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Conversion of Spin State and Protein Structure in *Rhodospirillum rubrum* Ferric Cytochrome *c'* Coupled with Dissociation of Dimer†

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ABSTRACT: Correlation between the spin states of *Rhodospirillum rubrum* cytochrome *c'* and its protein structure was studied by employing proton magnetic resonance, ultracentrifugation, and low-angle laser light scattering. ^{1}H NMR studies showed that the cytochrome *c'* is in high-spin states at up to pD 11 and in low-spin states at pD higher than 12 or in the presence of 2-propanol or sodium dodecyl sulfate ($NaDodSO_4$) at neutral pD. The protein signals in the 360-MHz ^{1}H NMR spectrum are widely spread up to pD 11 but show a coalesced pattern in the strongly alkaline pD region (>12) or in the presence of 2-propanol or $NaDodSO_4$. Therefore, it was concluded that the protein structure is rigid in the high-spin states and relaxed in the low-spin states. The molecular weight of the cytochrome *c'* was examined by sedimentation equilibrium and low-angle laser light scattering methods under various conditions. It was deduced that the cytochrome *c'* in the high-spin state has dimer form, whereas it takes monomer form in the low-spin state. This indicates that there is a correlation between spin states and the monomer–dimer conversion. A relaxed structure which is triggered by the dissociation of the dimer form into monomers could be primarily responsible for the low-spin state. The biological significance of the correlation is discussed in connection with the membrane-bound type of *R. rubrum* cytochrome *c'*.

Cytochrome *c'* constitutes a class of electron transport proteins which are widely distributed in phototrophic and aerobic bacteria (Bartsch, 1978). The heme is covalently bound to cysteine residues, as in other *c*-type cytochromes, but the properties of the proteins are quite different. The visible absorption spectrum of cytochrome *c'* at neutral pH resembles that of myoglobin rather than that of cytochrome *c*. Three and two spectroscopically distinguishable forms were observed in the oxidized and reduced states, respectively, by the use of electronic absorption (Horio & Kamen, 1961; Imai et al., 1969a), circular dichroism (Imai et al., 1969b; Rawlings et al., 1977), resonance Raman (Strekas & Spiro, 1974; Kitagawa et al., 1977), and nuclear magnetic resonance (Emptage et al., 1981; La Mar et al., 1981) spectroscopies. In the case of the oxidized state, type I characterized by the Soret band at 390 nm appears at acidic and neutral pH. The spin state of this form has been controversial. Originally it was assigned to a high-spin state (Horio & Kamen, 1961; Moss et al., 1968; Ehrenberg & Kamen, 1965; Tasaki et al., 1967) but afterwards interpreted as a quantum mechanical admixture of the intermediate-spin ($S = {}^3/_2$) and high spin ($S = {}^5/_2$) states on

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the basis of EPR[1] (Maltempo et al., 1974). Recent studies (Rawlings et al., 1977; Kitagawa et al., 1977; Emptage et al., 1977, 1981) again favor the high-spin state. Type II with the split Soret bands at 402 and 370 nm is seen in the pH range from 9 to 12 and is unambiguously assigned to a high-spin state. A typical *c*-type form (low spin) characterized by the Soret band at 407 nm appears in the strongly alkaline pH region (higher than pH 12), which is called type III. In the reduced state, a myoglobin-like form (type a) and a normal *c* type (type n) are observed in the regions up to pH 11 and higher than pH 12, respectively.

Conversions from type I to type II and from type II to type III are reversible (Horio & Kamen, 1961). It is also reported that the presence of much organic solvent (Imai et al., 1969a,b) or a small amount of sodium dodecyl sulfate ($NaDodSO_4$) (Kitagawa et al., 1977) at neutral pH induces a direct conversion from type I to type III. These observations are interesting in view of the unclarified biological significance of cytochrome *c'*. A low-spin state which is formed under physiological conditions could be particularly important if the protein is involved in an electron transport system. Actually, some cytochrome *c'* of *Rhodospirillum rubrum* is known to be bound to chromatophore membranes as a low-spin species (Kakuno et al., 1971).

In the present work, the factors determining the spin states of ferric cytochrome *c'* from *R. rubrum* have been investigated in detail by employing nuclear magnetic resonance and sedimentation equilibrium. Cytochrome *c'* of *R. rubrum* is in a dimer form under physiological conditions (Cusanovich, 1971). The single subunit is composed of 126 amino acids (M_r 14000), and its sequence was determined (Meyer et al., 1975). Although the crystal structure of this protein is not yet known, the X-ray analysis of cytochrome *c'* from *R. molischianum* which is also a dimer protein was recently reported (Weber et al., 1980, 1981). The monomer is composed of four α-helices but has no β-sheet. The intersubunit interaction is maintained by hydrophobic amino acid residues in α-helices. As anticipated from the amino acid sequence, the fifth ligand of the heme iron is a histidine residue. Our work suggests that there is a correlation between high-spin–low-spin and dimer–monomer conversions in the *R. rubrum* cytochrome *c'*.

Materials and Methods

Cytochrome *c'* was purified from *R. rubrum* according to a reported method (Bartsch et al., 1971). Proton magnetic resonance spectra were recorded on JEOL FX-100 and Bruker WM 360wb NMR spectrometers equipped with temperature control units. The spectra were taken for deuterium oxide solutions at 30 °C, and chemical shifts were read relative to sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS). The protein concentration was about 0.1 mM unless otherwise mentioned. The solvent peak was saturated by using homogated decoupling. Potassium phosphate buffer (0.1 M) was used for all measurements except pD titration and $NaDodSO_4$ addition experiments. In the latter cases, no buffer and 0.1 M sodium phosphate buffer were used, respectively. The pD value was determined with Radiometer pH meters 26 and PHM 84 with Ingold 4030-02 electrodes. Direct readings are given in the text. The values higher than pD 10 were corrected by the use of a Radiometer GK2321 electrode. Deuterium oxide (99.8%) and 2-propanol-d_8 were purchased from CEA (Commissariat à l'Energie Atomique, France).

Sedimentation equilibrium experiments were performed with a Beckman Spinco analytical ultracentrifuge Model E equipped with an absorption scanner at about 14000 rpm. Light at 390, 407, or 420 nm was used. The protein concentration was about 0.13 mg/mL (OD $\approx$ 0.4). Apparent molecular weight was determined according to

$$M_r(\text{app}) = \frac{2RT}{(1-\bar{v}\rho)\omega^2}\frac{\mathrm{d}\ln C(r)}{\mathrm{d}r^2} \quad (1)$$

where R, T, $\bar{v}$, ρ, ω, r, and $C(r)$ have the usual meanings (Williams, 1963). Partial specific volume $\bar{v}$ was assumed to be 0.742 which was calculated from the amino acid composition (Cohn & Edsall, 1943). In the case of pH 12.5, 0.720 was used as $\bar{v}$ which is more suitable for random coil (Kakiuchi & Williams, 1966). In the presence of 30% 2-propanol, a run without sample was carried out to confirm that no distortion is caused in the base line by centrifugal force. The value of $\bar{v}$ was assumed to be 0.777 which was corrected only for the density of the solvent. The sample solution was dialyzed against the solvent overnight. The molecular weight in the presence of 0.1% sodium dodecyl sulfate was determined by a modified version (S. Maezawa and T. Takagi, unpublished results) of the low-angle laser light scattering technique combined with high-performance gel chromatography reported by Kameyama et al. (1982). The protein concentration was lower than 1 mg/mL in the light scattering cell. Light scattering was measured at 633 nm. The differential refractometer was equipped with a tungsten lamp. Since the cytochrome *c'* has absorption bands in the visible region, it could be a source of error in dn/dc measurements. This will be discussed elsewhere by S. Maezawa and T. Takagi.

Results

Proton Magnetic Resonance. The 100-MHz proton magnetic resonance (^1H NMR) spectra of ferric cytochrome *c'* of *Rhodospirillum rubrum* in the low-field region were obtained at different pDs. Typical patterns are presented in Figure 1. The four signals appearing in the lower field than 60 ppm at pD 5.7 were assigned to the heme methyl resonances (Emptage et al., 1981; La Mar et al., 1981). When the pD is raised, these signals move upfield continuously with a sigmoidal curve of $pK_a = 8.5$. At pD 12.0, the second components of the four peaks appear in a higher field region from 10 to 30 ppm; namely, a discontinuous change in the chemical shift takes place in this pD region. The general features are the same as the reported ones (Emptage et al., 1981). The chemical shift changes in two pD regions correspond to the conversions between types I and II and types II and III, respectively. La Mar et al. (1981) estimated the exchange rate between types I and II from the broadening of the signals to be about 5×10^4 s^{-1}. Estimated from the signal separation (Wüthrich, 1976), the conversion rate between types II and III is slower than 2×10^4 s^{-1}. The extremely low chemical shifts of the heme methyl signals of types I and II suggest that types I and II are in high-spin states as indicated by recent studies (Rawlings et al., 1977; Kitagawa et al., 1977; Emptage et al., 1977, 1981). The chemical shifts of type III signals are typical for a low-spin state (Wüthrich, 1976).

The 360-MHz ^1H NMR spectra of the protein region are shown in Figure 2. In neutral pD region signals are widely spread, but the signal pattern becomes simpler, and the intensity becomes stronger with the increase of pD as is evident in the methyl proton region. The spectrum at pD 12.9 is close to that of a denatured protein. The intensities of the retained NH protons of types I and II did not change at all for several

[1] Abbreviations: ^1H NMR, proton nuclear magnetic resonance; EPR, electron paramagnetic resonance; D_2O, deuterium oxide; $NaDodSO_4$ (SDS in the figures), sodium dodecyl sulfate; *R. rubrum*, *Rhodospirillum rubrum*; *Rps. palustris*, *Rhodopseudomonas palustris*.

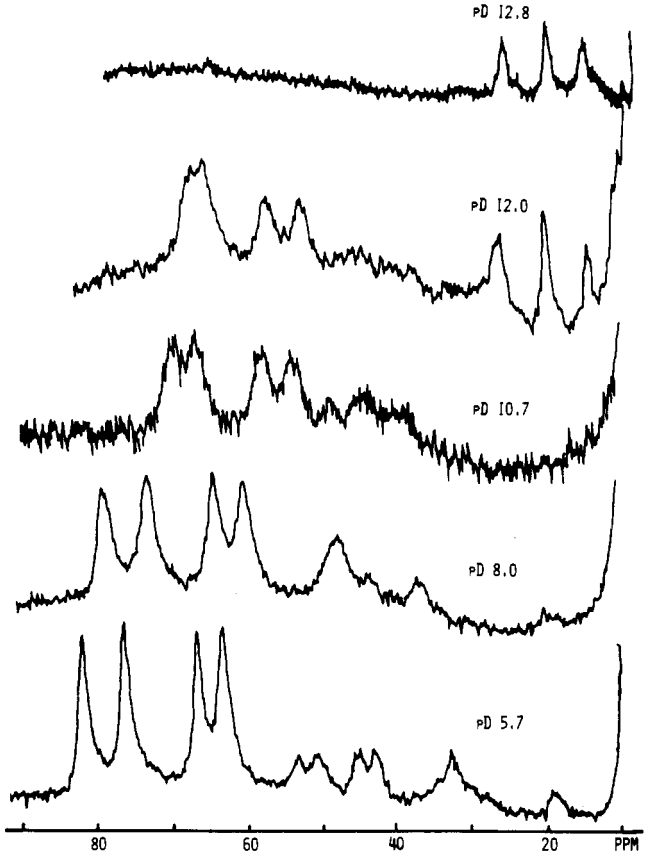

FIGURE 1: 100-MHz ^{1}H NMR spectra of *R. rubrum* cytochrome *c′* at different pDs. The protein concentration is 4.8 mM.

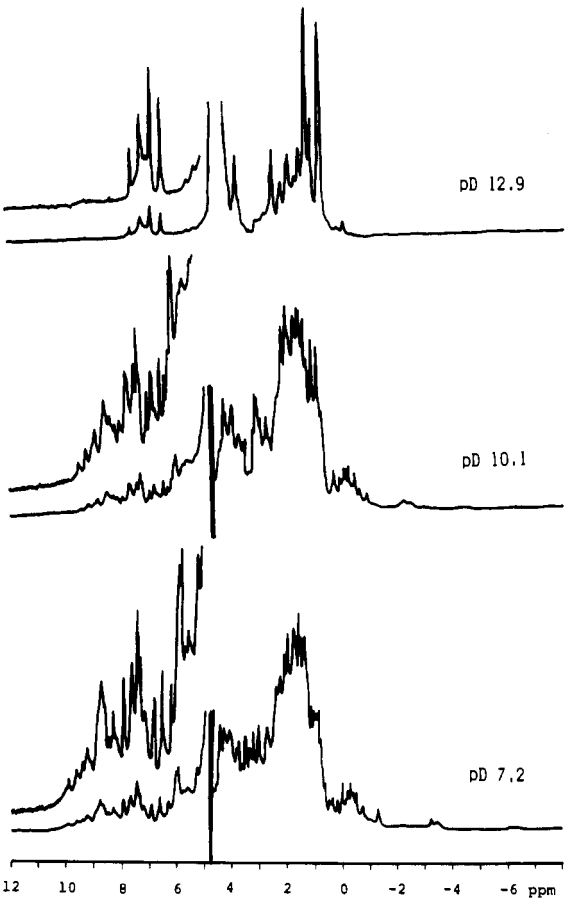

FIGURE 2: Protein region of 360-MHz ^{1}H NMR spectra of *R. rubrum* cytochrome *c′* at different pDs.

hours. From the spectral patterns and H–D exchange observations, it can be said that the structures of types I and II are rigid, while that of type III is flexible. The present observations are consistent with the previous report that the CD pattern showed a significant loss of the secondary structure at pH 12.3 (Imai et al., 1969b).

If the reversible structural conversions are taken advantage of, NH protons can be completely replaced with deuterons by raising pD to 12 and bringing it back quickly to neutral pD. The 360-MHz ^{1}H NMR spectra of such protein are given in Figure 3 for the region from 5 to 11 ppm. The aromatic protons are distributed in a range wider than those of simple proteins presumably due to the effect of the ring current and the paramagnetic center of the heme group. The monomer of the protein contains three tyrosine, four phenylalanine, two histidine, and one tryptophan residues (Meyer et al., 1975). The chemical shift changes as a function of pD are shown in Figure 4 for the aromatic signals in the region lower than 6 ppm. The plots suggest that there are two kinds of aromatic protons. Most of them are sensitive to the conversion between types I and II, but at least three signals (3, 10, and 11) are insensitive to it. Signals 10 and 11 show sigmoidal titration curves of about $pK_a = 11$. Thus they may be attributed to tyrosine residues. Since their titration curves are similar to each other, signals 10 and 11 could come from the same amino acid residue. The fact that most signals are sensitive to the spin-state change implies that most of the aromatic residues are located in the vicinity of the heme group as in the case of the cytochrome *c′* of *R. molischianum* revealed by X-ray analysis (Weber et al., 1980, 1981). Signals 9 and 12 are quite sharp and have one proton intensity in comparison with the tyrosine signals (10 and 11). Their titration curves are similar to each other and show an additional change in the acidic pD region besides the type I $\rightleftarrows$ type II conversion. They can be assigned to C2H and C4H protons of the histidine residue from their resonance positions and pH-sensitive behavior. His-41 is the only normal histidine residue because His-120 is the fifth ligand of the iron. The apparent pK_a of His-41 is estimated to be lower than pD 4 from the titration curves. It is abnormally low, and therefore, the histidine residue should be isolated from the solvent and/or involved in strong hydrogen bonding in type I. In an earlier work a small chemical shift change of the heme methyl signals at about pD 5 was attributed to the dissociation of His-41 (Emptage et al., 1981). If our assignments of His signals are valid, the change cannot be associated with the histidine residue. In the conversion between types II and III, new signals of the protein due to type III appear separately. Two proton signals at 6.51 and 6.89 ppm can be assigned to the deprotonated ring protons of free tyrosine residues in comparison with the chemical shifts of *N*-acetyltyrosine methylamide (Endo et al., 1981). From the signal separation of the tyrosine residue, the conversion rate between types II and III should be slower than 10^2 s^{-1} rather than 2×10^4 s^{-1} which was estimated from the heme methyl proton signals.

It was indicated by Kitagawa et al. that anionic detergent, sodium dodecyl sulfate, at a quite low concentration can convert the cytochrome *c′* directly from type I to type III, while cationic and nonionic detergents cannot at the same concentration. The effects of $NaDodSO_4$ shown in the 100-MHz ^{1}H NMR spectra of the heme signals at 1 mM protein concentration (for M_r 28 000) are illustrated in Figure 5. A change from type I to a low-spin state takes place at a $NaDodSO_4$ concentration between 0.15 and 0.4%. In the case of 0.1 mM protein concentration, 0.06% $NaDodSO_4$ is enough to induce the change (Figure 6). This suggests that there is a specific

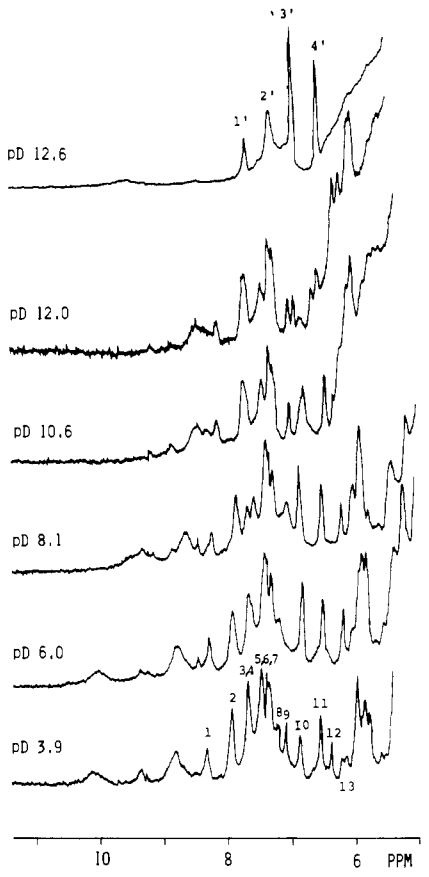

FIGURE 3: Aromatic region of 360-MHz ^{1}H NMR spectra of *R. rubrum* cytochrome *c'* at different pDs. All NH protons were replaced with deuterons.

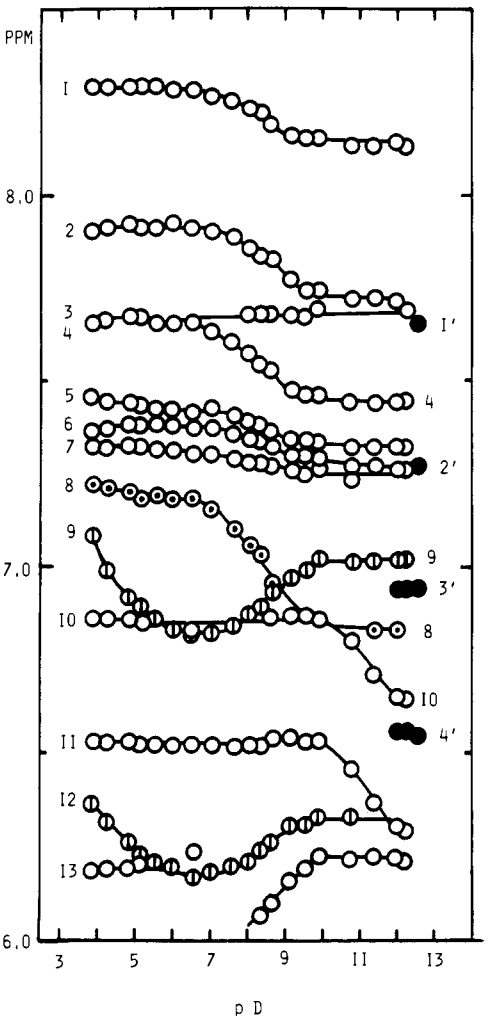

FIGURE 4: pD titration curves of the signals in the aromatic region. (○) Types I and II; (●) type III. (Φ) and (⊙) indicate the signals that can be traced referring to their signal shapes and intensities.

interaction between cytochrome *c'* and $NaDodSO_4$ molecules. Although the spectrum of the heme methyl region shows that cytochrome *c'* is in a low-spin state, its spectral pattern is not the same as that of type III at pD 12.8. The 360-MHz ^{1}H NMR spectra of the aromatic region in the presence of $NaDodSO_4$ are shown in Figure 6. The spectral pattern of the aromatic region becomes simpler on the addition of $NaDodSO_4$, and all of the retained NH protons were easily replaced by deuterons. This means that the protein structure is relaxed to a significant extent but is not in an identical state with that of type III. As can be seen in Figure 6, new signals grow at the expense of original ones with the increase of $NaDodSO_4$ concentration. On the basis of the discontinuity in the changes of the chemical shifts of the signals, the conversion between type I and the $NaDodSO_4$-induced low-spin form is slower than 2×10^2 s^{-1}. 2-Propanol was also reported to induce conversion to type III. This reagent is particularly interesting because it induces little change in the secondary structures (Imai et al., 1969b). The 360-MHz ^{1}H NMR spectra of the heme signals and the aromatic region in the presence of 30% 2-propanol-d_8 are given in Figures 5 and 6, respectively. The spectrum of the heme signals corresponds to that of a low-spin state but is different from either type III or the $NaDodSO_4$-induced low-spin form. The spectral pattern of the aromatic region is also simplified, and no retained NH proton remains, suggesting a flexible protein structure. The feature of the spectrum is, however, more complicated than that in the $NaDodSO_4$-induced and alkaline-induced forms.

Molecular Weight of Cytochrome c' in Each Spin State. The molecular weight of the monomer subunit of *R. rubrum* cytochrome *c'* is known to be 14000 on the basis of the amino acid sequence (Meyer et al., 1975). The apparent molecular weight of the cytochrome *c'* under different conditions was measured by the sedimentation equilibrium method to see if there was any form other than the dimer form. The obtained values are summarized in Table I.

Table I: Apparent Molecular Weight of Cytochrome *c'* of *R. rubrum*

solvent condition[a]	temp (°C)	M_r(app)
pH 6.8	20.1	27700
30% 2-propanol	4.9	12000
0.1% $NaDodSO_4$[b]	25.0	12000
pH 9.8	5.0	27800
pH 11.0	5.1	27300
pH 11.5	5.0	22650
pH 12.5	5.3	15200

[a] In 0.1 M potassium phosphate buffer. [b] In 0.1 M sodium phosphate buffer.

So that degradation of the protein could be avoided in the alkaline pH region, the temperature was kept at about 5 °C. Conversion between dimer and monomer takes place in accordance with the spin-state conversion between types II and III. As indicated by Cusanovich (1971) the cytochrome *c'* takes a dimer form in both types I and II, but it is shown that type III is in a monomer form. Thirty percent of 2-propanol also induced conversion to a monomer form. The apparent molecular weight in the presence of 30% 2-propanol was calculated under the assumptions that all components are

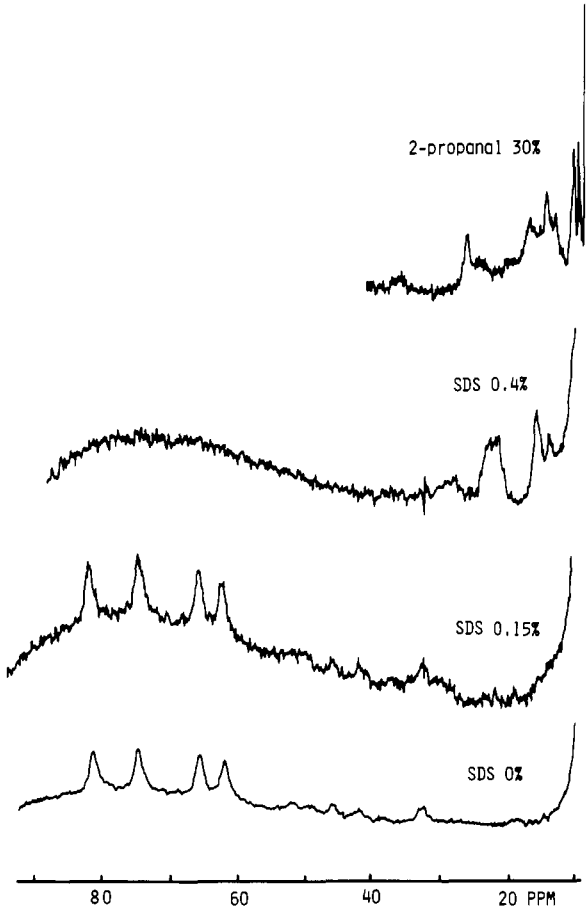

FIGURE 5: Effect of $NaDodSO_4$ or 2-propanol on the heme proton signals in the ^1H NMR spectrum of *R. rubrum* cytochrome *c′* at pD 6.9. The top spectrum is observed at 360 MHz, and the lower three spectra are at 100 MHz.

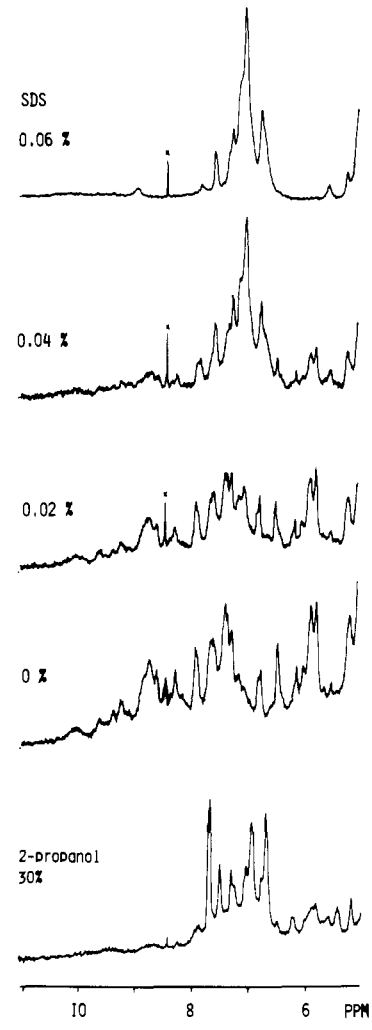

FIGURE 6: Effect of $NaDodSO_4$ or 2-propanol on the aromatic region of 360-MHz ^1H NMR spectrum of *R. rubrum* cytochrome *c′* at pD 6.9.

incompressible, that specific interactions between 2-propanol molecule and the protein are negligible, and that the redistribution of 2-propanol under centrifugal force is also negligible. These assumptions enable us to use eq 1 in a three-component system (Fujita, 1975). So that the effect of the centrifugal force could be minimized, the measurement was carried out at 10000 rpm. Actually, slight angular velocity dependence was observed since M_r 13000 was obtained as an apparent molecular weight at 17000 rpm. If there is a specific interaction between the protein and 2-propanol molecules, the real molecular weight will be larger than the obtained one because of large a $\bar{v}$ ($>1/\rho$) of the 2-propanol molecule (Fujita, 1975). Such effects, however, would not be so big as to affect the conclusion mentioned above. The molecular weight of the cytochrome *c′* in the presence of 0.1% $NaDodSO_4$ was determined by the low-angle laser light scattering technique combined with high performance gel chromatography. The technique gives the molecular weight of the protein in spite of the presence of bound $NaDodSO_4$ molecules (Kameyama et al., 1982). The obtained molecular weight shown in Table I clearly corresponds to the monomer.

As mentioned previously 2-propanol and $NaDodSO_4$ induce changes from type I (high spin) to low-spin states. Therefore it can be concluded from the present results that high- and low-spin states of *R. rubrum* cytochrome *c′* are associated with their dimer and monomer forms, respectively.

Discussion

Types I and II are considered to be in high-spin states. However, the reason for the conversion from type I to type II is rather controversial. Resonance Raman studies suggested that abnormality of type I as a high-spin state is associated with some structural constraint imposed by the protein on the heme group, and the conversion may be brought about by relaxation of the protein structure (Kitagawa et al., 1977). Six- and five-coordinations were also suggested for types I and II, respectively (Teraoka & Kitagawa, 1980), but reverse coordination schemes were proposed by NMR studies (Emptage et al., 1981; La Mar et al., 1981). No decisive evidence is available so far for the coordination. Spectral difference in Figure 2 implies that the conversion is associated with a partial relaxation of the protein structure which is accompanied by little change in the secondary structure of the protein as shown by CD studies (Imai et al., 1969a,b).

Type III at alkaline pH, the $NaDodSO_4$-induced form, and the 30% 2-propanol induced form are all in low-spin states. These reagents could simply denature the protein. However, their ^1H NMR spectra in the heme region are different from each other. This could be attributed to different structures of the protein and/or different ligands. In the case of type III, most of the secondary structure is lost, judging from CD (Imai et al., 1969b) and NMR (this work). We observed the CD spectrum of cytochrome *c′* in the presence and absence of 0.2% $NaDodSO_4$ at 18 μM protein concentration (the spectrum is not shown). A fairly large change was observed for the aromatic region (350–240 nm), but only a 15% increase in the molar ellipticity was revealed in the helix region

(240–200 nm) in comparison with the spectrum of the native one. The former result is in good agreement with the change observed in the aromatic region of the ^1H NMR spectrum (Figure 6). The CD experiments suggest that although the tertiary structure of the $NaDodSO_4$-induced form is lost to a great extent, most secondary structure is maintained. The aromatic region of the ^1H NMR spectrum of the 30% 2-propanol induced form is different not only from that of type III but also from that of the $NaDodSO_4$-induced form. CD studies showed no reduction of secondary structure (Imai et al., 1969b). These facts clearly indicate that type III and the $NaDodSO_4$-induced and 30% 2-propanol induced forms are in different states from the viewpoint of protein structure. In addition, there is no evidence that they are biologically inactive. Therefore, it is inadequate at this stage to define all of these forms as denatured states. As to the sixth ligand, there is no direct evidence whether it is the same for each type of monomer or not. A lysine residue was assumed as the sixth ligand for type III (Kitagawa et al., 1977). Since the $NaDodSO_4$- and 2-propanol-induced forms are found at neutral pH, it is unlikely that a protonated lysyl side chain coordinates the oxidized heme iron. $NaDodSO_4$ was ruled out as a ligand from experiments on a model system (Kitagawa et al., 1977). Accordingly, the same internal ligand is expected for both $NaDodSO_4$- and 2-propanol-induced forms. Small differences in NMR patterns of both forms may be due to the conformational difference of the apoprotein.

It has been shown in this work that conversion between high-spin and low-spin states is correlated with dimer dissociation. While the high-spin species, types I and II, are in dimer form as indicated by Cusanovich (1971), the low-spin species, type III, and the $NaDodSO_4$-induced and 30% 2-propanol induced forms are in monomer form. The fact that organic solvent and anionic detergent are efficient inducers of the dissociation of dimers suggests that hydrophobic interaction is a major factor stabilizing the dimer form. This idea is supported by the evidence of hydrophobic contact revealed in the crystal structure of the cytochrome *c'* of *R. molischianum* (Weber et al., 1980, 1981). Aromatic ring proton resonances of *R. rubrum* cytochrome *c'* are widely spread, probably due to the nearby location to the heme group. In the crystal structure of cytochrome *c'* of *R. molischianum* such a tendency can also be seen. The facts mentioned above suggest structural similarity in these two cytochrome *c'* proteins.

Since there is a monomer cytochrome *c'* (*Rps. palustris*) in a ferric high-spin state (Cusanovich, 1971), the low-spin state cannot be directly associated with the monomer form. The monomer forms of *R. rubrum* cytochrome *c'* (type III and the $NaDodSO_4$-induced and 30% 2-propanol induced forms) have much more flexible structure than its dimer forms (types I and II), as was concluded in ^1H NMR studies. The structural flexibility would allow the coordination of a new ligand, which leads to a low-spin state. The correlation between the spin state and the molecular weight suggests that intersubunit interactions play an important role for the dimer cytochrome *c'* to maintain the rigidity of the protein structure. The cytochrome *c'* of *Rps. palustris* is reported to be more resistant to organic solvents and more sensitive to urea than that of *R. rubrum* (Imai et al., 1969a). The hydrogen-bonding interaction seems to be more important than the hydrophobic interaction for the monomer cytochrome *c'* of *Rps. palustris* to maintain its rigid structure in contrast to the dimer cytochrome *c'*.

The existence of the low-spin states induced by $NaDodSO_4$ or organic solvent suggests that *R. rubrum* cytochrome *c'* might function not only in a high-spin state but also in a low-spin state in a biological system, depending on conditions. Kakuno et al. (1971) found the cytochrome *c'* bound to the *R. rubrum* chromatophore membrane. It could be removed from the membrane by a mixed solution of deoxycholate and cholate, but neither by salt solutions nor by urea. It was suggested that the bound cytochrome *c'* would interact with the hydrophobic region of the lipid bilayer. Furthermore, the visible absorption difference spectrum between the reduced and oxidized forms gives a typical *b*-type cytochrome pattern. That means the bound type of the cytochrome *c'* is in a low-spin state. This is supported by the fact that carbon monoxide cannot bind to the bound type cytochrome *c'*. These observations verified that there are two kinds of cytochrome *c'* in the *R. rubrum* chromatophore. Since anionic detergent interacts with cytochrome *c'* specifically and converts it to a low-spin state, acidic lipids could be a candidate which converts the free cytochrome *c'* in a high-spin state to the bound one in a low-spin state. A structural rearrangement would be induced by the specific interaction with acidic lipids. Such rearrangement would enable the hydrophobic part of the protein to bind to the hydrophobic region of the lipid bilayer. It was indicated that cytochrome *c'* can play a role in an electron transport system (Kakuno et al., 1975). For electron transfer, a low-spin form may be more appropriate than a high-spin form.

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Stereospecific Binding of Diastereomeric Peptides to Salmon Sperm Deoxyribonucleic Acid. Further Evidence for Partial Intercalation†

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ABSTRACT: A series of diastereomeric dipeptide amides, containing an N-terminal L-lysyl residue and a C-terminal L- or D-amino acid with a derivatized aromatic ring on the side chain, was synthesized to determine the dependence of (1) the chirality of the N-terminal amino acid α-carbon and (2) the length of the N-terminal amino acid side chain for intercalation of the aromatic ring. The nature of the complex between the peptide and DNA (i.e., electrostatic, intercalative, or a combination of these) was determined by UV and CD studies, viscometric titrations, and ^{1}H NMR studies. The results of these studies reveal distinct differences in the binding site of the aromatic rings of the various peptides. In particular, the results suggest that the α- and ϵ-amino groups of the lysyl residue bind electrostatically to adjacent phosphates on the DNA backbone in a *stereospecific* manner. As a result of this stereospecificity, the aromatic rings of the peptides with the L-L designation point toward the DNA helix, while those of the peptides of the L-D designation point away from the helix. This is completely consistent with previously reported work [Gabbay, E. J., Adawadkar, P. D., & Wilson, W. D. (1976) *Biochemistry 15*, 146; Gabbay, E. J., Adawadkar, P. D., Kapicak, L., Pearce, S., & Wilson, W. D. (1976) *Biochemistry 15*, 152]. The results also indicate a great dependence on the length of the side chain for intercalation of the aromatic ring. Specifically, if the side chain is long enough, and flexible enough, the aromatic ring can fully or partially intercalate, regardless of the chirality of the N-terminal amino acid α-carbon. However, if the side chain is too short, only partial intercalation is observed for peptides of the L-L designation, and no intercalation is observed for peptides of the L-D designation.

The recognition processes of proteins to specific sequences of nucleic acids is of considerable interest in several laboratories due to the number of different types of protein/DNA complexes found in the living cell. Because of the complexity of studying the interactions of two macromolecules, our approach has been to synthesize and study model systems using small oligopeptides and DNA of various AT/GC content (Gabbay et al., 1972, 1973). The model oligopeptides we have chosen contain aromatic amino acids whereby the aromatic moiety of the amino acid may partially intercalate into the base pairs of DNA. In particular, the results from previously presented studies (Adawadkar et al., 1975; Gabbay et al., 1976a,b) utilizing diastereomeric dipeptides, L-lysyl-L-phenylalaninamide (**1**) and L-lysyl-D-phenylalainamide (**2**), indicated that there is a stereospecific binding of the α- and ϵ-amino groups of the N-terminal L-lysyl residue to the nucleic acid backbone which then dictates the positioning of the aromatic ring either toward the interior of the helix for **1** or away from the helix for **2**. Due to steric constraints for **1**, the aromatic ring may only partially intercalate into the base pairs of the nucleic acid, resulting in a bend at the point of insertion. We wish to present further evidence in support of this mode of interaction of

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‡ Professor Edmond J. Gabbay died unexpectedly during the completion of this work. He was a bright scientist and a good friend, who will be sorely missed. It is for these reasons that this report is dedicated to his memory.

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