Table I. Association Constants and Complexation Shifts for Various Triply Hydrogen Bonded Complexes in Chloroform-d^a

complex	type	method used	proton(s) monitored	$\Delta\delta_{max}$ (ppm)	K_{assoc} (M^{-1})	$-\Delta G°_{298}$ (kcal mol^{-1})	ref
1·2	DAD·ADA				90	2.7	5
3·4	DAD·ADA				140	2.9	6
9·10	DAD·ADA	A	N9-H (**9**)	3.98	78	2.6	this work
9·11	DAD·ADA	A	N9-H (**9**)	4.10	70	2.5	this work
7·8	DDA·AAD				1.7×10^4	5.8	7
16·17 (5·6)	DDA·AAD	B			1×10^4	5.5	this work
14·15	DDA·AAD	B	N1-H (**15**)	1.63	9.3×10^3	5.4	this work
12·13[b]	DDD·AAA	B	NH_2 (**13**)	1.80	$\geq 10^5$	≥ 7	this work

[a] At 298 K. Duplicate runs gave K_{assoc} values that agreed within 5%. Method A: Titration with **9** at fixed concentration. Method B: Dilution of 1:1 complex. [b] Carried out in the presence of 2 molar equiv of 1,8-bis(dimethylamino)naphthalene (see ref 18).

Compound **15** was prepared by alkylating 9-deazaguanosine[12] with dioctyl 4-(bromomethyl)phthalate,[13] the long-chain ester groups providing high solubility in chloroform. Only an approximate association constant was reported[6] for the GC base pair (**5·6**) in chloroform, so a more accurate determination was made using 2′,3′,5′-tripentanoylguanosine (**16**)[14] and 4-ethylcytosine (**17**).[15]

The 4-aryl group in dihydropyridines **10** and **13** serves as a convenient tautomeric "switch". Thus, in dimethylformamide-d_7, **13** exists exclusively in the 1,4-dihydro form, while **10** is entirely in the 3,4-dihydro form, presumably due to a steric effect.[10] In chloroform-*d*, the ^1H NMR spectrum of **10** showed it to be in the 3,4-dihydro form, while that for **13** indicated a solvent-induced shift in the equilibrium to a ca. 67:33 mixture of 1,4-dihydro and 3,4-dihydro forms.[16] Interestingly, 10 equiv of **12** converted **10** (ca. 1 mM, $CDCl_3$) entirely into the 1,4-dihydro form. Likewise, 4 equiv of **9** converted **13** (ca. 5 mM, $CDCl_3$) from a 67:33 mixture to a 44:56 mixture of 1,4-dihydro and 3,4-dihydro forms.

Complexation studies were performed by ^1H NMR spectroscopy in chloroform-*d* under conditions where self-association of **9–17** was negligible. The association constants were determined using standard methods;[17] they are compiled in Table I. The alternating hydrogen-bonding motif (DAD·ADA) in **9·10** and **9·11** was weak with K_{assoc} values of 78 and 70 M^{-1}, respectively. The DDA·AAD complexes of **14·15** and the GC base pair (**16·17**) were significantly more stable with K_{assoc} values of 9.3×10^3 and 1×10^4 M^{-1}, respectively. The **12·13** (DDD·AAA) complex was by far the tightest examined. Indeed, only ca. 15% of uncomplexed **13** was observed when the concentration of the 1:1 complex was ca. 2×10^{-4} M. By accounting for the tautomeric equilibrium constant, the association constant can be estimated to be larger than or equal to 10^5 M^{-1}.[18] The difference in stability between complexes **9·10** and **12·13** is striking. Despite their structural similarity and identical number of hydrogen bonds, the latter complex is more stable by over 4 kcal mol^{-1}.

These results are consistent with Jorgensen's proposal that the variable stabilities of triply hydrogen bonded complexes originate in the arrangement of the hydrogen-bonding sites.[3,19] Combined with previous results,[3-7] the variations in K_{assoc} seen in Table I, and in particular the large difference in stability of complexes **9·10** and **12·13**, indicate that the number of hydrogen bonds alone will be a poor predictor of complex stability. For triply hydrogen bonded complexes the arrangement of donor and acceptor groups appears to correlate well with K_{assoc}, although not all triply hydrogen bonded complex stabilities are expected to fall in the narrow ranges found for the complexes in Table I.[19-21] Efforts are underway to incorporate some of these new complexes into supramolecular assemblies.

Acknowledgment. Funding from the NSF-PYI program (CHE 58202), the Searle Company, and a Camille and Henry Dreyfus Teacher-Scholar Award to S.C.Z. is gratefully acknowledged. T.J.M. acknowledges support from the Du Pont Company in the form of a graduate fellowship. The authors thank Professor W. L. Jorgensen for useful discussions and for sharing results prior to publication.

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(15) 9-Ethylcytosine was a generous gift of Prof. Nelson J. Leonard.
(16) The tautomers were in slow exchange at 300 MHz. The ester resonances in the ^1H NMR spectrum of **10** are doubled, with δ_{H-3} = 3.61 ppm and δ_{H-4} = 4.99 ppm ($J \approx 0$ Hz). Compound **10** appears to be a single diastereomer, which we tentatively assign as the cis isomer. The ^1H NMR spectrum of **13** is highly symmetrical, with δ_{H-4} = 4.88 ppm.
(17) For an excellent overview, see: Wilcox, C. S. In *Frontiers in Supramolecular Organic Chemistry and Photochemistry*; Schneider, H. J., Durr, H., Eds.; VCH: New York, 1991; pp 123-143.
(18) In the presence of acid this complex is unstable, undergoing clean hydride transfer from C-4 of **13** to C-10 of **12**. The binding constants were determined in the presence of 1,8-bis(dimethylamino)naphthalene.
(19) The present results do not indicate the origin of the variable stabilities, and other possibilities such as changes in the strength of the primary hydrogen bonds, differential solvation, alignment of molecular dipole moments, etc. cannot be ruled out.
(20) Recently, several new ADA·DAD complexes have been reported with K_{assoc} values somewhat higher than those in Table I: (a) Park, T. K.; Schroeder, J.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 5125-5127 (K_{assoc} = 670 M^{-1}, $\Delta G°_{298}$ = 3.9 kcal mol^{-1} and K_{assoc} = 960 M^{-1}, $\Delta G°_{298}$ = 4.1 kcal mol^{-1}). (b) Kelly, T. R.; Bridger, G. J.; Zhao, C. *J. Am. Chem. Soc.* **1990**, *112*, 8024-8034 (K_{assoc} = 440 M^{-1}, $\Delta G°_{298}$ = 3.6 kcal mol^{-1}).
(21) A complex of type DDA·AAD (very similar to **14·15**) has been reported to have an association constant of 126 M^{-1} ($\Delta G°_{298}$ = 2.9 kcal mol^{-1}): Hamilton, A. D.; Pant, N. *J. Chem. Soc., Chem. Commun.* **1988**, 765-766. This exception will be discussed in a full paper.

Molecular Harpoons: Membrane-Disrupting Surfactants That Recognize Osmotic Stress[1]

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Received January 22, 1992

In this paper we introduce a class of membrane-disrupting surfactants that has been designed to recognize osmotic stress in lipid bilayers. On the basis of their molecular shape and their pressure-sensitivity, we term such amphiphiles *molecular harpoons*. Results presented herein show that the ability of a harpoon to recognize a stressed bilayer is a function of its structure, its oligomeric state, and the strength of the osmotic gradient. The relevance of these findings to the creation of novel antimicrobial agents is briefly discussed.

When large unilamellar vesicles are first prepared via freeze–thawing and extrusion, the concentration of ionic solute within their aqueous interior equals that of the external phase.[2,3] The membrane is thus formed under isotonic conditions, and its compactness is maximized through hydrophobic interactions.[4]

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Table I. Membrane-Disrupting Power as a Function of Osmotic Stress

internal vesicle pressure[a] (P, atm)	surfactant activity (R_{50})[c] I	II	III	IV
0.0	0.063 ± 0.008	<0.001	<0.005	0.018 ± 0.001
+5.7	0.088 ± 0.004	0.023 ± 0.004	0.22 ± 0.16	0.030 ± 0.004
+13.8	0.12 ± 0.01	0.11 ± 0.01	0.63 ± 0.18	0.040 ± 0.003

[a] $P = RT(C_v - C_b)$,[5] C_v and C_b represent the osmolarity of the internal vesicular and external buffer solutions, respectively. Concentrations of CF used to produce internal pressures of 0.0, 5.7, and 13.8 atm were 79, 150, and 250 mM (270, 503, and 821 mOsm, freezing point depression). [c] Average from two series of experiments ±1 standard deviation.

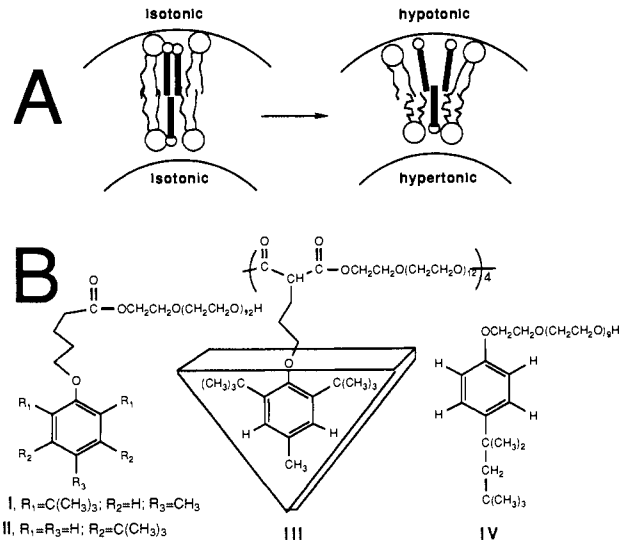

Figure 1. (A) Stylized illustration of a lipid membrane composed of phospholipid and cholesterol under isotonic conditions and under osmotic stress; darkened rectangular structures represent cholesterol. (B) The wedge-like segment of III is graphically highlighted.

Because water diffuses across lipid bilayers much faster than ionic solutes, dilution of these dispersions with pure water transforms the vesicles into a swollen state and places the bilayer under osmotic stress.[5] Under such conditions, water from the hypertonic side pushes against the inner leaflet, thereby increasing hydrocarbon exposure at the membrane–hypotonic interface (Figure 1). Recently, we have become intrigued with two fundamental issues that relate to osmotically-stressed bilayers: Can they be recognized by a membrane-disruptive surfactant? Can such recognition be fine-tuned? Not only are these questions of theoretical interest, but they also have important practical implications. In particular, the fact that microorganisms such as gram-positive and gram-negative bacteria are under considerable osmotic stress (i.e., 15–20 and 0.8–5 atm, respectively),[6] together with the fact that the plasma membrane of mammalian cells is relatively stress-free, raises the possibility that membrane-stress could serve as a definable target for chemotherapy. In this paper, we examine both of these questions.

Harpoons were conceived of from simple geometric considerations.[7,8] Specifically, we theorized that a membrane-disrupting surfactant, comprising a *rigid, wedge-shaped hydrophobic unit* attached to a hydrophilic chain, would be well-suited for recognizing stress in lipid bilayers. In particular we envisioned that a harpoon should favor insertion into a hypotonic leaflet, over an isotonic analogue, because of reduced steric hindrance. We further reasoned that a "blunt-tipped" harpoon might show higher selectivity, due to greater difficulty in wedging its way into an isotonic leaflet. With these ideas in mind, surfactants I, II, and III were selected as prototypes for investigation. Comparison of I with II was intended to clarify how the orientation of the hydrophobic tip affects a harpoon's ability to recognize a stressed membrane; surfactant III was chosen in order to examine the influence of oligomerization on stress-recognition. Triton X-100 (IV), which has compositional but not geometrical similarity to I, was also examined because of its extensive use as a membrane-disrupting agent.

Alkylation of 2,6-di-*tert*-butyl-4-methylphenol with 1-bromo-3-(2-tetrahydropyranyloxy)propane, followed by deprotection, tosylate formation, alkylation with diethyl malonate, and saponification, afforded 5-(2′,6′-di-*tert*-butyl-4-methylphenoxy)pentanoic acid (**1**). Subsequent decarboxylation, esterification with tridecaethylene glycol monotrityl ether, and hydrolysis yielded I. Alkylation of 3,5-di-*tert*-butylphenol with 5-bromovaleronitrile followed by hydrolysis, esterification with tridecaethylene glycol monotrityl ether, and hydrolysis afforded II.[9] Oligomerization of **1** with tridecaethylene glycol yielded III, having an average number of repeat units equaling 4.0, as estimated by gel permeation chromatography (polystyrene standards).

Large unilamellar vesicles (1000-Å diameter), containing 5-(6)-carboxyfluorescein (CF), were prepared from 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine/cholesterol (2/1) using established procedures.[2,10] After removal of non-entrapped CF by gel filtration (Sephadex G-50, isotonic buffer), the tonicity of the external phase was adjusted to 270 mOsm via the addition of either pure water (Milli-Q) or aqueous NaCl, and the dispersion was then incubated for 1 h. Aliquots were then added to varying concentrations of a given surfactant in 10 mM borate buffer [pH 7.4, 140 mM NaCl, 2 mM NaN_3 (270 mOsm)] in order to generate release profiles. For purposes of comparison, we report membrane-disrupting activity as R_{50} values, where R_{50} represents the ratio of phospholipid/surfactant (or repeat unit) that is needed to induce the release of 50% of the entrapped CF from a 5 μM dispersion of liposomes after 30 min at 25 °C.[10-13] Table I summarizes the principal results obtained.

Surfactants I and IV exhibited very modest sensitivity toward osmotic stress. On going from 0.0 to 13.8 atm, the potency of both surfactants increased by only a factor of 2. In striking contrast, the blunt-tipped harpoon (II) showed very high sensitivity toward stress, strongly favoring the disruption of hypotonic leaflets; attack on an isotonic analogue, or an outer *hypertonic* leaflet (internal pressure of −6.8 atm; $R_{50} < 0.001$), led to no significant release of entrapped CF. Although we anticipated that the stress-recognition by the oligomeric harpoon (III) might be greater than that by its corresponding monomer, due to cooperative effects, we were surprised at its magnitude. In particular, we did not anticipate the lack of activity observed under isotonic conditions. One possible explanation for this behavior is that there is competition between intramolecular hydrophobic association of III at the membrane surface and insertion of the pendant groups into the bilayer, and that hypotonic conditions favor the latter.

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Ongoing studies are aimed at (i) defining and exploiting stress-recognition in greater detail, (ii) clarifying the mechanism by which harpoons induce the release of vesicular CF, and (iii) examining the harpoon-lability of bacteria and also those microorganisms for which stress-recognition may be effectively expressed; e.g., enveloped viruses bearing highly curved outer membranes such as HIV.[14]

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Photoinduced Electron Transfer Mediated by a Hydrogen-Bonded Interface†

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Received October 15, 1991
. Revised Manuscript Received March 30, 1992

Long-range electron transfer (ET) in metalloproteins and enzymes is typically mediated by proton motion. Whereas the importance of proton-coupled electron transfer (PCET) in the primary processes of electron/hole pair separation and storage, as well as the function of a variety of biological assemblies including PS II,[1] cytochrome *c* oxidase,[2] and cytochromes,[3] is recognized, PCET has not been subjected to the rigorous experimental and theoretical treatment that has advanced the knowledge of long-range fixed-distance ET in organic and inorganic compounds,[4] proteins,[5] and enzymes.[6] One approach to assessing the role of proton motion on ET rates is to combine the strategy of photoinduced fixed-distance electron transfer[3-6] with that of photoinduced proton transfer.[7] The propensity of carboxylic acids to form cyclic dimers in low-polarity, non-hydrogen-bonding solvents[8] offers the opportunity to juxtapose an acceptor/donor pair via a hydrogen-bonded interface. We now report the electron-transfer kinetics for **1**, where ET is channeled through a protiated (**1**-H) or deuterated (**1**-D) dicarboxylic acid interface from a photoexcitable Zn^{II}PCOOH porphyrin (PCOOH = 13,17-diethyl-3,7,8,12,18-pentamethylporphine-2-acetic acid)[9] donor to the electron acceptor 3,4-dinitrobenzoic acid (DNBCOOH).

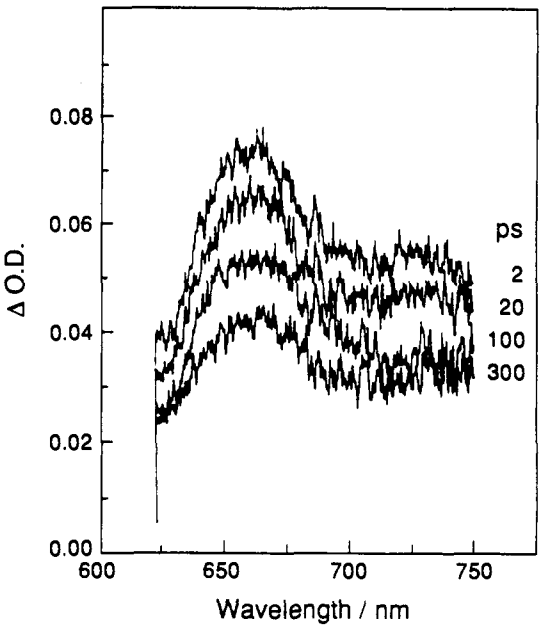
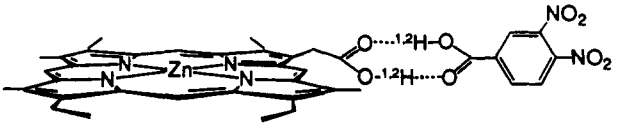


Figure 1. Time evolution of the transient difference spectrum of CH_2Cl_2 solutions of $ZnPCOOCH_3$ (1.5×10^{-3} M) in the presence of 3,5-$DNBCOOCH_2CH_3$ (5×10^{-2} M).



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Photoexcitation of ZnPCOOH(D) by the amplified output (λ_{exc} = 580 nm) of a Rhodamine 590 synchronously pumped (mode-locked Nd:YAG) dye laser (FWHM = 3 ps, E_p = 300 μJ)[10] produces the emissive $^1\pi\pi^*$ excited state with its characteristic absorption at 660 nm. The singlet decays monoexponentially, with lifetimes of 1.4 and 1.5 ns for ZnPCOOH and ZnPCOOD, respectively, to form a long-lived triplet state (τ_0 = 40 μs). The ZnPCOOH(D) donor is a powerful one-electron reductant from its $^1\pi\pi^*$ and is capable of reacting with a variety of organic acceptors including DNBCOOH(D), for which the driving forces for forward and back electron transfer are −0.73 and −1.37 V, respectively.[11] Indeed, addition of DNBCOOH(D) to dichloromethane solutions of ZnPCOOH(D) leads to the rapid quenching of the $^1\pi\pi^*$ excited state.

Steady-state and time-resolved experiments are consistent with a unimolecular quenching reaction of complex **1**, whose association constant is 552 M^{-1}.[12] The singlet lifetime of ZnPCOOH(D)

† A preliminary account of this work was presented at the conference Photoinduced Proton Transfer Dynamics in Chemistry, Biology, and Physics held at Florida State University, Tallahassee, FL, on 6–9 January 1991 in recognition of Michael Kasha's contributions in the area of proton transfer chemistry.

‡ Alfred P. Sloan Fellow and NSF Presidential Young Investigator.

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(10) A full description of the picosecond transient absorption instrument will be provided at a later time. The temporal calibration (zero time) of the instrument was obtained by overlapping the pump pulse with the red edge of the continuum pulse in our spectral window. In this manner, a signal arising from the effects of group velocity dispersion of the white light traveling through our optics (~2 ps) is observed only prior to zero time (Greene, B. I.; Hochstrasser, R. M.; Weisman, R. B. *J. Chem. Phys.* **1979**, *70*, 1247).

(11) The redox potential of excited-state donor, $E_{1/2}(ZnP^{+/*})$, is −1.30 V as calculated from $E(^1\pi\pi^*)$ = 2.1 eV and $E_{1/2}(ZnP^{+/0})$ = 0.80 V vs NHE (Kalyasundaram, K.; Newman-Spallart, M. *J. Phys. Chem.* **1982**, *86*, 5163), and that for the acceptor, $E_{1/2}(DNB^{0/-})$, is −0.57 V vs NHE (Mann, C. K.; Barnes, K. K. *Electrochemical Reactions in Non-Aqueous Systems*; Marcel Dekker: New York, 1970).

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