gioselective. Formation of neither the regioisomer nor the diarylated product could be detected by NMR analysis. Second, a variety of aryl bromides, rather than iodides, bearing an electron-donating, an electron-withdrawing, or an ortho substituent can be employed with almost equal success. Mildness of the reaction conditions tolerates the presence of a ketone or an ester functionality in the substrate, which is one of the most striking features of this method. Finally, the simplicity of the procedure, e.g., one-pot conversion of easily available silyl enol ethers to arylated ketones, is another advantage over other methods.

Registry No. 1a, 83511-78-8; **1b**, 73503-97-6; **1** (R = $CH(CH_3)CH_2CH_3$), 83511-79-9; **1** (R = $C(CH_3)_3$), 17510-46-2; **1** (R = $CH=C(CH_3)_2$), 6651-46-3; **1** (R = Ph), 13735-81-4; **2** (Ar = Ph; X = Br), 108-86-1; **2** (Ar = C_6H_4-*p*-OMe; X = Br), 104-92-7; **2** (Ar = Ph; X = I), 591-50-4; **2** (Ar = C_6H_4-*p*-Me; X = Br), 106-38-7; **2** (Ar = C_6H_4-*o*-Me; X = Br), 95-46-5; **2** (Ar = C_6H_4-*p*-Ac; X = Br), 99-90-1; **3** (Ar = Ph; R = C_7H_{15}), 32508-90-0; **3** (Ar = C_6H_4-*p*-OMe; R = C_7H_5), 66164-34-9; **3** (Ar = Ph; R = $CH_2CH_2CH(CH_3)_2$), 27993--43-7; **3** (Ar = C_6H_4-*p*-Me; R = $CH_2CH_2CH(CH_3)_2$), 83511-80-2; **3** (Ar = C_6H_4-*o*-Me; R = $CH_2CH_2CH(CH_3)_2$), 83511-81-3; **3** (Ar = C_6H_4-*p*-OMe; R = $CH_2CH_2CH(CH_3)_2$), 83511-82-4; **3** (Ar = C_6H_4-*p*-Ac; R = $CH_2CH_2CH(CH_3)_2$), 83511-83-5; **3** (Ar = Ph; R = $CH(CH_3)CH_2CH_3$), 27993-42-6; **3** (Ar = Ph; R = $C(CH_3)_3$), 6721-67-1; **3** (Ar = Ph; R = $CH=C(CH_3)_2$), 61799-54-0; **3** (Ar = Ph; R = Ph), 451-40-1; Bu_3SnF, 1983-10-4; $PdCl_2(P(o\text{-}MeC_6H_4)_3)_2$, 40691-33-6; (1-cyclohexen-1-yloxy)trimethylsilane, 6651-36-1; [(1-ethyl-1-propenyl)oxy]trimethylsilane, 17510-47-3; 2-phenylcyclohexanone, 1444-65-1.

High-Pressure NMR Studies of Hemoproteins. Pressure-Induced Structural Changes in the Heme Environments of Cyanometmyoglobin

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We are currently interested in the NMR of proteins under pressure with the aim to gaining an insight into details of protein structure in solution. We have previously studied the effects of high pressure on the spin states of heme iron[1] and on the ligand-exchange phenomena such as the acid–alkaline transition[2] of hemoproteins by the use of high-pressure high-resolution proton NMR at high field. We report here direct evidences for structural changes of the protein in the heme environments of cyanometmyoglobin (Figure 1) when subjected to high hydrostatic pressures.

A simple device for high-pressure NMR measurements and experimental details are described in our previous reports.[1] We followed the proton NMR spectrum of cyanometmyoglobin in H_2O solution[3] at various pH's and pressures with a special attention to exchangeable NH signals in the paramagnetically shifted region.

Figure 2 illustrates an example of the proton NMR spectra of cyanometmyoglobin (horse) in Tris·HCl buffer[4] pH 7.8 in H_2O at various pressures. Paramagnetically shifted resonances are shown in the spectra. It is of particular interest to note that the exchangeable proton signal at 18.6 ppm, which has been assigned[6]

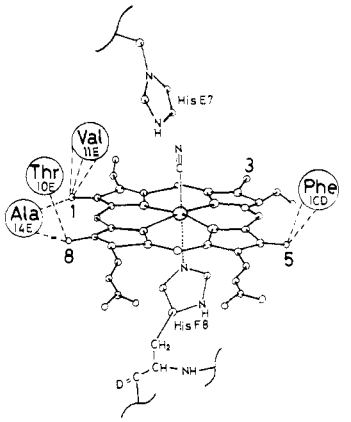

Figure 1. Heme environmental structure of cyanometmyoglobin based on the X-ray structure analysis. The dotted lines stand for the interatomic contacts within 3.9 Å.[10]

to the N_3H proton of distal histidyl (E7) imidazole, moves upfield upon pressurization, while the proximal histidyl (F8) NH proton resonance[6] at 16.4 ppm exhibits no pressure effect. The exchangeable proton peak e at 8.7 ppm, which was also assigned to the proximal histidine F8 peptide NH proton (Figure 1),[6] is also insensitive to pressure. These findings suggest that the distal region is more compressible than the proximal region in cyanometmyoglobin.

Another interesting feature in Figure 2 is the specific shift of the heme peripheral proton resonances, especially the 8- and 5-methyl signals[7] at 22.2 and 8.7 ppm, respectively, upon pressurization. With raising pressure from 1 to 900 atm, the 8- and 5-methyl peaks exhibit sizable upfield shifts accompanied by broadening, while the 1-methyl signal position remains at 13.4 ppm. When pressure is further increased above 1000 atm, these spectral changes for two methyl resonances appear to go back to those for lower pressures, with the distal histidyl N_3H proton peak exhibiting a continuous upfield shift. The single proton peak c at 13.1 ppm, previously assigned to the vinyl $C_\alpha H$,[8] shows noticeable downfield shift upon pressurization up to 900 atm, and beyond this pressure seems to move upfield toward the signal position at 1 atm. These spectral features of the heme peripheral proton groups at high pressures may allow us to expect that a pressure-induced structural change is proportional to pressure, as is manifested as a continuous shift of the distal histidyl N_3H peak, while this effect of a structural change could be exerted discontinuously on the heme peripheral proton groups. Nonbonded interactions between the heme periphery and amino acid residues in the heme vicinity may be modulated by pressure-induced local structural changes. These van der Waals contacts, which are presumably responsible for specific shift for the methyl and vinyl proton resonances, appear to be released at a specific pressure, say 1000 atm. It can be, at least, said that the pressure effects are localized to a particular region of the protein.

We have also examined high-pressure NMR of cyanide complexes of sperm whale myoglobin and its derivatives reconstituted with deuterioheme (the vinyl groups at the 2- and 4-positions are replaced by protons). Pressure-induced spectral changes for these cyanometmyoglobins at pH 7.5–8.1 were almost the same as those mentioned above for horse cyanomyoglobin. For the cyanide complex of deuteriomyoglobin, the distal histidyl N_3H signal at 18.3 ppm shifted 1.0 ppm upfield at 750 atm, and the pyrrole proton resonances located in the upfield region at −19.5 and −25.0 ppm experienced noticeable pressure-induced shifts of 0.5 ppm

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(3) Proton NMR spectra were recorded at 300 MHz on a Nicolet NT-300 spectrometer equipped with a 1180E computer system. Typical spectra of cyanomyoglobin consisted of 40000 trasients with 8K data points and a 5.8-μs 90° pulse after the strong solvent resonance in H_2O solution was suppressed by a 500-μs low-power 180° pulse.

(4) The pH of Tris·HCl buffer has been shown[5] to be independent of pressure up to 2000 atm, while the pH of phosphate buffer was shown to be decreased by 0.4 upon pressurization to 1000 atm. Therefore, we used Tris·HCl buffer throughout the present study unless otherwise noted.

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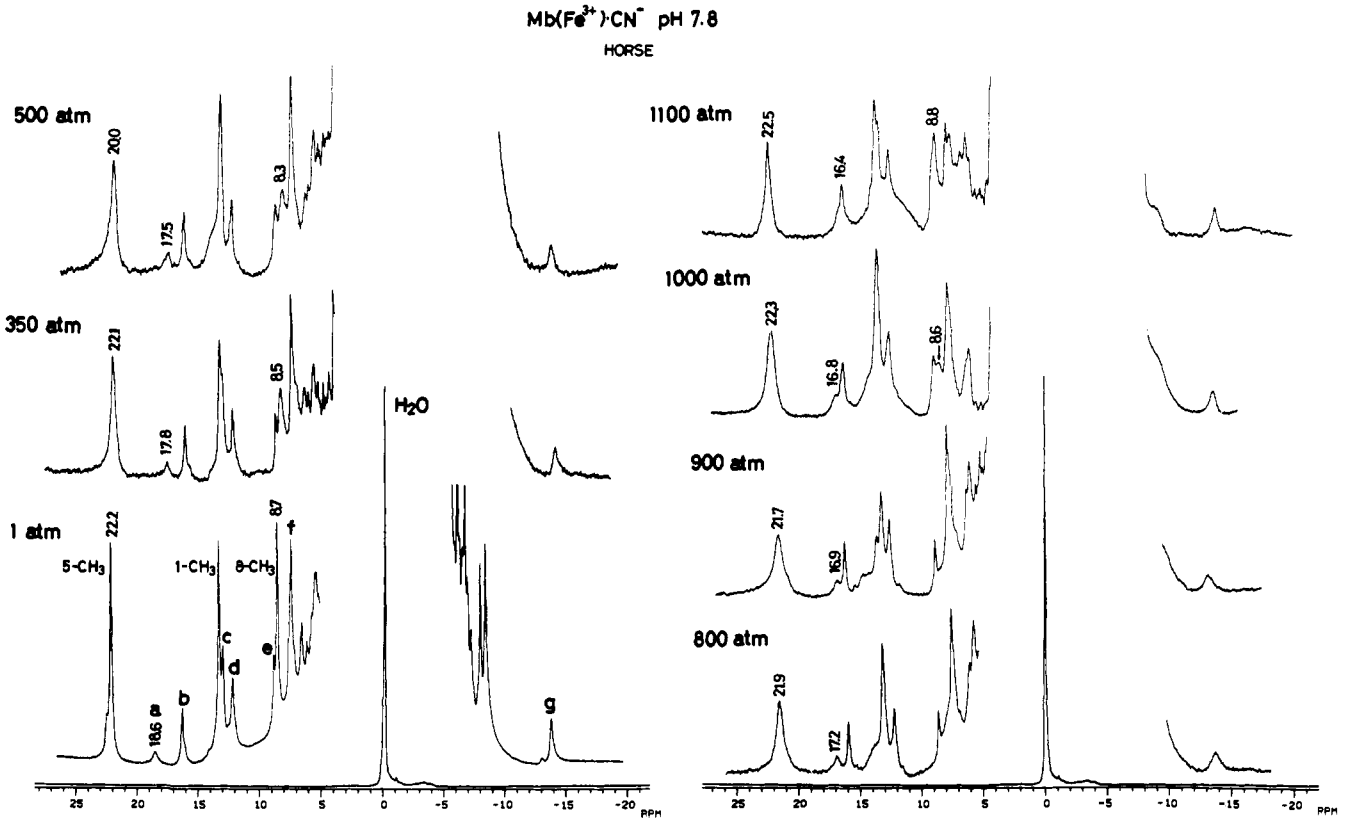

Figure 2. Pressure dependence of the 300-MHz proton NMR spectra of horse heart ferric cyanomyoglobin at 30 °C, pH 7.8 in 0.1 M Tris·HCl·H_2O buffer solution. The peaks a, b, and e are exchangeable proton signals. The numbering of the heme methyl groups is shown in Figure 1.

downfield and upfield, respectively, while the proximal histidyl NH peak at 16.0 ppm remained unchanged. It is therefore likely that the pressure-induced shifts of vinyl $C_\alpha H$ for the natural myoglobin and the pyrrole protons for deuteriomyoglobin are not caused by changes in the electronic structure of the heme but rather by the local structural alterations of the heme periphery resulting from the nonbonded interactions with nearby amino acid residues. We have also examined high-pressure NMR of other hemoproteins such as cyano horseradish peroxidase.[1b] No pressure-induced spectral changes were observed. Use of different buffer solutions led to the same results.

The distal histidyl N_3H proton is located near the heme iron closely enough to experience substantial pseudocontact shift. Decrease in the downfield paramagnetic shift for this resonance upon pressurization could be due to dislocation of this histidyl imidazole group in a way that the iron-N_3H distance increases and/or the N_3H moves far off the nodal axis of the heme plane. This structural change could occur by a swing away of the imidazole group from the distal histidine upon pressure-induced structural changes. According to an X-ray analysis of sperm whale cyanometmyoglobin,[9] the distal histidyl N_3H proton is hydrogen bonded to the coordinated cyanide.[6c] Some changes in this hydrogen bond interaction and in tautomeric equilibrium of the distal histidyl imidazole ring could not be ruled out as causes for the pressure-induced shift of the N_3H resonance. If this local structural change in the distal side of the heme is induced by pressure for other myoglobin derivatives, the ligand-exchange phenomena at the iron sixth coordination position are expected to be modulated by pressure. Our recent finding that the pK of the acid-alkaline transition of aquometmyoglobin is substantially changed at high pressure[2] could be interpreted in terms of this structural alteration. The hydrogen bond of the iron-bound water with the distal histidine in the acid form may be affected by this structural change upon pressurization and eventually lead to a change in pK associated with deprotonation of this bound water to produce the hydroxyl group at the iron sixth site (alkaline form).

In summary the present high-pressure NMR study of cyanometmyoglobin revealed that the pressure effects are localized to a particular region of the protein, especially the heme distal side.

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Electroreduction of Carbon Dioxide Catalyzed by Iron-Sulfur Clusters $[Fe_4S_4(SR)_4]^{2-}$

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Much attention has been focused on converting the cheap carbon resource carbon dioxide into organic substances. Carbon dioxide is electrolytically reduced to various organic acids, the distribution of which crucially depends on the reaction conditions such as electrode materials, solvent systems, and operational parameters.[1] Recently, Savéant and his co-worker[2] shed some mechanistic light on the product distribution. One of the problems

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