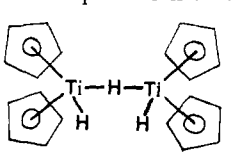
product of the reaction of **1** with $PhSiD_3$ to give a product with the expected reduction of the hyperfine interaction (a_H/a_D = 6.5141) as depicted in Figure 1b.

The features in Figure 1 exhibit a central multiplet (g = 1.9937) flanked by clearly resolved satellite splittings due to hyperfine interaction of the unpaired electron with Ti.[4] The central multiplet is composed of six lines, a doublet of triplets whose intensities correspond exactly to those expected for an unpaired electron interacting with two equivalent hydrogens ($a_{H(1)}$ = 0.97 mT) and a third hydrogen of different environment ($a_{H(2)}$ = 1.56 mT). The H(2) value is to our knowledge the highest value reported for Ti-H in a paramagnetic species. An important clue to the true nature of this compound is provided by a detailed analysis of the spectrum and by evaluation of the titanium hyperfine interaction constant (0.52 mT). The observed value is about one half of that normally observed for monomeric Ti(III) compounds and close to that expected for a single unpaired electron exchanging between two titanium centers.[5,6] The absence of features in the spectrum due to a triplet state (no ΔM_S = 2 transition at midfield in the frozen glass solution spectrum) on the one hand and the pattern of the isotope spectrum at room temperature on the other exclude the possibility of a dimer containing two Ti(III) centers and leave a mixed oxidation state Ti(III)/Ti(IV) dimer as the most reasonable alternative. This is further corroborated by the observation of an intense absorption band [λ_{max} = 655 nm; ϵ (minimum value) = 1200 per electron] in the electronic spectrum of the compound in hexane. Such absorptions are characteristic of mixed-valence compounds and conform to class III behavior in the classification of Robin and Day.[7]

Infrared spectra in Nujol of freshly prepared **3** show, in addition to the usual cyclopentadienyl bands due to the titanocene structure, a strong band due to Ti-H at 2000 cm^{-1} (shifted to 2010 cm^{-1} in hexane). In the deuterated compound the terminal Ti-D stretch is observed at 1490 cm^{-1} [ν(Ti-H)/ν(Ti-D) = 1.36]. A broad band of moderate intensity at 1220-1250 cm^{-1}, absent in the deuterated compound, may be attributed to a Ti-H-Ti mode.[8]

Reaction of **3** with carbon tetrachloride leads to its rapid destruction and to the formation of both Cp_2TiCl_2 and chloroform. This confirms that the integrity of the Cp ring is conserved in **3** and further supports the presence of an active hydride.

Taken together, the evidence outlined above leaves little doubt that the structure of the compound **3** is that shown below. The



3

molecule is shown in a cis configuration for ease of representation, but it is more likely to adopt the trans configuration.

The detailed chemistry of **3**, particularly with respect to its interesting catalytic properties, will be reported elsewhere.[9]

Acknowledgment. J.F.H. thanks the French government for a sabbatical leave fellowship to aid in carrying out this project. We are grateful to Dr. D. Gourier for advice on the interpretation of ESR spectra and to J. Henique for efficient technical assistance.

Registry No. 1, 1271-66-5; **3**, 88825-84-7; **3**-d_3, 88825-85-8; $PhSiH_3$, 694-53-1; $PhSiD_3$, 18164-03-9.

(4) Ti isotopes of mass 47 and 49 have spins (abundances) of $^5/_2$ (7.75%) and $^7/_2$ (5.51%), respectively. Analysis of the hyperfine interactions with Ti isotopes in dimeric complexes has recently been discussed in detail by Francesconi et al.[5] By their interpretation, the central sextet in our spectrum is due to an unpaired electron interacting with two titaniums (I = 0, I = 0), 76.06%. The satellites observable on the wings at moderate gains are due to Ti(I = 0 + I = $^5/_2$), 12.7%, and Ti(I = 0 + I = $^7/_2$), 9.61%. At high gain, satellite bands due to Ti(I = $^5/_2$ + I = $^5/_2$), 0.53%, Ti(I = $^7/_2$ + I = $^5/_2$), 0.82%, and Ti(I = $^7/_2$ + I = $^7/_2$), 0.30%, are observed. The hyperfine coupling constant is evaluated by measuring the separation between the clearly resolved satellites at low field.

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(9) The most important and novel catalysis effected by this and certain other titanium compounds is the polymerization of primary silanes to linear polysilanes. Details of this reaction will be published elsewhere.

Resonance Raman Study of Subunit Assembly Dependent Photoreduction of Heme of Extracellular Giant Hemoglobin

Teizo Kitagawa,* Sosuke Chihara, Kenzo Fushitani,[1] and Hideki Morimoto

Institute for Molecular Science
Okazaki National Research Institutes
Myodaiji, Okazaki, 444 Japan
Faculty of Engineering Science, Osaka University
Toyonaka, Osaka, 560 Japan

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Extracellular giant hemoglobin (Hb) with M_r = (3-4) × 10^6, found in various invertebrates[2a,b] and sometimes categorized as erythrocruorin, is composed of two superposed hexagonal disks made up of six subunits, ~110 Å in diameter.[3] The $^1/_{12}$ subunit assumes a molecular weight of M_r = (2-3) × 10^5. The effect of salts upon the O_2 affinity is opposite between the giant Hb and tetrameric Hb's of blood red cells;[4] cations such as Mg^{2+} and Ca^{2+} increase the O_2 affinity of the giant Hb while they have no effect on human Hb, and anions such as Cl^- and DPG lower the O_2 affinity of the latter while they have no effect on the former. It seems important to elucidate how the distinct molecular properties of the giant Hb depend upon the subunit assembly.

Resonance Raman (RR) spectroscopy yields the heme vibrational spectrum, which is sensitive to the electronic structure as well as the stereostructure of heme.[5,6] The technique has in fact revealed the quaternary structure dependence of the strain exerted on the Fe-histidine (F8) bond by globin of deoxyHb[7,8] and also the quaternary structure dependent photoreduction of metHb by laser light.[9] Accordingly, the RR spectrum can be used to explore whether the heme structure of the giant Hb depends on subunit assembly. We report here an unexpected observation that the heme of the giant Hb undergoes wavelength-dependent photoreduction when the Hb is intact or dissociated into the $^1/_{12}$ subunit, but not when the Hb is dissociated into smaller assemblies.

An extracellular giant Hb containing protoporphyrin IX was isolated from an annelid, *Travisia japonica*. The preparation procedures of the intact Hb (M_r = 3 × 10^6), the $^1/_{12}$ subunit (M_r = 2.4 × 10^5), an assembly (M_r = 26 000), and separated chains (M_r = 14 000-18 000) are described elsewhere.[10a,b] The dissociation into the $^1/_{12}$ subunit was reversed by incubation in 0.2 M sodium phosphate buffer, pH 6.0, in the presence of the native

* Address correspondence to this author at the Institute for Molecular Science.

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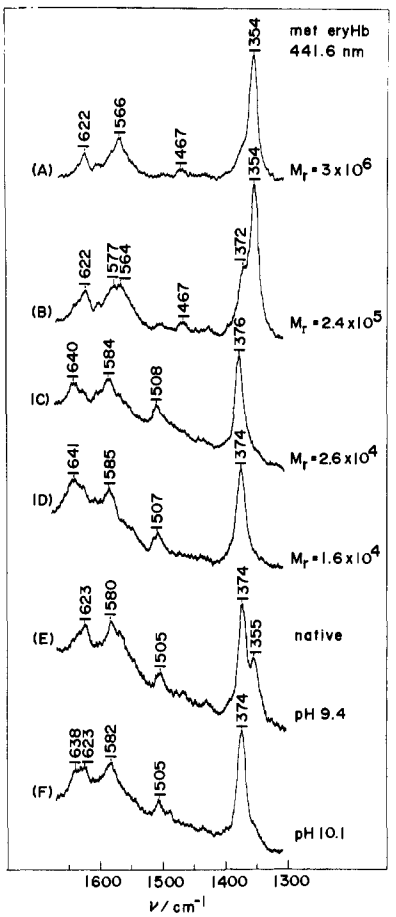

Figure 1. Resonance Raman spectra of an extracellular giant hemoglobin (eryHb) in the met form excited at 441.6 nm (40 mW): (A) intact form with $M_r = 3 \times 10^6$, (B) the $^1/_{12}$ subunit with $M_r = 2.4 \times 10^5$, (C) the assembly with $M_r = 26000$, (D) a separated chain with $M_r = 16000$ (subunit II_g in ref 10), (E) intact metHb was brought to pH 9.4, (F) intact metHb was brought to pH 10.1. The samples were contained in a stationary cell under an Ar atmosphere and the spectra were measured with JEOL-400D Raman spectrometer and He/Cd laser (Kinmon Electrics CDR80SG). The $^1/_{12}$ subunit was prepared by incubating the CO-bound form of the intact Hb in 0.1 M Tris/HCl, pH 8.0, and the 26000 assembly was obtained through DTT reduction of the $^1/_{12}$ subunit. Chains were obtained by DTT reduction of the $^1/_{12}$ subunit followed by ultragel filtration with ACA34 and succeeding chromatography on a DE32 cellulose (Whatman) column in the presence of DTT. The separated chain (II_g) with $M_r = 16000$ is one of the five smallest subunits, the N-terminal residue of which is Gly.[10]

molecules, although it was not always reproducible and an essential factor for the reconstitution is unknown. On the other hand, the $^1/_{12}$ subunit was restored from the 26000 assembly upon removal of dithiothreitol (DTT). Their deoxy or oxy form was obtained from the carbon monoxide bound form[10a,b] by repeated evacuation under light illumination and flushing with an Ar or O_2 gas, respectively. The met form was prepared by ferricyanide oxidation of the oxy form followed by gel filtration on Sephadex G-25, and its formation was confirmed with visible absorption spectrum. The molecular weight of the met forms of the native giant Hb, the $^1/_{12}$ subunit, and the 26000 assembly was reconfirmed with the gel filtration after the Raman experiments. Accordingly, possible rearrangements of the subunit assembly during the Raman measurements, which were performed under an Ar atmosphere unless otherwise stated, were insignificant in the present study.

The RR spectra of the met form of intact Hb (A), the $^1/_{12}$ subunit (B), the 26000 assembly (C), and the separated chain with $M_r = 16000$ (D) measured with a stationary cell and the 441.6-nm excitation line are shown in Figure 1. Spectra A and B are essentially the same as the spectra of their deoxy forms, even in the lower frequency region below 500 cm^{-1} (not shown). This implies the occurrence of photoreduction of the heme by laser

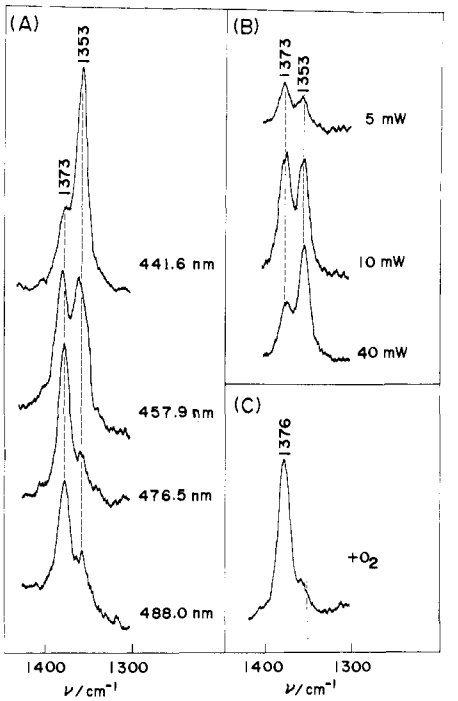

Figure 2. The resonance Raman spectra in the ν_4 region of the intact met giant Hb at pH 7.4: (A) the illumination-wavelength dependence of the intensity of the two ν_4 lines (1373 cm^{-1} for the met form and 1353 cm^{-1} for the deoxy form), (B) the laser-power dependence of intensities of the two ν_4 lines excited at 441.6 nm, (C) the ν_4 line of the 441.6-nm excited spectrum under the aerobic conditions. This indicates the formation of the oxy form. All spectra were obtained with the stationary cell.

irradiation. Spinning of the cell significantly retarded the photoreduction, although finally it was completely photoreduced under the anaerobic conditions. Under the spinning conditions the sample is illuminated by laser light for 1 μs at every 30 ms. Accordingly the rate of photoreduction is presumably slower than 1 μs, and it is irreversible. Spectra C and D are similar to the RR spectra of the ferric low-spin hemoproteins, although their deoxy form displayed the spectrum of the ferrous high-spin type similar to the intact Hb. The Fe–histidine stretching Raman lines[11-13] of the deoxy forms of all the four species were identified at 219–221 cm^{-1} like the high-affinity tetrameric deoxyHb.[7,8]

The oxidation state of the heme iron can be diagnosed by the ν_4 line that appears around 1355 and 1375 cm^{-1} for the deoxy and met forms, respectively.[14,15] The spectral pattern in the ν_4 region with the variant excitation wavelengths are illustrated in Figure 2A. When the intact Hb was illuminated at 488.0 nm or longer wavelengths, the 1355-cm^{-1} line markedly diminished. On the other hand, when the laser power was decreased at 441.6 nm, the relative intensity, I_{1353}/I_{1373} decreased as demonstrated in Figure 2B. When the met form was excited at 441.6 nm under aerobic conditions, the ν_4 line appeared at 1376 cm^{-1} as depicted in Figure 2C and the whole RR spectrum was remarkably close to that of oxy form. Consequently, it became evident that the giant Hb is extensively photoreduced by laser illumination around 441.6 nm but much less at 488.0 nm or longer wavelengths, and oxygen is bound to the photoreduced form under aerobic irradiation at 441.6 nm. The photoreduction takes place for the intact Hb and the $^1/_{12}$ subunit but not for the 26000 assembly and separated chains.

Spectra E and F in Figure 1 were observed for the intact metHb at pH 9.4 and 10.1, respectively. The photoreduction occurs only

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partially at pH 9.4 and no more above pH 10.1. This observation seems consistent with the fact that the giant Hb is dissociated into smaller assembly at pH 9,[16] and thus it appears less likely that an electron donor is an exogeneous impurity. Recalling that human metHb was partially photoreduced by laser irradiation at 441.6 nm only for the low-affinity quaternary structure,[9] we suggest that the photoexcitation of electrons creates a hole in the heme, to which an electron is transferred from the protein moiety with the specific conformation. The very similar phenomena were also observed for another giant hemoprotein, chlorocruorin with $M_r = 3 \times 10^6$, for which the photoreduction ceased to occur at alkaline pH.[17] Therefore, such feature seems inherent in the extracellular giant Hb's. Kinetics of photoreduction of this giant Hb are under investigation with time-resolved RR spectroscopy.

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A Novel Route for Stereospecific Construction of the A Ring of Anthracyclinones: Total Synthesis of (±)-γ-Citromycinone

Frank M. Hauser*† and Dipakranjan Mal

Department of Chemistry and Biochemical Sciences
Oregon Graduate Center
Beaverton, Oregon 97006

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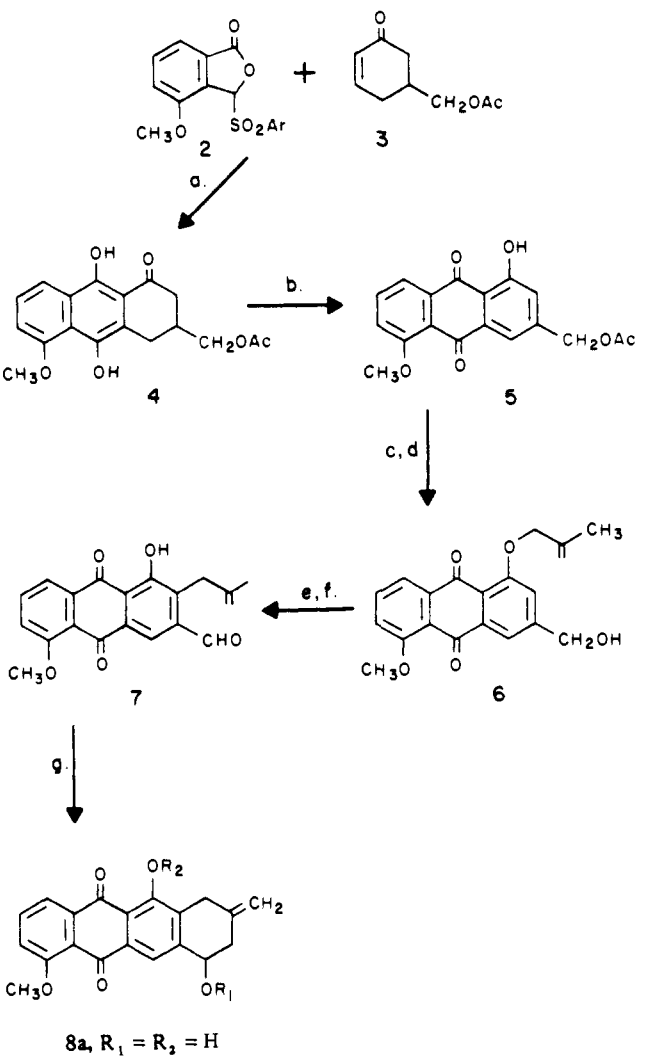
We have developed a conceptually new route for regio- and stereo-specific construction of the A ring of anthracyclinones with cis hydroxyl functionalities at C-7 and C-9.

The most commonly used sequence for constructing the A ring of such anthracyclinones has been to first fabricate an intermediate containing the 9-hydroxyl group then introduce the 7-hydroxyl through homolytic bromination and solvolysis.[1-3] The disadvantages associated with this procedure are well documented[4]—only moderate stereoselectivity is observed and the preparative value is frequently compromised by the low solubility of anthracyclinones in media that are compatible with the required bromination.

In contrast, our methodology directly generates the 7-hydroxyl group first, during the course of constructing the A ring, then utilizes this functionality to guide the stereospecific introduction of the second hydroxyl group at the 9-position. The key step in this sequence is the intramolecular ene reaction of the olefinic aldehyde **7** to regiospecifically furnish the *exo*-methylene alcohol **8a**.

As a demonstration of the efficacy of this methodology, we have performed the first total synthesis of γ-citromycinone. Because only small quantities of this rare anthracyclinone were originally

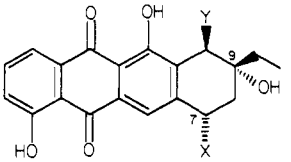
Scheme I



8a, $R_1 = R_2 = H$

[a] LiO-*t*-Bu, THF. [b] O_2, DMF, 100 °C, 12 h; 55% overall from **2**. [c] 2-(Chloromethyl)propene, K_2CO_3, KI, acetone; 95%. [d] NaOH, H_2O-THF; 98%. [e] $Na_2S_2O_4$, DMF, Δ; 94%. [f] $BaMnO_4$, CH_2Cl_2; 92%. [g] $SnCl_4 \cdot 5H_2O$, CH_2Cl_2; 93%.

isolated, a definitive structural assignment was not possible and two structures, **1a** and **1b**, were initially postulated.[5] Two groups[6,7]



1a, X = OH; Y = H
b, X = H; Y = OH

independently prepared **1b**; however, the mass spectral fragmentation pattern was inconsistent with that of γ-citromycinone, and the structure was revised to **1a**.[6,8,9]

The regiospecific preparation of the ene product **8a**, which serves

†Recipient of a Career Development Award, 1978-1983, from the National Cancer Institute of the National Institutes of Health (CA 00486).

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