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14. Data for cystallographic studies were measured on a MAC sciences MXC3 fourcircle diffractometer. The unit cell and other related parameters of compounds (5) are as follows; F.W=303.32; Colorless polyhedral; Crystal demension=1.80×0.80×0.40 mm^3; Space group C_{2h}^5-$P2_1/n$; a=18.214(5) Å, b=7.416(2) Å, c=12.037(3) Å, β=108.49(2) Å, V=1542.1(2) $Å^3$; Z=4; D_{calc}=1.337 g/cm^3; Radiation=Mokα, λ=0.71073 Å; Temperature=293(2)K; Final R=0.0539; Number of unique reflections=1982.

The Relationship between the C2 Proton NMR Signals of Coordinated Imidazole Rings of Cytochrome c_3 and Their Redox Properties

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Cytochrome c_3 are a unique class of heme protein which contains four hemes in a single polypeptide.[1] Crystal structures of cytochrome c_3 from *Desulfovibrio vulgaris* Miyazaki F (*Dv*MF) and *Desulfovibrio vulgaris* Hildenborough (*Dv*H) have been reported.[2,3] All of the 5th and 6th ligands of the four hemes are histidyl imidazoles. One of the remarkable features of these proteins are the extrmely low redox potentials of the four hemes in comparison with other *c*-type cytochromes. The crystal structure, however, did not give a clue to the extremely low redox potentials. The assignment of the C2 proton signals of ^{1}H NMR spectrum of cytochrome c_3 carried out in this work, which suggested the role of the imidazole rings in realizing the low redox potentials.

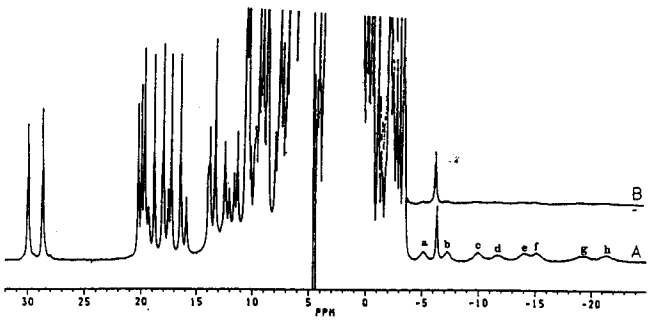

Figure 1. 400 MHz ^{1}H NMR spectra of *Dv*MF cytochrome c_3 at p^2H 7.0 and 30 ℃. (A) Non-deuterated. (B) Specifically deuterated at the C2 position of histidyl imidazole.

Desulfovibrio vulgaris Miyazaki F and *Desulfovibrio vulgaris* Hildenborough were cultured in medium C[1] and in a minimal medium[4] to obtain non-deuterated and deuterated cytochrome c_3 from *Desulfovibrio vulgaris* Miyazaki F, respectively. In the latter case, the C2 proton of the histidyl imidazole of cytochrome c_3 was specifically deuterated by replacing L-histidine of the minimal medium with deuterated L-histidine. Cytochrome c_3 were purified according to the reported procedure.[4] The purity was checked by the purity index (A_{552} (red)/A_{280}(ox)) and SDS-PAGE. 400 MHz ^{1}H NMR spectra were measured with a Bruker AM-400 NMR spectrometer. Chemical shifts are presented in parts per million (ppm) to the internal standard 2,2-dimethyl-2-silapentane-5-sulfonate (DSS).

There are nine histidine residues in the cytochrome c_3 from *Dv*MF, eight of which are ligands of the four hemes. ^{1}H NMR spectra of non-deuterated and deuterated ferricytochrome c_3 from *Dv*MF are presented in Figure 1.

In the spectrum of non-deuterated ferricytochrome c_3 from *Dv*MF (Figure 1A), eight extremely broad signals were observed in the region higher than 0 ppm, which disappeared on the deuteration of the C2 position of the imidazole rings (Figure 1B). Thus, they can be assigned to the C2 protons of the histidyl imidazoles coordinated to the heme irons. This means that all of the coordinated histidines could be detected as separate signals. They were designated as a-h from the low to high field. A sharp peak at about 8.9 ppm also disappeared. This can be ascribed to the C2 proton of non coordinated histidine (His-67), which agrees with the earlier assignment.[4]

The formal potentials of each heme (the microscopic redox potentials)of DvMF and *Dv*H cytochrome c_3 were estimated by the use of NMR.[5,6] Macroscopic redox potentials of cytochrome c_3 are reported to be −240, −297, −315 and −357 mV for *Dv*MF, and −263, −321, −329 and −381 mV for *Dv*H, respectively.[7] The macroscopic redox potentials of *Dv*H cytochrome c_3 are lower than those of *Dv*MF by about 24 mV except for the third redox potential. But, the crystal structure did not give a clue to the difference redox potential between both protein. The C2 proton signals of the coordinated imidazole groups were identified in a very high field region of the ^{1}H NMR as shown in Figure 2.

It scattered in a wide range from −5 to −22 ppm. The

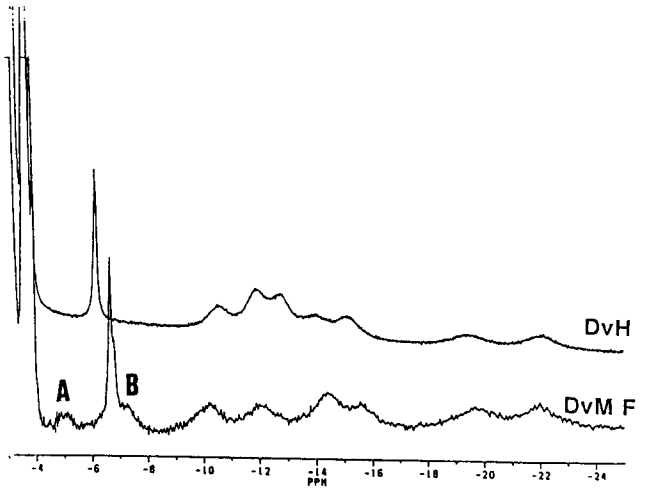

Figure 2. ^{1}H NMR spectra of cytochrome c_3 from *Desulfovibrio vulgaris* Miyazaki (bottom) and Hildenborough (top) in a high field region.

C2 proton signal of a histidyl imidazole group usually appears in the region from 7 to 9 ppm. Such extremely upfield shift can be explained either by the tilting of the axial ligand, or the imidazole nature of the ligand.[8,9] Since no significant tilting was found in crystal structure of both cytochrome c_3 molecules, the major reason of the upfield shift was ascribed to the imidazole nature of the histidine ligand. The imidazole nature of the ligand can explain, at least partly, the extremely low redox potentials of the cytochrome c_3 molecules in comparison with other *c*-type cytochromes. The spectrum of the C2 protons in the high field region was also reported for cytochrome c_3 from *Dv*H as shown in Figure 2(top).[10] It is shown in figure 2 along with that from *Dv*MF. It is clear from the figure that two lower signals (designated as A and B in Figure 2) of the C2 protons in *Dv*MF shift to the high field by several ppm in the spectrum of *Dv*H. Therefore, it is most plausible that two major differences with regard to the heme structure between both proteins could be responsible for the spectral changes of the C2 protons of the ligated imidazole groups. The upfield shift of the C2 proton signals can partly explain the lower redox potentials of the cytochrome c_3 from *Dv*H.

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Lyotropic Aggregation of Rod-Coil Oligomers-containing Poly(Ethylene Oxide)s

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The rod-coil diblock molecule is of considerable interest due to its capability of forming well defined supramolecular structures such as layered smectic phase and cylindrical micellar phase as the relative volume fraction of block varies.[1~5] Another interesting aspect of rod-coil molecules is of course the high immiscibility between rod and coil segments which allows block segregation to occur at relatively smaller molecular weights of each segment than in typical flexible diblock molecules. In previous publications,[6,7] we reported the synthesis of monodisperse rod-coil oligomers containing one rigid and one flexible poly(ethylene oxide) segment joined covalently to share the same molecular backbone and showed that the oligomers form a layered assembly with nanoscale dimensions. Complexation of the rod-coil molecules with $LiCF_3SO_3$, which might increase the relative volume fraction of poly(ethylene oxide) coil segment, gave rise to transformation of the layered assembly into the cylindrical micellar phase, most probably to relieve coil stretching penalty.[7] The supramolecular structure of these rod-coil oligomers may also vary as a function of oligomer concentration in a selective solvent in which the rod blocks are immiscible, while the flexible coil blocks are highly miscible, due to the variation of the relative coil volume fraction. This experimental system has therefore enabled us to study lyotropic aggregation behavior in a selective solvent which causes the relative volume fraction of coil segments to change, particularly in view of theoretical predictions.[1,4]

In this communication, we describe the lyotropic aggregation behavior of the monodisperse rod-coil oligomers containing poly(ethylene oxide)s as coil segments in aquous solution. Scheme 1 outlines the structure of the rod-coil molecules.