bonding MO[6] may be crucial for detecting such fluxionality. Attention to the nonbonding MO's of odd alternant polycyclic hydrocarbon ligands will open the way to explore further examples of stereochemical nonrigid behaviors.

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Ordering of Metal Chelates on the Basis of Bilayer Assembly

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We report herein strong intermetallic interactions of Cu(II) chelates formed at the surface of bilayer aggregates.

Synthetic bilayer membranes are two-dimensional arrays of molecules and are useful vehicles for ordering of part or whole molecules because of their structural flexibility. The molecular ordering produces various interesting properties. For instance, efficient energy migration among chromophores was implied in ammonium bilayers that contained anthracene[1] and carbazole[2] units, and large enhancements due to extensive dipolar coupling were observed in circular dichroism of chiral bilayer membranes.[3-5] These unique characteristics should be similarly applicable to preparation of ordered arrays of metal chelates.

Simon and others carried out extensive investigations on micellar and liquid-crystalline metal complexes[6,7] and found, among other results, a remarkable enhancement of the dioxygen stability constant for micellar cobaltous complexes.[8] We examined previously the interaction of metal ions with ligands at the bilayer surface, which caused subsequent changes of membrane physical states.[9,10] In these cases, however, the direct interaction of metal ions is not observed.

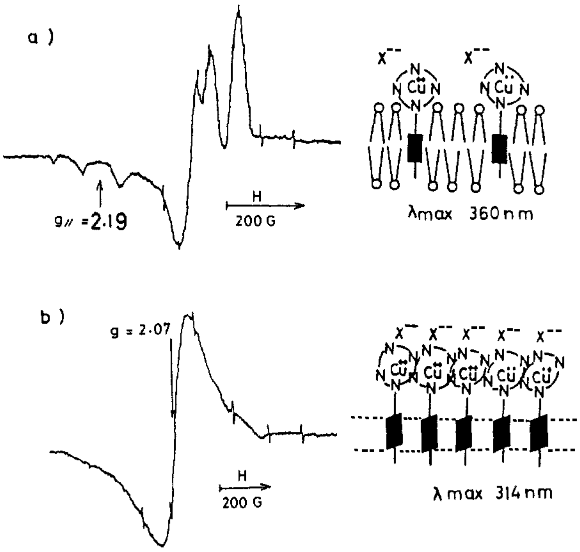


Figure 1. ESR spectra and schematic illustrations of Cu(II) chelates in the bilayer assembly. The ESR samples were prepared by quenching of aqueous bilayer dispersions by liquid nitrogen. (a) $[2C_{16}N^+2C_1]$ = 0.1 M; $[C_6AzoC_{10}\cdot 14N_4]$ = $[CuCl_2]$ = 1.0 mM. (b) $[C_6AzoC_{10}\cdot 14N_4]$ = $[CuCl_2]$ = 1.0 mM; [KCl] = 1.0 M.

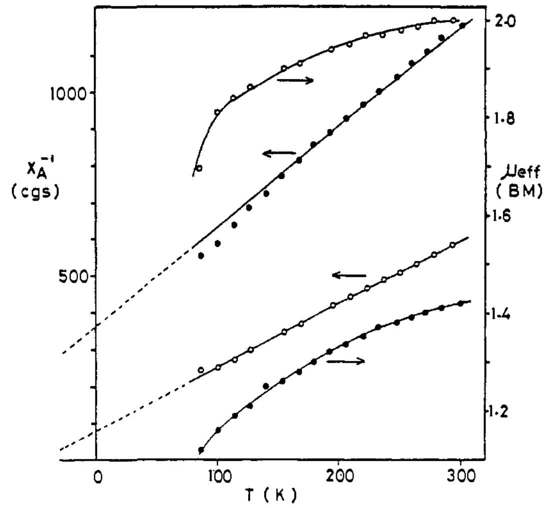
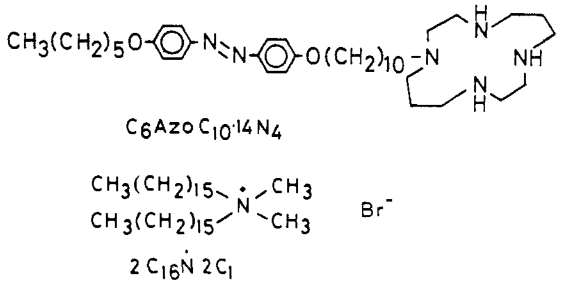


Figure 2. Temperature dependence of the magnetic property of bilayer powders: (○) $[(C_6AzoC_{10}\cdot 14N_4)_{1.0}Cu]Cl_2$; (●) $[(C_AzoC_{10}\cdot 14N_4)_{1.0}Cu]$-$SO_4$.

Chart I



We prepared a single-chain amphiphile $(C_6AzoC_{10}\cdot 14N_4)$[11] that contains the azobenzene moiety in the hydrophobic part and a cyclic tetramine (cyclam) as the hydrophilic head. The azobenzene unit was selected as reporter group, since its absorption spectra were shown to correlate with the membrane physical state[13,14] (Chart I).

The azobenzene amphiphile was dispersed in deionized water by sonication (Branson Cell Disruptor, 2 min, 40 W) together with

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(11) The azobenzene amphiphile was prepared from the corresponding bromide[12,13] and cyclam: NMR ($CDCl_3$) δ 0.93 (t, 3, CCH_3), 1.33 (m, 29, CCH_2C), 2.80 (m, 18, NCH_2), 4.03 (t, 4, OCH_2), 4.31 (m, 3, N-H), 6.95 (d, 4, aromatic), 7.85 (d, 4, Ar). Anal. Calcd for $C_{38}H_{64}N_6O_2\cdot 3H_2O$: C, 66.05; H, 10.21; N, 12.16. Found: C, 66.41; H, 9.63; N, 11.96.

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equimolar $CuCl_2$ and 1.0 M KCl. The dispersion (2-3 mM) contains rodlike aggregates with diameters of 30–40 Å and lengths of several hundred to 2000 Å, as confirmed by electron microscopy. Vesicles are not found. Differential scanning calorimetry gives an endothermic peak at 57 °C due to the gel-to-liquid crystal phase transition (T_c), as discussed in detail for related systems.[13] At room temperature the Cu(II)-containing bilayer gives λ_{max} at 314 nm, which implies the presence of the azobenzene cluster in the bilayer assembly. In the presence of 100 times excess of the matrix bilayer of $2C_{16}N^+2C_1$, the λ_{max} becomes located at 360 nm, irrespective of the physical state of the matrix (T_c = 28 °C). This indicates that there is no stacking among the azobenzene chromophore[14] and that the amphiphilic Cu(II) complex exists in the monomeric form in the bilayer matrix at all temperatures. These situations are illustrated schematically in Figure 1.

The electronic interaction of the Cu(II) complex was examined by ESR spectroscopy.[15] The aqueous dispersions were rapidly quenched from room temperature (293 K) to 77 K in liquid nitrogen. The monomeric Cu(II) complex in the $2C_{16}N^+2C_1$ matrix displays a typical anisotropic hyperfine splitting of copper ($g_{\parallel} = 2.19$, $|A|_{\parallel} = 200 \times 10^{-4}$ cm^{-1}), whereas the single-component bilayer gives a broad ESR signal (band width, ca. 800 G, $\bar{g}$ = 2.07). The latter spectrum results from the spin–spin interaction of the ordered Cu(II) ions. The results were the same when $CuSO_4$ was used in place of $CuCl_2$.

The metal–metal interaction in the $C_6AzoC_{10}\cdot 14N_4$–Cu^{II} bilayer was further examined by the measurement of magnetic susceptibility.[16] The powder samples were obtained by finely pulverizing cast bilayer films in order to avoid magnetic anisotropy. The structural similarity between aqueous bilayers and their cast films has been demonstrated for other azobenzene-containing bilayers.[17,18] Figure 2 shows the magnetic behavior of immobilized bilayers of the $C_6AZoC_{10}\cdot 14N_4$-Cu^{II} complex. Over the temperature range used in these experiments (80–300 K), the magnetic susceptibility obeys the Curie–Weiss law.

$$\chi_A(\mathrm{Cu}) = c/(T - \theta) \qquad (1)$$

where c is the Curie constant.

The Weiss constants (θ) are large and negative values: −44 K for $[Cu(C_6AzoC_{10}\cdot 14N_4)_{1.0}]Cl_2$ and −154 K for $[Cu(C_6AzoC_{10}\cdot 14N_4)_{1.0}]SO_4$. The θ values for ordinary Cu(II) complexes are 0 ± 10 K[19] and therefore, the θ value obtained for the bilayers suggests the presence of strong antiferromagnetic interactions among Cu(II) ions.

The effective magnetic moment was obtained from

$$\mu_{eff} = 2.828(\chi_A T)^{1/2} \qquad (2)$$

and its temperature dependence is plotted in Figure 2. The μ_{eff} of common Cu(II) complexes shows small temperature dependence (eg., for Cu(acetylacetone)$_2$, μ_{eff} = 1.89 μ_B at 90 K and 1.90 μ_B at 300 K).[19] In contrast, μ_{eff} of the bilayer powder increases considerably with temperature. This is again a strong indication of the antiferromagnetic interaction, such as reported for crystals of multinuclear metal chelates.[20,21]

In conclusion, the cooperative interaction of paramagnetic metal ions can be produced by complexation of metal ions with bilayer-forming ligands. The two-dimensional magnetic behavior has been studied for lamellar crystals and Langmuir–Blodgett films of manganese(II) alkanoates.[22-25] It is noteworthy, however, that the strong metal–metal interaction was observed for *aqueous dispersions* of metal/bilayer chelates. The interaction can be modulated readily by selection of appropriate metal/ligand combinations. Fundamental and application potentials of these systems are under investigation in these laboratories.

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[15] ESR spectra were measured by a JEOL JESME3 X-band spectrometer with 100-kHz magnetic field modulation. The magnetic field was calibrated by the splitting of Mn(II) in MgO.

[16] The molar magnetic susceptibility (χ_M) of the metal chelates was measured by the Faraday method in the temperature range 77–300 K. The molar susceptibility for Cu(II) ion (χ_A) was obtained by $\chi_A = \chi_M - \chi_L$, where χ_L is molar susceptibilities of ligands and counterions calculated by using Pascal's constants.

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Enzymatic Biotin-Mediated Carboxylation Is Not a Concerted Process

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The observation that the carboxylation of propionyl-CoA by the enzyme propionyl-CoA carboxylase to give *S*-methylmalonyl-CoA proceeds with retention of configuration at carbon, prompted Rêtey and Lynen[1] to propose that the transfer of the carboxyl group from *N*-carboxybiotin to propionyl-CoA involves a concerted electrocyclic transition state (Figure 1A). Later mechanistic[2-4] and spectroscopic[5] work supported the concerted route. In contrast, stepwise pathways in which a substrate proton is first abstracted and the resulting carbanion attacks either *N*-carboxybiotin[6] (Figure 1B) or CO_2 derived from it[7] (Figure 1C) have also been suggested, and these proposals (which avoid the steric and trajectory problems associated with the concerted route) are supported by work with a fluorinated substrate analogue[6] and by intermediate trapping experiments.[8] None of the experimental results, however, has distinguished unambiguously between the concerted and stepwise paths. We report here the use of the double isotope fractionation method[9,10] to make this distinction and find that *the carboxylation of pyruvate catalyzed by transcarboxylase*[11] *proceeds via a stepwise mechanism.*

Provided that substrate and product on–off steps are relatively rapid, the concerted mechanism involves the breaking of a carbon–hydrogen bond and the making of a carbon–carbon bond at the transition state, which will lead to both a ^2H and a ^{13}C primary kinetic isotope effect. A stepwise pathway requires at least one of these isotope effects but may show both if the two isotopically sensitive transition states (that for deprotonation, and that for carboxylation) are each partly rate-determining. If only one

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