anion and reionize the enzyme for another catalytic cycle.

This mechanistic sketch offers a dynamic explanation for the catalytic steps of CO_2 hydration. A 4-coordinate $Zn^{II}OH$ is expected on the basis of model studies to be less basic than a similar 5-coordinate complex.[10] The approaching fifth ligand increases the basicity and nucleophilicity of the $Zn^{II}OH$, facilitating attack on the previously bound CO_2. Carbonic anhydrase can thereby retain in the resting state a hydroxide ligand of pK_b near 7 while utilizing catalytically a 5-coordinate hydroxide of increased nucleophilic power. The dilemma of assigning a weakly basic pK_b of 7 to the Zn-bound OH while simultaneously invoking its participation as a nucleophile is resolved by the proposed mechanism. Moreover, a fundamental connection is established for the first time between the anionic inhibition of carbonic anhydrase and its catalytic mechanism.

We believe that the observed high pH uncompetitive inhibition of the CO_2 hydration activity of carbonic anhydrase is not readily accomodated by any previously proposed mechanism of action of the enzyme. We therefore offer this new proposal for carbonic anhydrase catalysis. In a forthcoming work we shall demonstrate more fully the congruence of this mechanism with previous results.

Acknowledgment. We are grateful to the National Science Foundation, the National Institutes of Health, and the Muscular Dystrophy Association for partial support of this research. We are also grateful to Donald B. Moore for his diligient assistance in all aspects of computer interfacing and programming and Drs. Conrad T. O. Fong, Kenneth W. Raymond, and Simo Sarkanen for helpful discussions.

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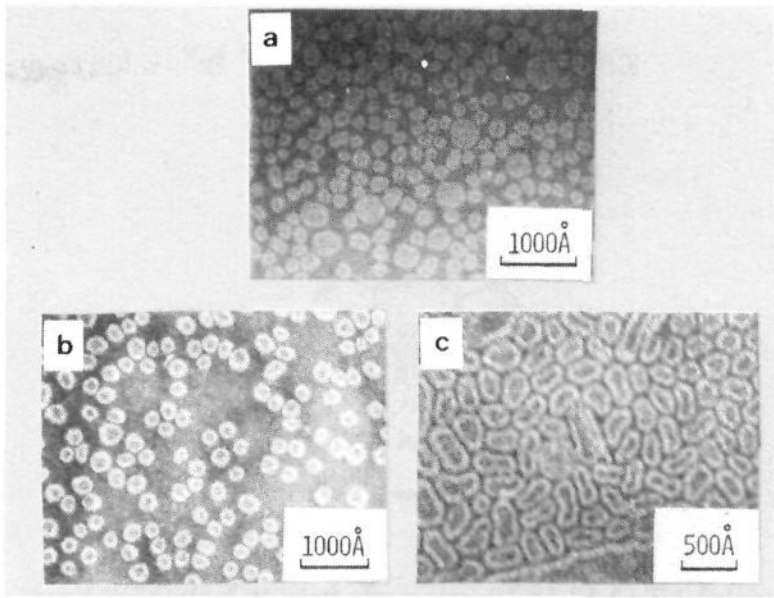

Figure 1. Electron micrographs of single-compartment vesicles negatively stained with uranyl acetate: (a) 5.0×10^{-3} M aqueous solution of **1** sonicated for 60 s with a probe-type sonicator at 30-W power (W-220F, Heat Systems-Ultrasonics) and allowed to stand for 10 min at 5 °C (magnification, ×75 000); (b) 5.0×10^{-3} M aqueous solution of **1** containing **2** (8.3×10^{-5} M) sonicated as above (magnification, ×64 000); (c) 5.0×10^{-3} M aqueous solution of **1** containing **2** (1.7×10^{-4} M) sonicated as above (magnification, ×107 000).

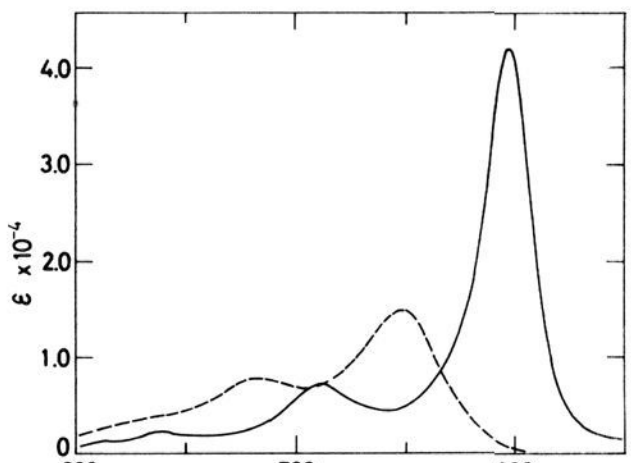

Figure 2. Electronic absorption spectra of **2a** (---) and **2b** (—) incorporated into single-compartment vesicles of **1** (1.0×10^{-3} M) in water containing KCN (5.0×10^{-4} M) at 20 °C.

Novel Substrate-Binding Property of Synthetic Membrane Vesicles Involving an Amino Acid Residue as a Molecular Component

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Currently, there is increasing interest in the behavior and characterization of naturally occurring[1] and synthetic bilayer membranes.[2] Although various probe techniques have been widely adopted to obtain information on the physical properties (microviscosity, micropolarity, fluidity, and so on) of these assemblies and particularly on the effects of their phase transitions thereupon,[3] there are only very scattered data as to the nature of hydrophobic substrate-binding characteristic of these bilayer assemblies. Recently, we have shown that amphiphiles involving an amino acid residue (Ala or His) interposed between a polar head group and an aliphatic double chain form *stable single-compartment vesicles* in aqueous media.[4,5] In the present work, we have studied the binding interaction of bilayer assembly, formed with cationic amphiphile **1**, with a hydrophobic Co(III) complex. The present bilayer aggregate provides two binding sites greatly different from each other in microenvironmental property. The substrate incorporated into these two sites is subjected to a novel distribution law, and its translocation between the two sites is practically prohibited due to the presence of a significant barrier provided at the so-called hydrogen-bonded region.

Amphiphile **1** (*N*,*N*-didodecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide),[6] like its zwitterionic analogue,[4] forms single-compartment vesicles with relatively uniform size (130–400 Å) in aqueous media upon sonication (Figure 1a). A Co(III) complex used here as a guest molecule is dicyano(8,12-

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(6) Prepared from reaction of $(CH_3)_3N$ with *N*,*N*-didodecyl-N^{α}-(6-bromohexanoyl)-L-alaninamide:[4] liquid crystal with final mp 195 °C; $[\alpha]^{20}_D$ −18.5° (*c* 0.91, EtOH); ^1H NMR ($CDCl_3$, Me_4Si) δ 0.88 (6 H, br t, $(CH_2)_{11}CH_3$), 1.25 (40 H, s, $CH_2(CH_2)_{10}CH_3$), 1.35 (3 H, s (sh), $CH(CH_3)$), ~2.00 (6 H, m, $NCH_2(CH_2)_3CH_2$), 2.26 (2 H, br t, $N(CH_2)_4CH_2CO$), 3.42 (9 H, s, $N^+(CH_3)_3$), 3.04–3.79 (6 H, m, $CH_2N^+(CH_3)_3$ and NCH_2-$(CH_2)_{10}CH_3$), 4.78 (1 H, br q, $CH(CH_3)$), and 7.04 (1 H, br d, NH). Anal. Calcd for $C_{36}H_{74}N_3O_2Br$: C, 65.42; H, 11.28; N, 6.36. Found: C, 65.07; H, 11.27; N, 6.09. Critical micelle concentration by surface tension method of the Wilhelmy principle, 1.5×10^{-5} M.

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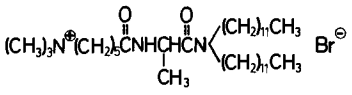

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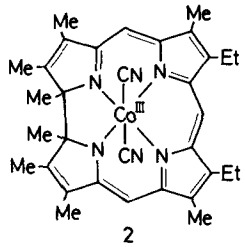

2

diethyl-1,2,3,7,13,17,18,19-octamethyltetradehydrocorrinato)cobalt(III) (**2**) which shows an intense intramolecular CT transition in a longer wavelength region, λ_{max} being sensitive to solvent polarity, extending from 739 nm in water to 789 nm in benzene, and the transition energy satisfactorily correlated with the solvent polarity parameter (Z value).[7] A dichloromethane solution of **1** and **2** was evaporated in vacuo to remove the solvent completely, water (3.03 mL) containing CN^- (5.0×10^{-4} M) was added to the residue, and the mixture [1.0×10^{-3} M in **1** and $(0.56–3.9)10^{-5}$ M in **2**] was subsequently sonicated to give a clear solution of single-compartment vesicles (Figure 1b,c). The electronic spectra indicated the presence of two spectrochemical species of **2**, one absorbing at 748 nm (**2a**) and the other at 796 nm (**2b**) (Figure 2). No species having intermediate spectral feature was detected. Both **2a** and **2b** are bound to the vesicular system, since **2** has only a very limited solubility in water (ca. 10^{-6} M) and, more directly, the vesicles containing **2a** and **2b** were characterized by gel-filtration chromatography on a colum of Sephadex G-50 with water containing CN^- as an eluant; both **2a** and **2b** were eluted together with vesicles at the void volume of column. On the basis of a correlation between λ_{max} and solvent polarity, **2a** is in a microenvironment somewhat more polar than in methanol (λ_{max} 754 nm)[7] and **2b** in that less polar than in benzene.[8] It is instructive to refer for comparison to the binding property of micellar hexadecyltrimethylammonium bromide (CTAB) for **2**, only a single species of **2** with λ_{max} at 756 nm, an intermediate value between 748 and 796 nm.

The distribution of **2** between **2a** and **2b** is quite unique as shown in Figure 3, where the amounts of **2a** and **2b** are plotted against the total concentration of **2** up to its solubilization limit range in the vesicles at [**1**] of 1.0×10^{-3} M. There is clearly a maximum for the incorporation of **2** in **2a** state, ca. 1×10^{-5} M.[9] When the total concentration of **2** is below this range, only **2a** is the vesicle-bound species. At greater concentrations of **2**, the distribution between **2a** and **2b** resulted in such a way that [**2a**] remains nearly at its maximal range and the residual portion is forced to exist as **2b**. Thus, we can control the molar ratio of **2b**/**2a** in the range 0–3 by adjusting the total concentration of **2**. It should be noted here that the ratio in a solution once prepared at a certain concentration of **2** remained unchanged over a month at room temperature, indicating almost complete inhibition of the interconversion between **2a** and **2b** (or translocation between two binding sites). It is evident that the present distribution behavior cannot be explained in terms of usual thermodynamic equilibrium; **2a** $\rightleftharpoons$ **2b**, K = [**2b**]/[**2a**]. When only **2a** is the vesicle-bound species (refer to Figure 3), **2a** remains as it is for the temperature range 0–100 °C without any significant spectral change. On the other hand, the system containing both **2a** (at saturation level, ca. 1×10^{-5} M) and **2b** (e.g., 2.4×10^{-5} M) exhibits an interconversion at elevated temperatures: e.g., most of **2b** was converted

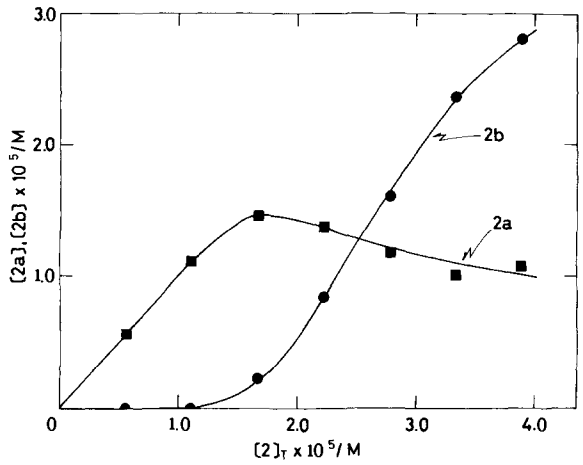

Figure 3. Distribution of **2a** and **2b** in single-compartment vesicles of **1** (1.0×10^{-3} M) in water containing KCN (5.0×10^{-4} M) at 20 °C; $[2]_T$ stands for the total concentration of **2** species.

to **2a** within 1 min upon heating at 92 °C. The resulting solution *oversaturated* with **2a** was then cooled down to 5 °C to rapidly restore more than 95% of the original **2b**, the original distribution feature of **2a** and **2b** being practically reestablished. The hydrogen-bonded region provided by the amino acid residue seems to be subjected to thermal disorder at higher temperatures so that the substrate flux from **2b** to **2a** is allowed.

Two additional observations are worthwhile for characterization of **2a** and **2b**. First, the addition of **2** to a presonicated solution of **1** only led to the formation of **2a** species. Second, **2a** and **2b** showed quite different reactivities from each other toward a chemical reagent in the bulk phase: the initial rate constants, $-d[\mathbf{2a}/\mathbf{b}]/dt/[\mathbf{2a}/\mathbf{b}]_0$, for anaerobic reduction of **2a** and **2b** with $HS(CH_2)_2OH$ (6.6×10^{-4} M) to the univalent cobalt species[10] at 3.8 °C are 7×10^{-3} and ca. 1.5×10^{-4} s^{-1}, respectively; [**1**] = 1.0×10^{-3} M, and [**2**] = $(0.56–1.68)10^{-5}$ M. The difference in reactivity may reflect the nature of microenvironments around **2a** and **2b**.

We can readily identify the binding sites for **2a** and **2b** in the vesicle: the binding site for **2a**, being relatively polar and exposed to the bulk phase, is located near the polar head group and that for **2b**, being as apolar as hydrocarbon and insulated to a significant extent from the bulk phase, is the hydrophobic double-chain interior. The barrier between these two sites is so effective that translocation of the guest molecule at room temperature is not allowed, while it is equally important to note that the substrate binding at different sites must not be independent processes but mechanistically coupled (Figure 3). The most plausible candidate for physicochemical identity of this barrier seems to be a hydrogen-bonded region composed of dimensionally aligned two amide groups of amphiphile **1**, in reference to the demonstration of importance of the hydrogen-belt region in biomembranes.[11] Such a hydrogen-bonded region may, by the same reason, be one of the major origins of the high stability of the present and analogous vesicles.[4,5] In fact, cosonication of **2** and a simpler vesicle-forming amphiphile (didodecyldimethylammonium bromide lacking in an intervening polar group),[2b] by the same experimental method described above, led to the incorporation of **2** as only one species with λ_{max} 742 nm without formation of another species comparable to **2b**. The distribution behavior shown in Figure 3 and the release and reuptake of **2b** upon heating–cooling cycles may also be understood on the basis of substrate- and temperature-induced disorder of the hydrogen-bonded region.

In conclusion, we have successfully identified the two different phases, which are separated by the so-called hydrogen belt, in the intramembrane region. An immediate application of this mem-

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(8) λ_{max} in *n*-hexane containing 1.5% (v/v) dichloromethane 793 nm.
(9) The overall distribution behavior in the presence of KCl (3.0×10^{-2} M) is similar to that in its absence (Figure 3), although the maximal amount of **2a** is reduced to ca. 0.6×10^{-5} M.
(10) For example: Murakami, Y.; Aoyama, Y.; Nakanishi, S. *Chem. Lett.* **1976**, 857–860.
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brane feature would be a specified arrangement of different substrates in different binding sites depending on their properties. We can also envisage a wider scope of other applications, in both mechanistic and practical senses, to barrier-dependent phenomena such as encapsulation and transport of various substrates.

Quadruply Bonded Tetramethyltetrakis(trialkylphosphine)dimolybdenum Compounds: Phosphine Exchange Kinetics, Acetone Formation with Carbon Monoxide, and Crystal Structure of $Mo_2Me_4(PMe_3)_4$

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Creation of coordinative unsaturation about a metal center is crucial for the generation of a catalytic cycle.[1] One of the most general methods for achieving such unsaturation is reversible dissociation of neutral ligands, such as phosphines. A reasonable amount is known concerning the energetics of phosphine exchange in mononuclear transition-metal systems,[2] but relatively little is known about polynuclear ones.[3] The quadruply bonded Mo(II) dimers are interesting candidates for such a study, since generation of vacant coordination sites about two metal atoms ca. 2 Å apart may allow them to cooperate in promoting reactions which are not otherwise possible. Unfortunately, little is known about the intimate reaction mechanisms which govern this class of molecules.[4] As part of our efforts to explore the reactivity of quadruply bonded dimers,[5-7] we have examined phosphine exchange kinetics and reductive cleavage by carbon monoxide in the alkyl compounds $Mo_2Me_4(PR_3)_4$.

Blue $Mo_2Me_4(PEt_3)_4$[8] was prepared from $Mo_2(O_2CCMe_3)_4$, MgMeCl, and PEt_3 in diethyl ether, followed by crystallization from diethyl ether at −10 °C. This complex undergoes ligand exchange with excess PMe_2Ph or PMe_3 in toluene solution within minutes at room temperature to give the known $Mo_2Me_4(PMe_2Ph)_4$ or $Mo_2Me_4(PMe_3)_4$, respectively.[9] The NMR parameters of all three complexes are similar, and they are doubtless isostructural. An X-ray crystal structure analysis of $Mo_2Me_4(PMe_3)_4$ has been performed,[10] and the molecular geometry (Figure 1) is in accord with that previously deduced spectroscopically.[9] The structure analysis reveals that the single crystals are composed of discrete binuclear molecules (Figure 1) which presumably contain a Mo–Mo quadruple bond.[10a] These crystals are essentially isomorphous with those of $W_2Cl_4(PMe_3)_4$[11] and have a solid-state packing arrangement which is quite similar to $Mo_2Cl_4(SEt_2)_4$.[12] Although the rigorous solid-state crystallographic symmetry of $Mo_2Me_4(PMe_3)_4$ is only C_2, it approximates rather closely to full D_{2d} symmetry with the two $MoL_2L'_2$ units having a nearly eclipsed (to within 0.8°) conformation. Relevant bond lengths and angles include Mo_1–Mo_2, 2.153 (1)Å; Mo–P, 2.513 (2,0,0,2) Å; Mo–C, 2.439 (5,18,18,2) Å; Mo–Mo–P, 102.5 (5,9,9,2)°; Mo–Mo–C, 115.3 (1,4,4,2)°.[10b]

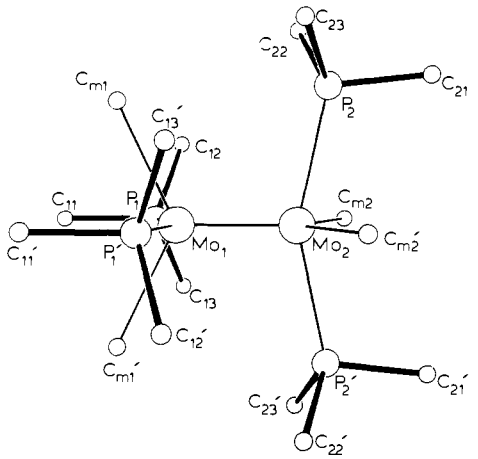

Figure 1. Perspective ORTEP drawing of the nonhydrogen atoms of $Mo_2Me_4(PMe_3)_4$. All atoms are represented by arbitrary-sized open circles for purposes of clarity. Atoms labeled with a prime are related to those labeled without a prime by the crystallographic twofold axis which passes through Mo_1 and Mo_2.

It is of interest to determine the kinetics and mechanisms of phosphine exchange in these binuclear clusters. But before an examination of the kinetic parameters is possible, the stoichiometry of the reaction and stereochemistry of the products must be established. This is accomplished conveniently by monitoring the $^{31}P\{^1H\}$ NMR spectra as a function of temperature during the progress of the reactions.

A toluene-d_8 solution of $Mo_2Me_4(PEt_3)_4$ (0.036 M) and PMe_2Ph (0.75 M) at −80 °C gives a spectrum that consists of two singlets due to these two species. Warming the sample to −50 °C gives free PEt_3, which eventually accounts for 25 ± 3%

† University of Nebraska.

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(8) Anal. Calcd: C, 46.4; H, 10.0. Found: C, 46.1; H, 9.78. ^1H NMR (PhMe-d_8, −50 °C): δ 0.32 (Mo–*Me* br), 2.18 ($P(CH_2CH_3)_3$, br), 1.11 ($P(CH_2CH_3)_3$ br); $^{13}C\{^1H\}$ NMR (PhMe-d_8, −50 °C): δ 3.86 (Mo–*Me* t, J_{pc} = 9 Hz), 16.29 ($P(CH_2CH_3)_3$ t, J_{pc} = 7 Hz), 9.46 ($P(CH_2CH_3)_3$, s; $^{31}P\{^1H\}$ NMR (PhMe-d_8, −50 °C): δ 19.9 (s). Positive chemical shifts are downfield of Me_4Si or 30% H_3PO_4.

(9) Andersen, R. A.; Jones, R. A.; Wilkinson, G. *J. Chem. Soc., Dalton Trans.* **1978**, 446–453.

(10) (a) Single crystals of $Mo_2Me_4(PMe_3)_4$ are monoclinic, space group $C_{2/c}$–C^6_{2h} (No. 15) with a = 18.507 (5), b = 9.462 (3), c = 17.513 (4) Å; β = 116.18 (2)°; Z = 4 (dimeric units). Three-dimensional X-ray diffraction data were collected for 3172 independent reflections having $2\theta_{MoK\alpha} < 55°$ on a Nicolet P1 autodiffractometer by using graphite-monochromated Mo Kα radiation and full (1° wide) ω scans. The solid-state structure was solved by using the "heavy-atom" technique, and the resulting structural parameters for all nonhydrogen atoms have been refined anisotropically to convergence [R (unweighted, based on F) = 0.044 for 2372 independent reflections having $2\theta_{MoK\alpha} < 55°$ and $I > 3\sigma(I)$] by empirically weighted full-matrix least-squares techniques on a Data General Eclipse S-200 computer using locally modified versions of the Nicolet E-XTL interactive crystallographic software system. (b) The first number in parenthesis following an averaged value of a bond length or angle is the root mean square estimated standard deviation of an individual datum. The second and third numbers, when given, are the average and maximum deviations from the averaged value, respectively. The fourth number represents the number of individual measurements which are included in the average value.

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