While far smaller than expected from the idea of independent blocks, the helix content of CGGY($_{16}$Tm$_{46}$) is far *larger* than predicted from the Zimm-Bragg single-chain theory using host-guest parameters.[5] This may be caused by the substantial number of *intra*chain salt bridges possible in subsequence 16-46, although these are not, overall, significant in the parent chain.[19] Studies are in progress on other tropomyosin subsequences designed to shed light on intrinsic local stabilities.

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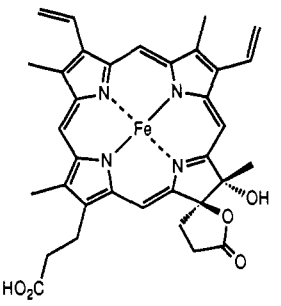

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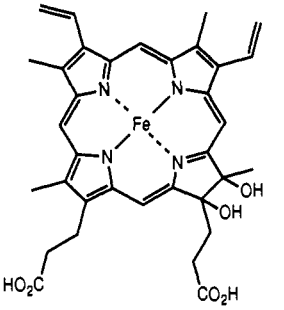

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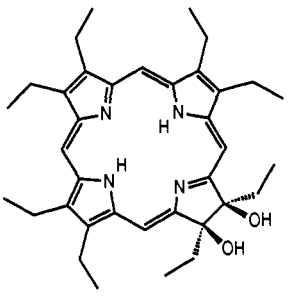

3

Molecular Structure of a Dihydroxychlorin. A Model of the Green Heme *d* and of a Photodynamic Therapy Sensitizer

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Heme *d* is the iron chlorin prosthetic group of bacterial terminal oxidases.[2,3] The molecular structures proposed for the green heme are alternatively a chlorin core with trans γ-spirolactone and hydroxyl groups at the saturated pyrrole ring[2] (**1**) or a dihydroxyprotochlorin IX[3] (**2**). **2** was shown[2] to readily cyclize to **1**. A similar spirolactone structure, but with the oxygens cis or on the same side of the pyrroline plane, has been suggested[4] for the green catalase HPII of *Escherichia coli*. In a different context, the burgeoning interest in the use of porphyrin derivatives for photodynamic therapy has focused attention on chlorins because they absorb at longer wavelengths than porphyrins and thereby allow deeper tissue penetration of incident light. Intriguingly, recent results have shown hydroxychlorins (and hydroxyporphyrins) to be effective photosensitizers for tumor eradication in vivo.[5,6] In particular, *cis*-dihydroxyoctaethylchlorin **3** (2,3-dihydroxy-2′,3′,7,8,12,13,17,18-octaethylporphyrin) showed better photonecrotic activity than the standard hematoporphyrin derivative used in clinical tumor phototherapy.[5] Chlorin **3** thus serves as a structural model both of one formulation of heme *d* and of the photodynamic sensitizer.

We present here a crystallographic determination of **3**. Not surprisingly, the molecule exhibits the structural characteristics of chlorins, i.e., elongated Cα-Cβ and Cβ-Cβ bonds in the pyrroline ring and significant distortion of the macrocycle, a feature also often found in hydroporphyrins.[7-12] Unexpectedly, the hydroxy groups of **3** form intra- and intermolecular hydrogen bonds, as evidenced by the short distances of 2.78 Å between the oxygens on the same molecule and 2.80 Å between the oxygens of adjacent molecules. The latter results suggest that if heme *d* is indeed a dihydroxychlorin, the macrocycle is likely to be anchored to its apoprotein via hydrogen bonds. As well, the propensity of **3** to form hydrogen bonds may contribute to its effectiveness,[5] and that of other hydroxyporphyrin derivatives,[5,6] as sensitizers in photodynamic therapy.

The molecular structure, atom names, and bond distances for **3**[13] are shown in Figure 1. The cis configuration of the hydroxyl

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(13) **3** was prepared by OsO_4 oxidation of octaethylporphyrin.[14] The compound crystallizes from chloroform/ethyl acetate with a half molecule of ethyl acetate of solvation $[C_{36}N_4O_2H_{48}\cdot{}^1/_2(C_4O_2H_8)]$, in the monoclinic space group $C2/c$, with a = 24.477 (8) Å, b = 14.696 (6) Å, c = 22.502 (16) Å, β = 114.08 (5)°, V = 7389.9 Å^3, Z = 8. Data collection at 200 K: Enraf-Nonius CAD4 diffractometer with graphite-monochromated Cu Kα radiation; scan range $4° \le 2\theta \le 100°$; 5018 reflections of the form h, k, $\pm l$ (excluding C-centering) measured, 4507 unique. The structure was solved using MULTAN 78 and refined with full-matrix least squares with anisotropic thermal parameters for the chlorin and isotropic thermal parameters for the molecule of solvation against the 2664 data with $F_o > 4\sigma(F_o)$ to final values of R_F = 0.068 and R_{wF} = 0.072. Hydrogens were included in calculated positions, except for those of the hydroxyl groups and the disordered solvation molecule. Additional refinement details are given in the supplementary material.

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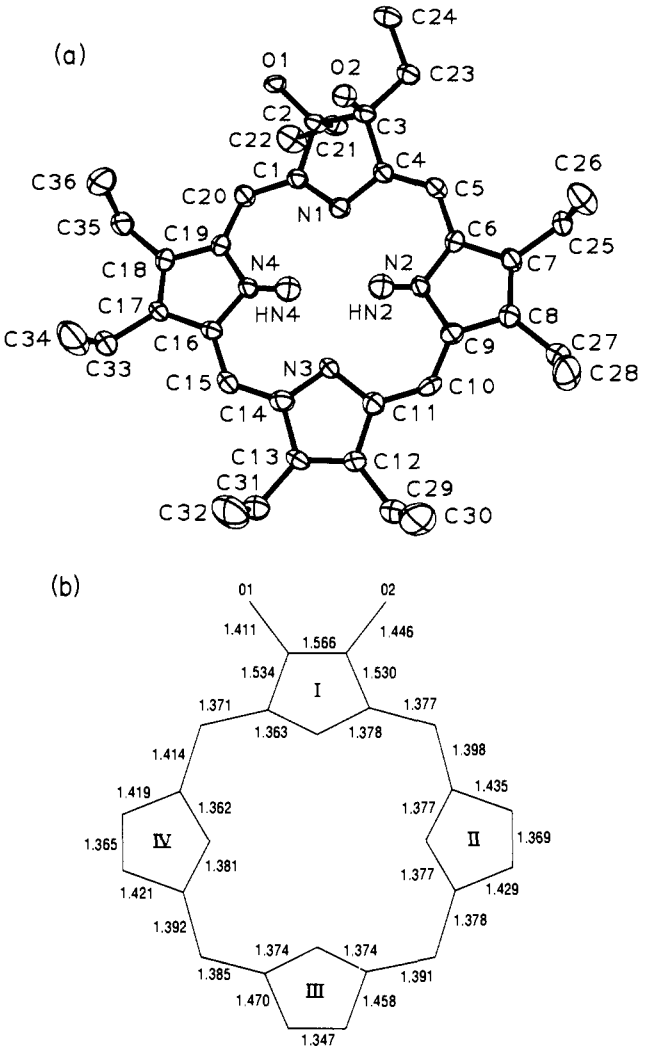

Figure 1. (a) Molecular structure of **3**. Ellipsoids enclose 50% probability except for hydrogens HN2 and HN4, which are not to scale. All other hydrogens are omitted for clarity. (b) Bond distances for **3**, in Å. The average esd of a typical C–C bond is 0.007 Å.

groups agrees with NMR results.[3,14] The molecule is highly symmetrical across the N1–N3 axis. The elongated Cα–Cβ bonds, 1.534 (7) and 1.530 (7) Å, and Cβ–Cβ bond, 1.566 (7) Å, of ring I are characteristic of pyrroline rings in hydroporphyrins,[7-12] as is the wide Cα–N–Cα angle of 107.7 (4)°. Although the protons on N2 and N4 were not located in the refinement, the average Cα–N–Cα bond angles of 109.8 (4)° in rings II and IV are diagnostic of protonated pyrrole rings.[15,16] For comparison, that angle in the unprotonated ring III is 104.6 (5)°. The wide Cα–N–Cα angles are offset by smaller N–Cα–Cβ angles. The C2–O1 and C3–O2 bonds of 1.411 (6) and 1.446 (6) Å are clearly single bonds and represent the only distances that vary by more than 3σ in opposite halves of the molecule. The asymmetric geometry of O1 and O2 is further evidenced by the differences in the Cα–Cβ–O and Cβ–Cβ–O angles, which average 114.1 (4)° around C2 and 107.8 (4)° around C3.

Deviations of the atoms of the macrocycle from the planes defined by the four nitrogens and the 24-atom core are included in the supplementary material. The molecule assumes an S_4 conformation and is markedly nonplanar, with the largest displacement of 0.64 Å at C3. Ring I is highly twisted with a dihedral angle of 31.2 (5)° about C2–C3 as compared to the average of

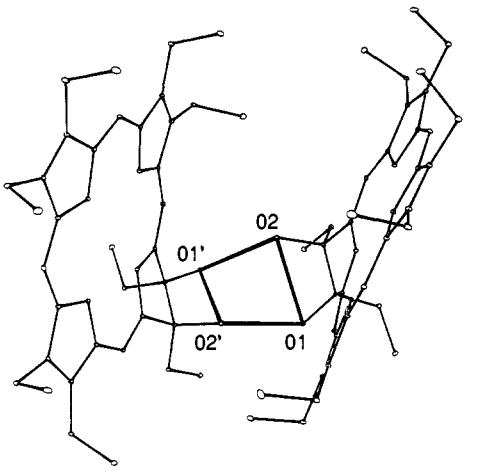

Figure 2. Hydrogen-bonded dimer of **3**. The intermolecular distances O1–O2′ and O1′–O2 are 2.80 Å. The intramolecular distances O1–O2 and O1′–O2′ are 2.78 Å.

1.2 (5)° in the pyrrole rings. As a consequence, the oxygens lie disparately above the planes of the nitrogens and the macrocycle. The displacements from the plane of the nitrogens are 0.34 and 2.04 Å for O1 and O2, respectively.

The asymmetry of the pyrroline conformation and of the bonding of the hydroxyl groups may reflect the most unusual feature of this determination. Symmetry-related molecules form dimers in "open clamshell" configurations with an angle of 29.3° between the two planes defined by the nitrogens of each monomer component of the dimer. The hinges of the clam are provided by intermolecular hydrogen bonding: the O1–O2′ and O1′–O2 distances between 2-fold-related molecules are 2.802 (5) Å, diagnostic of hydrogen bonding,[17] Figure 2. In addition, distances of 2.782 (5) Å between O1 and O2 within individual molecules are indicative of intramolecular hydrogen bonding, Figure 2. Furthermore, the disordered ethyl acetate molecule of solvation sits inside the clamshell with its keto oxygen located 2.848 (6) Å from O2 and its symmetry mate, O2′, in the dimer. One of the two crystallographically unique hydrogen bonds may thus be bifurcated and involve the solvent keto group as well. These concerted interactions clearly establish the tendencies of the hydroxyl groups of the chlorin to form hydrogen bonds. (Such bonds likely provide the driving interaction between the components of the dimer: the closest approach of ring I centers is 5.51 Å, and the center-to-center distance between molecules is 7.05 Å.) Such hydrogen bonding is not unique to **3**. We have observed three distinct modes of intermolecular aggregation mediated by hydrogen bonding between the 2-(1-hydroxyethyl) groups of bacteriopheophorbide *d* derivatives and their keto groups with O···O distances ranging between 2.80 and 2.89 Å.[18]

The propensity of hydroxychlorins (and presumably other hydroxyporphyrin derivatives) to form hydrogen bonds may thus help to anchor them within proteins and thereby contribute to the biological function of the green heme and to the effectiveness of photodynamic sensitizers bearing hydroxy groups. As has been found with other sensitizers,[6] dimerization may enhance the necrotic activity of **3** as well.

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Supplementary Material Available: Crystallographic details, a stereoscopic packing diagram, deviations from planarity, and final positional and thermal parameters for the non-hydrogen atoms of **3** (4 pages); observed and calculated structure factors for **3** (16 pages). Ordering information is given on any current masthead page.

Nonenzymatic Sequence-Specific Ligation of Double-Helical DNA

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Formation of a phosphorus–oxygen bond between phosphate and hydroxyl termini of DNA in aqueous solution requires chemical activation of the phosphate for nucleophilic substitution and positioning of the hydroxyl for attack on the activated phosphate in competition with water.[1-4] This esterification reaction is accomplished enzymatically by DNA ligases, which utilize energy from an ATP or NAD cofactor to activate the phosphates.[5] We report a *nonenzymatic* approach to ligation of double-helical DNA employing a single-stranded template to align two duplex strand termini in a local triple helix (Figure 1). A triple-stranded complex is formed by association of a pyrimidine oligodeoxyribonucleotide in the major groove of the Watson–Crick duplex with sequence specificity derived from Hoogsteen hydrogen bonding.[6-8] Juxtaposition of the two DNA termini by a guide sequence in a triple helix, accompanied by chemical activation of the terminal phosphates, promotes ligation of the double-helical DNA.

A 3.7 kilobase pair (kbp) blunt-ended linear DNA duplex possessing a 15 base pair purine tract for triplex formation at each end was constructed.[9] A 30 nucleotide template strand, complementary in a Hoogsteen sense to the continuous 30 base pair triplex site formed by apposing the termini of the double-helical DNA, was also synthesized. In a triple-stranded complex formed by association of the template strand with both ends of the double-helical DNA, the linear DNA molecule would be circularized. Upon chemical activation, the proximal 3′-hydroxyl and 5′-phosphate termini would be susceptible to covalent ligation on one or both strands (Figure 2).

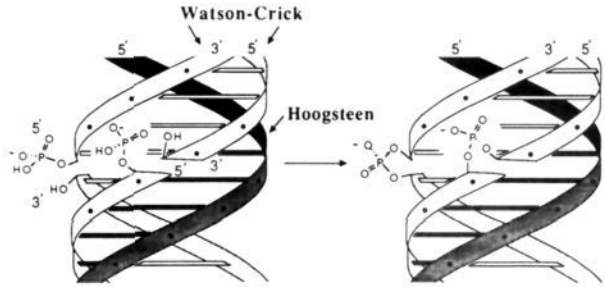

Figure 1. The 5′-phosphate and 3′-hydroxyl termini of two blunt-ended DNA duplexes can be aligned for condensation by association of an oligonucleotide template in a triple-helical complex.

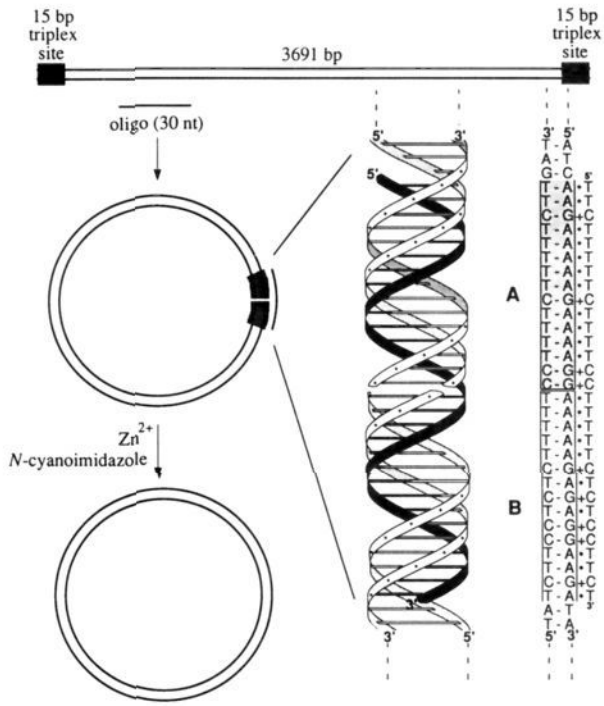

Figure 2. The substrate linear double-helical DNA can be covalently circularized in a reaction that requires single-stranded template, purine tracts on both ends of the double-helical DNA termini, and activation by *N*-cyanoimidazole (NCI) in the presence of Zn^{2+}.

A mixture of double-helical DNA (1.7 nM), 30-mer template (17 nM), and $ZnCl_2$ (20 mM) was allowed to react with the condensing agent *N*-cyanoimidazole (0.1 mM).[10,11] After 7 h (20 °C, pH 4.9), the reaction products resulting from single- and double-strand ligations were separated by agarose gel electrophoresis in the presence of ethidium bromide (Figure 3). Covalent closure of one strand of the linear DNA produces a circular molecule (form II) that migrates more slowly in the gel than the linear starting material (form III). If both strands of the plasmid are covalently closed, the circular DNA is positively supercoiled by intercalation of ethidium bromide (EB) contained in the gel. The positively supercoiled DNA (form I°) migrates more rapidly than the linear starting material (form III) and the negatively supercoiled DNA isolated from bacteria (form I).

Several DNA products from the chemical ligation reaction are observed (Figure 3, lane 7), with gel electrophoretic mobilities identical with those produced by treatment of the linearized

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