Acknowledgment. We thank the National Science Foundation (Grant CHE-8612063) for support of the research. NSF support for the Crystallographic Unit at Purdue (Grant CHE-8615556) is also gratefully acknowledged.

Supplementary Material Available: A listing of fractional coordinates, anisotropic thermal parameters, and complete bond distances and angles for **2** (18 pages); listing of observed and calculated structure factors for **2** (31 pages). Ordering information is given on any current masthead page.

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Helichrome: Synthesis and Enzymatic Activity of a Designed Hemeprotein†

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A number of model compounds have been synthesized[1] in an effort to mimic the active sites of enzymes by combining functional groups with appropriate host molecules such as cyclodextrins,[2a] crown ethers,[2b] and cyclophanes.[2c] This approach has been quite successful in elucidating general mechanisms[3] of enzyme catalysis and has provided useful information on the design of molecules which possess specified catalytic functions.

Synthetic polypeptides with defined tertiary structures are more attractive candidates for the assembly of model enzymes. Recent advances in recombinant DNA technology coupled with the rapid accumulation of X-ray structural data on native proteins have contributed to defining the anticipated folding in such polypeptides.[3] Moreover, several recent successful examples[4] of de novo designed small proteins also encourage the synthesis of polypeptide based model enzymes. Neither substrate binding nor catalytic activity have, however, been reported for such model proteins. We wish to report here the synthesis,[5] characterization, and catalytic activity of an artificial hemeprotein **1** (Figure 1).

The overall topology of the molecule **1** was carefully designed so that peptide **2** has a high potential to form amphiphilic α-helix[6] (Figure 2) and to create a hydrophobic pocket for substrate binding

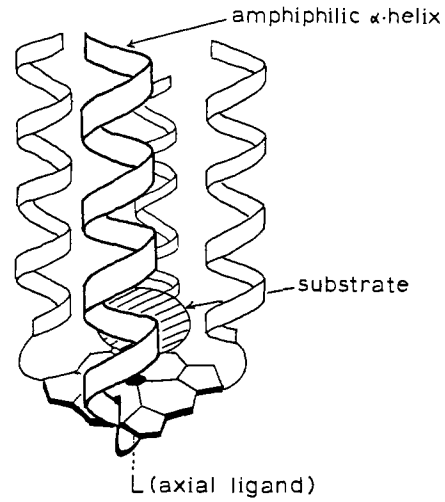

Figure 1. Proposed structure of helichrome **1** after folding of the peptide chains.

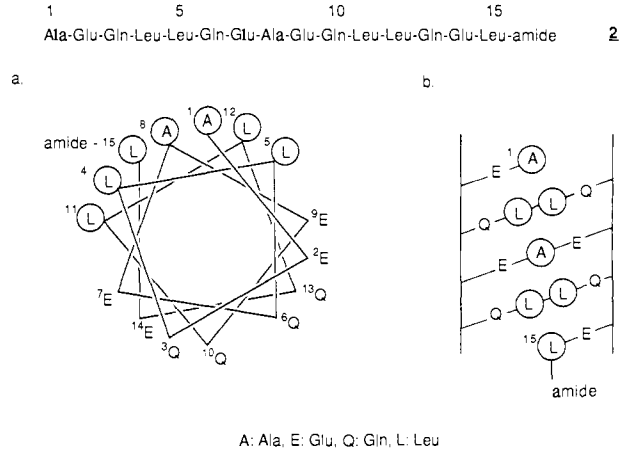

Figure 2. Amino acid sequence of peptide: (a) helix wheel and (b) helix diagram, in which the circle represents hydrophobic amino acids.

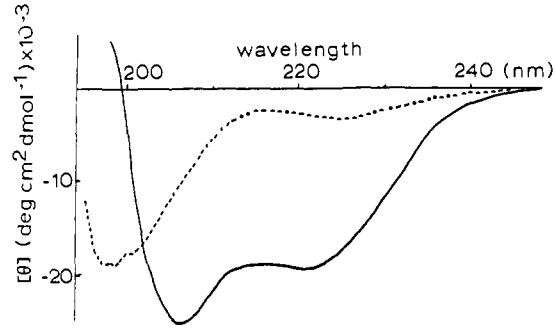

Figure 3. Circular dichroism spectra of helichrome (—, 7.95×10^{-5} M) and peptide alone (– –, 4.94×10^{-4} M) in 20 mM phosphate, 0.16 M KCl pH 7.5.

above the porphyrin ring after the expected folding of the peptide chains. The fully protected peptide segment **3** was synthesized[7a] via a fragment condensation of two small peptide segments (Boc-(1-7)-CO_2H and H_2N-(8-15)-$CONH_2$) which were prepared by utilizing oxime resin.[7b] After deprotection of the Boc group

† This paper is dedicated to the memory of Professor E. T. Kaiser.
‡ Deceased on July 18, 1988.

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(7) (a) Boc-Ala-Glu(OBzl)-Gln-Leu-Leu-Gln-Glu(OBzl)-oxime resin **6** was prepared by the stepwise peptide synthesis. The treatment of **6** with 1-hydroxypiperidine followed by Zn reduction in 90% AcOH and with leucine amide afforded N-terminus half (Boc-(1-7)-COOH) **7** and C-terminus half (Boc-(8-15)-$CONH_2$) **8**, respectively. A segment condensation of **7** and **8** after the deprotection of Boc group **8** gave the desired protected peptide segment **3** in 81% yield. (b) DeGrado, W. F.; Kaiser, E. T. *J. Org. Chem.* **1982**, *47*, 3258. DeGrado, W. F.; Kaiser, E. T. *J. Org. Chem.* **1980**, *45*, 1295.

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of **3** by treatment with trifluoroacetic acid, the activated porphyrin ester **5**[8] was reacted with the peptide segment in DMSO-DMF for 2 days at 50 °C. The precipitated products were collected (57%), treated with TMSOTf/thioanisole/trifluoroacetic acid reagent[9] in order to remove all protecting groups, and then subjected to reverse phase HPLC (Vydac C18 column, 20 mM Et_3N/H_3PO_4 pH 6.5, 30%-80% acetonitrile linear gradient for 30 min). A major peak at 63% acetonitrile was collected (37%) and was found to be the desired compound.[10]

Helichrome **1** is very soluble in buffer (over 1 mM in 20 mM phosphate, 0.16 M KCl pH 7.5) and is practically insoluble below pH 3 as expected from its peptide sequence. One of the most remarkable characteristics of **1** is the high α-helical content (ca. 70%) indicated by its CD spectrum in the aqueous buffer solution, whereas single peptide **2** alone exhibited a typical CD pattern of a disordered conformation[11] under identical experimental condition as shown in Figure 3. Helichrome **1** was found to be monomeric under the condition of the CD measurement based on both gel filtration on Sephadex G-50 and sedimentation equilibrium experiments (MW_{app} = 7200 ± 500); in accord with an intramolecularly folded state of the molecule. These observations strongly suggest that the close proximity of porphyrin-linked peptide segments induces the amphiphilic α-helical structure and then facilitates a spontaneous formation of the folded tertiary structure. Buffer solution of **1** showed a red-shifted fluorescence maximum at 617 nm, which indicated a moderately hydrophobic environment around the porphyrin ring[12] and provided further experimental support for the proposed structure of **1** in solution.

We next examined the aniline hydroxylase activity of the Fe(III) complex **1a**[13] of helichrome **1**. The formation of *p*-aminophenol was monitored[14] at varying aniline concentration and fixed concentrations of **1a** (3.1 μM), 7-acetylflavin (0.24 μM), and NADPH (2.5 mM) in 20 mM $N\alpha$-acetyl histidine buffer pH 7.0. A double-reciprocal plot of the rates for various concentrations of aniline was linear and provided k_{cat} = 0.02 min^{-1} and K_m = 5.0 mM. A series of control experiment showed that every component except 7-acetylflavin[15] in the reaction mixture was essential to the hydroxylase activity. Fe(III) coproporphyrin I (4.7 μM) showed negligible aniline hydroxylase activity[16] under the same conditions, demonstrating a significant contribution of the peptide segments to catalysis by **1a**, most probably by providing binding pocket(s) for the substrate(s). Furthermore, the observed hydroxylase activity of **1a** was completely inhibited by catalase (100 units) but not by superoxide dismutase (SOD) (10 units). Several hemeproteins[17] such as hemoglobin, indoleamine 2,3-dioxygenase, and L-tryptophane 2,3-dioxygenase have been reported to catalyze the hydroxylation of aniline in the presence of oxygen and an appropriate reducing system with k_{cat} and K_m values ranging from 0.02 to 0.65 min^{-1} and from 3.7 to 5.4 mM, respectively. Their activities are inhibited by both catalase (completely at 100 units) and SOD (ca. 50% with 10 units), suggesting possible involvement of peroxide type intermediates in the catalytic cycle.[17] Although our system requires further experimentation to define its catalytic mechanism, the above results clearly demonstrate that **1a** has a hydroxylase activity quite similar to that of native hemeproteins.

In conclusion, our preliminary work has established that helichrome **1** and its iron complex are a first generation model hemeprotein based on a synthetic peptide. It is worth noting that the folding process of such a synthetic protein could be simplified by the introduction of an appropriate organic compound as seen in the present system. Detailed mechanistic investigation of the catalysis by **1a** and further structural characterizations of **1** are now in progress.

Acknowledgment. This work was funded in part by National Science Foundation Grant CHE8418878 (E.T.K.). We thank Dr. Toshio Kokubo for many useful discussions and performing preliminary experiments for the synthesis of the activated esters of coproporphyrin I. We also thank Professor Stephen J. Benkovic for providing helpful suggestions for writing the manuscript and Dr. Mark A. Findeis for valuable discussions and reading the manuscript. All mass spectra were taken by Dr. Brian Chait at the Rockefeller University Biotechnology Mass Spectrometric Research Resources, supported by National Institutes of Health Grant RR-00862-14. CD spectra were measured on an Aviv 60DS supported by National Science Foundation Grant PCM8400268 in the laboratory of Dr. David Cowburn.

(8) Coproporphyrin I **4** was converted to the corresponding tetrahydroxysuccinimide ester **5** which was characterized by IR, MS, HPLC, and NMR after reacting with excess L-Ala-*tert*-butyl ester and was sufficiently pure (over 90%) for the next coupling reaction with the protected peptide segment.

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(12) Coproporphyrin I exhibited fluorescence maxima at 611, 616, and 620 nm in a buffer (1 mM phosphate, pH 7.0), 20% dioxane, and 50% dioxane, respectively.

(13) (a) Iron was incorporated by a reported procedure[13b] with slight modifications. Helichrome (**1**, 3 mg) was dissolved in AcOH-TFE (6:4, 1 mL) and reacted with excess $Fe(OAc)_2$ at 70 °C for 30 min. Solvent was removed under reduced pressure, and the residues were taken up with a buffer (5% $NaHCO_3$, 0.1 M EDTA, 0.5 mL). Excess iron and salts were removed by gel filtration on Sephadex G-25. Purity was confirmed by reverse phase HPLC, and no starting material was detected. **1a**: UV (20 mM $N\alpha$-acetylhistidine, pH 7.0) 394, 491, 518 (sh); and 610 nm. (b) Sano, S. In *The Porphyrins*; Dolphin, D., Ed.; Academic: New York, 1978; Vol. VII, p 377.

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Molecular Recognition of Alcohols by Layered Compounds with Alternating Organic and Inorganic Layers

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Vanadyl alkylphosphonates are a new family of compounds that can recognize primary alcohol molecules and selectively discriminate among various branched isomers. They are examples of layered solids with alternating inorganic and organic layers, intriguing materials that can exhibit useful sorptive and catalytic properties and can serve as microcrystalline bulk models for interfacial systems.[1-10] Vanadium alkylphosphonates undergo

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