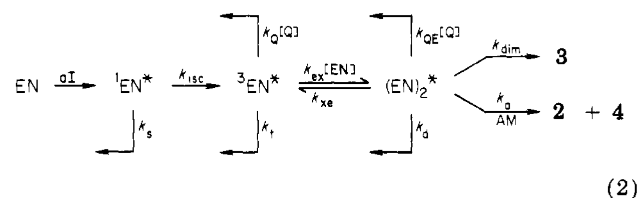
(generally 80–90%) were considerably better than those reported by Cookson and Hudec,[1] where their diminished yields may be the result of photolysis of the products under the conditions employed. Quenching of the reactivity of 0.13 M **1** in Et_3N as solvent was observed with added naphthalene (**5**) or piperylene (**6**) but not with fluorene.[18,19] In all cases the ratio of products (**2** + **4**)/**3** was identical with those in Tables I and II.

These data exclude any kinetic scheme where a ground-state enone competes with an amine molecule for an enone excited state (or two different states) to produce **3** or **4**. All such mechanisms require dependence of product ratios on enone concentration. Equation 2 represents a mechanistic scheme consistent with our



(2)

observations, where EN is enone **1**, AM is amine, Q is quencher, and k_s, k_t, and k_d represent the sum of all first-order decay rates for the singlet, triplet, and excimer. The product ratio (**2** + **4**)/**3** depends only on the ratio $k_a[AM]/k_{dim}$, and the plot of product ratios vs. amine concentration (Table I) has a slope $k_a/k_{dim} = 0.57$. Excimer formation has been represented as a reversible step.[2,20] The kinetic expression in eq 3 relates quantum yields and enone

$$\Phi_{dim}^{-1} = \Phi_{isc}^{-1}\frac{1}{k_{dim}\tau_{ex}}\left[\frac{k_t}{k_{ex}[EN]} + (1 - k_{xe}\tau_{ex})\right] \quad (3)$$

concentration, where τ_{ex} is the excimer lifetime. A plot of $1/\Phi_{dim}$ vs. $1/[EN]$ gives the relationship in eq 4 from the ratio of the slope

$$\text{slope/intercept} = k_t/k_{ex}[1/(1 - k_{xe}\tau_{ex})] \quad (4)$$

to intercept. The data in Table II yields a slope to intercept ratio of 0.11 while in the absence of amine that number is 2.7.[9] Since the bracketed term in eq 4 must be larger than 1, k_t/k_{ex} must be less than or equal to 0.11. Thus, in the absence of amine the excimer–triplet equilibrium allows triplet decay 73% of the time, while with apparent facile reaction between excimer and amine, triplet decay accounts for only 10% of all triplet pathways. The difference in excimer lifetimes is an additional term in the presence of amine (eq 5 and 6). Since $k_a/k_{dim} = 0.57$, the ratio k_a-

$$\text{without amine:} \quad \tau_{ex} = 1/(k_{xe} + k_{dim} + k_d) \quad (5)$$

$$\text{with amine:} \quad \tau_{ex} = 1/(k_{xe} + k_{dim} + k_d + k_a[AM]) \quad (6)$$

$[AM]/k_{dim} = 4.1$ in neat amine, and the extra term in the latter equation accounts for a substantially shorter excimer lifetime.

Our conclusion is that, at least in the concentration range studied, dimer **3**, adduct **4**, and reduced material **2** arise from competitive reaction from the triplet excimer. Little, if any, product arises by direct interception of the singlet or triplet enone. The structure of adduct **4** is itself inconsistent with a scheme involving hydrogen atom abstraction by the β carbon of the excited state followed by combination of the radicals. We favor a mechanism involving electron transfer to give a radical anion–radical cation pair which decays ultimately to give **2** and **4**. Data from a series of enones and tertiary amines will be presented separately in support of this scheme.

Acknowledgment. We acknowledge helpful discussions with Professor Peter Wagner, the assistance of Professor Jim Hinton in obtaining NMR data, and Research Corp. for financial support.

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Liposomal Membranes. 13. Transport of an Amino Acid across Liposomal Bilayers As Mediated by a Photoresponsive Carrier

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Azobenzene, stilbene, spiropyran, and rhodopsin are photoresponsive compounds that undergo a conformational change upon photoirradiation.[1-7] Hence, they have been utilized as a trigger or switch to photochemically control various phenomena such as substrate binding of crown ethers[2] or cyclodextrins,[3] activity of enzymes,[4] permeation of metal ions into liposomal membranes,[5,6] and morphology of synthetic bilayers.[7] In biological systems, of course, such a photoregulated process is well recognized as a primary stage in the photosynthesis and vision systems.[8] On the other hand, the transport of amino acids across cell membranes is really present in biological systems, where the carrier mechanism is generally preferred over the trans membrane channel mechanism.[9] In this communication, we show the first example of the photocontrolled transport of an amino acid across lipid membranes using the photospiran **1** embedded in liposomal bilayers of egg phosphatidylcholine (egg PC).

Upon UV irradiation in apolar organic solvents, the photospiran **1**, 1′,3′-dihydro-1′,3′,3′-trimethyl-6-nitrospiro[2*H*-1-benzopyran-2,2′-[2*H*]indole], is easily converted to the colored and ring-opened form (**2**) bearing a merocyanine dye skeleton (eq 1).[1] Both the

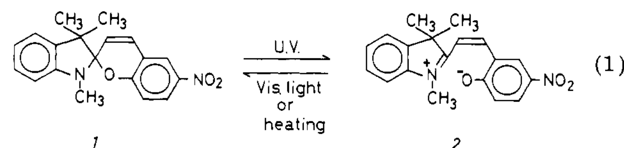

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Table I. Transport of Phenylalanine As Mediated by Photoresponsive Carrier 2 across Liposomal Bilayers of Egg Lecithin[a]

procedure	amino acid transported, %
during preincubation	nothing
after UV irradiation for 20 min and visible light irradiation for 5 min	17.2
while kept in the dark	nothing
after more UV irradiation for 20 min and visible light irradiation for 5 min	14.7

[a] 29 nmol of Phe was encapsulated in the interior cavity, and 164 nmol of 1 in the bilayers. The total concentration of egg PC as liposome was 1.0 mM. The pH values of the interior and exterior were 6.0 and 9.0, respectively.

ring-opened and -closed species are soluble in apolar solvents such as hexane and octanol, and this is evident from the fact that even when the hexane solution containing **1** was shaken with an aqueous buffered solution (pH 6.0) after UV irradiation for 10 min at 25 °C, the purple ring-opened species still remained in the hexane layer. The resulting zwitterionic dye **2** (in contrast to the neutral form **1**) is expected to form an ionic complex with a zwitterionic α-amino acid. Certainly, when phenylalanine (Phe) or its methyl ester (Phe–OMe) was placed in the water layer, the amino acid was transfered from the water phase to the organic phase upon UV irradiation.[10] This was examined by determining the decrease in the amino acid concentration of the aqueous phase fluorometrically by using fluorescamine.[11] Of course, no distribution of amino acid was observed in the dark or in the absence of **1**. Phe was more effectively transported by 2 than was Phe–OMe, and this revealed the greater importance of the ionic association between **2** and the amino acid as opposed to hydrophobic effects. These preinvestigations on a photocontrolled transfer of an amino acid from the water phase to the organic phase containing the photospiran **1** encouraged us to try the similar transport of an amino acid across liposomal membranes as mediated by **2**.

When a liposomal membrane is employed instead of a liquid membrane, it is necessary to ascertain the improbability of induced leakage of amino acid either by photochemical lysis of lipids or by physicochemical disordering of the photospiran-embedded bilayers upon photoirradiation. Neither a pH change of the liposome suspension accompanied by destruction of the phosphatidylcholine head group nor any degradates derived from lecithins was observed during and after the irradiation. This was confirmed by direct pH measurement of the liposome suspension and by repeated gel filtration of the irradiated liposome suspensions eluted from a Sephadex G-50 column. In order to detect possible physicochemical disordering of liposomal bilayers as caused by the photochromism of **1** embedded in the bilayers, we encapsulated a water-soluble fluorescent probe, pyranine (trisodium 8-hydroxy-1,3,6-pyrenetrisulfonate), instead of amino acid in the interior of the liposomes.[12] Since pyranine is not expected to form any stable complex with **2**, the leakage of pyranine under photoirradiation must result only from the physicochemical disordering of the bilayers. However, no leakage of pyranine under UV and visible light irradiation was observed.[13]

Table II. Transport of Phenylalanine Methyl Ester against a Concentration Gradient across Liposomal Bilayers As Mediated by 2 at 25 °C[a]

pH of interior	initial concentration: Phe–OMe in exterior, nmol	1 in bilayers, nmol	Phe–OMe in interior, nmol	transported Phe–OMe,[b] %
	→	→	→	
5.0	86	3.9	81	10.7 ± 1.0
6.0	73	8.2	44	13.8 ± 1.4

[a] Total concentration of egg PC was 1.0 mM. The pH of the exterior was kept constant at 8.5. An arrow indicates the direction of transport. [b] Amounts of Phe–OMe transported were determined after UV irradiation for 20 min and visible light irradiation for 5 min.

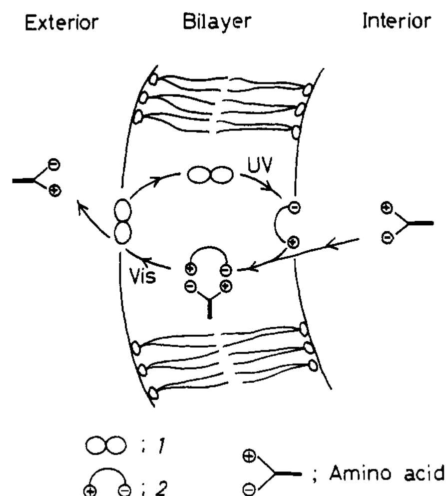

Figure 1. Schematic representation of amino acid transport across liposomal bilayers.

An aqueous suspension of single-walled liposomes containing the amino acid in the interior and **1** in the bilayer was isolated.[14] With use of aliquots from the liposome suspension, concentrations of amino acid, **1**, and egg PC were determined.[15] The amount of amino acid transported across the liposomal bilayers after photoirradiation was determined fluorometrically with fluorescamine.[11] The photospiran **1** exhibits a normal photochromism in bilayers (i.e., the photocoloration and thermobleaching cycle). Thermobleaching was effectively accelerated by irradiating visible light (500 nm). As expected, UV irradiation for 20 min followed

(10) A 1.5-mL mixture of aqueous buffered solution (pH 6.0) containing 1×10^{-4} M amino acid was vigorously shaken with 1.5 mL of hexane containing 1×10^{-3} M **1** on a Vortex mixer after UV irradiation for 10 min at 25 °C. The amino acid concentration in the water phase was immediately determined fluorometrically with fluorescamine according to the method described in the literature (ref 11). By this procedure, 30.0% Phe and 20.3% Phe–OMe were transported by 2, respectively, from the aqueous phase to the organic phase.

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(13) The leakage of pyranine from the interior cavity of vesicles was inspected fluorometrically after further gel filtration of the irradiated liposome suspension (UV (20 min) and visible light (20 min)) and passage through a Sephadex G-50 column (ϕ 1.4 × 24 cm). Pyranine emits at 510 nm by excitation at 410 nm.

(14) Egg PC was isolated and purified from fresh egg yolk by the same procedures as those described in the literature: (a) Singleton, W. S.; Gray, M. S.; Brown, M. L.; White, J. L. *J. Am. Chem. Soc.* **1965**, *42*, 53. (b) Sunamoto, J.; Kondo, H.; Yoshimatsu, A. *Biochim. Biophys. Acta* **1978**, *510*, 52. Small single-walled liposomes were prepared and isolated by essentially the same method as that described before: (c) Sunamoto, J.; Kondo, H.; Nomura, T.; Okamoto, H. *J. Am. Chem. Soc.* **1980**, *102*, 1146. Amino acid was encapsulated during dispersion of the thin film of egg PC and **1** into 4.0 mL of aqueous buffered solution (pH 6.0, 0.5 M phosphate) containing 0.1 M amino acid. The free amino acid was separated by gel filtration on a Sepharose 4B column (ϕ 1.8 × 60 cm) preequilibrated with 0.5 M borate (pH 8.5–9.0). Hence, the resulting liposomes have a pH gradient between the interior (pH 6.0) and exterior (pH 8.5–9.0). Even if fluorescamine were to permeate partly into the interior of the liposomes, it does not react with amino acid below pH 7.0.

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by visible light irradiation for 5 min at 25 °C[16] on the above liposome suspension brought about significant transfer of Phe from the interior of the liposomes to the exterior. Keeping the system in the dark at the same temperature did not lead to any spontaneous release of Phe (Table I). Additional transport of the amino acid took place by repeating the above procedures. This means that our present system functions repeatedly until an equilibrium concentration of Phe between the interior and the exterior of liposomes is attained. Decreasing the pH of the interior of the liposomes from 6.0 to 5.0, while keeping that of the exterior constant at pH 8.5, diminished the transport efficiency for Phe from 13.2 ± 1.2% to 6.2 ± 0.7%. This may be caused by protonation of the *p*-nitrophenolate moiety of the dye situated at the inner surface of liposomal bilayers, thereby impairing the formation of the ionic complex with zwitterionic Phe. When Phe–OMe was encapsulated in liposomes, substantial amounts of Phe–OMe were adsorbed on the outer surface of the liposomes even after gel filtration to eliminate free amino acid in the bulk aqueous solution. Under the circumstances, most interestingly, Phe–OMe was transported from the exterior to the interior of the liposomes against the concentration gradient (Table II). This was true also for the case when more Phe–OMe was added to the exterior. Though we cannot clarify the reason at the present time, it may be ascribed to the greater hydrophobicity of Phe–OMe compared with Phe and/or asymmetry in the distribution of **2** between the outer and inner leaflets of bilayers.[17] In any event, we have succeeded in the photocontrolled transfer of an amino acid across liposomal membranes accompanied by the photochromism of **1** embedded in the membranes, as schematically illustrated in Figure 1.

The fluidity change of the liposomal membranes caused by the photochromism of **1** was also investigated by use of the fluorescence depolarization technique.[18] 1,6-Diphenylhexatriene and *N*-dansylhexadecylamine[20] have been intercalated in liposomal bilayers as fluorescent probes in order to monitor the fluidity of membranes.[21] The former is localized in the hydrophobic domain of membranes,[19] while the latter binds close to the surface of membranes.[20] For both probes, the fluorescence polarization was increased with an increase in the concentration of the ring-opened species **2** upon UV irradiation. This suggests that the ring-opened species certainly immobilizes regions of both the surface and hydrophobic domains of bilayers, thereby possibly leading to a reduction in the diffusion of the complex and the photospiran **1** itself.

Several carrier-mediated transports of amino acids across liquid membranes (Pressman cells) have been reported.[22] However, none of them are photoresponsive, and the amino acids transported were not in the neutral form but were present as the ammonium or carboxylate salt. Thus, to our knowledge, the present system must be the first success in mimicking the transport of free amino acids across cell membranes. Most unfortunately, however, there still remain several disadvantages such as the restricted movement of the vehicle, with or without a passenger, in membranes, the competitive interaction of **2** between the amino acid and the phosphatidylcholine moiety of lecithins, and the photochemical instability of the photospiran **1** itself. Further investigation is in progress in our laboratories to enhance transport efficiency.

Registry No. 1, 1498-88-0; **2**, 18457-95-9; phe, 63-91-2; phe-OMe, 2577-90-4.

(16) In order to isolate ultraviolet light (250–350 nm) and visible light (>440 nm), we employed a liquid filter composed of 46% (w/v) $CoSO_4$–14% (w/v) $NiSO_4$ aqueous solution (20 mm thickness) and a sharp cutoff filter Y46 (Hoya Glass Works) were employed, respectively, with a Toshiba high-pressure Hg lamp SHL-100UV-2. The distance between the light source and sample cuvette was 10 cm.

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^{15}N NMR Study of [^{15}N]Actinomycin D Complexed with d(pGpC) and DNA

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^{15}N NMR has been used to characterize the solution conformation of several cyclic polypeptides such as gramicidin S[2] and viomycin[3] and others,[4] as well as linear peptides.[5–7] Those experiments have demonstrated that ^{15}N NMR is particularly well studied for probing hydrogen bonding and solvent exposure properties of peptide nitrogens and carbonyls. We have recently described the isolation and characterization of actinomycin D (Figure 2) isotopically labeled at each nitrogen with ^{15}N at greater than 90% enrichment.[8] In that report we assigned the ^{15}N NMR spectrum of [^{15}N]actinomycin in organic solvents as well as in water. We now present the first ^{15}N NMR spectrum of a drug in aqueous solution complexed with the dinucleotide d(pGpC) and with short segments of calf thymus DNA as part of our studies on the role of peptide conformation in the binding of actinomycin to nucleic acids.[9–11]

The effect of adding excess d(pGpC) is shown in Figure 1 and Table I. Actinomycin D is known to form a tight complex with d(pGpC) (one drug: two dinucleotides),[12,13] and this dinucleotide represents the preferred DNA binding site. The dimerization of actinomycin in water at millimolar concentrations is well documented.[14] Thus, for the spectrum of the free drug monomer, we refer to results obtained in a 93 mol% water/7 mol % methanol mixture.[8] The spectrum of the drug in aqueous buffer under conditions favoring dimer formation is included in Figure 1.

^{15}N NMR chemical shifts are sensitive to both solvent and conformation. While the relative importance of these effects has not been completely elucidated for actinomycin, our interpretation of the observed spectral changes is based on ^{15}N NMR results obtained on other peptide systems.[2–7] The large downfield shifts of the Pro resonance are consistent with the formation of the two

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