residue four-helix bundle metalloprotein. The availability of such a highly convergent and synthetically simple approach for the construction of protein-like structures should benefit future designs of functional macromolecules.

Acknowledgment. The Searle Scholars Program/The Chicago Community Trust and the Arnold and Mabel Beckman Foundation are gratefully acknowledged for their financial support. We are also grateful to colleagues M. Milik, D. Stout, and D. McRee for their contributions to computer modeling and graphics presentation, S. Kent, A. Jones, and M. Griffith for mass spectroscopic analysis, and R. LaBorde at The Scripps Institute of Oceanography and colleague K. Fernholz for their assistance in atomic absorption spectroscopy.

A Racemic Protein

Laura E. Zawadzke and Jeremy M. Berg*

Department of Biophysics and Biophysical Chemistry
The Johns Hopkins University School of Medicine
Baltimore, Maryland 21205

Received January 22, 1992

Most natural products exist as a single stereoisomer. In contrast, many synthetic chiral compounds exist as 1:1 mixtures of two enantiomeric forms. Such racemic mixtures may have desirable properties over the optically pure materials. For example, many racemic mixtures crystallize such that the two enantiomers are related by an inversion center.[1] This greatly simplifies the crystallographic phase problem.[2] In addition, the availability of the enantiomer of a natural product can provide a substance that is identical to that product except in a chiral environment.[3,4] We report herein the synthesis and characterization of a racemic protein via the synthesis of the two enantiomers separately.

We chose to study the rubredoxin protein native to the anaerobic bacterium *Desulfovibrio desulfuricans* 27774, hereafter RbDD.[5-8] RbDD consists of 45 amino acids including four cysteine residues that act to bind iron or other metal ions. This protein was chosen for study for several reasons, including its small size, its metal binding ability, and the relatively high hydrogenase-like activity of its Ni^{2+} complex.[7] The sequence in the proteins we have synthesized differs from the natural sequence[6] in that the amino terminus lacks a formyl group, and a nonliganding cysteine was changed to alanine. Synthesis was performed using a Milligen/Biosearch 9050 PepSynthesizer and *N*-fluorenylmethoxycarbonyl amino acid pentafluorophenyl esters as described previously.[9] RbDD was synthesized using either all L-amino acids or all D-amino acids. After reduction with β-mercaptoethanol (10 mM) for 2 h at 55 °C, the peptide isomers coelute on the reverse-phase HPLC column in a 27% to 32% gradient of water (0.1% TFA)/acetonitrile (0.1% TFA). Anaerobic metal titrations[10] clearly show that both L- and D-rubredoxin bind metal ions such as Co^{2+} and Fe^{2+} with high affinities with dissociation constants $<10^{-7}$ M. A representative titration is shown in Figure 1. The Fe^{2+} complexes could be oxidized to the characteristically red Fe^{3+} complexes upon exposure to air. As expected, no measurable differences between the L and D proteins were observed.

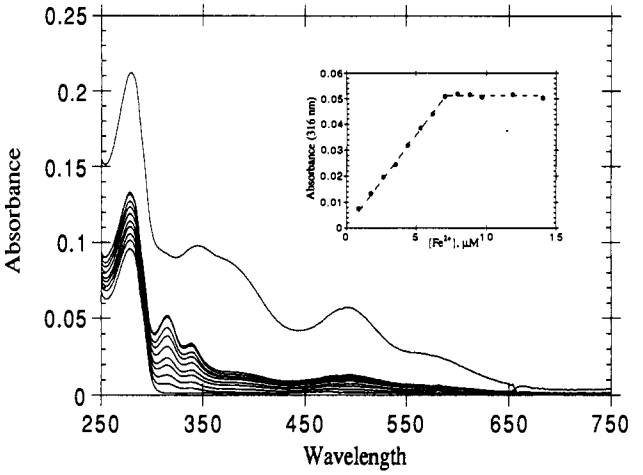


Figure 1. Fe^{2+} titration, Fe^{3+} saturation absorption spectra of D-RbDD. Similar spectra were obtained with $FeCl_2$ titrations of L-RbDD. L- and D-RbDD bind Fe^{2+} ($K_d \leq 5.8 \times 10^{-8}$) with subsequent oxidation to Fe^{3+} upon exposure to oxygen. Extinction coefficients determined from these titrations are for Fe^{2+}, $\epsilon_{316} = 6900\ M^{-1}\ cm^{-1}$, and for Fe^{3+}, $\epsilon_{490} = 7100\ M^{-1}\ cm^{-1}$. The titration curve is shown in the inset and depicts the tight-binding of D-rubredoxin for Fe^{2+}.

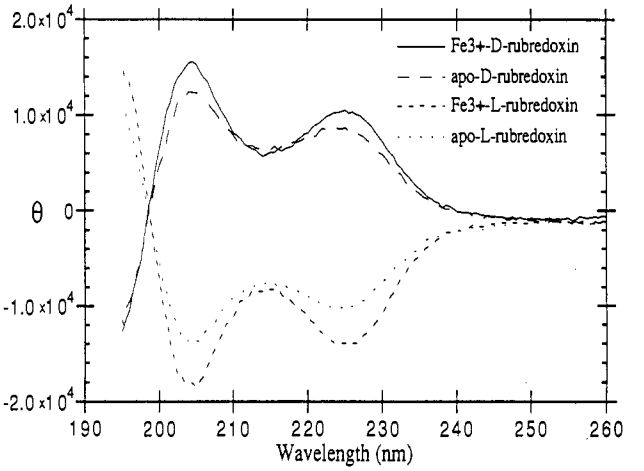


Figure 2. Circular dichroism spectra of Fe^{3+}-substituted RbDD and apo-RbDD isomers. Purified and reduced RbDD was dissolved in 5 mM Tris·HCl buffer to 9 μM, and 1.2 equiv of $FeCl_2$ was added anaerobically, with a final pH of 7.0. After oxidation of the metalated protein, 0.5-mL samples were transferred to a CD cuvette (Hellma No. 282 QS, 0.200-cm path length). Scans were taken from 260 to 195 nm, with five repeats to obtain an average spectrum. Units of θ are in deg·cm^2·(dmol of amino acids)$^{-1}$.

Circular dichroism (CD) spectra were taken using an AVIV Model 60DS circular dichroism spectropolarimeter. CD spectra of the reduced apoproteins as well as the Fe^{3+} complexes are shown in Figure 2. The spectra of both the apo and metalated forms are mirror images of each other. As further evidence for the different behavior of the enantiomers with regard to chiral probes, chymotrypsin digests were performed on both the apo and Fe^{3+} forms. RbDD has six potential chymotrypsin digestion sites within 45 residues. The metal-bound peptides were treated with chy-

(1) Brock, C. P.; Schweizer, W. B.; Dunitz, J. D. *J. Am. Chem. Soc.* **1991**, *113*, 9811.
(2) Stout, G. H.; Jensen, L. H. *X-ray Structure Determination: A Practical Guide*; Macmillan: New York, 1968.
(3) Urata, H.; Shinohara, K.; Ogura, E.; Ueda, Y.; Akagi, M. *J. Am. Chem. Soc.* **1991**, *113*, 8174.
(4) Mickus, D. E.; Levitt, D. G.; Rychnovsky, S. D. *J. Am. Chem. Soc.* **1992**, *114*, 359.
(5) Sieker, L. C.; Stenkamp, R. E.; Jensen, L. H.; Prickil, B.; LeGall, J. *FEBS Lett.* **1986**, *208*, 73.
(6) Hormel, S.; Walsh, K. A.; Prickil, B. C.; Titani, K.; LeGall, J.; Sieker, L. C. *FEBS Lett.* **1986**, *201*, 147.
(7) Saint-Martin, P.; Lespinat, P. A.; Fauque, G.; Berlier, Y.; LeGall, J.; Moura, I.; Teixeira, M.; Xavier, A. V.; Moura, J. G. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 9378.
(8) Stenkamp, R. E.; Sieker, L. C.; Jensen, L. H. *Proteins: Struct., Funct., Genet.* **1990**, *8*, 352.
(9) Green, L. M.; Berg, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 4047.
(10) Berg, J. M.; Merkle, D. L. *J. Am. Chem. Soc.* **1989**, *111*, 3759.

0002-7863/92/1514-4002$03.00/0

motrypsin (45 units/mL) for 5 h with no visible cleavage. The apoproteins were digested (0.75 units/mL) for 1 h at room temperature. Only the apo-L-RbDD was digested as determined by electrophoresis on a denaturing polyacrylamide gel.[11] The resistance of Fe^{3+}-L-rubredoxin to proteolysis is not surprising, in that it appears to be a very well-folded structure.[8] Even after boiling in reducing SDS–sample buffer and subsequent electrophoresis, the Fe^{3+}-bound forms of each isomer did not denature and appeared as red, comigrating bands on an SDS gel.

Initial crystallization studies of racemic RbDD (prepared by mixing equimolar amounts of the two enantiomers) have been performed. The goal is to produce crystals with one molecule in the asymmetric unit and an inversion center relating enantiomers. Although it has been suggested that there are entropic effects favoring racemate formation over spontaneous resolution,[12] Brock et al. have recently pointed out that these effects are extremely small.[1] There may be, however, enthalpic effects favoring racemate crystallization.[1] Clumps of small but apparently well-formed crystals have been obtained under a variety of conditions. CD studies of solutions produced by dissolving small clumps of crystals have revealed no significant CD, suggesting that these crystals do, indeed, contain the racemate. Using similar conditions, larger crystals suitable for diffraction studies have been grown. Characterization of these crystals by X-ray[13] and other methods is in progress.

Acknowledgment. Financial support for this work has been provided by the National Science Foundation and The Lucille P. Markey Trust. L.E.Z. is supported by the National Institutes of Health, National Research Service Award No. 5 F32 GM14070-02 from the National Institute of General Medical Sciences. J.M.B. is an A. P. Sloan Foundation Fellow.

(11) Laemmli, U. K. *Nature (London)* **1970**, *227*, 680.
(12) Jacques, J.; Collet, A.; Wilen, S. H. *Enantiomers, Racemates and Resolutions*; Wiley: New York, 1981.
(13) Mackay, A. L. *Nature* **1989**, *342*, 133.

Enhanced View of Structure and Binding for Cyclophane–Arene Complexes through Joint Theoretical and Experimental Study

William L. Jorgensen* and Toan B. Nguyen

Department of Chemistry, Yale University
New Haven, Connecticut 06511-8118

Elizabeth M. Sanford, Ito Chao, K. N. Houk,* and Francois Diederich*

Department of Chemistry and Biochemistry
University of California
Los Angeles, California 90024-1569

Received February 7, 1992

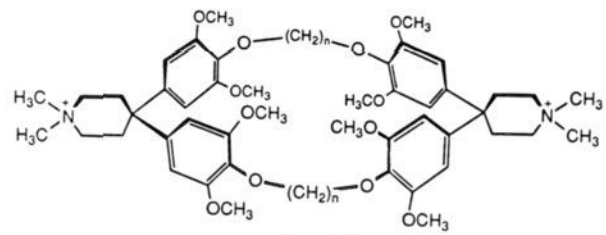
Cyclophane hosts are valuable models for exploring apolar binding in aqueous solution;[1-3] however, simple correlations with aromatic guest solubility or electron donor–acceptor indices are masked by solvophobic forces and specific substituent solvation.[2] Consequently, we have pursued theoretical means to help clarify the structures and solvation of complexes and the energetic contributions to binding.[4] We report here the first Monte Carlo simulations for cyclophane complexes, new experimental data that illustrate the predictive value of the methodology, and combined results that provide striking structural details.

Table I. Calculated and Experimental Free Energy Changes (kcal/mol)

p-xylene to	ΔG_G	ΔG_C	$\Delta\Delta G_b$ calcd[a]	$\Delta\Delta G_b$ exptl[b]
benzene	0.0	2.0	−2.0 ± 0.2	−1.5 ± 0.2[c]
p-cresol	−5.6	−5.7	0.1 ± 0.3	−0.4 ± 0.1[c]
hydroquinone	−11.4	−8.6	−2.8 ± 0.3	−2.2 ± 0.2[d]
p-dicyanobenzene	−7.7	−7.6	−0.1 ± 0.4	−0.1 ± 0.1[d]

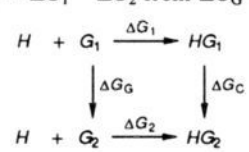
[a] At 298 K. [b] At 293 K. [c] This work. [d] Reference 2.

Figure 1. Sample configuration of host **1** with hydroquinone. Only water molecules hydrogen bonded to hydroquinone are shown.



1, $n = 3$
2, $n = 4$

An initial goal was to compute relative free energies of binding ($\Delta\Delta G_b$) for benzene derivatives with **1** in water. The BOSS program, which performs Monte Carlo statistical mechanics simulations, was enhanced to permit sampling for any bonds, bond angles, and dihedral angles.[5] The stretching and bending force constants come from the AMBER force field,[6] and the nonbonded interactions are described by the OPLS potentials.[7] All atoms are explicit except for hydrogens in CH_2 and CH_3 groups. Bond lengths were fixed except for the length of one ring-closure bond in the macrocycle. Internal coordinates in the benzene and piperidinium rings were not sampled; however, all remaining dihedral angles and the bond angles within the macrocycle were sampled. Statistical perturbation theory[8] was applied with the cycle below to compute $\Delta\Delta G_b = \Delta G_1 - \Delta G_2$ from $\Delta G_G - \Delta G_C$.[9]



Initially, *p*-xylene was energy optimized into the crystal structure

(1) Diederich, F. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 362. Diederich, F. *Cyclophanes*; Monographs in Supramolecular Chemistry, Vol. 2; Stoddart, J. F., Ed.; Royal Society of Chemistry: Cambridge, 1991.
(2) Ferguson, S. B.; Sanford, E. M.; Seward, E. M.; Diederich, F. *J. Am. Chem. Soc.* **1991**, *113*, 5410.
(3) Smithrud, D. B.; Wyman, T. B.; Diederich, F. *J. Am. Chem. Soc.* **1991**, *113*, 5420.
(4) Jorgensen, W. L. *Acc. Chem. Res.* **1989**, *22*, 184. Jorgensen, W. L. *Chemtracts: Org. Chem.* **1991**, *4*, 91.
(5) Jorgensen, W. L. *BOSS*, Version 3.1; Yale University: New Haven, CT, 1991.
(6) Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S., Jr.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765.
(7) (a) Jorgensen, W. L.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1988**, *110*, 1657. (b) Jorgensen, W. L.; Briggs, J. M.; Contreras, M. L. *J. Phys. Chem.* **1990**, *94*, 1683. (c) Jorgensen, W. L.; Severance, D. L. *J. Am. Chem. Soc.* **1990**, *112*, 4768. (d) Nguyen, T. B.; Jorgensen, W. L., to be published.
(8) Zwanzig, R. W. *J. Chem. Phys.* **1954**, *22*, 1420.
(9) Lybrand, T. P.; McCammon, J. A.; Wipff, G. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 833.

0002-7863/92/1514-4003$03.00/0