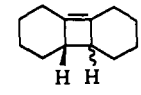
(*E*,*Z*)-**2**/(*E*,*E*)-**2** = 2.4 from *cis*- and *trans*-**1**, respectively. Comparison of these ratios with those observed from photolysis of *cis*-**1** [(*E*,*E*)-**2**/(*E*,*Z*)-**2** = 3.3] and *trans*-**1** [(*E*,*Z*)-**2**/(*E*,*E*)-**2** = 7.5] indicates that if ring opening occurs by the adiabatic disrotatory pathway, it does so only partially; in both cases, the relative yield of the formally allowed diene isomer is too high to be accounted for solely by this mechanism.

The degree of stereospecificity associated with photochemical ring opening of *cis*- and *trans*-**1** is substantially higher than that observed for any of the mono-,[3,4] bi-,[5-7] or tricyclic alkylcyclobutenes[8] that have now been studied, and the reaction is unusually efficient.[3,4,6] From both isomers of **1**, the major diene product is that of orbital symmetry allowed,[19] disrotatory ring opening. The present results should be compared to those obtained for the monocyclic analogues (*cis*- and *trans*-**3**) under similar conditions: these compounds afford mixtures of isomeric 3,4-dimethyl-2,4-hexadienes with only a slight preference for the formally allowed product(s).[4] The substantial difference between the ring-opening behavior of **1** and **3** is inconsistent with a nonconcerted mechanism for the reaction. In fact, the present results provide the strongest indication to date that *orbital-symmetry factors do play a role* in the photochemical ring opening of cyclobutenes.

3



4

Fully orbital symmetry controlled ring opening would be expected to proceed via a pathway involving partial disrotatory ring opening in the excited state followed by internal conversion to the ground state at a geometry corresponding to the pericyclic maximum in the ground state potential energy surface for disrotatory ring opening.[20] Evidence is available to suggest that the reaction is in fact initiated with disrotatory motions along the reaction coordinate, viz., the substantially higher quantum yield for ring opening of *cis*-tricyclo[6.4.0.0^{2,7}]dodec-1(2)-ene (*cis*-**4**) compared to that of the trans isomer.[8,21] Presumably, the ultimate formation of forbidden diene isomers[5-8] is due to some intervening process (of relatively minor importance in **1**) which diverts reactivity away from the "normal" pericyclic pathway. Possibilities include internal conversion to upper vibrational levels of ground-state cyclobutene, complete ring opening on the excited-state surface to yield singlet (vertical) excited dienes, or conversion to biradicaloid ground-state configurations.

The present results for **1** indicate that the overall course of the reaction, and its efficiency, can be altered as a result of specific structural features present in the cyclobutene (or perhaps the product dienes). One possible factor is ring strain (the activation energies for thermal ring opening of systems like **1** are 2-3 kcal/mol lower than those for the monocyclic analogues[17c]). However, the conformational rigidity of the product dienes or the alkyl substituents on the cyclobutene double bond might also play a role in altering the course of the reaction. Further studies of the effects of structural rigidity on the photochemical ring opening of cyclobutene derivatives are in progress.

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(18) For *cis*-**1**, the predicted ratio is given by $E,E/E,Z = (1 - \sum\phi_{EE\rightarrow}) / \phi_{EE\rightarrow EZ}$, where $\phi_{EE\rightarrow EZ}$ is the quantum yield for photoisomerization of (*E*,*E*)-**2** to (*E*,*Z*)-**2** and $\sum\phi_{EE\rightarrow}$ is the sum of the quantum yields for all the products of photolysis of (*E*,*E*)-**2**.

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Unusual Dynamic Features of the *trp* Repressor from *Escherichia coli*

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The *trp* repressor from *Escherichia coli* (a 25-kD symmetric dimer) is a DNA-binding protein that regulates the tryptophan biosynthetic pathway.[1] In the absence of Trp, the repressor has a low affinity for DNA and the transcription of the three operons involved in the de novo synthesis of Trp can proceed. At high Trp concentration, a stable ternary complex between the repressor, two Trp molecules, and operator DNA is formed, and the transcription of the *trp* operon is inhibited. Both the crystal structures[2,3] and the NMR solution structures[4,5] for both the aporepressor (Trp free) and the holorepressor (Trp bound) are known, and a structure of a DNA-repressor complex has been reported[6] and challenged.[7] The structural differences between the apo and holo forms of the repressor are relatively small, and the precise mechanism of repressor action is as yet not completely understood.

In an effort to better define this mechanism, we report herein amide-proton exchange rate data which point to significant differences in dynamics *between* and *within* the two repressor species. In preliminary one-dimensional NMR studies, the backbone NH population of the aporepressor could be classified into three classes according to their exchange rates in D_2O: rapid (lifetimes <10 min at 35 °C) = ~40% of total, intermediate (30-90 min) = ~10%, and slow (24-48 h) = ~50%. This was somewhat surprising, as the protein is 70% helical, and conventional wisdom would lead one to expect relatively slow exchange rates for all backbone NH's in helices. Approximate individual exchange rates were measured in a series of heteronuclear multiple quantum (HMQC)[8] experiments on a uniformly ^{15}N labeled holorepressor for a period of 27 h after dissolving in D_2O. A plot of individual lifetimes ($\tau = 1/k$) as a function of residue number is shown in Figure 1A, along with the secondary structure (helices A-F) of the monomer chain.[5] The striking result is that the exchange rates of backbone NH's in the DNA-binding region (D and E) are 2 orders of magnitude faster than for the ABCF dimeric core of the molecule.

A detailed comparison of the exchange rates in the presence and in the absence of tryptophan was carried out in similar experiments on a repressor selectively labeled with [^{15}N]leucine. We chose to label Leu because the 19 Leu residues are distributed throughout the repressor sequence with at least one residue residing in each of the α-helices. Furthermore, with only 19 amide protons to monitor, even the 1D ^{15}N-edited spectra were readily interpreted. Figure 1B shows the lifetimes of the leucine NH's of the aporepressor measured in a series of 1D ^{15}N-edited proton spectra recorded at 35 °C for a period of 44 h after dissolving in D_2O. Several of the Leu NH's had almost completely exchanged within the 10 min it took to dissolve the sample, put it in the spectrometer, and acquire the first spectrum ($\tau \leq 0.2$ h). For the holorepressor many of the exchange rates were slow enough that a series of 2D

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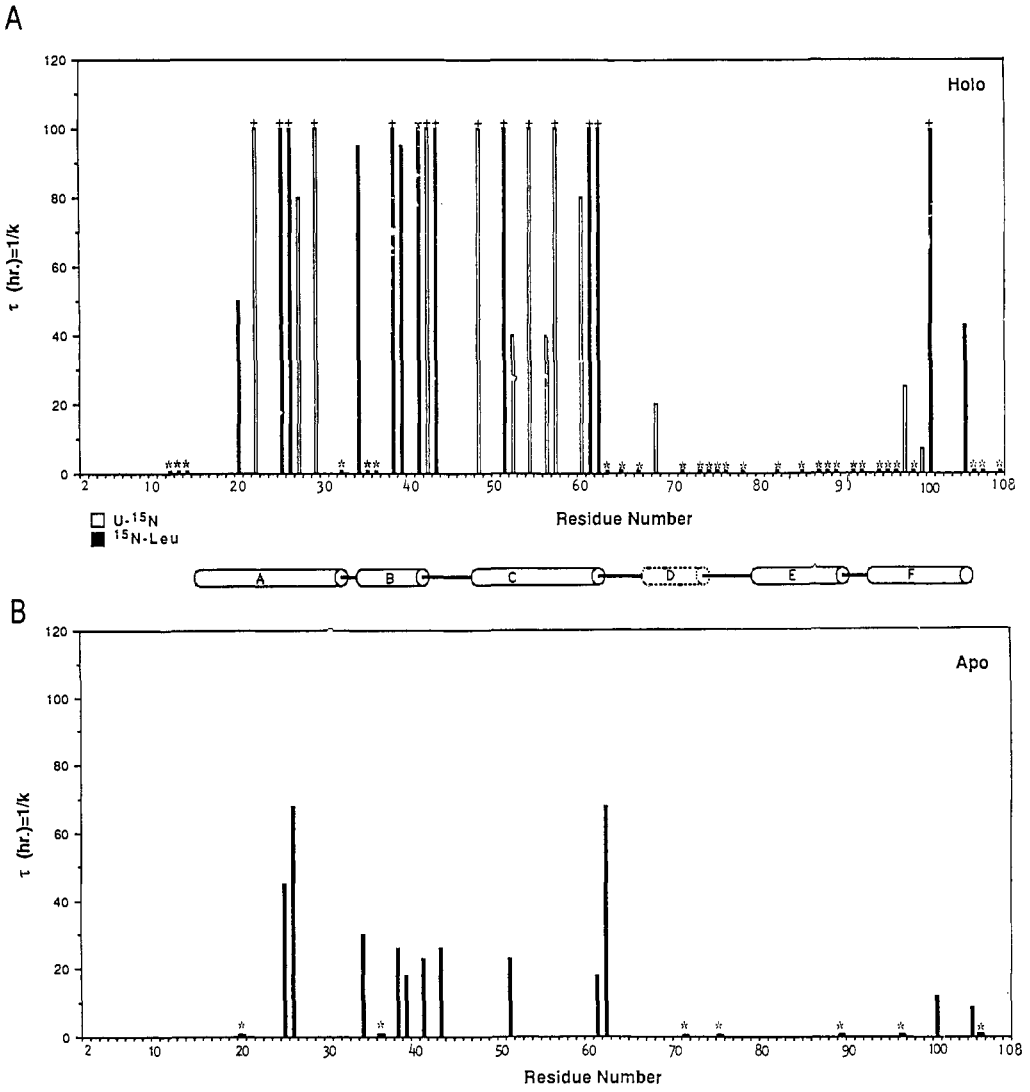

Figure 1. (A) Lifetimes ($\tau = 1/k$) of ^{15}N-labeled amide protons in uniformly ^{15}N labeled *trp* holorepressor (□) and selectively [^{15}N]Leu labeled *trp* repressor (■). Values >100 h are indicated with + and those <1 h with *. The values for U-^{15}N repressor were estimated from the decay of the 2D cross peak intensity in HMQC experiments (see text). The values for leucines in the holorepressor were calculated from the decay in peak intensities in the ^{15}N-edited ^{1}H spectrum of the first FID of a series of HMQC spectra of [^{15}N]Leu-labeled repressor. (B) Lifetimes ($\tau = 1/k$) of ^{15}N-labeled amide protons in [^{15}N]Leu-labeled *trp* aporepressor. These values were calculated from the decay in peak intensity in a series of 1D ^{15}N-edited ^{1}H spectra. Helical regions A–F[5] are indicated. Solution conditions for both samples were as follows: pH = 5.7, 500 mM NaCl, 50 mM sodium phosphate, 1 mM *trp* repressor (monomer concentration). The assignments for NH protons of all leucines except Leu71 have been previously reported.[5] The Leu ^{15}N resonances were assigned from a 2D HMQC-NOESY[9] spectrum of the holorepressor in which NOEs to neighboring sequential NH protons at each ^{15}N frequency were used to identify proton resonances correlated with each ^{15}N resonance. The only unassigned proton–nitrogen correlation was assigned to Leu71. Assignments for U-^{15}N holorepressor were based on previously reported[5] proton chemical shifts. Only those cross peaks that could be unambiguously assigned in the HMQC spectrum are reported here. Thus, blank spaces correspond to either Pro residues (no NH), Thr residues which are unlabeled because the protein was isolated from a Thr auxotrophic strain, or unassigned protons in the HMQC spectrum. Estimated error for leucine NH lifetimes is ~±10%.

HMQC experiments were recorded over a period of 101 h. The same residues that exchanged rapidly in the aporepressor also exchange fast in the holorepressor, although the rates are approximately 3 times slower for the latter. Figure 1 also shows that the slowly exchanging leucine NH's in the holorepressor are 1 order of magnitude slower than their counterparts in the aporepressor. These results clearly represent major differences in the dynamic behavior between the two species as well as major regional differences in the dynamics of each of the two structures.

The amide proton exchange rates observed for the DNA-binding region (D and E) are much more rapid than for the ABCF helices, in both the apo and the holo form. These results provide direct experimental evidence showing that helices D and E are the most flexible regions of the protein, as has been suggested by NMR,[4,5] crystallographic,[10,11] and molecular dynamics[12] studies. It may also explain the paucity of sequential and medium-range NOEs in this region of the repressor.[5] All of these findings point to extensive mobility of the DNA-binding domain. Binding tryptophan slows the exchange rates in all regions of the molecule. Tryptophan is also known to slow down the monomer–dimer equilibrium. This equilibrium lies far toward the dimer species for both the apo- and holorepressors, but the rate of monomer–dimer exchange is slower in the holorepressor.[13] An exact correlation between the rates of the two processes under different ambient conditions remains to be established.

Helices ABC′F′ (and A′B′CF)[2-5] thus form a *relatively* stable dimer core (average lifetimes of the Leu NH's in this region are 500 h for the holo form), while residues in the DNA-binding

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"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.

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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.

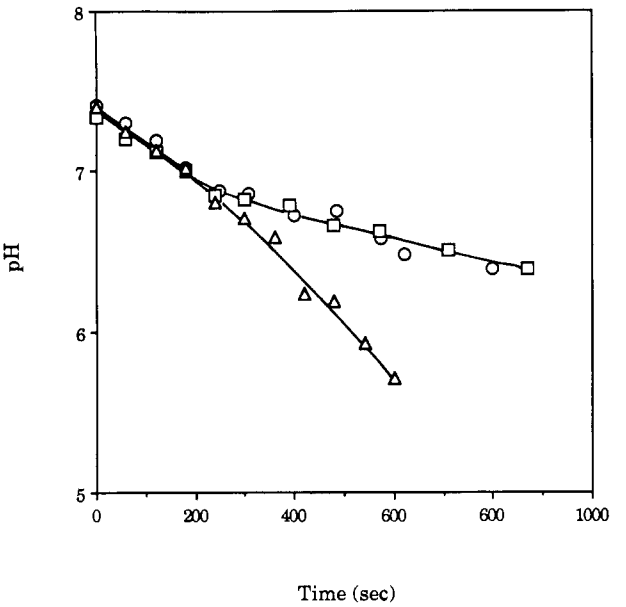


Figure 1. pH as a function of irradiation time for suspensions of **2a** and **2b** in 4 mM solutions 3,3'-dicarboxydiphenyliodonium hexafluorophosphate in 10 mM Tris buffer; (○) **2a** in 0.7 mg/mL PEAA, (□) **2b** in 0.7 mg/mL PEAA, (△) polymer-free suspension of **2b**.

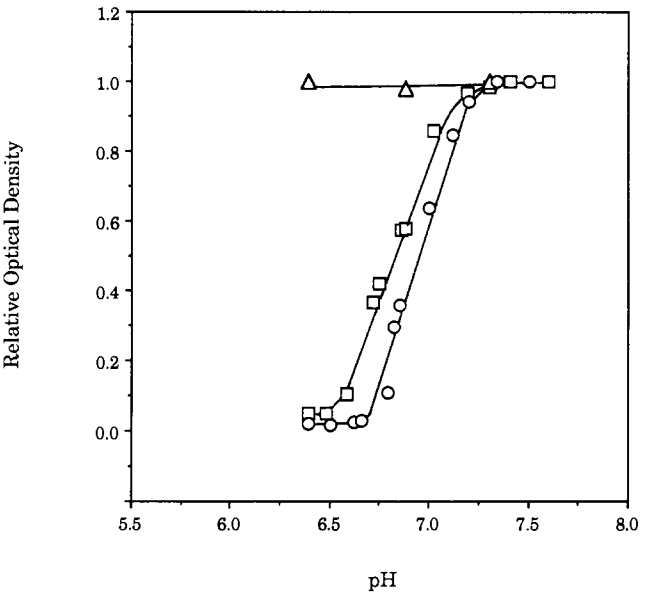
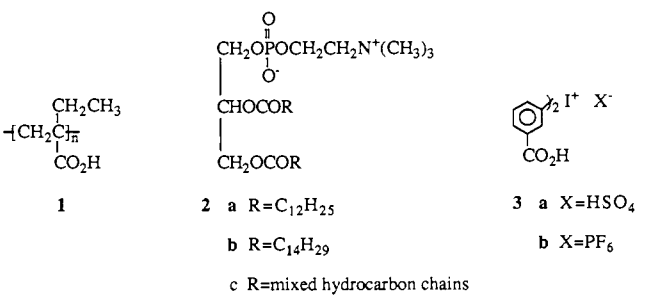


Figure 2. Optical density (relative to that before photolysis) at 600 nm as a function of pH for photolyzed suspensions of **2a** and **2b** in 4 mM 3,3'-dicarboxydiphenyliodonium hexafluorophosphate in 10 mM Tris buffer; symbols as in Figure 1. Estimated errors are ±0.02 units in pH and in relative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



1

2 a R=$C_{12}H_{25}$
b R=$C_{14}H_{29}$
c R=mixed hydrocarbon chains

3 a X=HSO_4
b X=PF_6

(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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