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^{1}H NMR studies of paramagnetic ferricytochrome *c*-551 from *Pseudomonas aeruginosa* at high pH: The role of histidine 16 in the spin transition

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Abstract

Cytochrome *c*-551 from the mesophile *Pseudomonas aeruginosa* is an electronic transfer protein that contains 82 amino-acid residues and a *c*-type heme as the prosthetic group with low spin Fe(II) in the reduced form and low spin Fe(III) in the oxidized form of cytochrome *c*-551. We have studied the electronic properties of ferricytochrome *c*-551 from *P. aeruginosa* at high pH (9–11.4) by means of paramagnetic ^{1}H NMR spectra and the T_1 and T_2 values of isotropically shifted proton resonances. We have also analyzed the temperature dependence of the hyperfine-shifts. Resonance assignment of some signals was based on 2D saturation transfer experiments, EXSY. These results indicate the existence of high-spin iron(III) cytochrome *c*-551 ($S = 5/2$, $^6A_1$) with penta or hexa-coordinated symmetry at high pH. A spin transition from low-spin iron(III) to high-spin iron(III) has been produced. This transition can be associated with the deprotonation of the axially coordinated amino-acid to iron, His-16. We also discuss the spin crossover between different ferricytochromes *c*.

Keywords: Paramagnetic NMR; Metalloprotein; Ferricytochrome *c*-551; *Pseudomonas aeruginosa*; Iron proteins; Spin transition

1. Introduction

Cytochromes are a group of electron transfer proteins present in a variety of prokaryotic and eukaryotic organisms [1,2]. Cytochrome *c*-551 (cyt *c*-551) from the mesophile *Pseudomonas aeruginosa* (*Pae*) participates in the denitrification process as an electronic donor to the nitrite reductase enzyme responsible for the reduction of nitrite. This electron transfer protein has a molecular weight of 8.7 kDa and contains a single polypeptide chain of 82 amino-acid residues. The function of cyt *c*-551 from *P. aeruginosa* is similar to the mitochondrial cytochrome c implicated in the respiratory chains of eukaryotic organisms [3].

In addition, the crystal structure of cyt *c*-551 from *Pae* was determined in 1982 at 1.6 Å resolution on reduced and oxidized forms of the protein [4]. The solution structure of ferrocyt *c*-551 from *Pae* has been determined from ^{1}H NMR data with an average RMSD of 1.33 Å [5]. Ferricyt *c*-551 contains a low spin Fe(III) ion and is equatorially bound to a protoporphyrin IX ring, Fig. 1A, and axially to the Nε2 nitrogen of His-16 and to the thioether sulfur of Met-61 amino-acid residues [4], Fig. 1(B). Previous reports have described the pH dependent redox potential associated with deprotonation of propionic acid number 17 of the

Abbreviations: NMR, nuclear magnetic resonance; 1D, one-dimensional; 2D, two-dimensional; NOE, nuclear Overhauser effect; NOESY, NOE spectroscopy; EXSY, exchange spectroscopy; cyt *c*, cytochrome *c*; PAGE, polyacrylamide gel electrophoresis; *Pae*, *Pseudomonas aeruginosa*.

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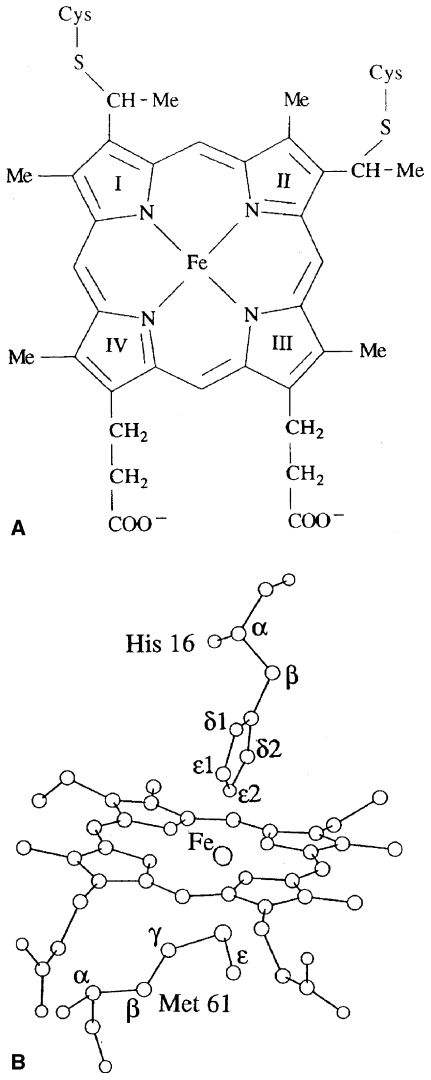

Fig. 1. The heme group of cytochrome *c*-551 from *Pae.* (A) Protoporphyrin IX ring. The nomenclature utilized for a protoporphyrin IX ring is of the IUPAC-IUB. (B) Spatial relationships of the heme group and the axial ligands, His-16 and Met-61.

protoporphyrin IX ring [6–8]. The pK_a value obtained is 6.2 for the oxidized form of the protein [6]. Additionally, data from EPR and electronic spectroscopy studies at pH >9.4 demonstrate the existence of a high spin species brought about by replacement of the iron ligand from the Met-61 thioether sulfur by other weak ligands, probably OH^- [9,10]. At higher pH values (pH >11) the existence of low-spin ferricyt *c*-551 has been proposed [9].

In the last two decades, paramagnetic ^{1}H NMR spectroscopy has been applied to different metalloproteins studies [11–13]. In particular information about coordination characteristics as well as the electronic properties for active sites in solution, by the study of isotropically shifted resonances and relaxation times, can be obtained. A number of previous reports have studied the assignment of heme protons on the oxidized form of the protein by means of heteronuclear multiple quantum coherence [14,15] or 2D ^{1}H NMR paramagnetic spectroscopy [16,17]. The present article reports on further research of the electronic properties and coordination geometry of ferricyt *c*-551 from *Pae* at high pH by using ^{1}H NMR of paramagnetic systems.

2. Experimental

2.1. Materials

All compounds used were analytical grade chemicals and purchased from Sigma and Merck. DEAE-Sephacel and CM-Sephadex (Pharmacia) were used for ion exchange chromatography. Sephadex-G100 (Pharmacia) was used for size-exclusion chromatography. D_2O (99.9%) was obtained from SDS Chemical. The protein was concentrated by using Amicon Model 8200 ultrafiltration cells equipped with PLBC 062 Millipore membranes and an ultrafree-15 centrifugal filter device from Millipore. Sample concentrations for ^{1}H NMR were 3–4 mM in protein dissolved in D_2O or in 95% H_2O/5% D_2O. Because the cyt *c*-551 sample is not totally in the oxidized form, an excess of potassium ferricyanide was added to the sample before the NMR experiments. The measurements were carried out with 20 mM potassium phosphate buffer at various pH values.

2.2. Bacterial growth and protein purification

Cytochrome *c*-551 was extracted from *P. aeruginosa* (CECT110) grown anaerobically at 35 °C in the medium described by Parr et al. [18] and purified in the partially oxidized state as described earlier [18,19]. The Bradford colorimetric assay [20] was used to determine the total amount of protein in the sample. Purified cyt *c*-551 shows a single band in PAGE and an UV–Vis spectral ratio in the reduced form of the protein $(A_{551} - A_{570})/A_{280}$ of 1.13–1.17 [21]. The cyt *c*-551 concentration was measured spectrophotometrically by means of the molar extinction coefficient for the reduced form of the protein $\varepsilon_{551} = 28.3 \times 10^3\ M^{-1}\ cm^{-1}$ [21].

2.3. Physical techniques

The optical spectra in the UV–Vis region were recorded on a Varian Cary 1 or a Shimadzu UV-2401PC recording spectrophotometer using 1 cm path length cuvettes. pH values of solutions were measured with a Crison micropH2000 pH meter provided with a Crison microelectrode. The pD measurements were corrected by the isotope effect.

NMR spectra were recorded on a Varian Unity 400 spectrometer operating at 400 MHz. One-dimensional spectra were recorded in H_2O or D_2O solvents using