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Photobiology of Bacteria

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Key words: photoactive proteins, photoreceptors, chromophores, energy transduction, light signalling, phototaxis, gene expression

Abstract

The field of photobiology is concerned with the interactions between light and living matter. For Bacteria this interaction serves three recognisable physiological functions: provision of energy, protection against excess radiation and signalling (for motility and gene expression). The chemical structure of the primary light-absorbing components in biology (the chromophores of photoactive proteins) is surprisingly simple: tetrapyrroles, polyenes and derivatised aromats are the most abundant ones. The same is true for the photochemistry that is catalysed by these chromophores: this is limited to light-induced exciton- or electron-transfer and photoisomerization.

The apoproteins surrounding the chromophores provide them with the required specificity to function in various aspects of photosynthesis, photorepair, photoprotection and photosignalling. Particularly in photosynthesis several of these processes have been resolved in great detail, for others at best only a physiological description can be given.

In this contribution we discuss selected examples from various parts of the field of photobiology of Bacteria. Most examples have been taken from the purple bacteria and the cyanobacteria, with special emphasis on recently characterised signalling photoreceptors in *Ectothiorhodospira halophila* and in *Fremyella diplosiphon*.

1. Introduction

Classification of photobiological processes

The aim of studies in photobiology is the characterization of the interactions between light and living matter (Song et al. 1991; Clayton 1977). Light plays various roles in the life of a microorganism (see Table 1). First, and of far most importance, light supplies the organisms that have learned to live the phototrophic mode of life with free energy for maintenance and growth. For this purpose a large array of antenna complexes and reaction centers has evolved during evolution. As a result, two mechanistically very different types of photosynthesis have come into being: (bacterio)chlorophyll based- and retinal-based photosynthesis (for a comparison of these two forms of photosynthesis: see Hellingwerf et al. 1994). Chlorophyll

based-photosynthesis itself developed into two clearly distinguishable forms: anoxygenic- and oxygenic photosynthesis.

Light, however, is not only useful; it is dangerous too. Upon absorption of a visible photon, for instance, in a photoactive protein the immediate surrounding of the light-absorbing molecule (the chromophore) heats up some 200° C between the picosecond and microsecond time domains (Li & Champion 1994). This notion makes it easy to imagine that photoactive proteins are prone to damage. However, even prior to this heating, other types of damage can occur. The excited singlet- and triplet-state of many chromophores can react with molecules like oxygen, to give rise to the highly reactive superoxides. These molecules may subsequently covalently modify proteins in a cell at random. In addition, radiation from the (near)UV-part of the visible spectrum may cause damaging photo-

Fig. 1. Representative examples of blue-, green- and red-light absorbing chromophores. (A): Part of the structure of the chromophore of the green fluorescent protein; (B) retinaldehyde and (C) bacteriochlorophyll *a*. For further details, see text.

chemical reactions, in particular in the nucleic acids of (micro)organisms. Significantly, these two pathways of light-induced damage of living organisms are counteracted by specific *light*-dependent protection- and repair-mechanisms, respectively.

The beneficial and detrimental effects of electromagnetic radiation urge many organisms to react specifically to their ambient light climate. This response can take two forms: cells may migrate to or from a particular environment, *via* active swimming in a phototactic process or *via* vertical migration, by exploiting differences in buoyancy, and/or they may induce or repress specific (sets of) genes. Three aspects of the light climate are important in this respect: its intensity, its periodicity and its spectral composition.

Table 1. Biological function of the absorption of visible radiation.

1)	Free energy conservation
2)	Photoprotection
3)	Signal transfer for motility
4)	Signal transfer for the regulation of gene expression

Particularly in the areas of phototaxis and photoregulation of gene expression recent progress in the elucidation of new types of photoreceptors has been obtained. This will be discussed in subsequent sections of this contribution.

Classification of chromophores

A mole of quanta of visible light contains an amount of free energy that ranges between 350 and 175 kJ, for near-UV and far-red photons, respectively. In order to be able to absorb these photons, photoactive proteins must contain a particular *chemical* structure, with an *electronic* structure in which a transition equivalent to this amount of free energy should be possible. Except for (far)UV irradiation, absorbed by the aromatic amino acids (Trp, Tyr and Phe), such transitions are not found in the basic constituents of proteins, the amino acids. The Dutch word for 'protein' makes this explicitly clear: eiwit (= 'egg's-white'). However, since Nature has decided that proteins should be the 'work-horses' of the living cell, even in photobiology, a large number of different prosthetic groups have evolved, either covalently or non-covalently attached to their respective (apo)protein, that confer the holoproteins with the ability to absorb light in the visible region of the spectrum of electromagnetic radiation. Comparison of the chemical structure of these chromophores makes it clear that they can be subdivided into a limited number of classes (see Table 2), with a significant correlation between their chemical structure and the sub-part of the visible spectrum in which they show maximal absorbance. A representative example from each of the three classes of chromophores is depicted in Fig. 1.

Most of the chromophores that we know have the basic structure of a tetrapyrrole: Four covalently linked heterocyclic pyrrole rings, either in linear (e.g. phytochrome) or in circular form (e.g. (bacterio)chlorophyll). The resulting very large conjugated

Table 2. Classification of the chromophores relevant for photobiology.

A:	Derivatized (hydroxylated) aromatic chromophores (stentorin, flavin [1,2])
B:	Polyene-containing chromophores (carotenes [1], retinals [3])
C:	Tetrapyrrole-containing chromophores ((bacterio)chlorophylls [1,2], phycobilins [1], phytochromes [3])

Numbers refer to the type of photochemistry displayed by the respective chromophores: 1: excitation transfer; 2: electron transfer and 3: photoisomerization.

structure that is formed in these tetrapyrroles allows specifically these chromophores to absorb photons from the red and infrared part of the spectrum (*via* their so-called Q_Y transition). In addition, these chromophores absorb at shorter wavelengths, *via* their Q_X and Q_Z transitions.

The second class of chromophores has a polyene structure. Linear polyenes with approximately ten carbon-carbon double bonds give rise to an electronic structure with π electrons that is optimally suited to absorb the middle part of the visible spectrum (approx 500 nm). Light from this part of the spectrum is in many aqueous environments physiologically by far the most important part, because of its ability to penetrate these environments and because of its relative abundance in the spectrum of light emitted by the sun, in combination with the filter effect of the atmosphere (Seliger 1977). Part of the polyenes, i.e. many of the carotenoids, have a very typical optical absorbance spectrum, with a main transition that shows a three-fold degeneracy, due to vibrational fine structure. With increasing chemical derivatization at the two extremes of a carotenoid molecule, in particular with increasing oxygenation, or with an increased conformational constraint (like for instance the formation of a *cis*-isomer), the three bands merge into one. The second major class of polyenes, the retinals (of which the all-trans form is the chromophore of the (archaeal) rhodopsins), typically show an a-symmetric absorption band with sidebands visible at the high-energy shoulder of the main transition, at a characteristic spacing of approximately 30 nm.

A third, slightly heterogeneous, class then remains, which contains mostly members that can be characterised as 'blue-light' receptors. Most members of

this class have a structure with (a) substituted benzene ring(s). The most important substituent is the hydroxy group. Stentorin is a member of this class, as well as, the chromophore of the green fluorescent proteins (a.o. from jelly fish). It may well be that the chromophore of the yellow protein(s) turns out to be the bacterial representative of this subclass. However, other substituents may be present as an alternative, like in the flavins, and the aromatic ring may even be heterocyclic, like with the pterins.

Both phototrophic- and non-phototrophic bacteria produce a large number of additional proteinaceous pigments (melanins, siderophores, antibiotics, etc.). For some of these the biological function is known, for others at this moment we can only speculate. For none of these, however, a photobiological role has been described.

The photochemistry of photobiology

The classification of the photochemistry of the reactions that are relevant for photobiology is very straightforward: Light-absorption induces either: (i) excitation transfer, (ii) electron transfer or (iii) photoisomerization. Representatives of all three classes of chromophores, in some form or another, can display all three types of photochemistry (see Table 2), except for light-induced electron transfer, which is not found among the polyenes and photoisomerization which has not (yet) been observed among the hydroxylated aromatics.

Thermodynamically, light is best understood from black-body radiation. A black body emitting light in the visible wavelength region will have a temperature between 4,000 and 7,000 K (T_{hv}). These high-temperature conditions allow description of visible radiation as an equilibrium process, making it thermodynamically straightforward to separate contributions to the free energy of individual photons in the form of free enthalpy and entropy. Physiological conditions in biology, however, necessitate much lower temperatures (i.e. close to room temperature (300 K; T)). Consequently, the maximal thermodynamic efficiency of the conversion of light energy is inherently low (i.e. $\eta^{max} \leq 1 - T/T_{hv}$). A second, and often overlooked, consequence is that the free energy of a photon is a function of photon density (Westerhoff & Van Dam 1987).

The chromophores of the pigmented proteins that play a role in photobiology generally have a high extinction coefficient (in the order of 10^4

Fig. 2. Effects of the protein environment on chromophore photo-energetics. The free energy surfaces of both the groundstate S_0 and first excited state S_1 of the free chromophore (solid line) are affected (dotted line) by binding of this molecule to the apoprotein. This results in a redshift of the groundstate absorbance, an increased quantum yield of photo-isomerization, and energy storage in the primary photoproduct.

$\text{mM}^{-1} \cdot \text{cm}^{-1}$). This means that these molecules have a comparatively large optical cross-section for capturing photons. The underlying explanation for this characteristic is the fact that the photochemically active entity, in all three basic types of chromophore, is the (conjugated) diene (note that even the p-hydroxyphenyl group has a significant amount of diene character). The orbitals of a π -electron in the ground- and excited state of a conjugated diene have a relatively large overlap integral and consequently a high probability of transfer (in squared form this factor is often referred to as the Franck-Condon factor).

Photobiological processes generally function with a high quantum yield, often near unity. This is true for light-induced excitation transfer, light-induced electron transfer and for photoisomerizations. These latter reactions in organic chemistry mostly have a quantum yield that is far smaller. The explanation for this must be in the apoprotein surroundings of these chromophores. The apoproteins in many cases cause a redshift in the absorbance maximum of the chromophore (like e.g. the 'opsin-shift'). It is therefore reasonable to assume that in many photoactive proteins the apoprotein lowers and shifts the excited state singlet energy

surface, relative to the ground state energy surface of the two isomeric states of the photoisomerization reaction (see Fig. 2).

In exciton- and electron-transferring chromophores a lowering of the energy level of the first excited state is often attributed to chromophore-chromophore (exciton) interactions, however, apoprotein-induced redshifts also contribute (Van Grondelle et al. in press).

Scope of this review

The field of photobiology for a long period has lagged behind with respect to the insight into the molecular characteristics of its major components, compared to the detailed knowledge available about the machinery that facilitates the chemotrophic mode of life. This situation has changed into a lead with the resolution of the three-dimensional structure of the bacterial reaction centers. However, for characterization of the components involved in photosignaling, rather than in light-energy transduction, an additional barrier often has to be taken, which is the low abundance of these proteins. The recent revolution in molecular genetics, however, is of great help to overcome this problem and has even allowed the characterization of a number of genes that code for so far unidentified photoreceptors.

In this review we want to discuss recent developments in the four areas that are relevant for photobiology (see also Table 1): energy transduction, photoprotection, phototaxis and photoregulation, with an emphasis on the latter two aspects, because of the recently identified photoreceptors that have a function in these areas. In this discussion we limit ourselves to the Bacteria. This implies that we will not discuss the archaeal rhodopsins. This topic, however, will be dealt with in the contribution of D. Oesterhelt. In addition, in view of the contribution of R. Blankenship, we will only briefly discuss the properties of photochemical reaction centers.

2. Light as the energy source

Classes of phototrophic bacteria

Traditionally phototrophic bacteria have been split up into two major categories: anoxygenic and oxygenic phototrophic bacteria. The oxygenic phototrophic bacteria are all members of a single and phylogenetically coherent group within the Gram-negative bacteria: the cyanobacteria. Within this family the strains are clas-

sified within one of the five specific subgroups that have been introduced, based on morphological criteria. These organisms are characterized by the presence of two separate photosystems, the presence of a specific class of antennae, the phycobilisomes, and the absence of chlorophyll b. During the past few years, however, a number of organisms have been described that do possess chlorophyll b. Initially these were classified as a separate group, the Prochlorales. The idea, however, is emerging that these organisms are closely related to the cyanobacteria, be it that phycobilisomes have never been detected in the Prochlorales (Matthijs et al. 1994).

The group of anoxygenic phototrophic bacteria is more diverse (for a recent review, see Imhoff 1992). Initially these were classified in a matrix of organisms, all belonging to the Gram-negative bacteria too, that use either an organic electron donor or an inorganic (sulphide) and organisms that do or do not have specific antennae: the chlorosomes. The four resulting families were: the *Rhodospirillaceae*, the filamentous green bacteria, the purple sulphur bacteria and the green-sulphur bacteria. On the basis of the deposition of elemental sulphur the purple sulphur bacteria were subdivided in two families: the *Chromatiaceae* and the *Ectothiorhodospiraceae*. However, within the past few years a number of organisms have been described that do not fit into this scheme. These are (1) the organisms that can perform *anoxygenic photosynthesis* only under *aerobic* conditions (*Protaminobacter*, *Erythrobacter*, *Rhizobium*, etc.) and (2) the Gram-positive phototrophic bacteria (*Heliobacterium* and *Heliobacillus*).

Classification schemes for microorganisms have witnessed a drastic change in emphasis from morphological and physiological criteria to phylogenetic relationships (Woese 1987). Furthermore, detailed molecular biological analysis in recent years has revealed a close relationship between various pairs of phototrophic and non-phototrophic bacteria. This is particularly true for members of the so called Proteobacteria (or: purple bacteria; compare f.i. *Rhodobacter* and *Paracoccus*), but striking examples can also be found among the cyanobacteria (c.f. *Spirulina*, *Thiospirillum* and *Saprospira*; Schlegel 1986). We therefore think that the currently most rational and meaningful classification follows the phylogenetic rationale and discriminates within the Kingdom of the Bacteria (or: the Domain of the Bacteria) 11 groups, six of which contain members that are phototrophic (see Table 3).

The resolution of the three-dimensional structure of the reaction centers of *Rhodopseudomonas viridis*

Table 3. Groups within the domain of the bacteria that contain members that are phototrophic.

chemotrophic	phototrophic
Thermatoga	Green non-sulphur bacteria
Deinococci and relatives	Green sulphur bacteria
Spirochetes	Bacteroides-Flavobacteria
Planctomyces and relatives	Gram-positive bacteria
Chlamydiae	Cyanobacteria
	Purple bacteria (α - γ subgroups)

and *Rhodobacter sphaeroides* (Deisenhofer et al. 1984; Allen et al. 1987a,b), in combination with the results of sequencing analyses plus site-directed mutagenesis of cytochrome-b/c₁ and -b₆/f complexes (Trumpower 1990; Gennis et al. 1993), has led to an enormous increase in our understanding of the pathways of electron transfer in anoxygenic- and oxygenic photosynthesis (see Figs. 3 and 5). The basic idea emerging from these investigations is that in anoxygenic photosynthesis light-induced cyclic electron transfer is catalysed by two large intrinsic membrane protein complexes (i.e. the reaction center and the cytochrome b/c₁ complex), which communicate via a lipophilic- (a quinone) and a water-soluble redox carrier (a c-type cytochrome), at the low and high redoxpotential side of the reaction center, respectively. This description fits best to the purple non-sulphur bacteria and the obligate aerobic bacteria. In the purple- and green sulphur bacteria there may be a significantly competing linear flux of electrons to NAD(P)H.

All phototrophic bacteria investigated so far contain a cytochrome b/c₁ or -b₆/f-like complex (for review, see Trumpower 1990), even the alkaliphilic representatives like the *Ectothiorhodospiraceae* (Leguijt et al. 1993), although the evidence for the presence of b/c₁ complexes in cyanobacteria remains questionable. Reaction centers come in two types (Nitschke & Rutherford 1991): With either quinone-type- or with (bacterio)chlorophyll and iron-sulphur clusters as electron acceptors, respectively. These two classes function as models for the two types of reaction center in oxygenic photosynthesis. In this latter process the major electron flux is directed from water to NADPH. Two reaction centers (PSII and PSI) function in this pathway in series, connected via a cytochrome b₆/f complex and plastocyanin or c-type cytochromes. Here we will not

Fig. 3. Schematic representation of light-driven cyclic electron transfer and proton motive force generation in *Rhodospirillum rubrum* as based on the recent structural and genetic data (see text for details). Abbreviations used: LH_{1/2}, light-harvesting complex 1 or 2; BChl, bacteriochlorophyll; BPh, bacteriopheophytin; Q_A and Q_B, primary and secondary quinone electron acceptors; Q_P, quinone pool in the membrane; cyt b_L, cyt b_H, cyt c₁ and FeS, low and high potential cytochromes b, cytochrome c₁ and the Rieske protein of the cytochrome bc₁ complex. [eH] symbolizes steps where both electrons and protons are transferred.

extensively discuss these electron transfer pathways, but rather point out some major unsolved aspects.

Topics in bacterial photosynthesis

Obligate aerobic anoxygenic phototrophs

Among the recently added members of the collection of anoxygenic phototrophic bacteria are quite a number that have some unconventional properties. One is the complex regulation of bacteriochlorophyll synthesis. This metabolic route in these organisms is not repressed by oxygen. However, this trait is also observed in a number of the more 'classical' purple bacteria (e.g. in *Rhodospirillum rubrum*; De Bont et al. 1981). A unique trait of the obligate aerobic phototrophic bacteria, however, is that they are able to

conserve light energy *only* when oxygen is around. Photosynthesis in these organisms is strictly dependent on the presence of oxygen. The best characterized examples of such bacteria are *Erythrobacter* and *Proteobacter* (see e.g. Okamura et al. 1985).

In spite of a rather detailed characterization of the photosynthesis machinery in these organisms, a molecular interpretation concerning the obligate aerobic character of their photosynthesis can not (yet) be given. With respect to all assayed characteristics, this photosynthesis machinery is similar to the one of *Rhodospirillum rubrum*. This is even true, for instance, for the midpoint potential of the primary quinone of reaction centers from *Erythrobacter* (Takamiya et al. 1987). Initially it was thought that a significantly increased value of this midpoint potential might explain the non-

functionality of the reaction centers under anaerobic conditions.

An alternative working hypothesis may be that the affinity of the reaction centers of obligate aerobic anoxygenic phototrophic bacteria for quinone compares unfavourably with the affinity of quinol for the same site. If so, quinol would function as an inhibitor of anoxygenic photosynthesis. Under anaerobic conditions, which will lead to increased quinol levels, this then would lead to prohibition of functional turnover of the reaction centers.

Lateral organization of the cyclic electron transport chain in the membrane

In the strictly anaerobic-phototrophic- and Gram-positive bacteria, like *Heliobacterium*, the photosynthesis machinery is supposedly arranged randomly and homogeneously in the cytoplasmic membrane which surrounds the cytoplasm of these cells. In *Rhodobacter* and related organisms this situation must be much more complicated. First, it is known that the cytoplasmic membrane can be strongly invaginated, particularly when cells grow in low light intensities. It differentiates into the true cytoplasmic membrane and intracytoplasmic membranes, which are densely packed with the various components of the machinery carrying out anoxygenic photosynthesis (i.e. antenna complexes, reaction centers, cytochrome *b/c*₁ complexes and ATP-synthetases). These intrinsic membrane complexes cannot freely diffuse in the membrane, as simple calculations, based on the rate of diffusion of intrinsic membrane proteins, can show.

Based on measurement of the kinetics of photooxidation of cytochrome *c* in intact *Rb. sphaeroides* cells, plus the considerations given above, it has been proposed that supramolecular protein assemblies (for a recent review, see Joliot et al. 1993) are the functional units in anoxygenic photosynthesis. In particular, the existence of super-complexes composed of one reaction center, one cytochrome *b/c*₁-complex and two molecules of cytochrome *c*₂ has been postulated.

Joliot et al. compared the possible mechanism behind the regulation of the different aggregation states with the process of state transitions in oxygenic photosynthesis. Interestingly this line of thinking can be extended to the recently discovered redox sensitive LH-kinase (Ghosh et al. 1994) which could indicate a mode of regulation of lateral organization of the components involved in light-induced electron transport very similar to that of the state transitions.

Experiments, recently reported by Vermeglio et al. (1993), indicating that cytochrome *c*₂ in intact cells of *Rhodobacter sphaeroides* is divided over two separate compartments, show that the organization of the photosynthetic machinery in anoxygenic photosynthesis is even more complex. The presence of different (sub)cellular compartments in *Rhodobacter sphaeroides* has been suggested previously (Crielaard et al. 1988) in order to explain the large discrepancy between two different methods for determining the transmembrane electrical potential in this organism.

Linear versus cyclic electron transfer in photosynthesis

The interaction between the linear (to O₂) and cyclic electron transport chain in purple non sulphur bacteria like e.g. *Rb. sphaeroides* and *Rb. capsulatus* has been well documented. The interaction between the (obligatory) linear and cyclic electron transport chain in purple sulphur bacteria, like e.g. *Ectothiorhodospiraceae* and *Chromatiaceae*, and the destiny of electrons donated by sulphide, still needs further investigation. As can be seen in Fig. 4, which shows the recent model for photosynthetic electron transport in *E. mobilis*, electrons from sulphide are donated into the electron transport chain in a *light dependent* reaction via a *c*-type cytochrome (*C_L*). From *C_L* the electrons are passed on to the primary donor and after excitation by light finally, via the primary (*Q_A*) and secondary (*Q_B*) electron acceptors in the photosynthetic reaction center, end up in the membrane soluble quinone pool (*Q_P*). To avoid overreduction of the electron transport chain, in these strict anaerobic bacteria (also no alternative electron acceptor has been demonstrated), electrons somehow have to be incorporated into cell material. In view of the redox-midpoint potential of the quinone pool (90 mV) the most likely route to accomplish this is in an *uphill* reaction (via NADH-dehydrogenase) to NAD⁺. NADH could subsequently be used in the (in this bacterium not yet demonstrated) Calvin cycle in the process of CO₂-fixation.

The energy necessary to drive this reaction can be provided via the proton motive force (Δp) as has been shown in numerous other bacteria. The proton motive force in this bacterium is generated via photosynthetic *cyclic* electron transport which involves the proton pumping *b/c*₁-complex. It is clear that in bacteria a well regulated balance has to be maintained between cyclic (Δp -generating) and linear (Δp -consuming *but* ΔE_{red} -generating) photosynthetic electron transport.

Fig. 4. Schematic representation of light-driven electron transfer and sulphide oxidation in *Ectothiorhodospira mobilis* cells as suggested by Leguijt (1993). Solid lines enclose membrane-bound components: the reaction center, membrane bound *c*-type cytochromes (denoted c_L and c_H), the cytochrome bc_1 complex (denoted Cytochrome b/c) and NADH dehydrogenase (denoted NADH dh.), respectively. The dashed lines indicate that the two cytochromes are present in the vicinity of the RC instead of being RC-bound. The numbers in square parentheses refer to E_m values. Both question marks indicate hypothetical soluble redox proteins. Abbreviations used: P, primary donor; Q_A and Q_B , primary and secondary quinone electron acceptors; Q_p , quinone pool in the membrane; Δp , transmembrane electrochemical H^+ gradient that drives 'reverse' electron flow; b_L , b_H , c_1 and FeS, low and high potential cytochromes *b*, cytochrome c_1 and the Rieske protein of the cytochrome bc_1 complex, respectively.

The mechanism behind this type of regulation remains to be studied.

Electron transfer pathways in cyanobacteria

Cyanobacteria are oxygenic photoautotrophic prokaryotes which feature a combination of chloroplast like photosynthetic electron transfer and of mitochondrial-type respiration within a single prokaryotic cell. These electron transfer systems are constitutive and secure ATP synthesis during daytime and in darkness, via photo- and oxidative phosphorylation, respectively. An extensive number of electron transfer pathways has been postulated to explain the connection between the various processes (Fig. 5; Mattheijs & Lubberding 1988; Scherer 1990). Cyanobacteria contain two different types of membranes. First, the cytoplasmic membrane, which is not involved in photosynthetic electron transfer. Second, the photosynthetically active thylakoid membrane, which is situated in the cytoplasm. The two types of membrane are not interconnected (Mattheijs & Lubberding 1988). Although some of the respiratory activity has been associated with the

cytoplasmic membrane, the thylakoid membranes contain by far most of the respiratory activity. The cyt b_6f complex and ATP-synthetase (the latter is situated on the cytoplasmic side) of the thylakoid membranes are used in common between light- and respiration-driven phosphorylation.

Photosynthesis is the key activity of cyanobacteria, its electron flux capacity exceeds respiration 3 to 10 fold. The light reactions of photosynthesis involve two photosystems, PSII (P680) and PSI (P700). Light in cyanobacteria is characteristically harvested by the phycobilisomes (PBS), massive structures localized on the periphery of the thylakoid membranes at the cytoplasmic side (Tandeau de Marsac & Houmard 1993). These antennae are easily discernable on electron micrographs and are composed of long rods composed of polypeptides with attached phycobilin-type chromophoric groups. These pigments (i.e. phycocyanin (PC) and allophycocyanin (APC)) provide cyanobacteria with their specific blueish colour. However, the red phycoerythrin (PE) may in part replace phycocyanin (see further below).

Fig. 5. Schematic representation of the electron transfer pathways in cyanobacteria, relevant for photosynthesis. In this figure the relevance of cyclic electron transfer pathways, in addition to the basic 'Z-scheme' of photosynthetic electron transfer, is indicated. For detailed explanation of the various numbered pathways: see text. Dashed lines (9 and 10) represent postulated pathways.

Oxygen evolution from water involves a very strong oxidant, Z, a tyrosine unit of polypeptide D1 (Vermaas et al. 1988), and the so called S(torage)-states. The oxygen-evolving complex contains 4 Mn-centers, which can be present in different valency states, to serve the extraction of 4 electrons from water (Debus 1992). The liberated protons are released in the thylakoid lumen. Cyanobacterial PSII reaction centers and the minimal cores derived of these, consisting of cyt b_{559} and the D1 and D2 polypeptides, (the latter two show similarity to the L and M subunits of purple bacteria) are widely used as model systems for chloroplast PSII, in studies on the mechanism of charge separation and photoinhibition (cf. 1a in Fig. 5; Shipton & Barber 1991). Passage of electrons from the primary stable acceptor Q_A to Q_B and onwards to PQ and the cyt b_6f complex is denoted by 1b and 3 (Lee & Whitmarsh 1989). Reactions 1a,b and 3 create a proton gradient,

the thylakoid lumen side becomes acidic. Reaction 2 has recently become topic of extensive studies (Busser et al. 1990; Barber & De Las Rivas 1993). The re-reduction of P_{680} by an electron from Q_B , via cyt b_{559} has been suggested to serve as a protective mechanism against photoinhibition. An alternative route of cyclic electron transfer encompasses the respiratory terminal cytochrome c oxidase (see reactions 3 and 4b; Matthijs & Lubberding 1988). This pathway may have a function in the poisoning of the electron transfer chain. Electrons in this way may proceed either via reaction 4a to the reaction center of PSI or may be donated to the oxidase in 4b. The mechanism of distribution of electrons has not been studied in great detail, several soluble electron carriers (c-type cytochromes, plastocyanin) are likely to be involved and their specificity for either of these systems is topic of current research (Jeanjean et al. 1993). Investment of light energy in

PSI (reaction 5, note the similarity with the RC of green bacteria), effects another charge separation and reduces FeS centers, which are adequate to reduce ferredoxin, the first stable acceptor of PSI (Golbeck & Bryant 1991). Oxygen, although being a principal product of oxygenic photosynthesis, is at various sites also disturbing conservation of light energy. This occurs for example in the so called Mehler reaction, which is indicated as reaction 6. The enzyme FNR (ferredoxin:NADP⁺ oxidoreductase) catalyzes electron transfer to NADP⁺ via ferredoxin. Recent observations have indicated that the FNR enzyme from a number of cyanobacteria differs from the rather conserved FNR in chloroplasts (for example of pea and spinach). The enzyme in cyanobacteria (3 species tested) has an N-terminal extension of 14 kDa (Schluchter & Bryant 1992; Matthijs et al. unpublished). The physiological function of this hydrophobic extension has not yet been revealed. The major site of regulation between light and dark metabolism is linked with the Calvin cycle, glucose-6-phosphate dehydrogenase and 6-phosphogluconate operate exclusively in darkness.

Cyanobacteria are free-living organisms and need to invest ATP for transport of inorganic nutrients from the environment. The energetic demand involved requires additional ATP synthesis, the source of which has been projected to be cyclic electron transfer around PSI. This antimycin A sensitive reaction (shown as 9) has also been designated to chloroplasts. We still lack part of the information, however, on the protein(s) involved in the transfer of electrons from ferredoxin to PQ, the so called FQR activity (Manasse & Bendall 1993). Recent studies have indicated that this type of cyclic reaction in cyanobacteria may not occur at all, or only at a minor scale, a conclusion very well in line with the puzzling observations on the effectiveness of antimycin A inhibition in cyanobacteria (Jeanjean R, personal communication). Instead, NADH oxidation via a membrane-bound *E. coli*-type NADH dehydrogenase has been shown to play an important role in cyanobacteria *in vivo* (Mi et al. 1992). The dehydrogenase is rather specific for NADH. To sustain NADPH oxidation at an appreciable rate, transhydrogenase activity (here shown as reaction 11) would be required (Jackson 1991). However, such activity may be absent in cyanobacteria (Scherer 1990).

In darkness, the oxidative pentose phosphate cycle is the major route via which adenine nucleotide coenzymes (principally NADP⁺) are (re)reduced in cyanobacteria. Freshly isolated thylakoid membranes oxidize NADPH 3 times faster than NADH. This

activity is very labile, in contrast to the very stable NADH oxidation activity (Matthijs et al. 1984). Current research in our laboratory is projected to link the N-terminal extension of FNR with its proposed additional role as NADPH dehydrogenase, indicated as reaction 10. Following either step 10 or 12, electrons proceed to PQ from which electrons can proceed to cytochrome c oxidase (Matthijs et al. 1984). Direct oxidation of PQ, which is basic to chlororespiration in chloroplasts (Peltier & Schmidt 1991) may also occur in cyanobacteria (not indicated in the scheme). This reaction is wasteful in the sense that it generates ATP only at a single site. This latter goal (i.e. maximal ATP yields) is reached if electrons flow to oxygen via cytochrome c oxidase, a 3 subunit Cu-containing oxidase of the aa₃ type (reactions 3 and 4b).

Modelling of cyclic electron transfer

Using the extensive set of kinetic data available on the (early) processes of anoxygenic photosynthesis it may become possible to formulate a *quantitative* description of the kinetics and thermodynamics of the light-driven electron transfer and coupled proton translocation that is at the basis of this process. In order to provide such a description, an appropriate definition of all the electronic states of the cyclic electron transport chain has to be available. Since no detailed structural information for the bc₁-complex is yet available, such a model can only be presented for reactions occurring in and around the photosynthetic reaction center. A twelve-state model delineating these reactions is presented in Hellingwerf et al. 1994. This model describes the route of electrons through the reaction center and the redox reactions coupled to re-reduction of the primary donor and oxidation of the secondary acceptor (or, more precisely, replacement of the secondary acceptor). Nearly all rate constants (for the transitions between the different electronic states) incorporated in this model have been taken from the literature; a small number has been determined through subsequent experiments.

Preliminary measurements in a system with solubilized reaction centers (in the presence of cytochrome c and ubiquinone-0) have shown that under certain conditions the model presented here satisfactorily describes the (concentration of) the various redox states. However, re-evaluation of a number of rate constants and mechanisms in the model may significantly improve the fit of the model to data.

A very important extension of the 12-state model will have to be the incorporation of proton motive force dependency. It has been shown (Molenaar et al. 1988) that one of the components of Δp , the membrane potential ($\Delta\psi$) exerts back-pressure on the overall rate of electron transport through the reaction center. Such an effect is to be expected in view of the fact that electron transport within the reaction centers proceeds vectorially against an oppositely directed electrical field. This effect has also been shown to be present in PSII reaction centers during oxygenic photosynthesis (Dau & Sauer 1992). Fortunately, experiments which have to accompany such a mathematical description can be performed without the need for the presence of a bc_1 -complex. It has been shown that after reconstitution of RC's into liposomes it is possible to generate a $\Delta\psi$ over the liposomal membrane in the absence of a bc_1 -complex. In this model system, translocation of protons is achieved by the addition of a water soluble quinone (UQ_0) and (membrane impermeable) cytochrome c. UQ_0 shuttles protons (bound upon reduction on the inner side of the membrane) from the inside of the liposomes to the external medium, where protons are liberated upon reduction of cytochrome c by ubiquinol.

In the future these studies may be extended by including the characterization of mutant forms of reaction centers, obtained via site-directed mutagenesis (Jones et al. 1992) and via the description of the reconstitution of reaction centers and a cytochrome b/c_1 -complex into liposomes.

3. Protection against excess visible irradiation

Role of carotenoids

In most light-harvesting- and reaction center complexes carotenoids are bound non-covalently to the protein-pigment complex, usually in their all-trans form (for review, see Cogdell & Frank 1988), be it that exceptions have been described like: the carotenoid in the reaction center of purple bacteria and the bulk of the carotenoids in heterotrophically grown *Scenedesmus obliquus* (Sandmann 1991), which are in a cis conformation. Carotenoids are also present in the screening pigment layers in cyanobacteria (scytonemin) and even in non-phototrophic bacteria.

The carotenoids may have one or more of three different functions: (i) they may play a role in the assembly or stabilization of the pigment/protein complexes;

(ii) they may be part of the antenna that channels excitons to the reaction center(s) and (iii) they may protect the organism against excess radiation (Ramirez 1992).

The protective function of carotenoids can be understood from the fact that many pigments, in particular (bacterio)chlorophylls, when excited to a singlet- or triplet-state can react with molecular oxygen, to form the highly reactive singlet oxygen. Carotenoids can prevent this in two ways: (i) They can channel excitations from (bacterio)chlorophylls to heat, via the involvement of a triplet state which spontaneously and rapidly de-excites to the ground state; (ii) when still some singlet oxygen is formed, these species can directly be quenched by carotenoids. Most likely, pathway (ii) becomes important when, due to overexcitation of an organism, pathway (i) becomes limiting. However, the latter pathway is of major importance for protection of non-phototrophic bacteria.

In the antenna function, the distance between the carotenoid and the (bacterio)chlorophyll is of decisive importance. The efficiency of energy transfer is only high (i.e. close to 100%) when the two pigments are at van der Waals distance. Upon an increase of this distance to 5 Å the transfer efficiency decreases to less than 5%. The efficiencies of transfer from carotenoids to bacteriochlorophyll in the purple bacteria vary considerably. Values in the range of 30 to 90% have been reported. With these values it is important to keep in mind that the spectral range of light absorbed by carotenoids very closely matches the wavelength region of sunlight with high potency to penetrate aqueous environments. This makes it quite complicated to discuss the efficiency of anoxygenic photosynthesis, for instance in comparison with retinal-based photosynthesis (cf. Hellingwerf et al. 1994).

Surprisingly, to the carotenoid that is in close association with the monomeric bacteriochlorophyll in the 'non-productive' branch of electron transfer in reaction centers of purple non-sulphur bacteria, a structural, rather than a protective role has been assigned (Ramirez 1992).

Oxygenic photosynthesis makes use of an advanced set of additional physiological protection mechanisms. A first example is the so-called zeaxanthin-cycle. This term refers to a cyclic process of oxidation and reduction of carotenoids, in which the different carotenoids have a widely varying efficiency of energy transfer to chlorophyll, allowing the organism to adjust the rate excitation of the two photosystems (see e.g. Schubert et al. 1994). As far as we are aware this process does not play a role in Bacteria and therefore will

not further be discussed. A second example is the so-called state transitions. Many organisms that carry out oxygenic photosynthesis are able to regulate the distribution of excitons over the two photosystems *via* migration of the major antenna complex (LHC) laterally in the membrane (for a review, see Allen 1992). This regulation may be induced by the spectral composition of the available radiation or by the relative requirement, in intermediary metabolism, of ATP and NAD(P)H, which may make it necessary to adjust the ratio of the rate of linear electron flow versus the rate of cyclic electron flow. The lateral migration of LHC is brought about by a reversible phosphorylation of antenna polypeptides, which in turn is regulated by the redox state of the cytochrome b_6/f -region of the linear electron transfer chain. This mechanism is important in chloroplasts, but also in the Prochlorales and (other) cyanobacteria. Reversible phosphorylation of components involved in the conversion of light-energy into a proton gradient (antenna complexes, cytochrome b/c_1 -complex, etc.) has been reported in purple bacteria too (Cortez et al. 1992; Ghosh et al. 1994). The physiological function of the latter process is less clear (see above).

Function of photolyases in DNA repair

Many Bacteria contain an enzyme that is able to repair damage in their DNA, caused by UV light (for a review, see Sancar & Sancar 1988 and Kim et al. 1992)). Short wavelength irradiation (250 to 300 nm) can cause covalent linkage of neighbouring thymidine residues, via the formation of a cyclobutane ring. Such damage can be repaired by so-called photolyases, enzymes that use light energy in the wavelength region between 300 and 500 nm to catalyse reversible electron transfer, to and from the cyclobutane ring, respectively. This order of electron transfer reactions (the alternative would be first an oxidation of the cyclobutane ring, followed by rereduction) is supported by *in vitro* studies with model compounds. The light-driven forward and backward electron transfer leads to the reformation of two unimpaired and separate thymidines. Of the bacterial enzymes, the photolyases of *Escherichia coli* and *Streptomyces griseus* have best been characterized. In addition, the enzymes from *Halobacterium halobium* and *Saccharomyces cerevisiae* have been studied in detail.

Analysis of action spectra has revealed that two pathways of electron transfer to the cyclobutane ring exist in the photolyases (Kim et al. 1992). The first

pathway makes use of a unique tryptophan residue, which is situated in the active site of the photolyase (very near the thymidine-dimer and possibly involved in its binding; Trp-277 of the *E. coli* enzyme), as the electron donor. In view of its action spectrum, however, this pathway can only be of marginal physiological significance. Because of this, it has been suggested that the more important function of this tryptophan is to bind the cyclobutane ring via (hydrophobic) stacking interactions. In the second and most important pathway, light activates an electron from a reduced flavin ($FADH_2$), which subsequently (and reversibly, see above) is transferred downhill to the cyclobutane ring. In this second pathway, in addition, a second chromophore functions as an antenna. This second chromophore can be either a pterin (methenyltetrahydrofolate, like in *E. coli*) or a deazaflavin (like 7,8-didemethyl-8-hydroxy-5-deazaflavin in *Streptomyces griseus*). This second chromophore thereby intensifies the absorbance characteristics of the photolyases in the region around 375 nm, resp. extends its absorbance range up to 450 nm.

The structure of photolyases has been strongly conserved, even across the Kingdom barrier, supporting the idea that this enzyme catalyses an essential function in all three kingdoms.

4. Light-signalling for a motility response

Different forms of (photo)taxis and motility

Phototaxis in Bacteria (in contrast to some Archaea, see e.g. the contribution of D. Oesterhelt), is a typical example of a black box. We know that light goes in and a response in terms of a net change in direction of the bacterium is the result. However, we know very little of the molecular details of the reactions that couple these two processes. A lot of energy will have to be invested to resolve these processes. Fortunately, a black box can absorb various forms of radiation energy.

The topic of phototactic movement is complex, first of all because various forms of movement exist. Cells can migrate by (i) swimming, (ii) gliding and (iii) floatation. The latter process is predominantly regulated via the buoyancy of the cells, adapted for instance via (light-dependent) synthesis of polysaccharides or gas-vesicles. In addition, several non-motile phototrophic bacteria (particularly of the green-sulphur bacteria) associate with heterotrophic bacteria, leading to a conglomerate which displays phototactic respons-

es (Imhoff 1992). In the following we will limit the discussion to the first two forms of phototactic processes.

The aspect of the light-climate detected in a particular response also is quite heterogeneous: The direction, intensity or colour of the light may be detected. Furthermore, the cells may respond with (i) an in- or decrease in velocity of movement or (ii) a change in direction. A quite complex and detailed terminology to discriminate all these responses has evolved (for a review, see Häder 1987; Armitage 1988).

Photoreceptors involved in phototaxis

Several examples are available in which the pigments involved in the free energy transduction in photosynthesis are simultaneously functioning as the photoreceptors in phototaxis. This is particularly true for *photokinetic* effects. In several purple bacteria it has been shown that the cyclic electron transfer chain directly mediates (via the proton motive force) this photokinesis (e.g. Brown et al. 1993). In gliding motility in cyanobacteria this applies to *Nostoc* and *Phormidium*. Surprisingly, however, in both genera only part of the electron transport chain appears to be involved in this process: PSII for the former and PSI for the latter (for a review, see Häder 1987). These observations underline the importance of elucidation of the role of cyclic electron transfer in the free energy metabolism of cyanobacteria. Also the photophobic response in *Phormidium*, i.e. the reversal of movement upon a decrease in intensity of illumination, is mediated via the photosynthetic pigments. Transmembrane fluxes of protons and Ca^{++} ions are supposed to be intermediates in this response. The pigments involved in true phototactic orientation (i.e. the movement towards and away from a light source) in organisms like *Cylindrosporum* and *Phormidium* appear to be different from the photosynthesis machinery. These pigments, however, have only poorly been characterized (Häder 1987), although it is known that a red-light absorbing photoreceptor must be involved.

Several purple non-sulphur bacteria react upon a lowering of the light intensity with an reversal of the direction of movement or a reorientation (i.e. show photophobic taxis). *Rhodobacter sphaeroides*, through the work of Armitage and co-workers, is the best studied example of these. This organism shows tactic responses that deviate significantly from tactic responses in enterobacteria: (i) it is methylation independent; (ii) metabolism of the attractant is a prerequisite, (iii)

flagella of this organism do not alternate the direction of rotation but rather alternate between stops and rotation in one of the two possible directions (Packer & Armitage 1993) and (iv) attractants cause a significant photokinetic effect. The phototactic response in this organism makes use of the same signal transduction machinery as chemoattractants, except for the initial part: the photoreceptor. Evidence is quite firm that also in this response in *Rhodobacter* the photosynthesis machinery functions as the photoreceptor. The signal from this cyclic electron transfer system is most probably transferred to the flagella via intermediary metabolism. The effect of activation of this response system is accumulation of cells in a light spot. In this response, in addition, some, as yet unsolved form of adaptation has to be involved (Armitage 1992).

A bacterial rhodopsin-like photoactive protein

Recently, a new type of photoactive protein has been identified in a number of halophilic, purple phototrophic bacteria: the photoactive yellow proteins (PYP's). These proteins have a strong yellow colour ($\lambda_{max} = 446 \text{ nm}$; $\epsilon_{446} = 45000 \text{ M}^{-1}\text{cm}^{-1}$) and are photoactive: After absorbance of a blue photon the protein engages in a cyclic chain of dark reactions, resulting in the reformation of the yellow groundstate after approximately 1 second (Meyer et al. 1987). Until now, three representatives of this class of photoreceptors have been characterized: a PYP has been isolated from *Ectothiorhodospira halophila*, *Rhodospirillum salexigens* and *Chromatium salexigens* (Meyer 1985; Meyer et al. 1990). These three proteins display strong amino acid sequence homology (Van Beeumen et al. 1993; Hoff et al. submitted). Using a specific polyclonal antiserum against PYP from *E. halophila*, a cross-reacting protein has been detected in a large range of Bacteria, even in non-motile and non-phototrophic bacteria (Hoff et al. submitted).

E. halophila displays a new type of negative phototaxis with a wavelength dependence that correlates with the absorbance spectrum of PYP (Sprenger et al. 1993). This has led to the working hypothesis that PYP is the photoreceptor for negative phototaxis in this organism. However, molecular genetic proof for this hypothesis has not yet been obtained.

The photochemistry (Meyer et al. 1989; Meyer et al. 1991; Hoff et al. 1992; Miller et al. 1993) and crystal structure (McRee et al. 1989) of PYP have been described in some detail. The protein consists entirely of β -strands, forming a β -clam structure, well

known from a number of eukaryotic proteins and completely different from the 7 α -helix bundle found in the rhodopsins. In the reported 3-dimensional representation of the structure of PYP a retinal molecule was depicted by the two plates of β -sheet. Also the photochemical characteristics of PYP suggested that the protein contains retinal as a chromophore. Therefore, PYP was proposed to be the first bacterial retinal protein. However, the analysis of small peptide-chromophore complexes derived from PYP has unambiguously shown that retinal is *not* the chromophore of PYP. The structure of this photoactive cofactor is currently under investigation.

5. Light as a signal in the regulation of gene expression

Regulation of carotenoid and bacteriochlorophyll synthesis

Regulation of carotenoid and bacteriochlorophyll synthesis in purple non-sulphur bacteria, like *Rhodospirillum rubrum* is mediated – most likely – through the free energy status of the cell. Upon depletion of oxygen, and with sufficient electron donor present, pigment synthesis is initiated. The synthesis of these pigments is closely coupled to the synthesis of the polypeptides of the photosynthesis machinery. High light intensities repress synthesis of the LH_{II}-part of this machinery (for review, see e.g. Coomber et al. 1990). Apart from the photosynthetic pigments no evidence is available for the involvement of additional photoreceptors.

Regulation of carotenoid synthesis in *Myxococcus xanthus* is regulated via a complex pathway, involving at least three regulatory proteins and a hierarchical cascade, at the transcriptional level of the expression of the carotenoid-synthesizing enzymes. An intermediate of the heme-biosynthetic pathway (protoporphyrin IX) is supposed to be the photoreceptor in this response (McGowan et al. 1993).

Regulation in chromatic adaptation: A bacterial rhodopsin

In so-called complementary chromatic adaptation (CCA), cyanobacteria can change the pigment composition of their antennae (phycobilisomes, also see above) in order to optimally adapt to the prevalent light conditions. If green light prevails, the red coloured C-phycocyanin (PC) is synthesized; in orange or red

Fig. 6. Relief of the nicotine-inhibited synthesis of C-phycocyanin by the addition of retinal. Visible absorption spectra were recorded of cell suspensions of the cyanobacterium *Fremyella diplosiphon*, 60 h after actinic illumination had been switched from orange- to green light. A: control cells; B: cells treated with 150 μ M nicotine; C: cells treated with 150 μ M nicotine, 10 mM Dithiothreitol and 100 μ M retinal. Further details of these experiments will be presented elsewhere (Schubert et al. in preparation).

light the blue coloured C-phycocyanin (PC; Grossman et al. 1993). The genes involved in this adaptive response have been mapped and the information available on gene activation occurring in CCA (Ferdinand and Grossman 1990), including phosphorylation-dependent binding of activator protein in the promoter area of genes encoding antenna polypeptides (Sobczyk et al. 1993), suggests the involvement of a Two-component signal transduction system, for which a sensor protein has been identified via complementation analysis of CCA-deficient mutants (Chiang et al. 1992). The protein identified in this complementation, RcaC, shows similarities with other sensor proteins of Two-component family. The identity of the actual

light-sensing component, however, remains to be elucidated. The involvement of phytochrome, a major photosensing pigment in plants, in CCA is highly unlikely in view of the wavelengths involved; phytochromes until now have only been detected in eukaryotic (micro)organisms. Rhodopsins on the other hand would cover the relevant spectral region.

Recent work in our department has provided evidence, supporting the conclusion that a retinal-protein (i.e. a rhodopsin) is involved in light sensing in CCA (Schubert et al. in preparation). First, the presence of retinal in the cyanobacterium *Calothrix* sp. could be demonstrated. Second, inhibition of retinal synthesis with nicotine (an inhibitor of β -carotene synthesis, the direct precursor of retinal) caused full inhibition of PE synthesis in *Calothrix* upon transfer from orange- to green light. Third, the inhibition of chromatic adaptation by nicotine could be reversed by exogenous retinal (Fig. 6). These results provide the first evidence that rhodopsins may also be functioning in the Kingdom of the Bacteria.

6. Conclusions

The basic mechanism of energy conservation in oxygenic and anoxygenic photosynthesis has been resolved and the detailed structure of many of the pigment/protein complexes involved is being elucidated, following the break-through in the increase in our insight in the structure of reaction centers of purple bacteria *via* the resolution of their crystal structure (Deisenhofer et al. 1984; Allen et al. 1987a,b).

Of only a limited number of photoreceptors involved in photosignalling, information of comparable detail is available. Photoreceptors have best been characterized in the Archaea, i.e. the archaeal rhodopsins, be it that information on the photoactive yellow protein is rapidly accumulating too (see Section 4).

The mechanism of many light-dependent processes in Bacteria, responsible for the response to a change in light quality and/or light quantity remains to be uncovered (e.g. McGowan et al. 1993). The use of action spectroscopy, in combination with the molecular genetic analysis of mutants impaired in a particular response (Ahmad & Cashmore 1993), provides a powerful strategy to tackle these remaining problems.

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