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 × 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.

$$\chi_A(Cu) = c/(T - \theta) \quad (1)$$

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

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

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.

Acknowledgment. We are grateful to H. Yanagi for the preparation of an amphiphilic ligand and to Y. Hisaeda for his advice on the ESR measurement.

(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.

(17) Shimomura, M.; Kunitake, T. *Polym. J. (Tokyo)* **1984**, *16*, 187–190.
(18) Shimomura, M.; Kunitake, T. *Thin Solid Films* **1984**, *121*, L89–L91.
(19) Figgis, B. N.; Harris, C. M. *J. Chem. Soc.* **1959**, 855–860.
(20) Figgis, B. N.; Lewis, J. *Prog. Inorg. Chem.* **1964**, *6*, 37–237.
(21) Gruber, S. J.; Harris, C. M.; Sinn, E. *J. Chem. Phys.* **1968**, *49*, 2183–2191.
(22) Haseda, T.; Yamakawa, H.; Ishizuka, M.; Okuda, Y.; Kubota, T.; Hata, M.; Amaya, K. *Solid State Commun.* **1977**, *24*, 599–602.
(23) Pomerantz, M.; Aviram, A. *Solid State Commun.* **1976**, *20*, 9–11.
(24) Aranaz, D. M.; Lomer, T. R. *J. Phys. C* **1969**, *2*, 2431–2433.
(25) Asaolu, L. A.; Blott, B. H.; Khan, W. I.; Melville, D. *Thin Solid Films* **1983**, *99*, 263–264.

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

(1) Rêtey, J.; Lynen, F. *Biochem. Z.* **1965**, *342*, 256–271.
(2) Rose, I. A.; O'Connell, E. L.; Solomon, F. *J. Biol. Chem.* **1976**, *251*, 902–904.
(3) Prescott, D. J.; Rabinowitz, J. L. *J. Biol. Chem.* **1968**, *243*, 1551–1557.
(4) Moss, J.; Lane, M. D. *Adv. Enzymol.* **1971**, *35*, 321–442.
(5) Mildvan, A. S.; Scrutton, M. C.; Utter, M. F. *J. Biol. Chem.* **1966**, *241*, 3488–3498.
(6) Stubbe, J.; Abeles, R. H. *J. Biol. Chem.* **1977**, *252*, 8338–8340. Stubbe, J.; Fish, S.; Abeles, R. H. *J. Biol. Chem.* **1980**, *255*, 236–242.
(7) Sauers, C. K.; Jencks, W. P.; Groh, S. *J. Am. Chem. Soc.* **1975**, *97*, 5546–5553.
(8) Kuo, D. J.; Rose, I. A. *J. Am. Chem. Soc.* **1982**, *104*, 3235–3286.
(9) Belasco, J. G.; Albery, W. J.; Knowles, J. R. *J. Am. Chem. Soc.* **1983**, *105*, 2475–2477.
(10) Hermes, J. D.; Roeske, C. A.; O'Leary, M. H.; Cleland, W. W. *Biochemistry* **1982**, *21*, 5106–5114. Hermes, J. D.; Morrical, S. W.; O'Leary, M. H.; Cleland, W. W. *Biochemistry* **1984**, *23*, 5479–5488. Hermes, J. D.; Tipton, P. A.; Fisher, M. A.; O'Leary, M. H.; Morrison, J. F.; Cleland, W. W. *Biochemistry* **1984**, *23*, 6263–6275.
(11) EC 2.1.3.1, isolated from *Propionibacterium shermanii* (A.T.C.C. 9615), by the method of: Wood, H. G.; Chiao, J.; Poto, E. M. *J. Biol. Chem.* **1977**, *252*, 1490–1499. Grown according to: Leadlay, P. F. *Biochem. J.* **1981**, *197*, 413–419.

0002-7863/86/1508-0328$01.50/0

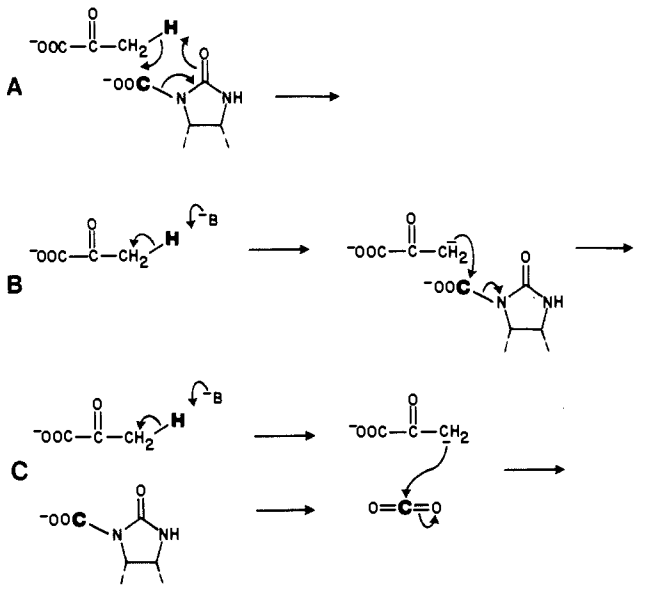

Figure 1. Pathways for biotin-dependent carboxylations. The carbon and the hydrogen directly involved in the isotope effects are shown in heavy type.

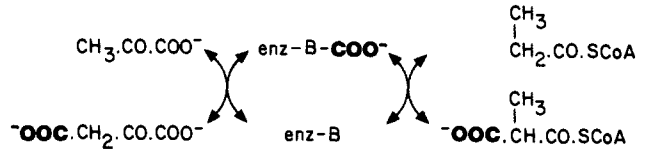

Figure 2. Reaction catalyzed by transcarboxylase. The enzyme is enz, and biotin is B.

isotope effect is observed, therefore, the reaction must be stepwise, but if both are seen, the reaction could be either concerted or stepwise. In 1975, Cheung et al.[12] showed that when pyruvate-d_3 is the substrate for transcarboxylase (Figure 2), a ^2H kinetic isotope effect is observed. We have now measured the ^{13}C isotope effect in the pyruvate carboxylation half-reaction[13] catalyzed by transcarboxylase to be 1.0227 ± 0.0007.[14] These results alone do not allow us to distinguish between concerted and stepwise paths. The distinction is possible, however, from a determination of the ^{13}C isotope effect using deuterated pyruvate. If the reaction is stepwise, deuteration of the pyruvate will slow the deprotonation step, making the subsequent carboxylation step less rate-limiting and thus lowering the observed ^{13}C isotope effect.[9,10,15] If the reaction is concerted, the overall reaction rate will be slowed by deuteration, but the ^{13}C isotope effect will remain unchanged.[9,10,15]

When pyruvate-d_3[16] is the substrate in the transcarboxylase reaction, the ^{13}C isotope effect is 1.0141 ± 0.001. That is, deuterium substitution in the pyruvate *reduces* the ^{13}C isotope effect from 2.3% to 1.4%, which means that *proton removal and carboxy group addition occur in different steps in the transcarboxylase-catalyzed reaction.* The effect of pyruvate deuteration on the ^{13}C kinetic isotope effect can be predicted[15] from the size of the observed ^{13}C isotope effect for protiopyruvate (1.0227), the measured deuterium isotope effect (1.4), and the ground-state fractionation factors for pyruvate[17] and the enzyme base that abstracts the proton.[15] The predicted value is 1.0136, which is gratifyingly close to our observed value of 1.0141. While the present experiments do not distinguish between the two stepwise paths (Figure 1B,C), the concerted pathway (Figure 1A) can, at last, be eliminated from further consideration.[18]

Acknowledgment. We are grateful to Joel Belasco for germinal contributions to the double isotope fractionation test, to Jeff Hermes and Peter Leadlay for helpful discussions, and to the National Science Foundation for support.

(12) Cheung, Y. F.; Fung, C. H.; Walsh, C. T. *Biochemistry* **1975**, *14*, 2981-2986. These workers obtained a deuterium isotope effect of 2.1 in V_{max} and in V_{max}/K_m. We have redetermined this quantity using pyruvate-d_3 under conditions similar to those used for the ^{13}C isotope effect measurement[13,14] and find a value of 1.4 ± 0.1. This discrepancy presumably derives from differences in pH, buffer, solvent composition, and cosubstrates.[14]

(13) To ensure that the measured ^{13}C isotope effect only relates to the half-reaction involving pyruvate carboxylation, the other half-reaction was maintained at equilibrium, and pyruvate was added at a rate such that pyruvate carboxylation was always at least 10 times slower than the carboxylation of acetyl-CoA.[14]

(14) Malonyl-CoA and acetyl-CoA were used for the second half-reaction instead of *S*-methylmalonyl-CoA and propionyl-CoA. The former substrates react about 0.7 times as fast as the latter pair (see also: Wood, H. G.; Jacobson, G., Gerwin, B. I.; Northrop, D. B. *Methods Enzymol.* **1969**, *13*, 215-230). The exchange rate between [^{14}C]acetyl-CoA and malonyl-CoA under the conditions used was at least 10 times faster than the rate of oxalacetate formation. Malonyl-CoA (250 μmol) and acetyl-CoA (250 μmol) were incubated with transcarboxylase[11] (20 units) at 30 °C for 30-45 min in 0.35 M potassium phosphate buffer, pH 6.8 (41.5 mL). The reaction was initiated by addition of pyruvate (6 μmol) and 1 μmol was added each minute for 44 min. The product oxalacetate was concomitantly converted to malate with NADH (125 μmol) and malate dehydrogenase (250 units). The reaction was followed by monitoring the change in absorbance at 340 nm. At the end of the reaction, malate was isolated by the procedure of O'Leary et al. (O'Leary, M.H.; Rife, J.E.; Slater, J.D. *Biochemistry* **1981**, *20*, 7308-7314), after removal of inorganic phosphate by precipitation as Li_3PO_4 and removal of excess Li^+ on an Amberlite IR-120(H^+) column. The purified malate was decarboxylated according to the procedure of: Hermes, J. D.; Roeske, C. A.; O'Leary, M. H.; Cleland, W. W. *Biochemistry* **1982**, *21*, 5106-5114. The CO_2 was isolated (O'Leary, M. H. *Methods Enzymol.* **1980**, *64*, 83-104). The ^{13}C:^{12}C ratio (corrected for ^{17}O content) was determined by isotope ratio mass spectrometry (Kruger Enterprises, Cambridge, MA).

(15) On the reasonable basis that each isotope fractionates independently of the other, we know that $[^{13}(V/K)_H - 1]/[^{13}(V/K)_D - 1] = {}^D(V/K)/{}^DK_{eq}$.[10] From the measured values of $^{13}(V/K)_H$ of 1.0227 and of $^D(V/K)$ of 1.4 and the knowledge of $^DK_{eq}$ (the deuterium starts in pyruvate with a fractionation factor of 0.84,[17] and ends up on an enzymic base with a presumed fractionation factor of 1.0), we can calculate the value of $^{13}(V/K)_D$ to be 1.0136.

(16) Cook, P. F.; Blanchard, J. S.; Cleland, W. W. *Biochemistry* **1980**, *19*, 4853-4858.

(17) Cleland, W. W. *Methods Enzymol.* **1980**, *64*, 104-125.

(18) Attwood and Cleland (Attwood, P. V.; Cleland, W. W., private communication) have recently arrived at the same conclusion for pyruvate carboxylase, by measuring the ^{13}C isotope effects for the enzymic decarboxylation of oxalacetate in solution in H_2O and in D_2O.

η^5 to η^3 Conversion in Indenyliridium Complexes

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Recently, several reports have suggested that ring slippage of cyclopentadienyl (Cp) metal complexes in which the Cp ring changes its bonding mode from η^5 to η^3 may be the mechanism by which a coordination site is opened in certain reactions.[1] Indenyl metal (IndM) complexes are more reactive for ligand substitution than their cyclopentadienyl analogues, presumably because the η^3 mode of bonding is stabilized by aromatization of the benzene ring.[2] This enhanced reactivity has been termed the "indenyl effect".[2] Even though there is a structurally characterized

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(1) (a) Rerek, M. E.; Basolo, F. *Organometallics* **1983**, *2*, 372. (b) Yang, G. K.; Bergman, R. G. *Organometallics* **1985**, *4*, 129. (c) Rest, A. J.; Whitwell, I.; Graham, W. A. G.; Hoyano, J. K.; McMaster, A. D. *J. Chem. Soc., Chem. Commun.* **1984**, 624.

(2) (a) Hart-Davis, A. J.; Mawby, R. J. *J. Chem. Soc. A* **1969** 2403. (b) Hart-Davis, A. J.; White, C.; Mawby, R. J. *Inorg. Chim. Acta* **1970**, *4*, 441. (c) White, C.; Mawby, R. J. *Inorg. Chim. Acta* **1970**, *4*, 261. (d) Jones, D. J.; Mawby, R. J. *Inorg. Chim. Acta* **1972**, *6*, 157. (e) Eshtiagh-Hosseini, H.; Nixon, J. F. *J. Less-Common Met.* **1978**, *61*, 107. (f) Rerek, M. E.; Ji, L.-N.; Basolo, F. *J. Chem. Soc., Chem. Commun.* **1983**, 1208. (g) Ji, L.-N.; Rerek, M. E.; Basolo, F. *Organometallics* **1984**, *3*, 740.

0002-7863/86/1508-0329$01.50/0