"helix-turn-helix" region (Leu 71, 75, and 89) have NH lifetimes of <0.5 h. The faster exchange of residues 20, 36, 96, and 105 can be rationalized by noting that these are all either in the first turn of their respective helices (20, 36, 96)[5] or the last residue at the end of a helix (105).[5] For region D, which in solution structure calculations[4,5] appears relatively open, one might argue that ready solvent accessibility, rather than the opening of a helix, is the main factor in determining the high exchange rate. However, for region E, which in the holorepressor appears as a well-defined helix, it would be difficult to maintain that *anything but* the more frequent opening of helix E as compared to A, B, C, or F is the factor determining the more rapid exchange. Even if the limiting step in determining the measured rate were the intrinsic NH exchange rate,[14] the measured rate represents a lower limit for the rate of helix opening. It should be noted that these results are not inconsistent with helix E comprising part of the corepressor binding site.[3,4] Although Trp binds tightly to repressor (K_D = 15 μM),[15] under the conditions of the NMR experiment the ligand is in intermediate to fast exchange on the NMR time scale,[5] as defined by the chemical shift difference between the free and bound forms. Thus, the corepressor is exchanging in and out of the repressor 5000-10000 times faster than the estimated rate of opening of helix E.

Acknowledgment. This work was supported by NIH Grants RR02300 and GM33385.

Supplementary Material Available: Table of actual exchange rates and ^{15}N chemical shifts for [^{15}N]Leu *trp* apo- and holorepressor (1 page). Ordering information is given on any current masthead page.

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Photoinduced, Polyelectrolyte-Driven Release of Contents of Phosphatidylcholine Bilayer Vesicles

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We report herein a new approach to the photoinduced release of contents of lipid bilayer vesicles.[1] The method builds on prior work from this laboratory, in which it was shown that adsorption of the hydrophobic polyelectrolyte poly(2-ethylacrylic acid) (PEAA, **1**) on phosphatidylcholine (PC, **2**) membranes causes conversion of the lipid from vesicular to mixed micellar form.[2] The adsorption of PEAA and the attendant vesicle-to-micelle transition are driven by increasing proton concentration, such that PC vesicles that are resistant to leakage at pH 7.4 can be induced to release their contents rapidly and quantitatively upon mild acidification. In the work described herein, we have used 3,3'-dicarboxydiphenyliodonium salts (**3**) as water-soluble, photosensitive proton sources.[3,4] Irradiation of vesicular PC suspensions prepared in dilute aqueous solutions of **1** and **3** results in rapid loss of vesicle contents.

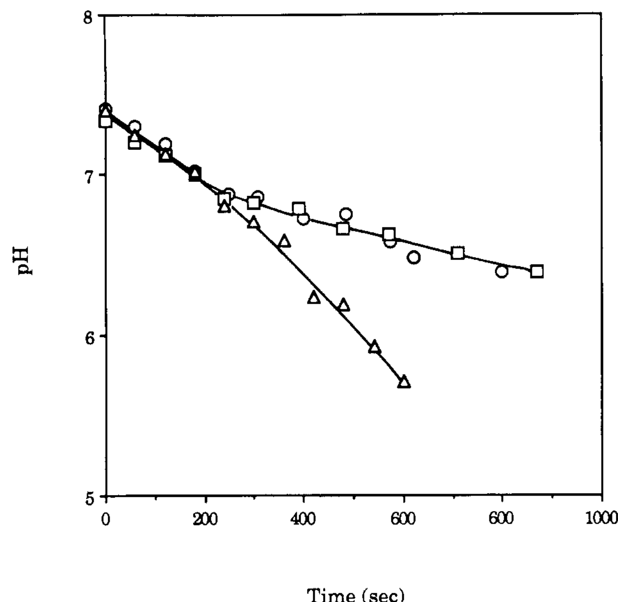


Figure 1. pH as a function of irradiation time for suspensions of **2a** and **2b** in 4 mM solutions 3,3'-dicarboxydiphenyliodonium hexafluorophosphate in 10 mM Tris buffer; (O) **2a** in 0.7 mg/mL PEAA, (□) **2b** in 0.7 mg/mL PEAA, (Δ) polymer-free suspension of **2b**.

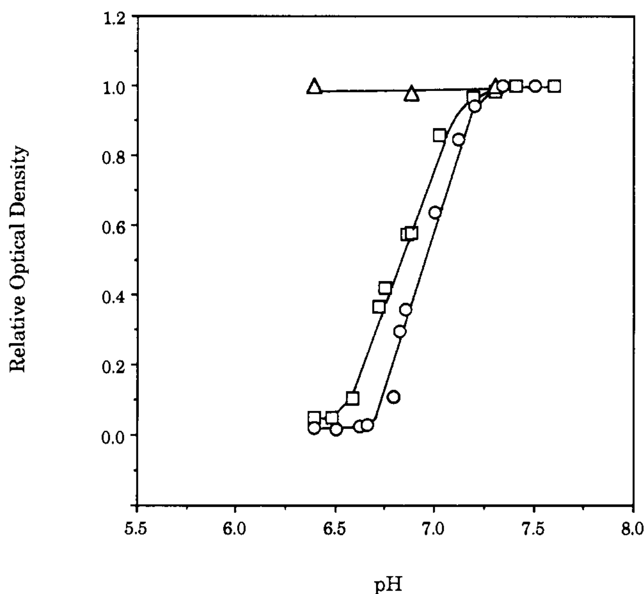
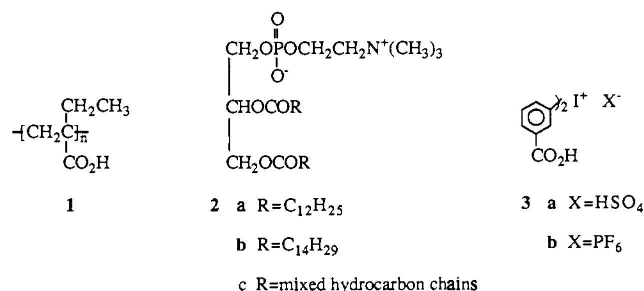


Figure 2. Optical density (relative to that before photolysis) at 600 nm as a function of pH for photolyzed suspensions of **2a** and **2b** in 4 mM 3,3'-dicarboxydiphenyliodonium hexafluorophosphate in 10 mM Tris buffer; symbols as in Figure 1. Estimated errors are ±0.02 units in pH and in relative optical density.



(1) Photoinduced reorganization of bilayer vesicles has been accomplished previously by (i) photoisomerization of azobenzene or retinoic acid derivatives incorporated into the bilayer (Kano, K.; Tanaka, Y.; Ogawa, T.; Shimomura, M.; Kunitake, T. *Photochem. Photobiol.* **1981**, *34*, 323. Pidgeon, C.; Hunt, C. A. *Photochem. Photobiol.* **1983**, *37*, 491. Morgan, C. G.; Thomas, E. W.; Sandhu, S. S.; Yianni, Y. P.; Mitchell, A. C. *Biochim. Biophys. Acta* **1987**, *903*, 504), (ii) photolytic cleavage of bilayer-forming amphiphiles (Fuhrhop, J. H.; Bartsch, H.; Fritsch, D. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 804. Haubs, M.; Ringsdorf, H. *Nouv. J. Chim.* **1987**, *11*, 151), or (iii) photoinduced polymerization and phase separation with conversion of the remaining lipid to a nonlamellar phase (Frankel, D. A.; Lamparski, H.; Liman, U.; O'Brien, D. F. *J. Am. Chem. Soc.* **1989**, *111*, 9262).

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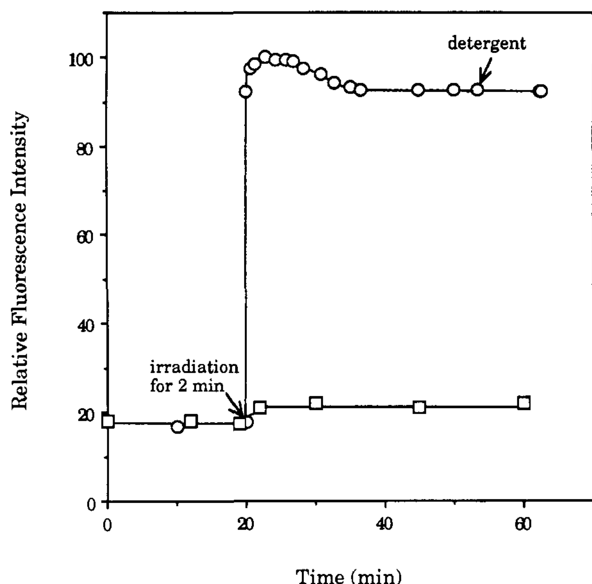

Figure 3. Fluorescence intensity of calcein-loaded suspensions of **2c** suspended in 1.6 mM **3a** (1 mM Tris buffer, 100 mM NaCl); (O), PEAA 0.2 mg/mL; (□), polymer-free control. The initial pH of sample was 7.7, the final pH 6.69. Arrows indicate times of irradiation and detergent addition.

Figure 1 shows the time course of acidification upon irradiation of suspensions of **2a** and **2b** prepared in 4 mM aqueous solutions of **3b**.[5] In the absence of PEAA, pH decreases nearly linearly with irradiation time, from an initial value of 7.4 to a value of 5.7 in 10 min under these irradiation conditions. Addition of PEAA increases the buffer capacity of the solution and slows the rate of pH depression; nevertheless, acidification is sufficient to drive the vesicle-to-micelle transition, as shown by an abrupt loss of the turbidity of the suspension (Figure 2).[6]

Figure 3 confirms that photoinduced acidification of PC suspensions can be used to effect release of vesicle contents.[7] Calcein was used as the marker dye, and release is reported as an increase in fluorescence emission as the dye is diluted into the extravesicular space.[8] As shown in Figure 3, the initial vesicle preparation (at pH 7.7) is stable with respect to leakage, but irradiation for 2 min results in quantitative release of marker. Irradiation for shorter times can be used to effect partial release of contents, as shown by a further increment in fluorescence emission upon addition of lytic concentrations of Triton X-100.

Acknowledgment. This work was supported by U.S. Army Research Office Grant DAAL03-88-K-0038.

(4) 3,3'-Dicarboxydiphenyliodonium disulfate (mp 162–4 °C) and hexafluorophosphate (mp 173–8 °C) were prepared by the method of Beringer and co-workers (Beringer, F. M.; Drexler, M.; Gindler, E. M.; Lumpkin, C. C. *J. Am. Chem. Soc.* **1953**, *75*, 2705).

(5) A solution of 15 mg of **2** in 3 mL of $CHCl_3$ was evaporated to dryness in a long-necked, round-bottomed flask on a rotary evaporator. After drying under vacuum for ca. 6 h, the lipid film was hydrated with 15 mL of 10 mM Tris buffer containing 50 mM NaCl and 0.01% NaN_3 with vortex agitation at a temperature slightly above the lipid melting transition. Rates of pH depression of aqueous solutions of iodonium salts (4 mM) in the absence and in the presence of PEAA (0.7 mg/mL) were measured in quartz cells of 0.4-cm inner diameter upon irradiation at 30 °C in a Rayonet minireactor (Southern New England Ultraviolet Co.) equipped with a merry-go-round sample holder and four 254-nm RPR mercury lamps. The sample cells were periodically withdrawn and shielded from the light, and pH was measured immediately. The sample of PEAA used in this work was of number-average molecular weight ca. 2×10^4 (Schroeder, U. K. O.; Tirrell, D. A. *Macromolecules* **1989**, *22*, 765) and is nearly atactic (Seki, K.; Tirrell, D. A. *Macromolecules* **1984**, *17*, 1692).

(6) For measurement of suspension turbidity, solutions of PEAA (0.7 mg/mL), 3,3'-dicarboxydiphenyliodonium hexafluorophosphate (4 mM), and lipid (0.7 mg/mL) in 10 mM Tris buffer were irradiated as described above. The sample cells were periodically withdrawn, shielded from the light, and kept in a constant-temperature bath (at 24 °C for **2a**, 30 °C for **2b**) to give a total of 30 min of incubation time. Optical density at 600 nm and pH were then measured at 25 ± 0.2 °C.

(7) Calcein-loaded vesicles of **2c** were prepared by sonication of a 15 mg/mL lipid suspension in a 250 mM solution of the dye in 1 mM Tris containing 100 mM NaCl. Small vesicles were isolated by gel filtration on Sepharose CL-4B. Lipid concentration was determined by the method of Charles and Stewart (Charles, J.; Stewart, M. *Anal. Biochem.* **1980**, *104*, 10) and adjusted to 0.04 mg/mL. PEAA was added to a concentration of 0.2 mg/mL and **3b** to a concentration of 1.6 mM. A 2-mL aliquot of the suspension was irradiated for 2 min in a quartz cuvette with a 254-nm Pen-Ray UVP lamp (4500 mW/cm² at 2.5 cm). Fluorescence intensity at 520 nm was recorded immediately on a Perkin-Elmer MPF-66 spectrometer thermostated at 25 °C (excitation at 495 nm; 5-nm slit widths). Maximum fluorescence intensity was obtained by addition of 0.05 mL of a 15% solution of Triton X-100.

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Multiring Interlocked Systems: Structure Elucidation by Electrospray Mass Spectrometry

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Interlocked rings (catenanes) have been a synthetic challenge in chemistry for decades.[1,2] They have experienced a revival of interest since new synthetic strategies have been developed based either on the three-dimensional template effect of a transition metal[3] or on preorganized donor–acceptor stacked arrangements.[4]

Systems consisting of several interlocked cycles may lead to long multilink chains (olympics), molecular collar chains, etc. and are thus especially appealing. The template synthesis of catenands (coordinating interlocked rings) opens the door to an infinity of topologically novel molecular systems, and in particular, it allows relatively easy synthesis of [3]-catenands[5,6] consisting of two peripheral rings separately interlocked to a central cycle.

The most efficient procedure relies on a cyclodimerization reaction, as represented in Figure 1. Besides the expected cyclodimer ($n = 1$), a substantial proportion of higher homologues may also be found.

Applying the strategy of Figure 1 to coordinating fragments bearing terminal acetylenic functions and using copper(I) as templating species, we prepared two series of cyclooligomeric compounds. The first family contains 30-membered peripheral rings interlocked to a large central cycle, whereas the second one contains 27-membered peripheral rings. Unexpectedly, we observed in both series the formation of higher homologues ranging from $n = 1$ to $n = 5$.

The present report will be restricted to the description of the various catenates consisting of up to six peripheral 27-membered rings interlocked to a large central cycle (up to 132 atoms). The various compounds studied are represented in Figure 2.[7]

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(7) **1**, **2**, and $[1,2,Cu]^+ \cdot BF_4^-$ were prepared as previously reported.[6,9] Chromatographic separation (silica; CH_2Cl_2 and small amounts of CH_3OH as eluent) afforded 3^{2+} (23%), 4^{3+} (23%), and 5^{4+} (10%) as pure isolated BF_4^- salts. These compounds are deep red glassy solids. They display very similar ¹H NMR properties and have identical elemental analyses. They can thus only be distinguished from one another by mass spectrometry. In addition, a mixture of 5^{4+}, 6^{5+}, and 7^{6+} (6%, 8%, and 5%, respectively) was also obtained. The yields indicated in ref 9 for 3^{2+} and 4^{3+} are erroneous due to the rigorously identical ¹H NMR spectra of these two compounds.

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