of molecular recognition and selectivity in electrochemical reactions.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research. We also acknowledge and thank the National Science Foundation for supporting this research under Grant CHE-8807846.

Conformational Selectivity in Molecular Recognition: The Influence of Artificial Receptors on the Cis–Trans Isomerization of Acylprolines

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Received February 25, 1991

The cis–trans isomerization of acylprolyl groups (Figure 1) is a kinetically significant step in protein folding[1] and, as such, has been the subject of intense scrutiny.[2] This interest has increased with the recent identification of a family of enzymes that catalyze this interconversion and the important role that two of their number, cyclophilin[3] and FK506 binding protein,[4] play in immunosupression. The peptidyl prolyl isomerases (PPIases) have been investigated by spectroscopic,[5,6] synthetic,[6] and kinetic[7] methods, and a catalytic mechanism involving amide-bond distortion has been proposed. Our interest lay in modeling this process, and we report herein that different synthetic receptors can discriminate between the two rotamers and so influence the acylproline s-cis–s-trans equilibrium.

In most acylprolines the s-trans rotamer is favored over the s-cis due to the steric demands of the acyl group.[2c] In succinamide diacid **1**[8,9] the s-cis:s-trans ratio is approximately 1:3.5. During this equilibrium the carboxylic acid groups move from 6.9 Å apart in *s-trans*-**1b** to 5.5 Å apart in *s-cis*-**1a**.[11] This suggested that an artificial receptor with appropriately spaced carboxylic acid binding groups might selectively complex *s-cis*-**1a** and so shift the equilibrium toward the less favorable rotamer. We have previously established[12] that terephthaloyl receptor **2** (NH–NH distance, 7.32 Å) binds strongly to aliphatic dicarboxylic acids (as in Figure 2). Molecular mechanics calculations[11] showed that **2** is well-suited to bind selectively to *s-cis*-**1a** compared to *s-trans*-**1b** (Figure 3). Addition of 1 equiv of **1** to a $CDCl_3$ solution of **2** resulted in a change in the s-cis:s-trans ratio to 3:2.[13] That this selective stabilization is due to binding is seen by the large downfield shifts (from 8.5 to 11.1 ppm)[12] of the amide NH resonances of **2**, as expected for the formation of a tetra-hydrogen-bonded complex of the type shown in Figure 3. Also, a downfield shift (0.4 ppm) of the succinamide αCH_2 confirms the position of the substrate within the cavity. The small preference shown by **2** for *s-cis*-**1a** may be due to the flexibility of the succinamide chain which, when

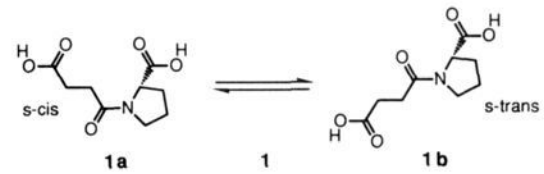

Figure 1.

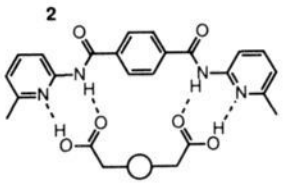

Figure 2.

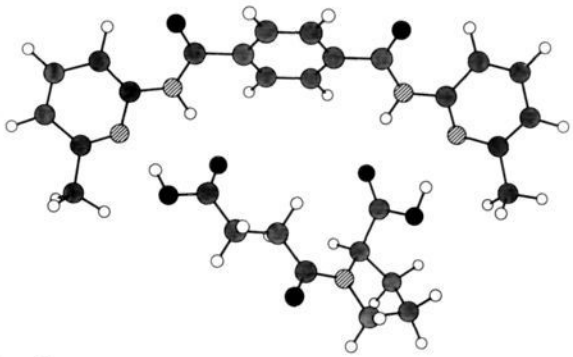

Figure 3.

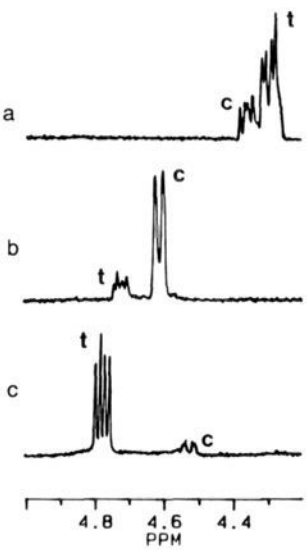

Figure 4. The proline αH region of the ^1H NMR spectra of (a) proline fumaramide **3**,[15a] (b) a 1:1 mixture of **2** and **3**,[15b] and (c) a 1:1 mixture of **6** and **3**.[15b]

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(1) Brandts, J. F.; Halvorson, H. R.; Brennan, M. *Biochemistry* **1975**, *14*, 4953. Fischer, G.; Schmid, F. X. *Biochemistry* **1990**, *29*, 2205.
(2) (a) Grathwohl, C.; Wüthrich, K. *Biopolymers* **1976**, *15*, 2025. (b) Hetzel, R.; Wüthrich, K. *Biopolymers* **1979**, *18*, 2589. (c) Nishihara, H.; Nishihara, K.; Uefuji, T.; Sakota, N. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 553.
(3) Takahashi, N.; Hayano, T.; Suzuki, M. *Nature* **1989**, *337*, 473. Fischer, G.; Wittmann-Liebold, B.; Lang, K.; Kiefhaber, T.; Schmid, F. X. *Nature* **1989**, *337*, 476.
(4) Harding, M. W.; Galat, A.; Uehling, D. E.; Schreiber, S. L. *Nature* **1989**, *341*, 758.
(5) Hsu, V. L.; Handschumacher, R. E.; Armitage, I. M. *J. Am. Chem. Soc.* **1990**, *112*, 6745.
(6) Nakatsuka, M.; Ragan, J. A.; Sammakia, T.; Smith, D. B.; Uehling, D. E.; Schreiber, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 5583.
(7) (a) Harrison, R. K.; Stein, R. L. *Biochemistry* **1990**, *29*, 1684. (b) Albers, M. W.; Walsh, C. T.; Schreiber, S. L. *J. Org. Chem.* **1990**, *55*, 4984.
(8) Formed from proline and succinic anhydride.
(9) Proline diacids of type **1** are interesting both as derivatives of AspPro and as ACE inhibitors[10] related to captopril and enalapril.
(10) Rich, D. H. *Comprehensive Medicinal Chemistry*; Sammes, P. G., Taylor, J. B., Eds.; Pergamon Press: Oxford, 1990; Vol. 2, p 404.
(11) Using MacroModel v.2. Still, W. C. Columbia University.
(12) Garcia-Tellado, F.; Goswami, S.; Chang, S. K.; Geib, S. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **1990**, *112*, 7393.
(13) Rotamer ratios were measured by integration of proline αH signals in the ^1H NMR spectra, and assignments were made by ^{13}C NMR shift comparisons.[14]
(14) Dorman, D. E.; Bovey, F. A. *J. Org. Chem.* **1973**, *38*, 2379.

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in a gauche conformation, would allow *s-trans*-**1b** to form two, albeit less favorable, bidentate hydrogen bonds to **2**.

To test this we have prepared the more rigid fumaramide **3**, which has an unaffected s-cis:s-trans ratio of 1:4.[15a] However, in a 1:1 mixture with **2** in $CDCl_3$, **3** shows a strong (4:1) preference for the s-cis rotamer, as expected for the formation of a complex of type **4**.[16] The shift in equilibrium is clearly seen in the proline αH region of the ^1H NMR spectra of **3** and **2**–**3** (Figure 4, parts a and b). The rotamer assignments were made by chemical shift comparisons[2c,14] and by the observation (from the s-cis form) of an intramolecular NOE between the resonance at 4.62 ppm (Figure 4b) and that of the fumaramide αCH. In the *s-cis*-**3** complex the fumaramide αCH also shows an intermolecular NOE with the terephthaloyl protons and is shifted 0.33 ppm further downfield than in the *s-trans*-**3** complex, reflecting its position close to the periphery of the terephthaloyl spacer (as in **4**). *s-cis*-**3** is able to form two bidentate contacts with **2** while *s-trans*-**3** presumably forms a single contact between the more acidic amino acid carboxylate and one aminopyridine in **2**.

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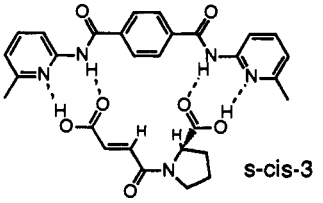

s-cis-3

The conformational selectivity can be varied by changing the position of the aminopyridine binding groups. Addition of biphenyl receptor **5** (NH–NH, 11.7 Å) to **3** leads to a much smaller downfield shift in the amide NH resonances (1.7 ppm), no shift in the fumaramide αCH, and a reversion of the s-cis:s-trans ratio back to 1:4. These results suggest that **5** is too long to form any

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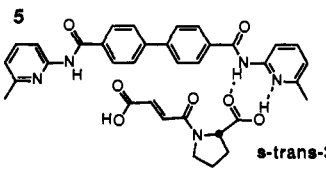
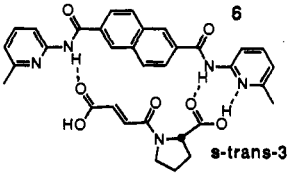


s-trans-3

6



s-trans-3

more than a single bidentate contact with either *s-cis*- or *s-trans*-**3** and so has little effect on either the ratio or the binding-induced NMR shifts. In contrast, naphthyl receptor 6 (NH–NH, 9.39 Å) has a positioning of binding groups intermediate between **2** and **5**. Molecular modeling[11] has suggested that, in addition to the single bidentate contact of **5**, **6** can form a third hydrogen bond to the s-trans fumaramide carboxylate. A 1:1 mixture of **6** and **3** shows a substantial shift in the equilibrium toward the trans rotamer (s-cis:s-trans, 1:8), as seen in the ^1H NMR proline αH region (Figure 4c). Further support for this complex comes from the 0.24 ppm downfield shift of the fumaramide βCH and the observation of an intermolecular NOE between it and the naphthyl protons.

The 32-fold difference in rotamer equilibrium between **2**–**3** and **6**–**3** is less than might be expected for the change in hydrogen-bonding environment, suggesting that these simple hosts are not yet optimized for their substrates. Design improvements as well as modifications to recognize the twisted amide transition state are currently underway.

Acknowledgment. We thank the National Institutes of Health (GM 35208) and the Office of Naval Research for financial support of this work. We are also grateful to the Spanish Ministerio de Educacion y Ciencia for postdoctoral fellowships to C.V. and F.G.-T., the Fulbright Commission for a fellowship to F.G.-T., and Prof. Russell Petter for many valuable discussions.

(15) (a) Measured in $CDCl_3$–CD_3OD (8:2) due to the insolubility of the diacid in $CDCl_3$. (b) Measured in $CDCl_3$.

(16) The K_a for **2**–**3** was too high to be measured accurately by ^1H NMR and was estimated at 10^4–10^5 M^{-1}.

Specific Enzyme-Induced Decapsulation

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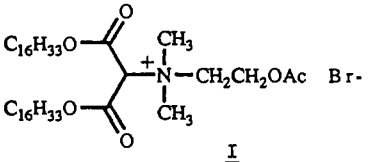
Received April 8, 1991

Little difficulty exists in finding chemotherapeutic agents that kill pathological cells; the problem lies in identifying agents that do so without damaging normal cells as well.[1] One possible route to selectivity plays on the fact that certain pathological cells produce excessive amounts of a particular enzyme (e.g., bone cancer/alkaline phosphatase;[2] neuroblastoma/acetylcholinesterase[3]). If such an enzyme is capable of breaking open specially designed vesicles that encapsulate a cytotoxic drug, then selective therapeutic activity toward the enzyme-exuding cell can be achieved.[4] Since this mechanism of selectivity depends on the encapsulating vesicle, and not on the drug within, the choice of drug becomes less restrictive.

We describe herein compound I which, in concert with acetylcholinesterase (AcE),[5] models the above process.[6,7] Since I



I

has an ionic headgroup plus two long hydrocarbon tails, it possesses the two features necessary for vesicle formation.[8] And since I also incorporates an acetylcholine-like moiety, it reacts with AcE to form a primary alcohol (eq 1, Scheme I). The hydroxyl can then engage in an intramolecular attack upon the carbonyl (eq 2, Scheme I) to eject one of the two tails. But single-chain amphiphiles do not form bilayers and, in fact, they are often used to destroy vesicles.[9,10] Consequently, the vesicular system will experience an enzyme-induced "lesion" and ultimate destruction. Vesicular contents would, naturally, escape.

Compound I was synthesized by monobromination of dihexadecyl malonate (Br_2, refluxing CCl_4, 87%)[11] followed by reaction with 2-(dimethylamino)ethyl acetate[12] (5-fold excess of amine, no solvent, 45 °C, 48%). The product was purified by three or four crystallizations and characterized by ^1H and ^{13}C NMR, IR, elemental analysis, and FAB-MS.

Vesicles of I were formed by bath sonication of a 5-mg film of I with 2.0 mL of buffer at 25 °C for 5 min and then at a temperature 5–10 deg above the phase-transition temperature of 46 °C (a value determined by differential scanning calorimetry). Quasi-elastic light scattering[13] on the clear solutions revealed that

(1) Sneader, W. *Drug Discovery: The Evolution of Modern Medicines*; Wiley: New York, 1985; Chapter 16.

(2) Bodansky, O. *Biochemistry of Human Cancer*; Academic Press: New York, 1975; p 80.

(3) Kimhi, Y.; Mahler, A.; Saya, D. *J. Neurochem.* **1980**, *34*, 554. Stieger, S.; Buetikofer, P.; Wiesmann, U. N.; Brodbeck, U. *J. Neurochem.* **1989**, *52*, 1188.

(4) Phospholipid vesicles are degraded in nature by phospholipases. The goal here is, in effect, to mimic this process with synthetic lipids and with enzymes normally intended for other purposes.

(5) Quinn, D. M. *Chem. Rev.* **1987**, *87*, 955.

(6) This work is abstracted from the Ph.D. Thesis of D. E. Johnston, Jr. (Emory University, 1990), with a title identical with that of this article. Full experimental details are contained therein.

(7) The CA index name for I is 2-propanaminium, *N*-[2-(acetyloxy)ethyl]-1,3-bis(hexadecyloxy)-*N*,*N*-dimethyl-1,3-dioxo-, bromide.

(8) Kunitake, T.; Okahata, Y. *J. Am. Chem. Soc.* **1977**, *99*, 3860.

(9) Zaslavskii, B. Y.; Borovskaya, A. A.; Lavrinenko, A. K.; Lisichkin, A. Y.; Davidovich, Y. A.; Rogozhin, S. V. *Chem. Phys. Lipids* **1980**, *26*, 49.

(10) Sunamoto, J.; Kondo, H.; Yoshimatsu, A. *Biochem. Biophys. Acta* **1978**, *510*, 52.

(11) Palmer, C. S.; McWherter, P. W. *Organic Syntheses*; Gilman, H., Ed.; Wiley: New York, 1941; Collect. Vol. I, p 245.

(12) Purchased from Kodak.

(13) Menger, F. M.; Lee, J.-J.; Aikens, P.; Davis, S. *J. Colloid Interface Sci.* **1989**, *129*, 185.

0002-7863/91/1513-5467$02.50/0