olefinic carbon resonances in such an environment. Indeed, the ^{13}C NMR spectrum of cylinder **3** shows but a single olefinic carbon resonance in $CDCl_3$ saturated with (+)-2,2,2-trifluoro-9-anthrylethanol,[13] while two olefinic carbon peaks are resolved ($\Delta\delta$ = 1.9 Hz at 62.9 MHz) in the spectrum of Möbius strip **2** under identical conditions. The chirality of compound **2** represents a novel example of optical isomerism. There are no chiral centers, and no molecular rigidity is required to keep the optical antipodes of **2** nonequivalent. They owe their distinct character to connectivity only, and yet they have identical connectivity.

In conclusion, we have prepared and characterized the first molecular Möbius strip: a unique structure with novel and esthetically pleasing symmetry properties. Studies on resolution of compound **2**, cleavage of the double bonds of **2** and **3**, and extension of the synthesis to other topologically interesting structures, including more highly twisted cylinders, catenanes, knots, and multiply looped catenanes, are in progress.

Acknowledgment is made to the donors of The Petroleum Research Fund, administered by the American Chemical Society, for partial support of the research. We also thank the National Science Foundation (Grant No. CHE80-11391) and Monsanto Agricultural Products Co. for financial assistance and the University of Colorado Computing Center for blocks of computer time. We thank Mike McNeil for obtaining mass spectral data and Catherine Fenselau, of the Middle Atlantic Mass Spectrometry Laboratory, a National Science Foundation Shared Instrument Facility, for assistance. We are especially indebted to Professor Kurt Mislow and his group for helpful discussions concerning the symmetry of the Möbius molecule.

Registry No. **1**, 81617-29-0; (±)-**2**, 81617-30-3; **3**, 81617-31-4; **6**, 81617-32-5; **7**, 81617-33-6; **8**, 81617-34-7.

Supplementary Material Available: Spectral and analytical data on all new compounds reported, a listing of atomic positional and thermal parameters, and a drawing for the crystal structure of cylinder **3** (5 pages). Ordering information is given on any current masthead page.

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Picosecond Study of the Photodissociation of a Model Hemoprotein Compared to Hemoglobin

J. A. Hutchinson,† T. G. Traylor,‡ and L. J. Noe*,†

Departments of Chemistry, University of Wyoming Laramie, Wyoming 82071 and University of California, San Diego La Jolla, California 92037

Received January 19, 1982

We communicate the results of a comparative study of picosecond photodissociation experiments on the CO derivatives of hemoglobin, hemoglobin subunits, and unstrained chelated protoheme **1**-CO shown in Figure 1. These results suggest that the differences in the initial stages of the photodissociation for these molecules may be correlated with the geometry of the heme pocket, possibly the position of the proximal imidazole, although other geometrical effects cannot be ruled out at this time. Our objective in this study is to establish grounds for additional experiments of this type on the CO and O_2 forms of modified model compounds with the aim of determining the effect of heme geometry and/or strain on the photodissociation process. Such studies will be of importance in gaining additional insight,[1,3] from the persepctive of photodissociation dynamics,[4,5] into effects of tertiary heme structure on the affinity of the heme for the sixth axial ligand in the T and R forms of hemoglobin.[6,7]

The picosecond absorption experiments reported here were conducted by pumping the Q band of the various heme-porphyrin complexes with use of the second-harmonic 530-nm light generated from a mode-locked neodymium-phosphate glass laser. The absorption changes in the Soret region were recorded over a 42-nm band width at certain fixed-time delays between the pump and probe pulses. Our double-beam sample-reference system was equipped with a triple spectrograph and a channel-plate intensified dual wide-area diode array detector. This detector was interfaced to a computer-based data acquisition system.[2] A comprehensive analytical and experimental evaluation of this apparatus can be found elsewhere.[8] The hemoglobin and isolated chains were prepared by using standard procedures.[9-11] Purity of the CO, O_2, and deoxy derivatives was checked spectroscopically as well as by using electrophoresis. All samples were maintained at 5 °C at pH ~7.45 in 0.025 M Bistris-Tris buffer. The sample concentrations were adjusted to give a Soret absorbance of ~1.2 in a 1-mm path for HbCO and ~1.0 for **1**-CO. Traylor and co-workers discuss the synthesis and purification of unstrained protoheme **1**, which we used in CTAB solution at ambient temperature. They have also shown that the spectroscopic, thermodynamic, and kinetic properties of unstrained chelated protoheme are almost identical to R-state hemoglobin.[3]

Figure 1 shows our results on the initial stages of the photodissociation for protoheme **1**-CO and for HbCO at various delays, between the 530-nm optical pump pulse and the Soret interrogation pulse, ranging from -8 to 24 ps. Each of these pulses has a width (FWHM) of ~8 ps. The spectra recorded from -8 to +8 ps are in a temporal range where there is varying, but considerable, pump-probe pulse overlap. For the spectra recorded at negative delay, the peak of the probe pulse precedes the peak of the pump pulse. Earlier work in this area[4,5] has shown that the difference spectrum[12] having a minimum centered at 418 nm is due to the bleaching of the CO compound, while the spectrum with maximum at ~436 nm corresponds to the appearance of the deoxy compound and/or photoproduct. Taking into account these facts and the noise limitations stated in Figure 1, comparison of the spectra at various delays reveals some important similarities and differences between these compounds in the early stages of photodissociation. The -7 and -8 ps spectra indicate that both compounds have undergone measurable bleaching with very little

† University of Wyoming.
‡ University of California.

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(12) The optical density of the difference spectra is given as $\Delta(OD) = [\log (I_0/I)_{\text{with op}} - \log (I_0/I)_{\text{no op}}]$ (op = optical pump).

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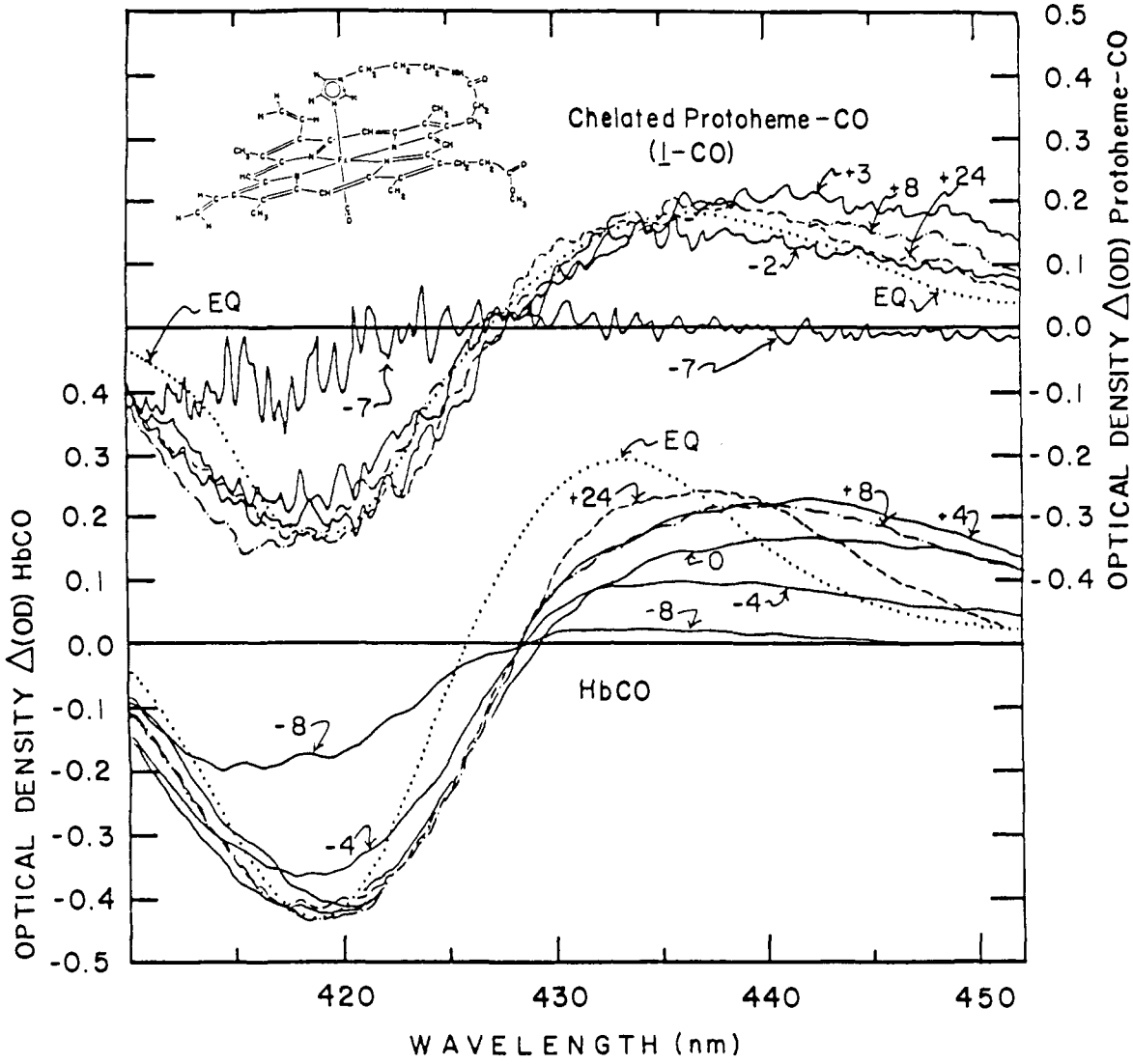

Figure 1. Picosecond difference spectra of **1**-CO (top) and HbCO (bottom) at various delays between the 530-nm excitation pulse and Soret interrogation pulse. The delays are indicated on the spectra. The equilibrium, EQ, difference spectrum for **1**-CO is taken from other results[7] and scaled to the picosecond data. The HbCO equilibrium curve is taken on a separate sample differing in concentration from sample used for the laser experiments. The laser data are averages of at least four pairs of (excitation vs. no excitation) experiments. The estimated error in the **1**-CO data ranges from ±0.05 OD at 410 nm to ±0.02 at 450 nm; for HbCO the error is ±0.02 to ±0.01 optical density units over this 40-nm range. The energy of the excitation pulse is no greater than 3 mJ in these experiments.

formation of deoxy photoproduct. For HbCO, the bleaching is complete by ~0 ps, but the photoproduct spectrum continuously evolves up to 24 ps, accompanied by a gradual sharpening and blue shift. Within experimental error, our photodissociation results for the CO forms of Hbα and Hbβ are identical with those of HbCO.[13] In **1**-CO the bleaching is complete by ~0 ps as well, but the deoxy spectrum is fully established by 3 ps. For all these compounds, the deoxy spectrum developed by 24 ps remains unchanged to 3 ns without further evolution and/or evidence of geminate recombination. Clearly the deoxy photoproduct established in HbCO by 24 ps is not the same as ground-state deoxy-Hb. We observe major differences in the formation of the stable deoxy spectrum between these compounds; namely, the intermediate(s) in the photodissociation pathway to the deoxy compound is longer lived in HbCO than in **1**-CO.

As this is a preliminary study and we have not yet completed experiments outside the 42 nm wide Soret region of Figure 1 or on the O_2 or other CO synthetic compounds, we have not determined the identity of this intermediate. That the dissociative pathway of HbCO and **1**-CO proceeds through an excited Hb* or 1* intermediate is a possibility. Cornelius and associates[5c] have proposed an excited Mb* state in the photodissociation of MbO_2. For MbCO they observe the bleaching and appearance of the deoxy spectrum to be instantaneous; but in MbO_2 the appearance of the deoxy spectrum is delayed with regard to the bleaching such as we observe in HbCO and in **1**-CO. They have identified a short-lived 12-ps spectral intermediate near 455 nm as being associated with an excited Mb*. Since the MbO_2 spectrum shows much more of this intermediate than MbCO, these investigators suggest that MbO_2 photodissociates through an excited state of Mb* whereas MbCO produces ground-state Mb directly, these observations being consistent with a mechanism involving a multiphoton photolysis–relaxation process. However, a parallel investigation by Reynolds et al.[5b] found both MbCO and MbO_2 to bleach within 3.5–5 ps accompanied by an 11-ps delay in the appearance of the deoxy spectrum for both molecules. These investigators suggest a mechanism involving a ligated intermediate state, τ = 7–10 ps, in the dissociative pathway of both MbO_2 and MbCO. This possibility must also be considered in the interpretation of our results. In particular, one could account for the bleached starting material of HbCO and **1**-CO that has not yet appeared as deoxy photoproduct if it is trapped in a low-lying crystal field $^3T_1$ state of the CO–heme complex.[4c,d]

The differences that we see in the photodissociation of HbCO and **1**-CO may be linked to important structural differences in the heme pocket between these compounds. Recent theoretical

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results by Gelin and Karplus[14a] and structural results by Baldwin and Chothia[14b] support a proposal that the affinity of the sixth ligand for the heme is critically dependent on the tertiary heme structure that originates from the position of the proximal imidazole. This imidazole and the F helix to which it is bound undergo major structural movements on going from deoxy to ligated Hb; these changes for the α and β subunits include a 1-Å translation of the F helix across the prophyrin face and a movement of the imidazole from a position that is asymmetric with respect to the porphyrin nitrogens to one that is more symmetric. The fact that the initial spectral events of CO photodissociation to 3 ns for Hb, Hbα, and Hbβ appear to be identical and yet significantly different from those of either unstrained protoheme **1** or MbCO[5c] suggests that tertiary heme structure may be of a major factor affecting photodissociation. The geometry of the heme in HbCO is very much different from that of the linear imidazole-Fe-CO geometry found in **1**-CO. In addition to the bent FeCO geometry,[15] HbCO has about 60 interactions between the globin and the heme of 4 Å or less.[16] The predissociative geometry of the heme, particularly the position and strain on the proximal imidazole, may be very important in determining the relative position of certain critical Fe d-d and/or charge-transfer states[17] that govern the process of photodissociation.

Acknowledgment. We are grateful to the Research Corp. and to the National Science Foundation (Grant PMC 7816199 to L.J.N.) for support of this research.

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Oxygen Chiral Phosphodiesters. 7. Stereochemical Course of a Reaction Catalyzed by Staphylococcal Nuclease

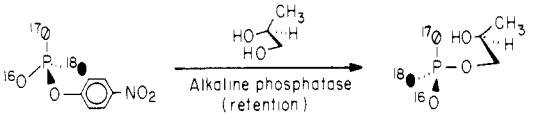
Shujaath Mehdi and John A. Gerlt*†

Department of Chemistry, Yale University
New Haven, Connecticut 06511

Received February 1, 1982

Of the phosphodiesterases that have been characterized with respect to the stereochemical course of the displacement reaction at phosphorus,[1,2] high-resolution X-ray structural data are available only for ribonuclease A.[3] This structural information, coupled with chemical data, has allowed the catalytic roles assumed by the active-site amino acid functional groups to be described. In contrast, the mechanisms of the metal ion dependent phosphodiesterases cannot be described in comparable detail. For this reason, we have determined the stereochemical course of a hydrolysis reaction catalyzed by staphylococcal nuclease. This enzyme requires calcium ions for both DNase and RNase activities. Extensive research reported by the laboratories of Anfinsen and of Cotton has provided a detailed structural description of the enzyme, with the complete amino acid sequence[4] and a 1.5-Å X-ray structure of an enzyme-inhibitor complex[5] being available. Dunn, DiBello, and Anfinsen have described a detailed kinetic study of the hydrolysis of thymidine 3′-phosphate 5′-(4-nitrophenyl phosphate) (NPpTp); these investigators also reported that attempts to trap a covalent adduct between the enzyme and substrate were unsuccessful.[6] More recently, Cotton, Hazen, and Legg have proposed a mechanism for the hydrolysis reaction catalyzed by the nuclease that is based upon the geometric relationship between the calcium ion, thymidine 3′,5′-bisphosphate (pdTp), and active-site residues found in the 1.5-Å X-ray structure.[5] The carboxylate of glutamate 43 was suggested to act as a general basic catalyst in the attack of a water molecule on the 5′-phosphorus atom of a nucleotide ester bound in the active site. In this mechanism, the calcium was postulated to assist in properly positioning the carboxylate group via an intervening water molecule and to neutralize the phosphate ester charge by a direct ionic interaction. This proposal implies that the hydrolysis reaction should proceed with inversion of configuration at phosphorus. In accord with, but not proving, this mechanism, we have found that the nuclease catalyzes the hydrolysis of one of the diastereomers of thymidine 5′-(4-nitrophenyl [^{17}O,^{18}O]phosphate) ([^{17}O,^{18}O]-NPpT) in $H_2{}^{16}O$ to yield 4-nitrophenyl [^{16}O,^{17}O,^{18}O]phosphate ([^{16}O,^{17}O,^{18}O]-pNP) with *inversion* of configuration at phosphorus.

Scheme I



The R_p diastereomer of [^{17}O,^{18}O]-NPpT[2c,7,8] was hydrolyzed in $H_2{}^{16}O$ at pH 8.8 and 42 °C in the presence of 10 mM Ca^{2+} by using the nuclease (Worthington) as catalyst. The progress of the reaction was followed by HPLC, and after the reaction was approximately 80% complete (16 h), the [^{16}O,^{17}O,^{18}O]-pNP was

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