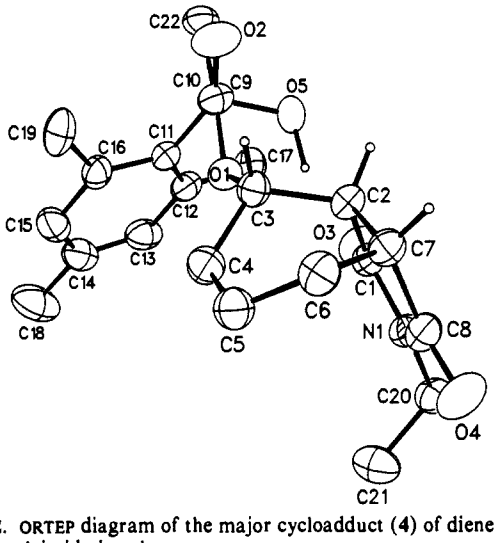

Figure 2. ORTEP diagram of the major cycloadduct (**4**) of diene **1b** with *N*-ethylmaleimide in toluene.

The elegant asymmetric Diels–Alder reactions of Masamune et al. provide a classic example of the use of *intramolecular* hydrogen bonding.[10] The present work shows that *intermolecular* hydrogen bonding with the dienophile carbonyl is preferred over intramolecular (with the ester carbonyl), and that equally high, and synthetically very useful, selectivity can also be obtained.[11]

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Supplementary Material Available: Tables of X-ray data, refined atomic positional and thermal parameters, bond distances, and bond angles (8 pages). Ordering information is given on any current masthead page.

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Layered Arrangement of Oriented Myoglobins in Cast Films of a Phosphate Bilayer Membrane[1]

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We describe herein a novel method for the layered arrangement of protein molecules by using synthetic bilayer membranes. Artificial organization of protein molecules should lead to many interesting possibilities. It is a key technology for developing protein-based electronic devices, and it can provide pseudonatural multienzyme systems as a new methodology in biotechnology.

Some years ago, Fromherz reported adsorption of cytochrome *c* to an arachidic acid monolayer and discussed its orientation based on polarized absorption spectra.[2,3] Two-dimensional crystallization of proteins on a monolayer was recently developed by Uzgiris and Kornberg.[4] Ringsdorf and co-workers used their approach to prepare 2D crystals of a streptavidin–biotin lipid monolayer.[5]

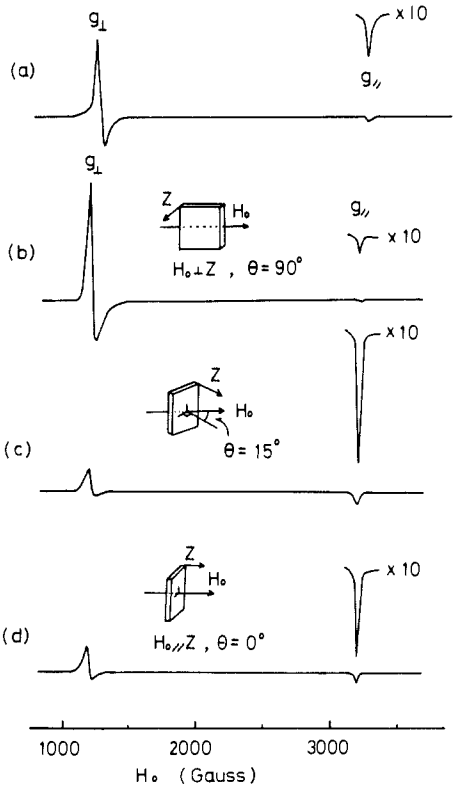
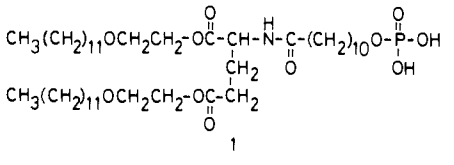


Figure 1. ESR spectra: (a) met-Mb powder; (b–d) met-Mb immobilized in a cast film of **1** ([met-Mb]/[**1**] = 1/160, pH = 7.5, 10 mM Tris·HCl). Spectral conditions: microwave power 5 mW, microwave frequency 9044 MHz, modulation frequency 100 kHz, modulation amplitude 7.9 G, time constant 0.03 s, scan time 4 min, temperature 4 K.

Matsumoto and co-workers prepared 2D crystalline monolayers of ferritin and F_1-ATPase on a clean mercury surface by self-association of the protein molecule.[6] Some membrane proteins were anisotropically immobilized in multilayer films of biolipids.[7] It is desirable to establish a more general methodology to be used for organizing protein molecules (water soluble as well as membrane bound) in controlled orientations.

As a first step toward this goal, we conducted immobilization of myoglobin in cast films of synthetic bilayer membranes. The heme group in myoglobin is a convenient probe for absorption spectral detection of denaturation and ESR spectral detection of protein orientation. Cast films of certain synthetic bilayer membranes were shown to possess highly regular multibilayer structures in which molecular orientation (microscopic anisotropy) is converted to macroscopic anisotropy.[8-12]

Synthetic amphiphile **1** was dispersed in 10 mM Tris·HCl buffer by sonication (pH = 7.5, 20 mM). Metmyoglobin (met-Mb, from horse heart) was then dissolved by gently shaking at room tem-



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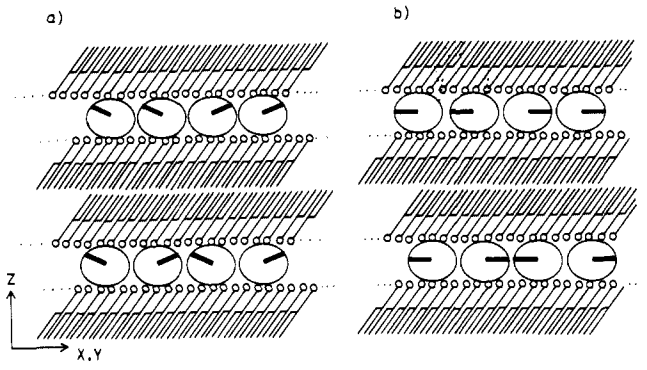

Figure 2. Schematic illustration of met-Mb molecules immobilized in cast multibilayer films: (a) cast from Tris·HCl buffer; (b) cast from phosphate buffer. The protein molecules show at least one-dimensional ordering along the Z axis. The heme plane is expressed by filled rectangles.

perature ([met-Mb] = 0.06–0.5 mM, μ = 0.02). Multilayer films were obtained by spreading the dispersion on Fluoropore membranes (Sumitomo Electric) and allowing it to stand at 20–25 °C for a few days. Low-temperature ESR spectra were measured with a JEOL JES-RE2X X-band spectrometer equipped with a liquid helium cryostat with 100-kHz magnetic field modulation.

The cast film containing met-Mb was self-supporting and showed a gel-to-liquid crystal phase-transition behavior very similar to that of the bilayer alone. This confirms that the bilayer structure is maintained even in the presence of immobilized met-Mb. An ESR spectrum of met-Mb powder is characteristic of high-spin iron(III) porphyrin (Figure 1a)[13] and is composed of two components, $g_\perp$ = 5.9 and $g_\parallel$ = 2.0, where $g_\parallel$ (g_{ZZ}) represents the principal value of the g tensor that is parallel to the normal of the heme plane (i.e., perpendicular to the heme plane), and $g_\perp$ ($g_{XX} = g_{YY}$) represents the principal value of the g tensor that is perpendicular to the normal of the heme plane (i.e., parallel to the heme plane). It is interesting that ESR spectra of the cast film show strong anisotropy depending on the angle (θ) between the normal of the film plane (Z axis) and the applied magnetic field H_0: Figure 1b–d. When the film plane is parallel to the magnetic field (θ = 90°), a strong signal of the $g_\perp$ component appears at 1000 G but the $g_\parallel$ signal is weak. As angle θ decreases from 90° to 0°, the $g_\parallel$ component is intensified, assumes a maximum at θ = 15–20°, and is then slightly decreased. The $g_\perp$ signal shows complementary changes. The observed angular dependence clearly indicates that the heme plane of met-Mb is oriented at an angle of 15–20° against the bilayer surface.[14]

This specific orientation is maintained even when the ratio of met-Mb over amphiphile ([met-Mb]/[**1**]) is varied from 1/40 to 1/330. The overall dimension of myoglobin is about 45 × 35 × 25 Å, and it is presumed that 30 basic amino acid residues and 20 acidic amino acid residues are distributed on the protein surface, forming a few charged domains.[17] The molecular cross section of **1** is ca. 50 Å^2 as estimated from CPK model building and from the data of its surface monolayer.[18] The ratio of [met-Mb]/[**1**] = 1/40 corresponds to a situation where met-Mb molecules cover almost completely the polar bilayer surface in the multibilayer film.

Denaturation apparently does not occur with met-Mb included in the cast film. Its ESR data are in good agreement with those of native met-Mb: see above. Absorption maxima of met-Mb in the cast film (Soret band, 409 nm; Q band, 500, 540, and 630 nm) coincide with those of met-Mb itself dissolved in Tris buffer. The active site of the protein is not disturbed seriously by immobilization under the current conditions.

The orientation of met-Mb is dependent on buffer and the ionic strength of the casting solution. When phosphate buffer (pH 7.5, 10 mM) was used instead of Tris·HCl buffer, the ESR spectra of the resulting cast film showed the strongest $g_\parallel$ signal at θ = 0°. This anisotropy corresponds to the orientation of the heme plane parallel to the plane of the cast film. On the other hand, an increased ionic strength of the casting solution (μ = 0.2 instead of the original 0.02) caused some loss in the orientational specificity. The electrostatic interaction of the polar groups at the protein surface and the phosphate head group of the bilayer membrane appears to determine the protein orientation.

In conclusion, myoglobin molecules were incorporated into multilamellar films of a phosphate bilayer membrane without detectable denaturation. The myoglobin molecule can fill the polar interbilayer region and maintains at least the one-dimensional order due to electrostatic interactions with the bilayer surface. This is schematically illustrated in Figure 2. The present approach is distinguished from the previous examples in that two-dimensional placement of proteins is attained by simple solvent casting. Improved anisotropy was found relative to those of membrane-bound proteins in a matrix of biolipids.[7] This indicates an advantage of synthetic bilayer membranes as matrices. We envisage a wide range of biochemical and biotechnological applications.

Acknowledgment. We are grateful to Dr. Y. Ishikawa for ESR simulation.

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Peptidyl-Prolyl Cis–Trans Isomerase Activity of Cyclophilin Studied by One-Dimensional ^{1}H Nuclear Magnetic Resonance Spectroscopy

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Recent advances in multidimensional NMR[1] spectroscopy have made it possible to study and determine the structures of many biologically interesting molecules at atomic resolution.[2] Additionally, lest one forget, NMR is also uniquely useful in studying the dynamic and kinetic properties of these biomacromolecules. In fact, certain functional properties of molecules whose large size is not amenable to detailed structure elucidation by even three-

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(1) Abbreviations: BzFAP, benzoyl-Phe-Ala-Pro; CsA, cyclosporin A; CyP, cyclophilin; NMR, nuclear magnetic resonance; PPIase, peptidyl-prolyl cis–trans isomerase; Succ-AAPF-pNA, succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide.

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