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The distribution of soluble metallo-redox proteins in purple phototrophic bacteria

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A comparison is made of types and distribution of cytochromes and certain ferredoxins (HiPIP) among photosynthetic bacteria. These are subdivided as to the type of reaction center each species is believed to contain. The proteins listed are assumed to be of periplasmic origin. Interrelationships suggested by the comparison are discussed.

The comparative approach to a biochemical topic first requires identification of the type and variety of objects to be compared. Here is compiled a list of soluble heme or iron-containing proteins isolated and, in the main, highly purified from phototrophic purple bacteria. The list is not comprehensive; it largely reflects observations made in the laboratories of Drs. Kamen and Cusanovich. Nevertheless the list is large enough to suggest a number of investigations into the nature and function of these proteins. The proteins are probably all derived from the periplasm of these Gram-negative organisms and therefore most likely engage as mobile redox carriers between immobile transmembrane electron transfer complexes [1,2] or as reactants in extracytoplasmic substrate oxidation [3]. However, the possible location in the periplasm and the functional role have been described for relatively few of these proteins.

It has proven remarkably difficult to devise specific functional tests for these proteins. As an alternative, the more abundant proteins have been exploited as interesting variations on the cytochrome theme and subjected to varied physico-chemical characterization by an international cadre of specialists.

For Table I, reaction center types were identified either by direct isolation or from simple redox difference spectra made with membrane suspensions washed to remove much of the adventitious soluble components. At high redox potential ($E_h > 100$ mV), Group I organisms display an α-band (about 550–554 nm) characteristic of *c*-type cytochromes and at low redox potential ($E_h < 0$ mV), a cytochrome *b* α-band (560 nm), suggestive of the predominating presence of a cytochrome bc_1 complex. Group II organisms display at high redox potential an α-band (ca. 555–558 nm) and at low redox potential an α-band (about 550–554 nm), typical of the cytochrome spectrum of reaction centres, which contain the tetraheme cytochrome *c* complex such as is found in *Rhodopseudomonas viridis*. Cursory study indicates that one or the other cytochrome spectrum is dominant in these difference spectra and are therefore of diagnostic value. With the possible exception of *Rhodospirillum molischianum*, those organisms which possess menaquinone [4] cluster within Group II – an observation which may also be of diagnostic value in determining the nature of reaction center in a bacterial species.

Further ordering of the entries in the table involves sequential grouping of the most similar patterns of cytochrome occurrence within each group. Limited space precludes full description of the variety of metalloproteins compared. All Group I organisms as well as 6 of 20 Group II organisms possess cytochrome c_2. This cytochrome is defined on the basis of amino acid sequence analysis [5], which also serves to identify the constitutive iso-cytochromes c_2 listed. Similarly a normally non-constitutive iso-c_2 has been found to be produced in *Rhodobacter sphaeroides* from which the normal c_2 gene has been deleted [11].

High-spin cytochrome c' occurs almost ubiquitously, suggestive of an essential function; its occurrence is limited to anaerobically grown cells inasmuch as low levels of dioxygen repress c' production even when photopigment production is undeterred. The low-spin

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cytochromes c' are structural homologs of high-spin c' [6,7], in both of which the heme binding site is located near the C-terminus. Met 16, rather than the H_2O found in the high-spin form, is presumed to act as sixth ligand to the heme group to account for the low-spin character of the absorption spectra. In these cytochromes the α peak is always shifted to the red at about 554–558 nm as compared with about 549–552 nm observed with cytochromes c_2 in which the heme group is located near the N-terminus. Aerobic growth enhances the level of *Rb. sphaeroides* cytochrome *c*-554 several-fold [6].

Under the heading 'TYPICAL PSEUDOMONAS CYTS.' are collected purple photosynthetic bacterial cytochromes which are structural analogs to several types of cytochrome produced under denitrifying growth conditions by *Pseudomonas* species. Excepting the purported cytochrome *c* peroxidase proteins, these cytochromes have been found only among Group II organisms which fail to produce cytochrome c_2; (c.f. Ambler [5] for structural comparisons). Cytochrome *c*-551 may serve the role of cytochrome c_2 in the absence of that cytochrome. The cytochromes designated c_4 are membrane-bound di-heme proteins, typically displaying an asymmetric α peak. They can be solubilized by organic solvents, such as acetone [8] or butanol [9]. Cytochromes c_5 are mono-

Table I

Distribution of soluble iron-containing redox proteins in purple phototrophic bacteria

+ Indicates presence, confirmed by sequence or less often inferred from spectral data. + + indicates isozymes. − is indicated only where one or more of several closely related species has this protein and the others do not. A blank space indicates that the protein has not yet been observed, but is still remotely possible. Abbreviations: SHP, sphaeroides heme protein; CAT, catalase; CP, catalase-peroxidase; BF, bacterioferritin; low pot. *c*, heterogeneous group of low-potential cytochromes containing 1, 2, or 4 hemes; FC, flavocytochrome *c*; low-spin iso-*c'*, low-spin isozymes of cytochrome *c'*; Ps. Fd, typical *Pseudomonas* 7-Fe-S ferredoxin as opposed to the nitrogenase-associated ferredoxins, which are presumed present in all species; CCP, *Pseudomonas* cytochrome *c* peroxidase.

	c_2	c'	Low spin iso-c'	HiPIP	FC	Low pot. *c*	Typical pseudomonas cyts.					Misc.	Ref.
							c-551	c_4	c_5	CCP	*c*-552		
I. Species with simple reaction center													
Rps. palustris 37	+	+	+ +			+						BF, CP	[a]
Rps. palustris 6	+	−	+ +										[b]
Rps. rutila	+	+										Ps. Fd	[b]
Rb. sulfidophila	+	+	+			+							[a]
Rb. sphaeroides	+ +	+	+			+						SHP, BF, CAT	10, 11
Rb. capsulatus	+	+								+		bound *b*-561	[a]
Rb. blastica	+	+								+			[b]
R. rubrum	+	+				+						BF	12
R. photometricum	+	+											[b]
R. molischianum	+	+											13
Rm. vannielii	+	−		+									[b]
II. Species containing reaction center associated tetraheme cytochrome													
Rps. viridis	+	−											[b]
Rps. acidophila	+	−											[b]
Rp. globiformis	+	−		+									14
R. salexigens	+	+				+							15
R. centennum	+		+									*b*-557	[b]
Rps. marina/agilis	+	+	+	+ +		+						Ps. Fd, 2Fe Fd	16
Rps. sp. H1R		+				+			+			*c*-550	[a]
R. salinarum		+		+ +		+							17
Rc. tenuis		+		+		+	+	+			+	BF	18
Rc. purpureus		+		−			+						[b]
Rc. gelatinosus		+		+			+	+					19
E. halophila		+		+ +		+			+				20, 21
E. halochloris		−		−					+				20, 21
E. abdelmalekii		−		−					+				[b]
E. vacuolata		+		+ +				+				sol. *b*-558	[b]
E. shaposhnikovii		+		+ +				+				sol. *b*-558	22, 23
C. vinosum		+		+	+	+		+				SHP, CP	24
C. gracile		+		+	+	+		+					25
T. roseopersicina		+		+	+	+		+					25
T. pfennigii		+		+	−			+				*c*-552 (545)	26

[a] Bartsch, R.G., unpublished data.
[b] Meyer, T.G., unpublished data.

heme cytochromes, which in *Pseudomonas* are also membrane-bound due to an N-terminal extension. The phototrophic homologs are completely water soluble. Two diheme cytochromes have been found which are structurally similar to *Pseudomonas* cytochrome *c* peroxidase, but as yet no assay condition has been found by which that enzymatic capability can be demonstrated. The *Thiocapsa pfennigii* *c*-552(545) may also fall in this group. *Rhodocyclus tenuis* cytochrome *c*-552 appears to be a structural analog of *Ps. stutzeri* cytochrome *c*-552 (Meyer, T.E., unpublished data). Functions have not been identified for any of this group of proteins.

With the exception of very limited production by *Rhodomicrobium vannielii*, HiPIP is limited to Group II occurrence where it may substitute for cytochrome c_2 as a secondary electron donor to the reaction center. The occurrence of iso-HiPIP's is noted in some species.

Flavocytochrome *c* is limited to 3 of the 4 purple sulfur bacteria which produce periplasmic [3] sulfur inclusions. The cytochrome does not occur in *T. pfennigii*.

Within the heterogeneous low-potential cytochrome group, different structural forms appear to exist. All are characterized by redox potentials below 0 mV and all the absorption spectra mimic that of cytochrome c_3 of *Desulfovibrio* species, yet the only authentic tetraheme member is that produced by *Rhodopseudomonas* sp. H1R. In this cytochrome, distribution of the hemes over a peptide chain of but 84 residues (c.f. Ref. 27) may necessitate a new mode of folding to engage a remote His as ligand for a terminal heme *c*. The other cytochromes in this group contain either one or two hemes (cf. Ref. 28). The absorption spectra of all these cytochromes strongly suggest that all hemes will prove to be bis-His liganded.

In the miscellaneous category are relegated various heme proteins not conveniently categorized or discussed.

The collection of heme and related soluble proteins inventoried here is a result of what was once projected as a study in bacterial photosynthetic electron transport systems. The diverse assortment of cytochromes found in the photosynthetic bacteria diverted us to a comparative study of these proteins. This comparison is expected to provide insight into functional or taxonomic relationships among the photosynthetic bacteria and also to hint at interspecies relationships. This report is a partial update of an earlier compilation [24] which contains many details omitted here.

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