Functional characterization of PccH, a key cytochrome for electron transfer from electrodes to the bacterium *Geobacter sulfurreducens*

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**ARTICLE INFO**

Article history:
Received 17 April 2013
Revised 27 June 2013
Accepted 1 July 2013
Available online 11 July 2013

Edited by Miguel De la Rosa

**ABSTRACT**

The cytochrome PccH from *Geobacter sulfurreducens* (Gs) plays a crucial role in current-consuming fumarate-reducing biofilms. Deletion of *pccH* gene inhibited completely electron transfer from electrodes toward Gs cells. The *pccH* gene was cloned and the protein heterologously expressed in *Escherichia coli*. Complementary biophysical techniques including CD, UV–visible and NMR spectroscopy were used to characterize PccH. This cytochrome contains one low-spin c-type heme with His–Met axial coordination and unusual low-reduction potential. This reduction potential is pH-dependent, within the Gs physiological pH range, and is discussed within the context of the electron transfer mechanisms from electrodes to Gs cells.

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**1. Introduction**

Electron transfer towards extracellular terminal acceptors is one the most remarkable features of the bacterium *Geobacter sulfurreducens* (Gs), by which it can reduce toxic or radioactive metals and convert renewable biomass into electricity [1]. These capabilities opened a potential window for *Geobacter*-based applications in bioenergy production and bioremediation [1,2]. Examples of such applications include degradation of hydrocarbon contaminants in soils, reduction of insoluble Fe(III) and Mn(IV) oxides, precipitation of uranium in contaminated aquifers and electron transfer from electrodes to Gs cells. In this process, the reducing power of an electrode, maintained at a sufficient negative electrically conductive pili [4]. More recently, it was discovered that Gs cells may be different than current-consuming biofilms [5]. In this process, the reducing power provided by an electrode, maintained at a sufficient negative electrochemical potential, can be used by the cells to synthesize valuable organic compounds, thus opening new perspectives in the field of bioremediation and biofuel production [6,7]. To develop these applications, understanding the mechanisms by which microorganisms can accept electrons from electrodes is essential. However, these mechanisms are still poorly understood. To shed light on this, Strycharz and co-workers [8] used microarray analysis to compare gene transcript abundance in current-consuming versus current-producing Gs biofilms. In the first case, Gs cells mediate the transfer of electrons from a graphite cathode poised at −293 mV to the terminal electron acceptor fumarate (30 mV). In the second case, a graphite anode poised at 507 mV was used as extracellular electron acceptor for the oxidation of acetate (−280 mV) (the redox potentials refer to the standard hydrogen electrode, SHE). The results showed that genes encoding for outer membrane cytochromes (e.g. OmcZ) or PilA that are essential for efficient current-producing cells, had a much lower abundance in current-consuming biofilms [8]. On the contrary, a putative periplasmic c-type cytochrome encoded by gene GSU3274 (hereafter designated *pccH*) showed clearly the largest transcript abundance [8]. Deletion of gene *pccH* completely inhibited electron transfer from electrodes, but had no influence on electron transfer to electrodes [8]. Overall, this study suggested that the routes for electron transfer from electrodes to Gs cells may be different than the ones for current production. Compared to *pccH*, the other genes encoding redox-active proteins showing higher transcript abundance in current-consuming cells were by far less abundant and their cellular localization could not be predicted with confidence.

**Abbreviation:** PccH, *Geobacter sulfurreducens* cytochrome encoded by gene GSU3274

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Apparently, none of these other genes encoded outer membrane redox-active proteins. Therefore, to date only cytochrome PccH was unequivocally identified as crucial for Gs cells to be able to accept electrons from electrodes [8]. In the present work, we undergo for the first time a biochemical characterization of cytochrome PccH, which was proposed to serve as an intermediary in electron transfer between the outer cell surface and the inner membrane on Gs cells.

2. Materials and methods

2.1. Amino acid sequence analysis and DNA manipulation

Genomic DNA from Gs was provided by Prof. D.R. Lovley (University of Massachusetts, Amherst). Sequence data for Gs cytochrome pccH gene (GenBank accession number AAR36664) were obtained from Kyoto Encyclopedia of Genes and Genomes Web site, under the accession number T00155. Cytochrome PccH was predicted to be located on the bacteria’s periplasm [8,9]. However, there was no consensus in the prediction of the signal peptide cleavage site of the protein by several bioinformatics tools. SignalP 4.1 server [10] predicts no signal peptide cleavage site; Signal-3L [12] after residue 19 and PrediSi (PREDiction of SIgnal peptides) (http://www.predisi.de/home.html) and PSORT Prediction [http://psort.ims.u-tokyo.ac.jp/form.html] predict the signal peptide cleavage site at residue 21. Thus, in order to not exclude any possibility, the pccH gene was cloned including the region containing all putative signal peptide cleavage sites. Primers were purchased from Invitrogen, restriction enzymes and T4 DNA ligase from Fermentas. All PCR products were purified using Wizard PCR Purification System (Promega). Digested vector and plasmids were purified using E-gel Electrophoresis System (Invitrogen) and NZYMInprep kit (NZYTech), respectively. Phusion High-Fidelity DNA polymerase (Finnzymes) was used for amplification from genomic DNA and Taq DNA polymerase (VWR) for colony PCR. Escherichia coli DH5α cells were used during DNA manipulation.

2.2. Cloning of cytochrome PccH gene, site-directed mutagenesis, production and purification of recombinant proteins

The vector used for cloning was pVA203, which is a derivative of the plasmid pkIVLen004 containing the lac promoter, the OmpA leader sequence and the gene for mature triheme cytochrome PpcC from Gs [13,14]. The resulting plasmid was designated pCS3274. The cloning procedure is described in Appendix A (Supplementary methods). To identify the putative methionine axially coordinated to the heme, each methionine residue in the sequence of cytochrome PccH (M46, M48, M67 and M84) was independently replaced by alanine. Therefore, four PccH mutants were produced: PccHM46A, PccHM48A, PccHM67A and PccHM84A, using the NZY-Mutagenesis kit NZYTech and PccH expression vector pCS3274 as a template. Oligonucleotides were designed by the QuikChange Primer Design program (Agilent Technologies). The presence of desired mutations was confirmed by DNA sequencing in both strands. Proteins were expressed, produced and purified as described in Appendix A (Supplementary methods).

2.3. UV–visible analysis, quantification and extinction coefficient of PccH

UV–visible absorption spectra for all fractions obtained in the chromatographic steps were acquired, at room temperature, on an UV–visible scanning spectrophotometer Ultraspec 2100pro (Amersham Biosciences, Switzerland) with quartz cuvettes with 1 cm path length (Helma). Fully reduction of samples was achieved by adding sodium dithionite (Sigma) in small increments from a 2 M stock. Protein concentration was determined by measuring the absorbance of the reduced PccH α-band at 552 nm, using the extinction coefficient of 32.5 mM⁻¹ cm⁻¹ determined in the present work. The quantification and extinction coefficient determination of PccH are described in Appendix B (Supplementary methods).

2.4. Molecular mass determination and heme quantification

The theoretical molecular mass of cytochrome PccH was calculated according to the amino acid composition of the mature protein (http://ca.expasy.org/tools/pi_tool.html) plus the molecular mass of one heme c group [15]. The experimental molecular mass determination and heme quantification of PccH are described in Appendix C (Supplementary methods).

2.5. Circular dichroism spectroscopy

Circular dichroism (CD) spectra of PccH (0.2 mg/mL prepared in 20 mM NaCl at pH 7) were recorded at 25 °C in the far UV region (200–260 nm) using a JASCO J-810 spectropolarimeter with a Pel-tier-thermostated cell support using a 0.1 cm path–length cell quartz. The conformational stability of PccH was assessed by performing temperature denaturation, monitored by far-UV CD at 220 nm, which reports on the stability of the secondary structural elements. For thermal-induced denaturation, a heating rate of 3.0 °C min⁻¹ was used, and temperature was increased from 25 °C to 95 °C. The fraction of unfolded protein (fU) was monitored by CD spectroscopy and calculated according to the expression \( f_U = \frac{(\theta_U - \theta_0)(\theta_U - \theta_0)}{(\theta_U - \theta_0)} \), with \( \theta_U \) corresponding to the ellipticity at 220 nm of the protein in the native folded state, \( \theta \) the ellipticity at a defined temperature, and \( \theta_0 \) the ellipticity at 220 nm of the completely unfolded state.

3. NMR studies

3.1. Sample preparation

Cytochrome PccH samples with ~140 μM for 1D NMR studies and 2 mM for 2D NMR studies were prepared in 45 mM phosphate buffer with NaCl (100 mM final ionic strength) in 92%H₂O/8%H₂O or in 2H₂O (99.96% atom). Reduction of the samples was achieved by first flushing out the air from the oxidized sample with argon and then by adding sodium dithionite in small aliquots from a degassed 2 M stock solution to the NMR tube with a gas-tight syringe through the rubber septum. NMR spectra were obtained before the addition of sodium dithionite and after its removal by ultrafiltration methods (Amicon Ultra) to confirm that the protein integrity was not affected.

3.2. NMR spectroscopy

All NMR spectra were recorded at 25 °C on a Bruker Avance 600 MHz spectrometer equipped with triple-resonance cryoprope. For 1D 1H NMR spectra a total of 64 K data points were collected to cover a sweep width of 42 kHz. The following set of 2D NMR experiments was acquired: fully oxidized protein: 2D 1H–13C-HSQC; 2D 1H–TOCSY (45 ms mixing-time) and 2D 1H–NOESY (80 ms); sample with a mixture of reduced and oxidized protein: 2D 1H–EXSY (25 ms). 2D NMR spectra were acquired with a sweep with of 26 kHz in 1H dimension and 45 kHz in 13C dimension. 1H chemical shifts are reported in parts per million (ppm) calibrated using the
water signal as internal reference and the $^{13}$C chemical shifts calibrated through indirect referencing [16]. Spectra were processed using TOPSPIN (Bruker Biospin, Karlsruhe, Germany).

3.3. Redox titrations followed by visible spectroscopy and determination of reduction potentials

Redox titrations of PccH were followed by visible spectroscopy at 25 °C inside an anaerobic glove box (MBrAun) kept at <1 ppm oxygen, as described previously [17]. Samples with 30 μM protein concentration were prepared in the pH range 4–9 using sodium acetate, sodium phosphate and Tris–HCl buffer solution adjusted with NaCl to a final ionic strength of 100 mM. The detailed description of the methodology used is described in Appendix D (Supplementary methods).

4. Results and discussion

4.1. Production of cytochrome PccH

The gene pccH encodes for a periplasmic monoheme c-type cytochrome with 150 amino acids, including a signal peptide composed by 21 residues and one typical heme c-binding motif (Fig. 1). The pI/Mw tool program on the ExPASy Server (http://web.expasy.org/compute_pi/) predicted, from the amino acid sequence of PccH. The far-UV CD spectrum of this cytochrome is indicated in Fig. 2A. In the native state, the spectrum is typical of a folded protein with high α-helix content, featuring intense negative bands at

![Fig. 1](image)

**Fig. 1.** (A) SDS–PAGE analysis of pure cytochrome PccH (lane 1) stained with Coomassie blue. Lane M corresponds to the molecular weight markers. The numbers on the left refer to molecular weight in kDa. (B) Amino acid sequence of PccH from *G. sulfurreducens*. The signal peptide and mature protein residues are boxed green and blue, respectively. The heme binding motif is highlighted (black). (C) Alignment of PccH mature sequence with putative cytochrome c family proteins: *Pp*, *Pelobacter propionicus*; *Ts*, *Thioalkalivibrio nitratireducens*; *Ts*, *Thioalkalivibrio sulfidophilus*; *Lc*, *Leptothrix cholodnii*; *Rf*, *Rhodofex ferrireducens*; *Pp*, *Polaromonas* sp. The numbers refer to the gene that encodes each cytochrome. The conserved residues in the proteins are boxed: heme attached residues (blue) and non-heme attached residues (light blue).

4.2. Analysis of the cytochrome PccH amino acid sequence

Using the amino acid sequence of the mature PccH, we searched the non-redundant amino acid data base of NCBI using the basic local alignment search tool (BLAST) [18]. This cytochrome shows the highest homology with putative monoheme cytochrome c family proteins isolated from the following bacteria: *Pelobacter propionicus* (70% sequence identity), *Thioalkalivibrio nitratireducens* (54%), *Thioalkalivibrio sulfidophilus* (51%), *Leptothrix cholodnii* (51%), *Rhodofex ferrireducens* (48%), and *Polaromonas* sp. (47%). The sequence alignment of these proteins is depicted in Fig. 1C. For all cytochromes, the heme binding motif CXXCH is placed at the N-terminus region, a typical feature of cytochrome c class I members [19]. To the best of our knowledge, there are no biochemical data for any of these cytochromes.

4.3. Spectroscopic characterization of cytochrome PccH

CD and NMR spectroscopy were used to probe the folding of PccH. The far-UV CD spectrum of this cytochrome is indicated in Fig. 2A. In the native state, the spectrum is typical of a folded protein with high α-helix content, featuring intense negative bands at

![Fig. 2](image)
208 nm and 220 nm (Fig. 2A, solid line). An additional negative band at 233 nm in the CD spectrum is attributed to aromatic residues [20], for which the five tryptophan residues of PccH (Fig. 1) contribute the most. To monitor the thermal stability of the protein we also carried out a characterization of the temperature-induced unfolding followed by far-UV CD spectroscopy (Fig. 2B). Increasing the temperature resulted in a progressive $\alpha$-helix to random coil transition with loss of the folded spectral features (Fig. 2A, dotted line). The value of midpoint thermal unfolding ($T_m$) was $78 \pm 1 \, ^\circ C$ (Fig. 2B). At the end of the thermal denaturation a notable protein precipitation was observed, suggesting the occurrence of non-reversible modifications at high temperature. The proper folding of PccH was also corroborated by the well-dispersed and narrow signals observed in the 1D $^1H$ NMR spectra of the protein in the reduced and oxidized states (Fig. 3). The line widths of the NMR signals ($<10$ Hz and $<60$ Hz in the reduced and oxidized states, respectively) are typical of a monomeric protein in solution [21].

We then proceed to the identification of the heme spin-state and respective axial ligands by means of UV–visible and NMR spectroscopy. NMR is a very powerful technique to identify the spin-state of the heme groups and their axial ligands. The spectral regions are quite distinct for cytochromes containing high- or low-spin hemes. In the oxidized state, the 1D $^1H$ NMR spectra of high-spin cytochromes shows extremely broad signals above 40 ppm, which typically correspond to the heme methyl substituents (see inset (A) in Fig. 3). In contrast, in low-spin cytochromes, the methyl signals are mainly found in the region 8–35 ppm. Similarly, in the reduced form, 1D $^1H$ NMR spectra in both cases are also quite distinct [22]. The reduced NMR spectra of high-spin cytochromes show wider spectral regions (typically from $-15$ up to $30$ ppm) compared to low-spin ones (typically from $-5$ to $11$ ppm). In the case of PccH, the 1D $^1H$ NMR signals cover the regions $-3$ to $30$ ppm and $-5$ to $12$ ppm in the oxidized and reduced forms, respectively and, hence, are typical of a low-spin cytochrome (Fig. 3). Thus, from the NMR studies it can be concluded that PccH is diamagnetic when reduced (Fe(II), $S = 0$) and paramagnetic when oxidized (Fe(III), $S = 1/2$).

The reduced 1D $^1H$ NMR spectrum of a low-spin cytochrome is also very helpful in identification of the heme axial ligands, particularly in the case of a methionine residue. The typical pattern of heme axial methionine signals includes a three-proton intensity peak at approximately $-3$ ppm, and up to four resolved one-proton intensity peaks in the low-frequency region of the spectrum [19]. Such pattern is clearly observable in the reduced spectrum of cytochrome PccH indicating that the heme group is axially coordinated by a methionine (see upper spectrum in Fig. 3). The side chain signals of the heme axial methionine are also expected to be observed in the upfield region of the oxidized 1D $^1H$ NMR spectrum. However, due to their proximity to the unpaired electron of the heme iron, the signals are extremely broad to be detected. The only exception is for the three-proton intensity $\varepsilon CH_3$ group, which despite its broadness is still observable, in the oxidized spectrum in the region $-9$ ppm to $-25$ ppm, as reported in the Biological Magnetic Resonance Data Bank (http://www.bmrb.wisc.edu/) for c-type cytochromes with His–Met axial coordination. Therefore, the signal with three-proton intensity at $-11.48$ ppm in the oxidized spectrum of PccH can be tentatively assigned to axial methionine $\varepsilon CH_3$ group (see bottom spectrum in Fig. 3). To further confirm this, a 2D $^1H$ EXSY NMR spectrum was acquired for a partially oxidized PccH sample. In this spectrum, a connectivity between the $\varepsilon CH_3$ proton signal at $-3.06$ ppm, with proton signal at $-11.48$ ppm further confirms that the latter can be assigned to an axial methionine $\varepsilon CH_3$ group (Fig. S1). On the other hand, the signals corresponding to the aliphatic $\alpha CH$ and $\beta CH_2$ groups of an axial histidine are typically shifted to low-field region in the oxidized spectra. The combined used of 2D $^1H$–$^{13}C$ HSQC, 2D $^1H$–$^2H$TOCSY and 2D $^1H$–$^15N$-NOESY NMR spectra acquired in the oxidized form of several mono- and multiheme c-type cytochromes are reported in the literature and established typical regions for heme methyl, heme propionate and axial His $\beta CH_2$ and $\alpha CH$ groups in the 2D $^1H$–$^{13}C$ HSQC (for a review see [23]). Therefore, in the present work this methodology was used to assign the heme axial histidine aliphatic protons of PccH (Fig. S2) and further confirm that the heme group of PccH has His–Met axial coordination.

The heme spin-state and heme axial ligand features observed for cytochrome PccH were additionally corroborated by UV–visible spectroscopy. The optical absorption spectrum of PccH has maxima at 525 nm, 411 nm, 352 nm and 280 nm (Fig. 4). Upon reduction
the protein shows the Soret, β and α bands at 417 nm, 523 nm and 552 nm, respectively (Fig. 4). This spectral pattern is typical of low-spin hexacoordinated hemes [19]. The band at 695 nm observed in the UV–visible spectrum (see inset in Fig. 4) is indicative of His–Met axial coordination [19] in the oxidized form. The molar extinction coefficients of these peaks are summarized in Table 1.

4.4. Specific assignment of PccH heme axial ligands

Cytochromes c can bind one or several c-type hemes through two thioether bonds involving the sulphydryl groups of two cysteine residues present in the CXXCH signature for heme attachment. The heme iron ion is always axially coordinated by the histidine side chain present in this motif (for a review see [24]). Therefore, in PccH the histidine present in the heme binding motif (H21 – see Fig. 1) is the natural candidate for the proximal ligand of the heme. On the other hand, up to four methionine residues (M46, M48, M67 and M84) can bind the PccH heme group at the distal position. In order to uniquely identify the sixth ligand of PccH, each methionine was replaced by alanine by site-directed mutagenesis. The 1D 1H NMR spectra acquired in both oxidized and reduced PccH mutants were compared to those obtained for the wild-type cytochrome. With exception of mutant PccHM84A, in all other mutants the typical signal of the methionine εCH₃ proton is clearly observed (see insets (B) and (C) in Fig. 3) showing that M84 binds axially to PccH heme group. Therefore, the heme binding motif CXXCH is placed at the N-terminus region and the sixth ligand, provided by a methionine residue, about 40 residues further on towards the C-terminus, which constitute a typical feature of class I cytochromes [19].

4.5. Redox titrations of cytochrome PccH

Redox titrations of PccH followed by visible spectroscopy were performed in the physiological pH range for Gs growth: pH 6, 7 (not shown) and 8 (Fig. 5). No hysteresis was observed, as the reductive and oxidative curves are superimposable, indicating that the redox process is fully reversible. The reduction potential values obtained were +1 mV (pH 6), −24 mV (pH 7) and −35 mV (pH 8) versus SHE. The decrease in the reduction potential values with pH leads to a progressive stabilization of the oxidized form. The influence of the pH on the heme reduction potential (redox-Bohr effect) can be explained in a pure electrostatic basis since the progressive deprotonation of an acid/base group in the vicinity of the heme
is expected to lower its affinity for electrons with the concomitant decrease of the reduction potential values. Also, on a pure electrostatic basis, the pK_{red} value of the protonatable group responsible for the redox-Bohr effect is expected to be higher compared to its value in the fully oxidized protein (pK_{ox}). The pH dependence of the reduction potential in the pH range 4 to 9 was used to determine the pK_{red} (6.6) and pK_{ox} (5.0) values of PccH (Fig. S3). As mentioned, the redox-Bohr effect usually relates with deprotonation/protonation events of a protonatable group in the vicinity of the heme. This was confirmed by the changes observed on the 1H chemical shift of heme methyls with pH (Fig. S4). The observed redox-Bohr effect in the pH range 5 to 8, indicates that electron transfer is thermodynamically coupled to proton transfer in the physiological pH range for Gs growth and might be functionally relevant.

Compared to other class I cytochromes, which have reduction potential values typically in the range +200 to +350 mV [25], the reduction potential of PccH is considerably lower. Previous studies carried out on cyanide derivatives of horseradish, cytochrome c, lignin and manganese peroxidases showed that the strength of the Fe–N2 bond, between the heme iron and the nitrogen atom of the axial histidine, correlates with the lowering of the heme reduction potentials (for a review see [26]). Therefore, a shorter Fe–N2 distance, with the concomitant reinforcement of the interaction, could be one of the factors contributing to the low reduction potential of PccH. Such particular electronic properties of the heme iron are most probably at the origin of the different pattern of 1H chemical shift for axial methionine and heme methyl resonances compared to other class I cytochromes (Fig. 3). In addition to the nature of the axial ligands, other factors such as the heme solvent exposure or electrostatic interactions with residues in the neighborhood of the heme have been shown to also modulate the reduction potential [27]. Therefore, the structural determinants responsible for the low-reduction potential of PccH cannot be further addressed in detail in the absence of a structural model. Nonetheless, it is striking to note that the available data on other c-type cytochromes from Gs containing His–Met axially coordinated hemes, also display unusual low-reduction potential values [28,29], a feature that allow these proteins to be redox active in the typical negative working potential ranges observed for Gs cells [30].

### 4.6. Implications

The discovery that Gs cells can accept electrons directly from electrodes for the reduction of terminal electron acceptors has prompted the investigation on the exploration of this feature to a wide-range of applications. This work reports the first biochemical characterization of the cytochrome PccH, a key protein in this process. PccH contains one c-type heme with His–Met axial coordination, which were identified as H21 and M84.

The cytochrome PccH was previously suggested to function as an intermediary in electron transfer between the outer cell surface and the inner membrane. Gene knockout studies revealed that the absence of PccH inhibited the capacity of Gs cells to accept electrons from electrodes. Also, the microarray analysis revealed that pccH was the gene showing the highest transcript abundance when Gs cells use a graphite cathode (poised at −293 mV) as the sole electron donor to reduce fumarate (+30 mV versus SHE) to succinate [8]. The thermodynamic studies performed in the present work are relevant for the understanding of the electron transfer mechanism from graphite cathodes to Gs cells. The reduction potential values of PccH determined in the physiological pH range for Gs growth (~35 to +1 mV) show that this periplasmic cytochrome is thermodynamically suitable to bridge the electron transfer from biocathodes to more electro-positive electron acceptors, such as fumarate. Since outer membrane cytochromes are abun-

### Table 1

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<tr>
<th>Peaks in the electronic absorption spectra (nm)</th>
<th>Molar extinction coefficient (mM⁻¹ cm⁻¹)</th>
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<td>Oxidized</td>
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<tr>
<td>Soret</td>
<td>411</td>
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<tr>
<td>Others</td>
<td>280</td>
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<tr>
<td>Reduced</td>
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<td>Soret</td>
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<tr>
<td>β-band</td>
<td>523</td>
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Fig. 4. UV–visible spectra of the fully oxidized (solid line) and reduced (dashed line) forms of PccH. A more concentrated PccH sample was used to probe the region of 695 nm band (see inset), which is indicative of an axial methionine coordinated to the ferric heme.

Fig. 5. Redox titrations followed by visible spectroscopy for PccH at pH 8 (circles) and pH 6 (squares). The open and filled symbols represent the data points in oxidative and reductive titrations, respectively. The continuous lines indicate the results of the fits to the Nernst curves for one-electron reduction with −35 ± 5 mV (pH 8) and +1 ± 5 mV (pH 6). The reduction potentials are relative to SHE. As an example, the inset illustrates the α-band region of the visible spectra acquired in the redox titration at pH 8.
dant in Gs cells, it is conceivable that a yet unknown cell-surface-associated redox component accepts electrons from the biocathode, which are then transferred to more electro-positive electron acceptors within the periplasm, such as Pch, to cytoplasmic-associated redox components and, finally, to the terminal electron acceptor fumarate. The observation that the current-consuming capacity of Gs cells increased over time with the repeated addition of fumarate, suggests that energy might be conserved to support growth from direct electron transfer from electrodes [31]. The redox-Bohr effect observed in the pH range 6 to 8 indicates that Pch can also couple proton to electron transfer in the Gs physiological pH range and may be involved in these energy-conserving mechanisms.

Acknowledgments

We thank Prof. D.R. Lovley for providing genomic DNA from \textit{Geobacter sulfurreducens}, Dr. Douglas V. Laurents for support in the CD data collection and Prof. Teresa Catarino for helpful discussions. We also thank the anonymous referees for their valuable comments and their constructive suggestions. We acknowledge the MALDI-TOF-MS Service of the REQUIMTE, Chemistry Department, UNL. This work was supported by project grant PTDC/QUI/ 70182/2006 (to CAS) and the strategic grant PEst-C/EBB/LA0006/ 2011 (to REQUIMTE Laboratório Associado) from Fundação para a Ciência e a Tecnologia (FCT), Portugal. J.M.D. is recipient of grant SFRH/BD/89701/2012 from FCT, Portugal.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2013.07.003.

References


