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Characterization of the pH-Dependent Resonance Raman Transitions of Archaeal and Bacterial Rieske [2Fe−2S] Proteins

Toshio Iwasaki,*,† Asako Kounosu,† Derrick R. J. Kolling,‡ Antony R. Crofts,‡ Sergei A. Dikanov,§ Akihisa Jin,|| Takeo Imai,|| and Akio Urushiyama||

Department of Biochemistry and Molecular Biology, Nippon Medical School, Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan, Departments of Biochemistry and Veterinary Clinical Medicine, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, and Department of Chemistry, Rikkyo (St. Paul's) University, Toshima-ku, Tokyo 171-8501, Japan

Received December 27, 2003; E-mail: tiwasaki@nms.ac.jp

Proteins containing Rieske-type [2Fe−2S] clusters play important roles in many biological electron-transfer reactions such as aerobic respiration, photosynthesis, and biodegradation of various alkene and aromatic compounds.[1−4] The variation of the redox potential (E_m) of the cluster, and its pH dependence, reflects the presence of two different types of proteins with distinct biological electron-transfer functions: The high-potential Rieske proteins in cytochrome bc_1/b_6f complexes of the aerobic respiratory chain and photosynthesis have a pH-dependent $E_{m,acid\ pH}$ of ∼150−490 mV, and the low-potential proteins in a broad group of bacterial multicomponent terminal oxygenases and soluble Rieske-type ferredoxins have pH-independent potentials of ∼−150 to −50 mV up to ∼pH 10.[1−13] The redox-linked ionization of the high-potential Rieske center is relevant to its physiological function and has been characterized by circular dichroism (CD), electron paramagnetic resonance (EPR), and cyclic voltammetry.[7,8,14,15] It has been proposed that the pH dependence of the cluster E_m is associated with protonation of the two coordinate histidine imidazole ligands, although actual experimental evidence for this proposal is very weak, and it has been assumed that no structural change of the immediate cluster environment occurs even above pH 12.[7,8] A role for the histidine ligands in the pH dependence of E_m was based on the resonance Raman (RR) spectral transition of the 266−274-cm^{-1} band between pH 7.3 and 10.1, which had tentatively been assigned to the putative Fe−N_{imid} stretching vibrations of *Thermus thermophilus* high-potential Rieske protein,[16,17] but this was *inconsistent* with recent ^{15}N-labeling studies on archaeal and bacterial high- and low-potential proteins.[18] Our normal mode calculation suggests that this band has a dominant contribution from complicated deformational displacements in the polypeptide backbone involving some amide groups that extend beyond the liganding residues (at least by two residues).

Although the thermodynamic properties of several Rieske-type proteins have been well-characterized using protein film voltammetry,[7,8] the absence of solid spectroscopic data has precluded a deeper understanding of the redox-linked ionization of the Rieske protein family on a structure−function basis. We report here a comparative study of the pH-dependent RR spectral changes of three Rieske proteins with different redox properties: the high-potential Rieske cluster-binding domain solubilized by proteolytic cleavage from the hydrophobic N-terminal tail (ISP) of *Rhodobacter sphaeroides* cytochrome bc_1 complex ($E_{m,acid\ pH}$ = +308 mV; p$K_{a,ox1,2}$ = 7.6 ± 0.1 and 9.6 ± 0.1, respectively);[8,19] the high-potential, archaeal sulredoxin (SDX) from *Sulfolobus tokodaii* strain 7 ($E_{m,acid\ pH}$ = +188 mV; p$K_{a,ox1,2}$ of the visible CD transition = 8.4 ± 0.2 and ∼12, respectively (Figure S1))[20−22] with weak homology to the regular cytochrome bc_1-associated Rieske proteins (DDBJ accession number, AB023295); and the low-potential, archaeal Rieske-type ferredoxin (ARF) from *Sulfolobus solfataricus* strain P-1 ($E_{m,7}$ = −62 mV) with the canonical cluster-binding motif for the low-potential Rieske-type ferredoxins (DDBJ accession number, AB047031).[18b,23]

The RR spectra at 77 K of the oxidized [2Fe−2S] clusters in bacterial ISP (**A**−**C**), archaeal SDX (**D**−**F**), and ARF (**G**−**I**) at acidic to neutral pH are similar, showing at least eight bands in the 250−450 cm^{-1} region (Figure 1). These features suggest lower symmetry around the clusters than that around the $[2Fe-2S]^{2+}$ clusters of other enzymes with complete cysteinyl ligation, which typically exhibit six or seven bands under similar conditions.[16,18,24] The major variations among them are relative band positions and intensities in the 250−380 cm^{-1} region, where Fe−$S^{t(/b)}$ stretching vibrations are extensively coupled to deformations of the polypeptide backbone involving exchangeable amide groups N−H(D) that extend beyond the cluster ligands.[18b] They reflect the differences in relative (S^b−)Fe−S^t bond strengths, which are primarily defined by extensively coupling to the surrounding protein, in the following order: ARF > SDX > ISP.

The larger separation of two p$K_{a,ox}$ values for SDX (Figure S1) than for ISP[8,19] and the similar RR transitions for both proteins (**A**−**F**) demonstrate that (i) the ionizable group with p$K_{a,ox1}$ in the archaeal and bacterial high-potential Rieske proteins influences the visible CD transition but *not* RR bands in the 250−450 cm^{-1} region, indicating negligible conformational rearrangement of the immediate cluster environment around p$K_{a,ox1}$, and (ii) the large RR changes were detected at alkaline conditions near and above p$K_{a,ox2}$ for the two high-potential Rieske proteins. The changes of some vibrational modes in the 250−340 cm^{-1} region, including the 5-cm^{-1} upshift of the ∼270-cm^{-1} band as previously reported for *T. thermophilus* high-potential Rieske protein,[16,17] provide direct spectroscopic evidence for local conformational changes of the polypeptide backbone near the high-potential Rieske clusters at pH near and above p$K_{a,ox2}$. Moreover, the marked changes of vibrational modes in the 390−440 cm^{-1} region, in which contributions of Fe−S^b stretching modes become dominant in the Rieske protein system,[18] indicate some modification of the geometry of the [2Fe−2S] cluster core.

For the low-potential ARF, the visible CD changes occurred transiently around pH 11 and eventually led to irreversible, complete breakdown of the cluster around pH 12 (Figure S1). The RR spectra of ARF did not change significantly at least up to pH ∼10 (**G**,**H**). The cluster slowly broke down irreversibly above pH 10.9, and

† Nippon Medical School.
‡ Department of Biochemistry, University of Illinois at Urbana-Champaign.
§ Department of Veterinary Clinical Medicine, University of Illinois at Urbana-Champaign.
|| Rikkyo (St. Paul's) University.

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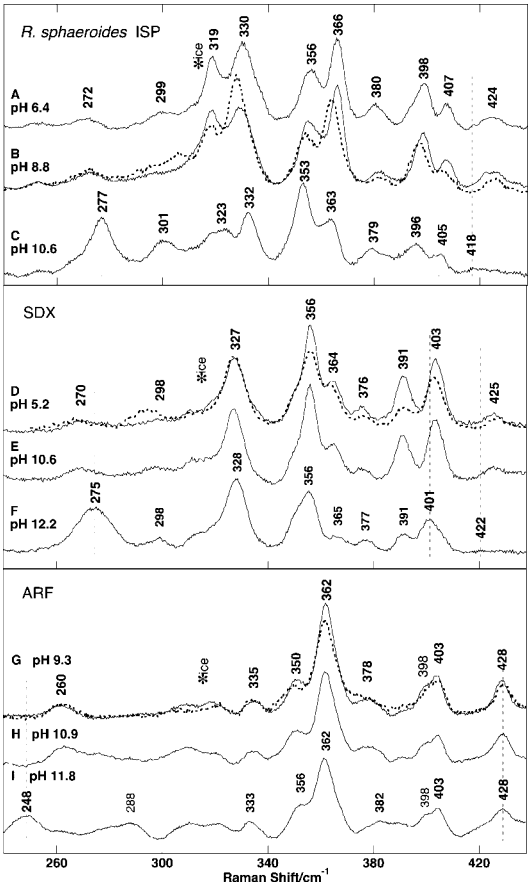

Figure 1. RR spectra of the bacterial ISP (**A**−**C**), archaeal SDX (**D**−**F**), and ARF (**G**−**I**) at different pH. The spectra of samples prepared in a D_2O buffer (dashed line) are also indicated in the figure. All spectra were recorded at 77 K using a Spex 750M Raman spectrometer (the slit width, 80 μm) fitted with a Spectrum-One CCD camera and a Spectra-Physics 2017 Ar^+ laser (488.0-nm excitation; output, 500 mW) by collecting 45° backscattering off the surface of a frozen sample. A multiscan signal-averaging technique was employed.

the RR spectra of ARF rapidly frozen at the initial point of the breakdown process at pH 11.8 showed substantial shifts of the vibrational modes in the 250−330 cm^{-1} region,[18c] but smaller changes in the 390−440 cm^{-1} region (**I**). Thus, in the initial stages of cluster degradation at alkaline pH, the geometry of the [2Fe−2S] cluster core is maintained, but the immediate environment undergoes conformational changes due to deprotonation of the polypeptide backbone amide groups.

In conclusion, contrary to previous reports,[16,17,21] deprotonation of the ionizable group with p$K_{a,ox1}$ (likely the histidine N_ϵ with a higher degree of conformational freedom) around physiological pH does not promote RR-detectable structural changes in the immediate cluster environment of the high-potential Rieske proteins (Figure 1). The absence of such a change may facilitate rapid proton−electron transfer of the Rieske center at the quinol-oxidizing Q_o site in cytochrome bc_1/b_6f complex due to a small structural reorganization energy. The RR changes of the high-potential proteins are in fact detected at pH *near and above* the p$K_{a,ox2}$. The resultant stable alkaline intermediate has the modified cluster environment due to deprotonation of some exchangeable amide groups in the polypeptide backbone, rather than simple changes of the Fe−N_{imid} stretching vibrations.[18c] In the low-potential ARF, this intermediate was not clearly observed because the cluster environment is rapidly destabilized upon deprotonation of many residues in the polypeptide backbone around pH 11−12, resulting in irreversible breakdown of the [2Fe−2S] cluster core (Figure S1).[18c]

The observed differences between the high- and low-potential Rieske proteins are probably attributable to the covalent stabilization of two cluster-binding loops by a conserved disulfide linkage in the high-potential proteins,[1−4] which is absent in ARF.[18b,23] Because the RR spectral changes (Figure 1) have frequently been invoked to explain the redox-linked ionization of the high-potential Rieske proteins,[12,17] interpretation of other data, e.g., the pH-dependent Mössbauer parameters,[25] is clearly warranted in the future studies.

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Supporting Information Available: Visible CD spectra, sample preparations for *R. sphaeroides* ISP, *S. tokodaii* SDX, and *S. solfataricus* ARF, and ref 18c (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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