > 420$ nm) of a photosystem that includes $Ru(bpy)_3^{2+}$ as a photosensitizer, EDTA as a sacrificial electron donor, and PAV-modified glutathione reductase in the presence of oxidized glutathione, GSSG, results in the formation of reduced glutathione,[6] GSH. Figure 1A shows the evolution of GSH in

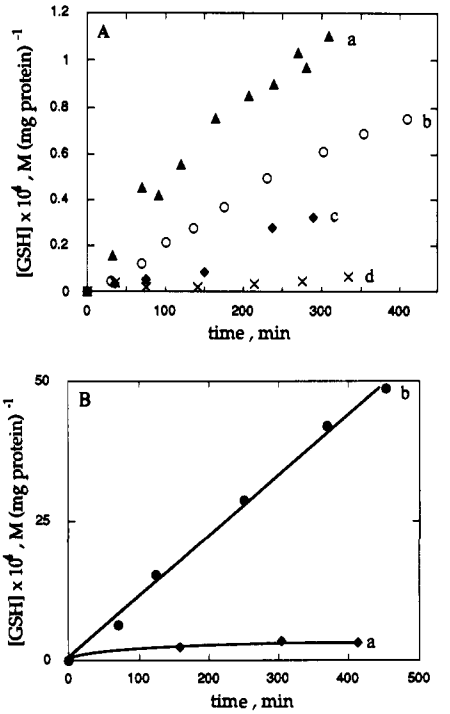

Figure 1. (A) Rate of GSH evolution (normalized per milligram of protein) under illumination ($\lambda > 420$ nm) in a photochemical system consisting of $[Ru(bpy)_3^{2+}] = 6.8 \times 10^{-5}$ M, [EDTA] = 0.01 M, [GSSG] = 0.01 M in 3 mL of 0.1 M phosphate buffer, pH 7.5. The loading degrees of PAV on the enzyme (mole/mole) in the various systems are (a) 3.9, (b) 1.8, (c) 1.4, and (d) 0.5. (B) Rate of GSH evolution (normalized per milligram of protein) in the photosystem described in part A: (a) in the system described in graph Aa; (b) in a photosystem composed of 1.66 g of the redox polymer which contains the immobilized relay-modified glutathione reductase.

photosystems that contain the modified enzyme at varying degrees of relay loadings.[7,8] It is evident that the higher the relay loading, the faster the reaction rate. Control experiments reveal that the biocatalyzed transformation does not occur in the dark, nor does it occur in the absence of any of the components constituting the photosystem. Laser flash photolysis experiments, following the excited-state lifetime of $Ru(bpy)_3^{2+}$ at different PAV-modified

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(5) A typical experiment involves dissolving 4.0 mg of PAV, 160 mg of HEPES (sodium salt), and 360 mg of urea in 3.2 mL of water. The solution is brought to pH 7.0 by adding 1 M HCl. Then 1 mL of the resulting solution is mixed with 1 mL of 0.05 M HEPES buffer solution, pH 7.1, containing 11.2 mg of the enzyme, and 13.3 mg of EDC is added. The reaction is stirred at 0 °C for 3 h, and the solution is dialyzed extensively against 0.1 M NaH_2PO_4 buffer, pH 7.5, until the enzyme solution does not contain any free PAV. The resulting solution contains 5.0 mg/mL of protein (assayed by the Biuret method), and the concentration of the protein-bound PAV is estimated to be 1.7×10^{-4} M, corresponding to 3.9 molecules of bound PAV/enzyme molecule. When lower loading values of the relay are desired, the concentration of PAV in the reaction mixture and the quantity of the coupling agent are decreased.
(6) The concentration of GSH is determined by Ellman's method; 1.9 mL of 0.1 M phosphate buffer, pH 7.6, and 0.1 mL of 5,5′-dithiobis(2-nitrobenzoic acid), BIS, reagent (1.6 mg of BIS/mL in 0.1 M phosphate buffer at pH 7.0) are added to 0.1 mL of the reaction sample. Absorbance at $\lambda = 412$ nm ($\epsilon = 13600\ M^{-1}\ cm^{-1}$) is measured after 2 min; cf.: McNiel, T. L.; Beck, L. V. *Anal. Biochem.* **1986**, *22*, 431.
(7) The concentration of the protein-bound PAV in the solution is estimated by the following method: 0.2 mL of the sample is diluted with 0.8 mL of 0.1 M phosphate buffer, pH 7.5. The solution is deaerated, and 5 mg of sodium dithionite is dissolved in it. The absorption at $\lambda = 602$ nm ($\epsilon = 11800\ M^{-1}\ cm^{-1}$) is measured after 5 min of further deaeration. The loading corresponds to the mole ratio of bound PAV to that of the protein; the molecular weight of glutathione reductase is taken as 118000; cf.: Colman, R. F. *Methods Enzymol.* **1971**, *17B*, 500.
(8) The PAV-modified glutathione reductase exhibits 72% of the activity of the native enzyme. The assay for this comparison consists of a photochemical system that includes $Ru(bpy)_3^{2+}$, 6.8×10^{-5} M, MV^{2+}, 1×10^{-2} M, GSSG, 1×10^{-2} M, and EDTA, 1×10^{-2} M, pH 7.5. Native glutathione reductase or the modified enzyme, 1.85 mg, is introduced into the system, and the formation of GSH is followed.

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