Biochimica et Biophysica Acta, 1016 (1990) 364–370
Elsevier

BBABIO 43189

Soluble cytochromes and a photoactive yellow protein isolated from the moderately halophilic purple phototrophic bacterium, *Rhodospirillum salexigens*

T.E. Meyer, J.C. Fitch, R.G. Bartsch, G. Tollin and M.A. Cusanovich

Department of Biochemistry, University of Arizona, Tucson, AZ (U.S.A.)

(Received 16 October 1989)

Key words: Cytochrome *c*; Yellow protein; Phototrophic bacterium; Electron transfer protein; (*R.* salexigens)

Three soluble cytochromes were found in two strains of the halophilic non-sulfur purple bacterium *Rhodospirillum salexigens*. These are cytochromes c_2, c' and *c*-551. Cytochrome c_2 was recognized by the presence of positive charge at the site of electron transfer (measured by laser flash photolysis), although the protein has an overall negative charge (p*I* = 4.7). Cytochrome c_2 has a high redox potential (300 mV) and is monomeric (13 kDa). Cytochrome c' was recognized from its characteristic absorption spectrum. It has a redox potential of 95 mV, an isoelectric point of 4.3, and is isolated as a dimer (33 kDa) of identical subunits (14 kDa), a property which is typical of this family of proteins. *R. salexigens* cytochrome *c*-551 has an absorption spectrum similar to the low redox potential *Rb. sphaeroides* cytochrome *c*-551.5. It also has a low redox potential (−170 mV), is very acidic (p*I* = 4.5), and is monomeric (9 kDa), apparently containing 1 heme per protein. The existence of abundant membrane-bound cytochromes *c*-558 and *c*-551 which are approximately half reduced by ascorbate and completely reduced by dithionite suggests the presence of a tetraheme reaction center cytochrome in *R. salexigens*, although reaction centers purified in a previous study (Wacker et al., Biochim. Biophys. Acta (1988) 933, 299–305) did not contain a cytochrome. The most interesting observation is that *R. salexigens* contains a photoactive yellow protein (PYP), previously observed only in the extremely halophilic purple sulfur bacterium *Ectothiorhodospira halophila*. The *R. salexigens* PYP appears to be slightly larger than that of *Ec. halophila* (16 kDa vs. 14 kDa). Otherwise, these two yellow proteins have similar absorption spectra, chromatographic properties and kinetics of photobleaching and recovery.

Introduction

Electron-transfer proteins have been well-characterized in fresh-water and marine species of purple phototrophic bacteria [1–4], but relatively little is known of halophiles. Soluble electron-transfer proteins in *Ectothiorhodospira halophila* [5] were reported to be similar to those of non-halophiles, but they generally have lower redox potentials and are very acidic. In addition to the electron-transfer proteins, a novel water-soluble photoactive yellow protein (PYP) was found in *Ec. halophila* [5–8]. There is an unresolved question as to whether the halophiles are more closely related to one another than to fresh-water and marine species, or whether they independently evolved ways to survive in high salinity environments. If so, what are the common features of adaptation to a halophilic environment? To investigate these questions further, we examined *Rhodospirillum salexigens*, which is a moderately halophilic non-sulfur purple bacterium, isolated from evaporating ponds of seawater along the coast of Oregon [9]. It requires a minimum of 5% NaCl for growth (the optimum is 6–8%) and it grows slowly at 20% [10]. We now find that the electron-transfer proteins are more like those of non-halophiles than they are to those of *Ec. halophila*, although *R. salexigens* is now the second halophilic species shown to produce photoactive yellow protein (PYP).

Methods

R. salexigens type strain WS68 (gift of Dr. J. Imhoff) and '*Ec. mobilis*' strain Yc6.1 (gift of Dr. H. Biebl, isolated from Solar Lake, Sinai, salt optimum 4–6%) were grown on a medium containing (per liter) 1 g yeast extract, 1 g sodium acetate, 1 g sodium malate, 1 g sodium glutamate, 60 g NaCl, 0.8 g $MgCl_2 \cdot 6H_2O$, 0.1 g $CaCl_2 \cdot 2H_2O$, 2 g KH_2PO_4, 0.5 g $(NH_4)_2SO_4$, and 1 ml Hutners trace elements, all of which was adjusted to pH

Abbreviations: FPLC, fast performance liquid chromatography; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; PYP, photoactive yellow protein.

Correspondence: T.E. Meyer, Department of Biochemistry, University of Arizona, Tucson, AZ 85721, U.S.A.

6.9. Cell yield was typically 3.6 g/l wet weight after 3 days phototrophic growth.

1 kg of cells were suspended in 3 vol. of 0.1 M Tris-HCl buffer (pH 7.5) and broken at 20000 lb/inch^2 in the Ribi Cell Fractionator (an automated French Press). Broken cells were centrifuged in the Beckman Spinco Type 45 Ti rotor for 4 h, the extract was desalted on Sephadex G-25, it was made 1 mM in Tris-HCl, adjusted to pH 7.5, and absorbed to a 7 × 11 cm column of DEAE-cellulose (Whatman DE-52). Chromatography is described in the Results section.

The redox potentials of cytochromes c_2 and c' were measured in 50 mM phosphate buffer (pH 7.0) using a spectroelectrochemical cell with ferricyanide and Fe-EDTA as mediators and methyl viologen and ferricyanide as reductant and oxidant. The potential of cytochrome *c*-551 was calculated based on the relative state of reduction of cytochrome and FMN in a photochemical titration using 20 mM phosphate (pH 7) buffer and 10 mM EDTA as the electron donor. The redox potential of FMN was assumed to be −205 mV [11].

The native molecular weight was measured using a Pharmacia FPLC system with a Bio-Rad BioSil TSK 125 column. Elution buffer contained 150 mM phosphate and 150 mM NaCl (pH 7.0). Elution peaks were monitored with a Hewlett-Packard Diode Array Spectrophotometer at two wavelengths, 280 nm and at the visible maximum. Protein was determined by the Pierce BCA protein assay method using bovine serum albumin as standard [12]. Laser flash photolysis of cytochrome c_2 and PYP was performed as described by Simondsen and Tollin [13] and Meyer et al. [6,14]. Subunit sizes were measured by SDS-PAGE using the Pharmacia Phast System, with 20% crosslinked gels, stained with Coomassie Blue R-250. The Bio-Rad low-molecular-weight standard kit, containing phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme, was used. Isoelectric points were also measured with the Phast system using Pharmacia IEF pH 4–6.5 gels. The Pharmacia pH 3–10 broad calibration kit was used as standard (containing in part: soybean trypsin inhibitor, β-lactoglobulin, bovine and human carbonic anhydrase B).

Results

The DEAE-cellulose column, which contained protein adsorbed from the bacterial extract as described in the Methods section, was developed with increasing increments of buffer up to 20 mM Tris-HCl, and then with additional 20 mM increments of NaCl up to 200 mM NaCl, and finally with 0.1 M increments of NaCl up to 0.5 M. Approx. 27 μmol of a high redox potential (judged by its presence in the completely reduced form) cytochrome *c*-552 was eluted at 60–120 mM NaCl for strain WS68. About 40 μmol cytochrome c' (judged by its characteristic absorption spectrum) was eluted at 160–300 mM NaCl. In a parallel experiment, about 20 μmol high-potential cytochrome *c*-552 and 25 μmol cytochrome c' were found in strain YC6.1. Other colored proteins were not apparent at this stage of purification because they were mixed with the principal components and present at about one-fifth their level.

The high redox potential cytochrome *c*-552 fractions from DEAE-cellulose were pooled, desalted, concentrated on a small DEAE-cellulose column, eluted with 0.5 M NaCl, and chromatographed on Sephadex G-75 (Pharmacia). Pooled cytochrome fractions from this column were then rechromatographed on DEAE-cellulose using a linear gradient of 0–150 mM NaCl in 20 mM Tris-HCl (pH 7.5). The high-potential cytochrome *c*-552 was eluted at 65 mM NaCl and was followed off the column by a low-potential cytochrome *c*-551 at 90 mM NaCl. The pooled high-potential cytochrome *c*-552 fraction was oxidized with ferricyanide and rechromatographed on DEAE-cellulose, where more of the low-potential cytochrome *c*-551 was separated. The combined amount of low-potential cytochrome *c*-551 from strain WS68 was about 4 μmol. A small amount of high-potential cytochrome *c*-552 (1 μmol) eluted at 110 mM NaCl (this minor component appears to be identical to the main component in all respects except DEAE-cellulose chromatography, and may be deamidated or otherwise denatured). It was not further characterized. The final purification step of the main high-potential cytochrome fraction was precipitation by ammonium sulfate (70–80% saturation). The pure cytochrome *c*-552 was recovered in an overall yield of about 65% and the best ratio of 280 nm to 416 nm absorbance was 0.19. Purification of the high-potential cytochrome *c*-552 from strain YC6.1 was similar, but it required 80–120 mM NaCl to elute it from DEAE-cellulose in the oxidized and reduced forms, respectively, which suggests that it may be a little more acidic. The isoelectric point of WS68 cytochrome *c*-552 is 4.75. The absorption spectra of the oxidized and reduced forms of cytochrome *c*-552 are shown in Fig. 1. The native size of the high-potential cytochrome *c*-552 is about 13 kDa. The subunit size is also 13 kDa. The redox potential of WS68 *c*-552 is 300 mV. Because these properties are characteristic of a number of high-potential cytochromes of various types, we measured the kinetics of reduction by FMN semiquinone, generated by laser flash photolysis, as a function of ionic strength [14], with the expectation that this technique would allow us to distinguish the particular type of cytochrome *c*. All known cytochromes c_2 have a positive charge at the site of reduction, even if the protein has an overall negative charge, whereas other types of bacterial cytochrome have an active site charge which is the same sign as the net protein charge [14]. It was found that the rate

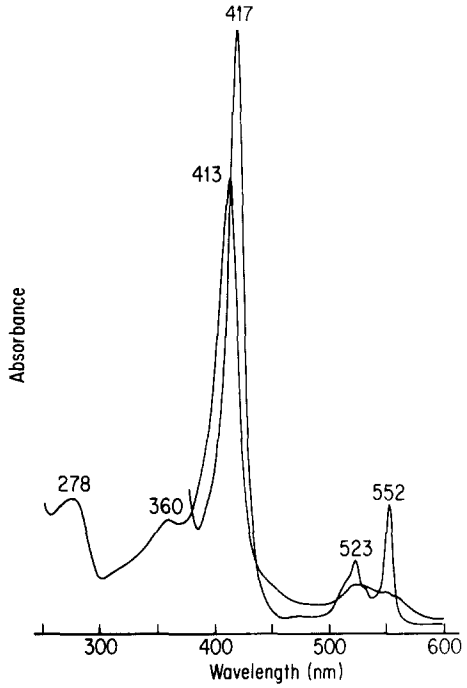

Fig. 1. Oxidized and reduced (with dithionite) ultraviolet-visible absorption spectra of *R. salexigens* cytochrome *c*-552.

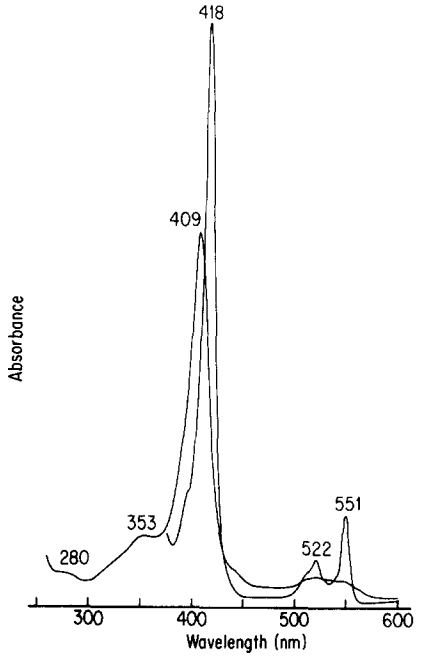

Fig. 3. Oxidized and reduced (with dithionite) ultraviolet-visible absorption spectra of *R. salexigens* cytochrome *c*-551.

constant for reduction of the *R. salexigens* cytochrome *c*-552 decreased with increasing ionic strength as shown in Fig. 2, indicative of a plus-minus charge interaction with FMN semiquinone. The magnitude of the charge at the WS68 cytochrome *c*-552 active site is estimated to be +1.0. Thus, the high-potential cytochrome *c*-552 from *R. salexigens* may be tentatively identified as a cytochrome c_2 because it has an active site charge opposite in sign to that of the whole protein, consistent with a concentration of positive charges near the edge of the heme such as has been observed in all other cytochromes c_2 [14].

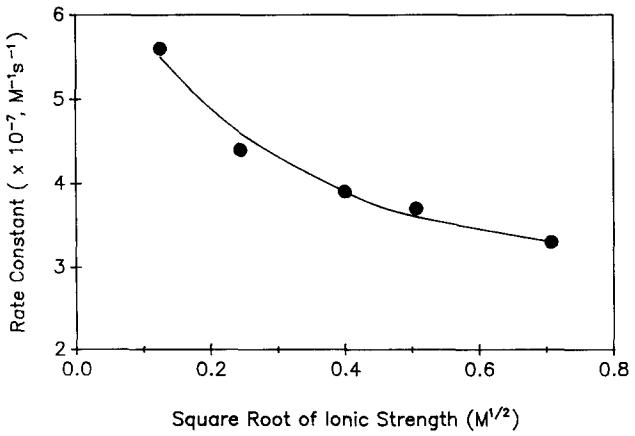

Fig. 2. Kinetics of reduction of cytochrome *c*-552 by FMN semiquinone. Solutions contained 1–10 mM EDTA and 5–222 mM phosphate (pH 7.0) plus about 70 μM FMN. The sample was illuminated with a pulse of light from a dye laser at 450 nm and kinetics were monitored at 575 nm as described by Meyer et al. [14] and Simondsen and Tollin [13]. Protein concentration was varied from 10 to 60 μM.

The low redox potential cytochrome *c*-551 from strain YC6.1 was purified by DEAE-Sepharose (Pharmacia) chromatography (0–100 mM NaCL gradient), hydroxyapatite chromatography (0–100 mM phosphate gradient, elution at 30 mM phosphate), and by reverse ammonium sulfate gradient chromatography (35%–0) on Phenyl-Sepharose (Pharmacia). Pure cytochrome *c*-551 was recovered in an overall yield of about 25%. The best ratio of 280 nm to 409 nm absorbance was 0.10. The absorption spectra of the oxidized and reduced forms are shown in Fig. 3. The native size measured by FPLC appears to be 6.5 kDa, but the subunit size measured by SDS-PAGE appears to be 9 kDa. We assume that the anomalously small native size is due to a hydrophobic interaction with the TSK column (which we have previously observed with a few other proteins, unpublished observations). The redox potential is −170 mV, and the isoelectric point is 4.5. There is apparently one heme per cytochrome (9.0 mg protein per μmol heme).

The pooled cytochrome *c′* fractions from the first DEAE-cellulose column of strain WS68 were concentrated and then chromatographed on Sephadex G-75. The cytochrome *c′* separated from a smaller yellow protein, which appeared to be similar to a photoactive protein isolated from *Ec. halophila* (Ref. 5; see below). The cytochrome *c′* was next precipitated by 70–80% saturation ammonium sulfate and finally chromatographed on DEAE-cellulose using a linear gradient of 100–200 mM NaCl in 20 mM Tris-HCl (pH 7.5). The cytochrome *c′* eluted at about 170 mM NaCl. The pure

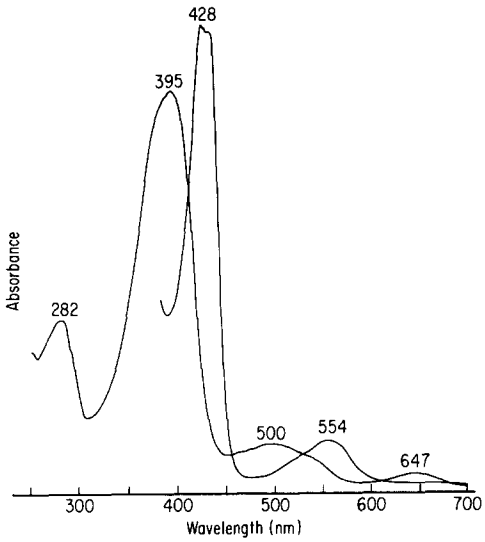

Fig. 4. Oxidized and reduced (with dithionite) ultraviolet-visible absorption spectra of *R. salexigens* cytochrome *c'*.

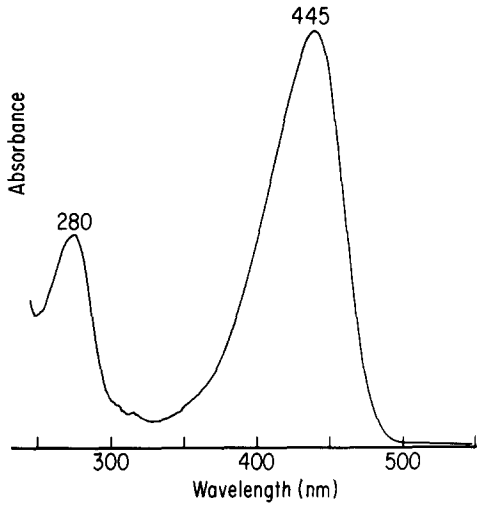

Fig. 5. Ultraviolet-visible absorption spectrum of *R. salexigens* photoactive yellow protein.

protein had a ratio of 280 nm to 400 nm absorbance of 0.44. It was recovered in an overall yield of approx. 45%. The absorption spectra of the oxidized and reduced forms are shown in Fig. 4. The native size is 33 kDa and the subunit is 14 kDa, which indicates that it is a dimer as isolated, like the majority of known type II cytochromes [15]. The redox potential is 95 mV and the isoelectric point is 4.3.

The yellow protein which was partially separated from cytochrome *c'* on Sephadex G-75 was adsorbed to hydroxyapatite in 100 mM NaCl and the column developed using a linear gradient of 0–100 mM phosphate plus a constant 100 mM NaCl (pH 7). The yellow protein and a small amount of low-potential cytochrome *c*-551 were eluted at the beginning of the gradient (which in retrospect should have been repeated with a lower final phosphate concentration). Residual cytochrome *c'* was left on the column. The yellow protein was made 30% saturated in ammonium sulfate and adsorbed to Phenyl-Sepharose. The column was developed with a 30% to 0% saturation ammonium sulfate reverse linear gradient. The yellow protein eluted at about 20% ammonium sulfate, before residual unidentified cytochrome and colorless proteins. About 1 μmol was recovered in approx. 25% yield (assuming the same extinction coefficient as *Ec. halophila* yellow protein, which is 45 $mM^{-1} \cdot cm^{-1}$ at 445 nm [6]). The best ratio of 280 to 445 nm absorbance for the yellow protein was 0.47. The absorption spectrum is shown in Fig. 5. The native size of the yellow protein is about 16 kDa. When *Ec. halophila* and *R. salexigens* yellow proteins were run side by side on SDS-PAGE, the latter appeared to be slightly larger (16 kDa vs. 14 kDa). The yellow protein was found to be photoactive when excited by a pulse of laser light at 445 nm, as shown in Fig. 6. After an initial bleach that was faster than the time resolution of the apparatus (about 1 μs), the visible peak bleached further with a rate constant of $1.2 \cdot 10^4$ s^{-1} and completely recovered with a rate constant of 4.7 s^{-1}. These kinetic properties are virtually identical to those of PYP from *Ec. halophila* [6]. However, *R. salexigens* PYP which had been dialyzed vs. water could be bleached by room light and the now biphasic recovery was at least two orders of magnitude slower ($4 \cdot 10^{-2}$ to $7 \cdot 10^{-3}$ s^{-1}). Protein denatured by dialysis could not be renatured by addition of salt. N-terminal sequence analysis of denatured PYP (Van Beeumen, J., personal communication) indicated several end-groups and suggests that proteolysis may have been the reason for alteration of kinetics. *R. salexigens* PYP is thus, to our knowledge, the first 'halophilic' protein to be characterized in phototrophic bacteria.

Photosynthetic membranes of strain YC6.1, isolated by high-speed centrifugation of broken cells, were resus-

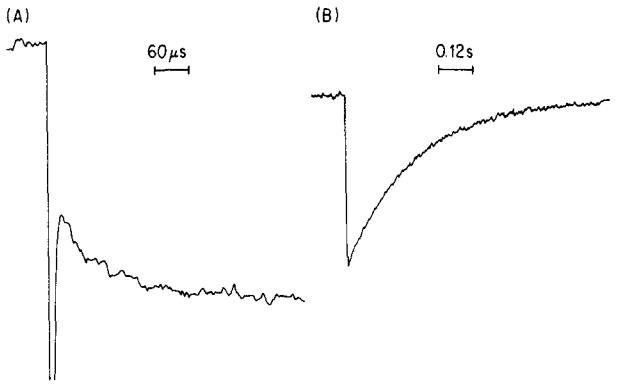

Fig. 6. Kinetics of laser-induced photobleach and recovery of *R. salexigens* yellow protein excited at and monitored at 450 nm [6,7] using the apparatus described by Simondsen and Tollin [13]. (A) BLeach of the yellow protein at 450 nm on a 0.5 ms time-scale (average of two flashes). The initial negative spike is a laser artifact. (B) Recovery measured on a 1 s scale.

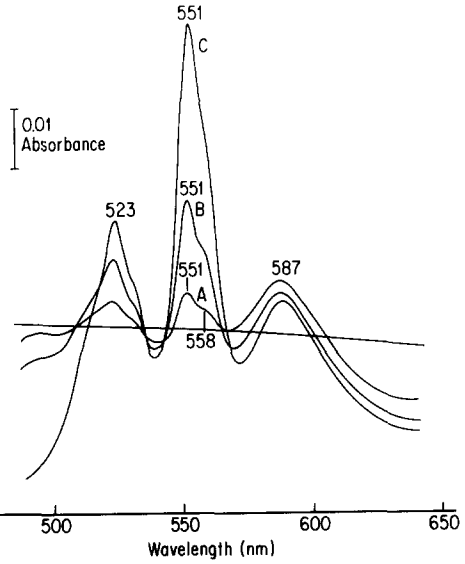

Fig. 7. Membrane difference absorption spectra for *R. salexigens*. (A) As is minus ferricyanide; (B) ascorbate minus ferricyanide; (C) dithionite minus ferricyanide. Spectra were recorded in 0.1 M phosphate (pH 7). All spectra were arbitrarily adjusted to zero absorbance at about 541 and 567 nm. The peak at 587 nm is due to changes in bacteriochlorophyll absorbance.

pended by homogenization in 0.1 M phosphate buffer (pH 7) and difference spectra recorded on an Aminco DW2 spectrophotometer as shown in Fig. 7. There was a relatively large amount of *c*-type cytochrome in the membrane, which was comparable to what we have observed previously with bacteria which contain the tetraheme reaction center cytochrome. Approx. 10% of the heme was present in the reduced form and an additional 35% was reduced in the presence of ascorbate, whereas the remainder was reduced only by dithionite. This indicates that approximately half the heme has a high redox potential (greater than 0 mV). The alpha peak maximum was at 551 nm and there was a shoulder at 558 nm. In the dithionite-minus-ascorbate difference spectrum (not shown), the maximum was still at 551 nm , but the shoulder was much less pronounced. This indicates that the lower-potential hemes absorb at shorter wavelength than the high-potential hemes, which is very similar to what is observed with *Chromatium vinosum* [16], *Rhodopseudomonas viridis* [17], *Thiocapsa pfennigii* [18], and *Rhodocyclus gelatinosus* [19] reaction center cytochrome *c*. The Soret maximum in the dithionite-minus-ascorbate difference spectrum of *R. salexigens* (not shown) is at 425 nm.

Discussion

Three soluble cytochromes were found in *R. salexigens*. The high-potential cytochrome *c*-552 was tentatively identified as a cytochrome c_2 because it has positive charge at the site of reduction, although the protein has a net negative charge overall. Asymmetric charge distribution is characteristic of the cytochromes c_2 which all have positive charge near the edge of the heme which is partially exposed to solvent [14]. The identification as a cytochrome c_2 must be verified by amino-acid sequence determination. Nevertheless, the presence of both cytochromes c_2 and c' in *R. salexigens* suggests a closer relationship to the freshwater bacteria *R. rubrum, Rb. capsulatus,* or *R. molischianum* than to *Ec. halophila*, the only other photosynthetic halophile examined to date. The soluble redox proteins present in *Ec. halophila* are HiPIP, cytochrome c' and a cytochrome *c*-551, which although it is a type I protein, is not a c_2 [5]. Type I cytochromes *c* are those which are homologous to mitochondrial cytochrome *c*.

It was previously noted that electron-transfer proteins from *Ec. halophila* were more acidic and the redox potentials were generally lower than those from freshwater bacteria [5]. The cytochromes from *R. salexigens* are also very acidic, although the redox potentials are more similar to those of non-halophiles. Specifically, *R. salexigens* cytochrome c_2 has a redox potential of 300 mV, whereas the average of 17 cytochromes c_2 is 343 ± 48 mV with extremes of 250 and 470 mV. The redox potential of *R. salexigens* cytochrome c' is 95 mV, whereas the average for 15 cytochromes c' is 46 ± 30 mV. The extremes are 0 and 150 mV. This suggests either that the redox potentials of proteins from halophiles may not be lower than those of non-halophiles, or that this is only a property of the extreme halophile, *Ec. halophila,* which has a salt optimum of 23%, versus *R. salexigens,* which has a 7% optimum.

Very low redox potential cytochromes such as the cytochrome *c*-551 found in *R. salexigens* are only occasionally observed in purple bacteria, such as *Rhodopseudomonas palustris* [20], *Rhodospirillum rubrum* [21], *Chr. vinosum* [22] and *Rb. sphaeroides* [3], and are usually minor components. There are no published amino-acid sequences, and therefore it is difficult to relate the *R. salexigens* cytochrome *c*-551 to the others, or even to say whether there is a single class of low-potential cytochromes in phototrophic bacteria. *Rb. sphaeroides* cytochrome *c*-551.5 has 2 hemes in a 16 kDa protein, which is proteolytically degraded on storage to an 8 kDa form without apparent alteration of spectral or redox properties [3]. *R. salexigens* cytochrome *c*-551 has 1 heme per 9 kDa protein which, if closely related to *Rb. sphaeroides*, may have been completely split in half by proteolysis during purification.

The membrane difference absorption spectra we measured for *R. salexigens* suggest the presence of a tetraheme reaction center cytochrome *c* based on the following evidence. Approximately half the heme has a high potential and the other half low. The wavelength maximum of the low potential heme is at shorter wave-

length than the high-potential heme. The large amount of *c*-type cytochrome in the membrane masks the absorbance due to any expected cytochrome bc_1 complex. These characteristics have previously been observed for the more thoroughly characterized tetraheme reaction center cytochromes from *Rps. viridis* [17,23], *Chr. vinosum* [16] *Rc. gelatinosus* [19,24] and *Cf. aurantiacus* [25,26]. Purified reaction centers from *R. salexigens* do not contain a cytochrome *c* [27], but then the cytochrome does not copurify with the reaction center in *Rc. gelatinosus* [24] or in *Cf. aurantiacus* [25, either.

The photoactive yellow protein from *R. salexigens* is interesting because it is only the second protein of this type to be characterized. The *R. salexigens* yellow protein has approximately the same size, the same chromatographic properties, and the same absorption spectrum as PYP from *Ec. halophila* [5]. In addition, they both have the same kinetics of photobleach and recovery [6]. A difference is that the *Ec. halophila* PYP is extremely stable, whereas the *R. salexigens* PYP is apparently denatured at low ionic strength. The denatured protein has several end-groups (Van Beeumen, J., personal communication), which suggests that PYP was degraded by proteolysis. We have no data for undenatured protein and cannot say whether there may have been a single end group or whether proteolysis may have occurred during purification or after dialysis. Nevertheless, we conclude that PYP is a 'halophilic' protein which is stable only at high ionic strength. The only other species known to have halophilic proteins which are unstable at low ionic strength is *H. halobium* [28].

We have observed proteins having absorption spectra similar to PYP in other species, but the one such protein which we have purified (from *Rps. marina*) is not photoactive, it has a larger molecular weight and is virtually all helical (unpublished observation). It is possible that the photoactive protein is characteristic only of halophilic phototrophs, but additional species will have to be examined to establish such a correlation.

How closely is *R. salexigens* related to *Ec. halophila*? As stated above, these are the only two species known to produce the photoactive yellow protein. However, only a small number of moderate and extreme halophiles have been characterized, so it is difficult to generalize. One might consider the possibility of culture contamination as the source of PYP in *R. salexigens.* However, *R. salexigens* and *Ec. halophila* have very different salt optima for growth (7% vs. 23%) and they utilize different sources of carbon (organics vs. CO_2), respectively. These considerations completely rule out culture contamination by *Ec. halophila* as the source of PYP in *R. salexigens.* In addition to the metabolic differences, *R. salexigens* and *Ec. halophila* differ in electron-transfer protein content. Cytochromes c_2 and c', which we have characterized in *R. salexigens*, are commonly found in non-sulfur purple bacteria such as *Rb. sphaeroides* [3], *R. rubrum* and *R. molischianum* [1]. *Ec. halophila,* on the other hand, has an electron-transfer protein complement more like other purple sulfur bacteria such as *Chr. vinosum.* HiPIP, cytochrome c' and a cytochrome *c*-551 not closely related to cytochrome c_2 are abundant in *Ec. halophila* [5]. The only similarities between *R. salexigens* and *Ec. halophila* besides PYP are cytochrome c' and a possible tetraheme reaction center cytochrome [29]. Thus, we conclude that these two species are more closely related to other purple bacteria than they are to one another. How, then, did *R. salexigens* acquire the PYP? The best explanation is gene transfer, in which case we expect the sequences of the PYPs to be a lot closer to one another than are the cytochromes c' from the same two species. Another explanation would be that PYP occurs at a low level in most purple bacteria, but is only expressed at detectable levels in the halophiles. In this scenario we expect the sequences of PYP's to be approximately as divergent as the cytochromes c'. These possibilities are now being tested both by sequence determination and by screening purple bacteria for cross-reaction with antibodies against PYP.

The function of PYP is unknown, but is expected to be related to its photoactivity. We have found, for example, that when *Ec. halophila* PYP is bleached by light, there is a conformational change, which exposes a hydrophobic site to solvent [7]. Thus our current hypothesis is that PYP may be a blue-light photoreceptor for negative phototaxis and may bind to a membrane transducer only in the bleached form, causing the bacteria to swim away from potentially damaging ultraviolet light. The three-dimensional structure of *Ec. halophila* PYP has been determined [8] and the gene is being cloned for eventual deletion to test for its effect on phototaxis.

Strain Yc6.1 (Bonn 9906) was originally thought to be a strain of *Ectothiorhodospira mobilis.* In fact, Stackebrandt et al. [30] found that the ribosomal RNA was similar to that of an *Ec. mobilis* strain (not the type strain). However, our results clearly show that strain Yc6.1 is very similar in electron-transfer protein content to *R. salexigens* type strain WS68 and unlike *Ec. shaposhnikovii* [31,32], which has also been reported to be similar to *Ec. mobilis* in rRNA sequence [30,33]. The electron-transfer proteins of *Ec. shaposhnikovii* are most like those of *Ec. halophila.* We cannot directly compare the rRNA sequence results because different strains were used in each study, but our results suggest that the work should be repeated using the same strains. Perhaps in the future more effort should be made to include type strains of each species examined. In addition, the amino-acid sequences of the *R. salexigens* cytochromes and yellow protein have to be determined in order to confirm our results showing that strains YC6.1 and WS68 are closely related. We do not exclude the possi-

bility that other bacteria currently thought to be *Ec. mobilis* may be strains of *R. salexigens* or vice versa.

Acknowledgement

This work was supported by grants from the NSF, DMB-8718678 (T.E.M.), and the NIH, DK15057 (G.T.).

References

1 Bartsch, R. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 249–279, Plenum Press, New York.
2 Meyer, T.E. and Kamen, M.D. (1982) Adv. Protein Chem. 35, 105–212.
3 Meyer, T.E. and Cusanovich, M.A. (1985) Biochim. Biophys. Acta 807, 308–319.
4 Henseler, A., Truper, H.G. and Fischer, U. (1986) FEMS Microbiol. Lett. 33, 1–8.
5 Meyer, T.E. (1985) Biochim. Biophys. Acta 806, 175–183.
6 Meyer, T.E. and Yakali, E., Cusanovich, M.A. and Tollin, G. (1987) Biochemistry 26, 418–423.
7 Meyer, T.E., Tollin, G., Hazzard, J.H. and Cusanovich, M.A. (1989) Biophys. J. 56, 559–564.
8 McRee, D.E., Tainer, J.A., Meyer, T.E., Van Beeumen, J., Cusanovich, M.A. and Getzoff, E.D. (1989) Proc. Natl. Acad. Sci. USA 86, 6533–6537.
9 Drews, G. (1981) Arch. Microbiol. 130, 325–327.
10 Golecki, J.R. and Drews, G. (1980) Eur. J. Cell Biol. 22, 654–660.
11 Draper, R.D. and Ingaham, L.L. (1968) Arch. Biochem. Biophys. 125, 802–808.
12 Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. and Klenk, D.C. (1985) Anal. Biochem. 150, 76–85.
13 Simondsen, R. and Tollin, G. (1983) Biochemistry 22, 3008–3016.
14 Meyer, T.E., Watkins, J.A., Przysiecki, C.T., Tollin, G. and Cusanovich, M.A. (1984) Biochemistry 23, 4761–4767.
15 Cusanovich, M.A. (1971) Biochim. Biophys. Acta 236, 238–241.
16 Kennel, S.J. and Kamen, M.D. (1971) Biochim. Biophys. Acta 253, 153–166.
17 Trosper, T.L., Benson, D.L. and Thornber, J.P. (1977) Biochim. Biophys. Acta 460, 318–330.
18 Seftor, R.E.B. and Thornber, J.P. (1984) Biochim. Biophys. Acta 764, 148–159.
19 Fukushima, A., Matsuura, K., Shimada, K. and Satoh, T. (1988) Biochim. Biophys. Acta 933, 399–405.
20 Meyer, T.E., Bartsch, R.G. and Kamen, M.D. (1971) Biochim. Biophys. Acta 245, 453–464.
21 Yoch, D.C., Carithers, R.P. and Arnon, D.I. (1978) J. Bacteriol. 136, 1018–1026.
22 Gray, G.O., Gaul, D.F. and Knaff, D.B. (1983) Arch. Biochem. Biophys. 222, 76–86.
23 Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1985) Nature 318, 618–624.
24 Clayton, B.J. and Clayton, R.K. (1978) Biochim. Biophys. Acta 501, 470–477.
25 Freeman, J.C. and Blankenship, R.E. (1989) Photosynth. Res., in press.
26 Meyer, T.E., Tollin, G., Cusanovich, M.A., Freeman, J.C. and Blankenship, R.E. (1989) Arch. Biochem. Biophys. 272, 254–261.
27 Wacker, T., Gadón, N., Steck, K., Welte, W. and Drews, G. (1988) Biochim. Biophys. Acta 933, 299–305.
28 Kerscher, L. and Oesterhelt, D. (1981) Eur. J. Biochem. 116, 587–594.
29 Lefebre, S., Picorel, R., Cloutier, Y. and Gingras, G. (1984) Biochemistry 23, 5279–5288.
30 Stackebrandt, E., Fowler, V.J., Schubert, W. and Imhoff, J. (1984) Arch. Mikrobiol. 137, 366–370.
31 Kusche, W.H. and Truper, H.G. (1984) Arch. Mikrobiol. 137, 266–271.
32 Kusche, W.H. and Truper, H.G. (1984) Z. Naturforsch. 39c, 894–901.
33 Ivanova, T.L., Turova, T.P. and Antanova, A.S. (1985) Arch. Mikrobiol. 143, 154–156.