OXYGENIC PHOTOAUTOTROPHS: PHOSPHORYLATION AND OLIGOMERIC STATE OF PHOTOSYSTEM 2. RESPONSES TO STRESS. A REVIEW

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Dedicated to the 50th anniversary of the foundation of the Department of Biochemistry, the first biochemical department in Czechoslovakia.

A review is presented on some aspects of metabolism and supramolecular structures typical of oxygenic photoautotrophs, in particular the higher plants: (i) The problem of photosystem 2 (PS2) heterogeneity, namely the PS2 core-protein phosphorylation and PS2 oligomeric state, are discussed. There are at least four proteins reversibly phosphorylated in the PS2 core. The phosphorylation is light-dependent and its exact function is unclear. The PS2 dimer, usually considered the native state, may not be the only form occurring *in vivo*. (ii) The reaction of plants to various stress factors is described. Heat shock, reactive oxygen species and toxic metal exposition have been chosen as stressor examples, since the response of plants to them is specific and different from animals. A review with 182 references.
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1. PHOTOSYSTEM 2

1.1. Introduction

The vast majority of the organisms living on Earth are energetically dependent on solar radiation (hereinafter denominated "light", even though not all of the visible wavelengths are able to drive photosynthesis and not all of the photosynthetically active radiation is in the visible range). However, only some of them are able to convert the energy of light into the chemical energy of reduced organic compounds. Such organisms are called phototrophs and the process of light utilization is called photosynthesis. Two basic types of photosynthesis are known at present: rhodopsin-based photosynthesis and chlorophyll-based photosynthesis.

The first type is known as the rhodopsin photosynthesis. The photosynthetic apparatus consists of a retinal-protein light-driven proton pump and an ATP synthase able to utilize the resulting cross-membrane H^+ concentration gradient.

Much more important, however, is the chlorophyll-based photosynthesis. It occurs in a wide variety of organisms, from photosynthetic bacteria to higher plants. Among these, of an outstanding ecological importance are the oxygenic photoautotrophs, i.e. plants, algae, cyanobacteria and oxyphotobacteria (prochlorophyta). These organisms are the exclusive oxygen suppliers to the atmosphere and their ancient ancestors (probably some types of cyanobacteria) have built-up today's oxygenic atmosphere.

Chlorophyll-based photosynthesis is a complex set of biophysical and biochemical pathways which can be divided into two main parts:

– The "light part", localized in a membrane, converting the energy of photons into the chemical energy of ATP or NAD(P)H and ATP.

– The "dark part", localized in the chloroplast stroma in eukaryotes or in cytosol in prokaryotes, utilizing the outputs of the light part in other metabolic processes, most importantly (and most frequently) for CO_2 fixation in the case of photoautotrophs. This part is not directly dependent on light energy.

This review deals exclusively with the light part of photosynthesis which itself is a complex process. In principle, it consists in an electron transport chain similar to that of mitochondrial respiration, leading to formation of a concentration difference of H^+ (proton concentration gradient) across the membrane. The membrane contains an H^+-driven ATP synthase, similar again to that of mitochondria, utilizing the "protonmotive force" of this concentration difference to synthesize ATP from ADP and inorganic phos-

phate. The electron transport chain of oxygenic phototrophs takes place in the membrane of thylakoids. These are membrane vesicles localized in chloroplast stroma of eukaryotic cells or in the cytoplasm of prokaryotic cells. The space enclosed in thylakoids is called lumen.

The overall electron transport chain of oxygenic photoautotrophs transfers electrons from water to the oxidized coenzyme $NAD(P)^+$ to form oxygen and reduced coenzyme $NAD(P)H + H^+$, i.e. just the opposite compared with mitochondria. However, the main "H^+-gradient-forming engines" of both respiration and photosynthesis are very similar: a complex of cytochromes and Fe-S-proteins receiving electrons from a quinol (two-electron and two-proton membrane-soluble carrier, usually ubiquinone in respiration and plastoquinone in photosynthesis) and passing them to a single-electron carrier (a soluble *c*-type cytochrome or plastocyanin). It is important at this point, that the quinone receives the electrons on one side of the membrane (where it acquires protons subsequently from the environment) while they are delivered to the cytochrome complex on the other side of the membrane, where the protons are released, as the next carrier is only able to accept an electron. Proton transport across the membrane is further enhanced by the Q-cycle, passing one of every two electrons back to the quinone[1–7]. On this level, the difference between photosynthesis and respiration is made by the driving force of the electron transport. In respiration, it is the high negative redox potential of organic compounds, easily reducing oxygen to water. In photosynthesis, it is the energy of photons allowing electrons to be transferred apparently against the redox potential gradient, oxidizing water and reducing CO_2 into organic compounds. The devices performing this little miracle are called photosystems and there are two types of them in oxygenic photoautotrophs: photosystem 1 (PS1) and photosystem 2 (PS2). The principle of their function is the same: a chlorophyll molecule that upon absorption of a photon becomes a strong reducing agent, with negative redox potential high enough to reduce the next electron acceptor in the above mentioned chain. On the other hand, the cation formed in this reduction has a potential positive enough to extract an electron from the appropriate donor, in the case of PS2 even from water.

PS1 and PS2 differ both in polypeptide composition and redox cofactors acting in the electron transport chain. Besides PS1 and PS2, there are other photosystems occurring in anoxygenic prokaryotic phototrophs, each of them belonging to one of the two classes (relative to PS1 or PS2). However, they all share certain homology in their core proteins, both in amino acid sequence and spatial arrangement. It is generally believed at present that photosystems have evolved from a unique common ancestor[8,9]. The

photosystems are usually connected to so-called antenna complexes. Their role is to absorb light and pass the excitation energy to the photosystem inner antennae (described later in this paper). Several types of antenna complexes exist in the wide spectrum of phototrophic organisms. Cyanobacteria typically use phycobilisomes[10], located on the thylakoid surface. A phycobilisome is a hemispheric hydrophilic body composed of a triangular core and six rods protruding radially outwards. Its basic building blocks are phycobiliprotein "monomers" grouped to trimeric discs, which are further stacked to hexameric units. The phycobilisome rods are cylinders of stacked hexamers. The core is composed of three hexameric units anchored to the membrane through a special "linker peptide". There are other "linkers" to keep together the core with the rods and yet other to link the individual hexameric units of the rods. The phycobilin "monomer" itself is composed of two subunits – α and β. These are polypeptides of the molecular weight (MW) of 17 and 18 kDa, respectively, containing the chromophores – phycobilins (linear tetrapyrroles covalently bound to the proteins). In higher plants, the antenna complexes are usually considered photosystem components. In this article, to reflect this usance, the term "PS supercomplex" will be used when dealing with a PS including the antennae. The term "PS core" will mean the PS complex without the antennae and without extrinsic hydrophillic components like the oxygen-evolving complex of PS2.

The antenna complexes of higher plants (called light-harvesting complexes, LHC) are trans-membrane hydrophobic proteins binding chlorophylls *a* and *b* (hereinafter Chl *a* and Chl *b*), encoded by nuclear *cab* gene family. They are polypeptides in the MW range 24–31 kDa. The structure of LHC proteins of both photosystems is highly homologous. The LHC of PS2 (LHC2) include the so-called "minor" (or "internal") PS2 antennae CP24, CP26 and CP29 and the "main" (or "external") LHC2 antennae (CP stands for chlorophyll-protein, the numbers represent the apparent molecular weights on SDS denaturating gel electrophoresis in kilodaltons). The former do not dissociate from the PS2 core complex *in vivo* and are thought to link it with the external antennae. The external antennae are trimers of the *Lhcb*1 and *Lhcb*2 gene products, proteins of MW about 25 kDa, each containing eight Chl *a*, seven Chl *b* and two lutein molecules[11–13]. They contain nearly 70% of the chlorophyll connected with PS2. The structure of LHC2 has been resolved by electron crystallography at 3.4 Å[14]. The LHC2 complex plays another important role in regulation of light-energy distribution between the two photosystems. It is reversibly phosphorylated/dephosphorylated by protein kinases/phosphatases depending on the redox state of

membrane plastoquinone pool. In the dephosphorylated state, it is attached to PS2, supplying there the excitation energy. Over-reduction of PQ pool activates a kinase phosphorylating LHC2, which is released from PS2 upon phosphorylation and associates even with PS1 to balance the energy income of the photosystems. PQ pool reoxidation stops kinase activation and phosphatases dephosphorylate LHC2 which then returns to PS2 [15].

1.2. Photosystem 2

Photosystem 2 (PS2) is a large membrane pigment-protein complex playing the role of light-driven water:plastoquinone oxidoreductase. PS2, due to its high redox potential, is the crucial improvement of the photosynthetic machinery that equipped its possessors with the ability to oxidize water and made it possible for the oxygenic photosynthesis to evolve. It is interesting to note that PS2, due to its sensitivity to some herbicides, is also a suitable sensor for monitoring these environmental pollutants[16,17]. It consists of three main parts, both from functional and structural points of view: oxygen-evolving complex (OEC), PS2 core and the light-harvesting antenna described above. For a general review, see[18–24].

1.3. Photosystem 2 Core Complex

The PS2 core complex constitutes the central part of PS2. Compared with the PS2 supercomplex, it does not include the LHC2 antennae and the oxygen-evolving complex polypeptides. In the center of the core complex, there is a heterodimer of polypeptides D1 and D2 (Fig. 1). These proteins are highly conserved in all the taxa of oxygenic photosynthetic organisms, especially in the amino acid residues involved in binding cofactors. Their MW is 32 and 34 kDa, respectively, and they contain five transmembrane helices each[25–27]. They carry all the redox components of the electron transport chain in PS2. These redox cofactors are:

– P_{680}, located on the D1/D2 heterodimer near the lumenal membrane surface. It is the primary electron donor after excitation with a light quantum. It was assumed to be a special chlorophyll *a* pair, because of PS2 homology with the purple-bacterial reaction centre[28]. According to X-ray structural model of PS2 [29], however, it is likely that P_{680} consists of four Chl *a* molecules[30,31], in Fig. 1 shown as P_{680} (the "original" pair) and $Chl_{D1,D2}$.

– A pheophytin molecule on the D1 protein, the primary electron acceptor from P_{680}.

– The tightly bound plastoquinone Q_A on D2 and the loosely bound plastoquinone Q_B on D1 protein. In the respective order, they are the subsequent acceptors after the D1 pheophytin. Q_B serves as the so-called two-electron gate, being tightly bound when in the semiquinone form and exchanging readily with the membrane plastoquinone pool in fully oxidized and fully reduced states.

– Two molecules of β-carotene (not shown in Fig. 1), scavengers of singlet oxygen[32], which is produced if the redox components acting after pheophytin are blocked. At least one of them, located on the D2 protein, close to cyt b_{559}, participates in the cyclic electron flow around PS2 [33–35]. In addition, the carotenes play an important structural role and they might even be important in the D1 turnover (mentioned below), its oxidation being the signal for D1 degradation[32,35].

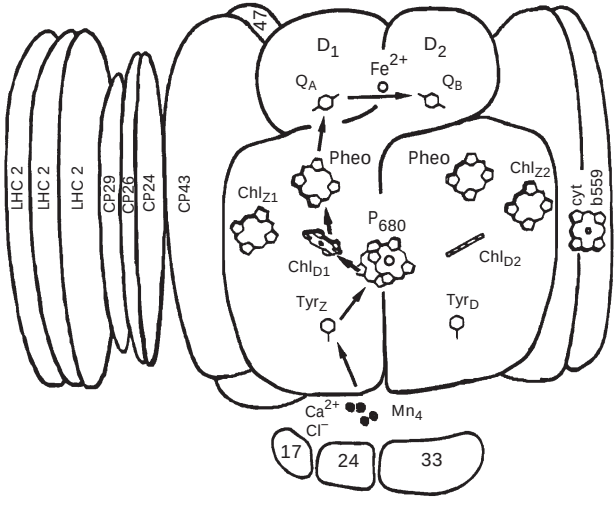

FIG. 1

A scheme of photosystem 2 central heterodimer with the redox cofactors. PS2 redox cofactors: P_{680}, the reaction center chlorophyll *a* pair, formerly identified with the primary donor; Chl_{D1}, Chl_{D2}, the chlorophyll *a* pair newly ascribed to P_{680}; Chl_{Z1}, Chl_{Z2}, accessory chlorophyll *a* molecules; Tyr_Z and Tyr_D, redox-active tyrosine Z and its counterpart on D2 protein; Pheo, pheophytin; Q_A and Q_B, secondary quinone acceptors of PS2; Fe, non-heme iron. PS2 subunits: D1, D2, components of the central heterodimer; CP43 and 47, the inner antennae; CP24, CP26, CP29, the minor light-harvesting antennae; LHC2, the main light-harvesting complex; 17, 24, 33, polypeptides of the oxygen-evolving complex of corresponding MW. The positions of PS2 subunits are schematic. Adapted from[10]

– Two accessory chlorophylls *a*, one on each of the two proteins (Chl_Z in Fig. 1). Their function probably is to route the excitons from the inner antennae to P_{680} and participate in the protective cyclic electron transfer around PS2, together with cyt b_{559} and a β-carotene molecule of the reaction centre[33,34].

– Tyr residue in position 161 of D1 (Tyr_Z), supplying electrons to P_{680}^+ from OEC.

– The manganese cluster of OEC, extracting electrons from water and passing them to P_{680}^+ via Tyr_Z. It binds to the lumenal surface of the D1 protein.

Each of the D1 components has its counterpart on the D2 protein, so that the arrangement of these components is nearly symmetric. However, they function in a very asymmetric way. Up to the quinones, the D2 branch is inactive.

Cytochrome b_{559} is another PS2 component. It is a heterodimer of two subunits (α and β) of MW 9 and 4.5 kDa, respectively. They have one transmembrane helix each and they are connected by means of the heme, ligated by one histidine from each subunit. There was no known function for the cytochrome besides the structural one for a long time, only a long-lasting suspicion that it might participate in the cyclic electron flow around PS2 in case of insufficient electron supply from water, as mentioned above. The suspicion becomes proved enough to accept it as a fact[32,35,36]. Cyt b_{559} is required for assembly of functional PS2 [37].

The D1/D2 heterodimer together with the cytochrome b_{559} and with the *psb*I gene product (a protein with one transmembrane helix and an unknown function) form the reaction centre complex, the smallest PS2 subset capable of stable charge separation. The D1 protein undergoes rapid turnover cycle, so being a probable important point of PS2 activity regulation[38].

The core complex further contains the so-called inner antennae – the polypeptides CP43 and CP47. These are hydrophobic proteins with six transmembrane helices[39,40], containing together 25–50 Chl *a* molecules[19]. In addition to the role of exciton transducers between LHC and the reaction centre, they have an important structural role. Interestingly, it has been found recently that a protein similar to CP43, called CP43′, functions as a PS1 antenna in some cyanobacteria under iron deficiency[41–45]. Another protein homologous to CP43, binding Chl *a* and Chl *b* plays the role of light-harvesting antenna in oxyphotobacteria, both of PS1 [46] and PS2 [47]. These findings have led to the suggestion that all the chlorophyll-binding proteins in phototrophic organisms evolved from a common ancestor[48].

There are about ten small, one-helix core subunits with MW less than 10 kDa. Their function and exact localization is usually not well known. The subunit psbH is required to stabilize PS2 complex and to maintain electron transport ability between the quinones Q_A and Q_B. It is reversibly phosphorylated, suggesting a regulatory role. It is supposed, as well as psbX subunit, to reside close to cyt b_{559} [49]. The psbS polypeptide is necessary for dissipative photoprotection of LHC complex, but it is associated rather with the PS2 core[50,51]. For the PS2 supercomplex structure, psbW subunit is of great importance. This product of a nuclear gene has been found to be necessary for dimerization of PS2 [52]. Another study shows that it can be detected only in dimeric PS2 [51].

1.4. Oxygen-Evolving Complex

The oxygen-evolving complex (OEC) is composed of three peripheral membrane proteins on the lumenal side of PS2 with MW of 33, 24 and 17 kDa and it is responsible for maintaining optimal conditions for the photosynthetic water oxidation and oxygen evolution carried out by the manganese cluster mentioned above. The cluster is able to bind two water molecules and to extract sequentially four electrons from them. The 33 kDa protein stabilizes the Mn cluster, while the other two proteins (not present in cyanobacteria) help to create a microenvironment with high effective concentration of Ca^{2+} and Cl^-. Both these ions are required for water oxidation and removal of the 24 and 17 kDa proteins increases significantly the Ca^{2+} and Cl^- concentrations needed for the maximum oxygen evolution rate[19,53].

1.5. Photosystem 2 Heterogeneity

PS2 is never completely homogeneous, it is always found in several different populations[54]. Its heterogeneity has been observed in several aspects:

1. PS2 α and PS2 β [55,56] differing in the rate of Q_A reduction and in the occurrence in stacked and non-stacked regions of the membrane, respectively.

2. Mathematical deconvolution of time dependence of the chlorophyll fluorescence induction curve complementary area in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) into several kinetic phases[57–59].

3. Disability of a certain fraction of PS2 to reduce the Q_B acceptor (non-B centers)[60,61].

4. By means of isoelectrofocusing of PS2 particles from spinach (BBY particles)[62] solubilized with a non-ionic detergent dodecyl β-D-maltoside, it

is possible to isolate four populations of PS2 core differing in total phosphorylation level[63,64]. Other differences between them are different affinities to photosynthetic herbicides[64–66], different electron transport activities[67] and different sensitivities to photoinhibitory conditions[67].

5. PS2 possibly occurs in two oligomeric forms: as a dimer and as a monomer.

1.6. Photosystem 2 Core Phosphorylation

Reversible protein phosphorylation has been known for a long time to be one of the most important regulatory mechanisms in animal cells[68]. During the last two decades, it has become clear that this mechanism is much more ubiquitous. The early studies of protein phosphorylation in plant cells dealt with phenomena having an analogy in animals, like ribosomal and nuclear protein phosphorylation[69]. The first known example of plant regulatory protein phosphorylation without any parallel in animals is the phosphorylation of the light-harvesting complex LHC2, described above in this review. Since that time, this item has become a textbook example of a plant metabolic process regulated by reversible protein phosphorylation.

Much less clear, however, is the role of phosphorylation of PS2 core proteins. Of these, D1, D2, CP43 and *psb*H are reversibly phosphorylated. It has been suggested that PS2 core-protein phosphorylation might be a mechanism of electron transfer regulation. It reduces the ability of quinones and other electron acceptors to bind to the Q_B site and, as a result, these PS2 centers become less efficient in electron transfer[65,70]. It has also been shown that phosphorylation of D1 protein is a light-dependent step in the process of its degradation and turnover[71,72]. Some papers also suggest that, under photoinhibitory conditions, it could be a signal to avoid D1 depletion from the membrane[73] and protect against strong illumination, as the stuctural changes induced by phosphorylation could facilitate the formation of dissipative PSII centres, inactive in electron transport[74]. It has been observed that under stress conditions (drought, excessive light), both the level of phosphorylation and the turnover rate of the D1 protein change[75]. The idea that phosphorylation and D1 turnover are strongly correlated is also supported by the hypothesis that phosphorylation could be responsible for the regulation of gene expression in higher plants[76]. Recent results indicate, however, that D1 phosphorylation is not the immediate cause of PS2 activity change[77]. Moreover, another factor controlling D1 phosphorylation seems to be the circadian rhytmicity[78].

The phosphorylation of CP43 has been shown to be dependent on light, too. The protein is exposed to kinases by a light-induced conformational change[79]. Some of the other types of PS2 heterogeneity may be correlated with the core phosphorylation[75,80].

1.7. Oligomeric State of Photosystem 2

A frequently discussed feature of PS2 is its oligomeric state *in vivo*[21,81,82].

There are two main approaches to address the problem. X-ray crystallography had not led to sufficient data due to low resolution of three-dimensional PS2 crystals diffraction[83,84]. Only recently, high-resolution X-ray studies of PS2 complex from cyanobacteria have been successful[29,85,86].

The other approach to study PS2 oligomerization is electron microscopy (EM). The earliest attempt to elucidate the thylakoid membrane structure by EM dates back to 1975 and it reveals the basic shape and order of the "particles" visible on the membrane surface[87]. Rögner et al.[88] used EM to study solubilized cyanobacterial PS2 by means of the single-particle image-averaging procedure. They observed monomeric and dimeric particles and established their MW to 300 and 500 kDa, respectively, by size exclusion gel chromatography. Since that time many other reports have been published dealing with EM of solubilized PS2 complex[89–97], suggesting its dimeric nature, with the exception of Haag et al.[94]

Another way of EM utilization in PS2 structural studies is analysis of its two-dimensional crystals. These are analyzed again by the single-particle (or unit-cell) image-averaging method. The results of this technique are contradictory. The teams working in this field can be divided into two groups: "dimerists" and "monomerists". The former mostly use 2-D crystals grown from detergent-solubilized PS2 complexes[98–104], the latter[105–109] work with crystals occurring in situ in PS2-enriched membrane particles obtained by mild detergent treatment, such as the famous BBY particles[62] or with the above-mentioned reconstituted crystals[110]. It has been proposed that dimeric PS2 might be artificially induced by a detergent during the isolation procedure or in the course of crystallization[111].

Several other reports support the dimeric nature of PS2 exploiting size-exclusion gel chromatography[88,112], sucrose density gradient centrifugation[52] or analysis of the fluorescence induction curve of DCMU-poisoned leaves grown in intermittent light[113]. Recently, it has been suggested that the PS2 oligomeric state may differ in the stacked and unstacked regions of the chloroplast thylakoid membrane[114].

To make an account of the current knowledge of this topic, it is necessary to consider that all the experiments attempting to resolve the monomer versus dimer question are based on PS2 preparations more or less detergent-treated, probably none of them deserving the denomination "native".

A method allowing determination of functional MW of a protein complex under native conditions is radiation target analysis (RTA). According to the target theory[115] (cited according to[116]), the biological activity of a protein irradiated with high-energy radiation decreases exponentially with the radiation dose. RTA is a method allowing determination of functional MW (the minimal size of a protein or a multisubunit protein complex needed to maintain its biological activity) of proteins exploiting the theoretical background of the target theory[117,118]. It is based on the drastic changes in protein molecular structure caused by ionizing radiation of sufficient energy (1 MeV or more), the decrease of biological activity being its consequence. The rate of this decrease depends on the size of protein molecules. The larger the molecule is, the higher is the probability of a hit by a high-energy radiation particle at a given radiation intensity, which also means a more rapid decrease of the biological activity. A semi-empirical equation[119] is used to calculate MW. The main advantage of this method is the fact that it is not necessary to purify the protein of interest, it is sufficient to obtain any preparation in which it is possible to measure its activity.

The RTA method has been used with PS2 several times. Takahashi and Asada[120,121] attempted to estimate the size of binding sites for manganese in PS2 and the size for binding the 33 kDa protein of OEC. Other authors used RTA to measure the molecular weight of PS2 [122–126]. They typically found values in the range 88–175 kDa for the water oxidation. Hsu et al.[122] used the chlorophyll fluorescence kinetics as the studied activity and found a MW of 266 kDa for PS2 α and 135 kDa for PS2 β. The former value corresponds approximately to MW usually reported for solubilized monomeric PS2 by gel chromatography[88], the latter is near to other results[127,128] obtained by RTA with PS2 particles (BBY)[62] isolated from light-adapted plants as well as to the results cited above. It has been suggested[128] that light adaptation decreases the functional MW of PS2 for most of its activities, both for thylakoids and for PS2 particles. Light is known to change the PS2 core phosphorylation state and phosphorylation is generally believed to participate in various forms of heterogeneity in PS2 activities and physical properties (see above). The RTA results support the idea of monomeric PS2 in thylakoid membrane. Unfortunately, RTA is not able to distinguish between native monomers and dimers "half-destroyed" by the radiation.

Thus, this finding is valid only from the "functional" point of view, i.e., meaning that each PS2 monomeric complex functions independently. An aggregation with a regulatory role, as suggested e.g. in[114,129], can hardly be excluded by means of this technique. As newer structural works show, PS2 is regularly found in the dimeric form. The dimer has always practically the same structure, which is true both for the core-complex from cyanobacteria[130,131] and the supercomplex from higher plants[91,96]. It is necessary to refer here to the newly described (and already mentioned) PS2 supercomplex from Oxyphotobacteria[47], where the PS2 dimer is associated with ten molecules of a Chl *a/b* binding antenna protein homologous with CP43. Another important finding is the existence of a PS2 subunit, occurring only in dimeric form, the psbW polypeptide[51,52]. This subunit might be a linker between the two halves of the dimeric PS2. All these results support the idea that PS2 is dimeric *in vivo*, even though the presence of its monomers is still possible depending on the membrane location or some regulatory influences.

2. HOW PLANTS RESPOND TO STRESS

2.1. Definition of Stress

The notion of stress was introduced into scientific research in order to allow the physiological response of organisms to extraordinary impacts from the inner and outer environment to be investigated and explained. Accordingly, Larcher[132] defined the effect of stress on plants as the exposure of plants to extremely unfavorable conditions. Thus, the life of the plant need not necessarily be endangered, but stress alerts the alarm response in the plant organism, i.e. its defense and adaptation reactions[133]. In many cases, a mild influence of stressors has a general stimulating effect and only their stronger influence will inhibit various metabolic processes.

As to the duration of the influence of stressors, three basic time scales can be delineated[134]:

a) Short-term responses, which occur within minutes of an environmental change and typically involve components which already exist in the organism. Short-term responses are generally reversible.

b) Long-term responses, which can begin within one hour, but usually are pronounced in a period of days or weeks after an environmental change. These responses typically involve altered patterns of gene expression and, in some cases, morphological changes. The responses are not immediately reversible and often lead to the development of a visually differ-

ent phenotype. Long-term responses represent acclimation if they provide advantages for the organism in the altered environment.

c) Adaptive responses. Evolutionary changes in genotypes may take place over many generations of a population, adapting the population to a modified environment.

2.2. Plants and Stress

This review is chiefly oriented to plants. Other photoautotrophs widely encountered in nature, e.g. cyanobacteria and photosynthetic bacteria will be more or less omitted in the following parts.

Higher plants are truly terrestrial organisms. By a series of adaptations, they have evolved to become the dominant life form on our planet, most other life forms being dependent on them.

With a wide variety of habitats and, differing from animals by lacking the ability to move, plants are exposed to biotic stresses due to the activities of various pathogens, feeding organisms, a multitude of environmental stresses including drought, salinity, extremes of temperature, solar radiation and further various abiotic stresses due to human activity. Plants are remarkably capable of surviving the effect of these stresses in numbers sufficient to complete their life cycles. Such stresses have created a strong selective pressure, resulting in the molecular, biochemical and physiological adaptations that have enabled them to survive under such adverse conditions.

In the following, we will only deal with those stressors and their responses in the plant organism, which are rather general not only among plants (for example, temperature stress and/or the heat shock, oxidative stress and the influence of heavy metals on the photosynthetic apparatus of photoautotrophs). The latter topic as well as other stress factors and their response is being investigated at several institutions of this country[135–141] including Department of Biochemistry, Faculty of Science, Charles University[135–138]. Readers interested in more detailed information are referred to the relevant literature[142–147].

2.3. Heat Shock

The heat shock response is a conserved reaction of cells to environmental stresses which is characterized by a rapid induction of the synthesis of heat-shock proteins (HSPs) and acquisition of thermotolerance[148]. Several classes of HSPs have been described in eukaryotes, including plants.

A comparison of the major HSPs in different organisms has shown that in general, they are highly homologous among eukaryotes. Similarly, homologous proteins were found in most prokaryotes. This indicates that HSPs play extremely important and common roles in all, or at least many life forms.

In addition to heat, however, other influences also lead to elevated expression of HSPs. It is interesting to note that, in different organisms, the expression of HSPs has been shown to be also affected by numerous chemicals, plant hormones, several heavy metals, some forms of physical treatment, anaerobiosis, high concentrations of salts and even low-temperature stress[149].

Different HSPs may have different functional properties, but they are all capable of interacting with other proteins and to act as molecular chaperons[149].

Molecular chaperons were originally defined by their ability to recognize and bind proteins that are kept, after proteosynthesis, in the inactive state, competent for activation by proper folding. Apparently, however, the chaperon activity is required by all living cells throughout their lifetime. Massive induction as a response to temperature stress indicates a much higher demand, probably resulting from an increase in protein denaturation, i.e. danger to the cell. Consequently, the cellular chaperon pool has to be replenished after stress.

Expression of HSPs appears to be regulated at the transcriptional levels, depending on one or more heat-shock transcriptional factors (HSFs).

The control of HSF activity as well as the multiplicity and biological role of the different HSFs not only in plants are still not understood and continue to be a subject of scientific interest (Fig. 2).

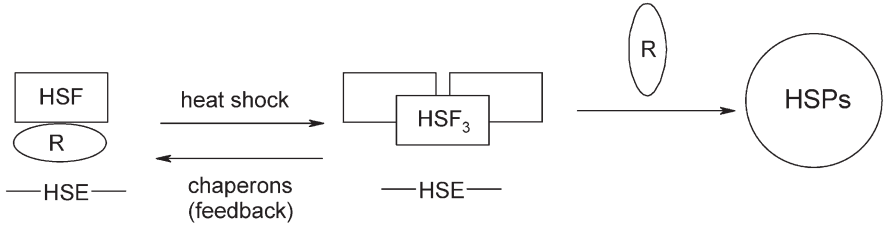

FIG. 2

Model of the mechanism of de-repression of HSF activity. Feedback regulation: repression of HSF trimerization (HSF_3) and DNA binding by transacting negative regulators (chaperon R) at normal temperature and de-repression upon heat stress via dissociation of R from HSF. HSF, heat-shock transcription factor; R, "repressor" chaperone; HSPs, heat-shock proteins; HSE, heat-shock promoter element (HSF binding sequence). Adapted from[148]

2.4. Reactive Oxygen Species

Under the influence of various environmental stresses, plants produce a family of reactive oxygen species (ROS) including singlet oxygen ($^1O_2$), superoxide (O_2^-) hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$)[150–154]. These reactive molecules are interconvertible and capable of damaging membranes, proteins and nucleic acids. These reactive oxygen species can form when plants are exposed to radical-forming air pollutants including ozone, SO_2, some halogenated hydrocarbons, NO and NO_2. Moreover, active oxygen can be formed when environmental stresses cause overreduction of chloroplast or mitochondrial electron transport chain. Chloroplasts, for example, produce active oxygen under conditions of high illumination and low temperature (the effect of spring frost on conifers)[150–152]. Low temperature also seems to limit the activity of the cytochrome part of the mitochondrial electron transport chain, and may result in formation of H_2O_2.

Among of the best known scavengers of singlet oxygen are doubtless carotenoids, particularly β-carotene. Out of the other antioxidants, let us mention at least enzymes of the superoxide dismutase (E.C. 1.15.1.1) type, ascorbate peroxidase (E.C. 1.11.1.11) or glutathione reductase (E.C. 1.6.4.2). We will discuss glutathione (GSH) in more detail, because it is an important plant stress-mitigating agent.

GSH is a tripeptide (γ-glutamylcysteinylglycine). Like their animal and microbial counterparts, plants have evolved to rely on unique properties of this substance to protect them from a wide spectrum of environmental stresses. The ability of GSH to protect plants against stress derives from the two chemical properties of the thiol group of cysteine. It can be oxidized and thus provides a source of reducing equivalents to buffer the plant from a number of oxidative stresses. In addition, the chemical reactivity of the thiol allows glutathione to complex with a range of organic and inorganic chemicals, protecting plants against their potentially toxic effects.

GSH is also involved in quenching free radicals[153–155]. The reaction involves the ascorbate/GSH cycle, where ascorbate is the ultimate electron donor for reduction of, e.g., H_2O_2 to water and GSH is an intermediate electron carrier (Fig. 3) where GSH reductase as well as ascorbate peroxidase and superoxide dismutase mentioned above, are also involved.

One of the probably most fascinating aspects of the protection afforded by GSH to plants is its ability to confer moderate levels of resistance to heavy metals (for a detailed description, see Chapter 2.5.), particularly cadmium and copper.

In this sense, glutathione can be rated among phytochelatins.

We have indicated in the preceding text and demonstrated by the example of GSH that individual stressors do not act separately, that instead they are interlinked and mutually bound in the response of the respective organism, cell or subcellular particle (Fig. 4).

According to the diagram in Fig. 4, metal uptake for example would have caused a decrease in the GSH level. This could lead to a higher level of reactive oxygen species and trigger the synthesis of signal molecules. One or several of these factors may trigger transcription of the genes for GSH synthesis.

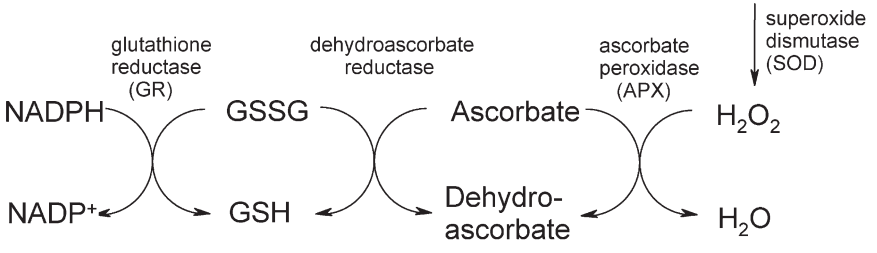

FIG. 3

The ascorbate/glutathione cycle for quenching reactive oxygen species. GSH, glutathione; GSSG, glutathione disulfide. Adapted from[156]

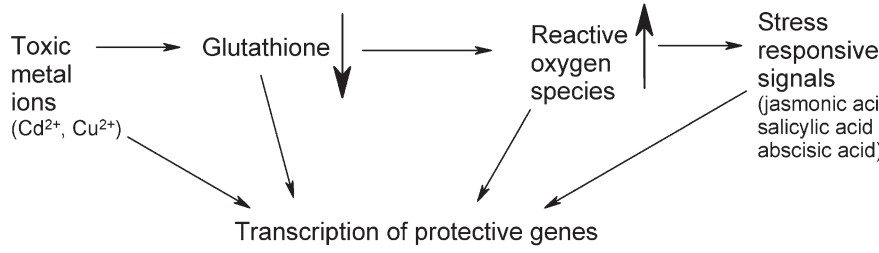

FIG. 4

A model of the signal transduction pathway by which plants respond to cadmium, copper and other toxic ions. Metal uptake brings about a decrease in glutathione level which may result in an increase in reactive oxygen species and start the synthesis of signal molecules. One or more of these factors may trigger transcription of the protective genes. Adapted from[156]

2.5. Heavy Metals

Much attention is at present devoted to problems linked with heavy metals, since occurrence of these metals in the environment has increased with industrial development. Heavy metals can be defined in general as metals of density greater than 5 g/cm^3. Quite a number of metals conform to this definition. Highly toxic metals (e.g. Cd, Hg, Pb, As) are to be found among them, as well as metals which are essential to many organisms in low concentrations (e.g. Cu, Mn, Co, Zn).

Elevated concentrations of toxic metals in the environment are a grave problem at present. Toxic metals are accumulated in different components of the environment, particularly in the vicinity of large agricultural and industrial centers. The elevated concentrations of toxic metal ions in water and soil are the reason for which they are taken up by root systems, accumulate in various parts of plants[157] and migrate along the food chain into animal organisms and human tissues. Under normal conditions, up to 75% of cadmium ions enter plant cells through the root systems, which thus probably are the first site of the negative influence of these ions[158,159].

Toxic metal ions can react with the photosynthetic apparatus – responsible for the basic metabolic process of photoautotrophs – on different levels, i.e. metals can accumulate in the main photosynthetic organ, the leaf, they can interact with cytosolic enzymes and organic compounds; even changes in the function of chloroplast membranes are possible as, e.g., Cd ions also trigger changes in the lipid composition of thylakoid membranes and finally, the function of supramolecular complexes of the individual photosystems is also influenced[160].

Cadmium ions also exert a negative influence on light-harvesting pigments, particularly chlorophyll. Stobart et al.[161] and Böddi et al.[162] found that Cd^{2+} inhibits the biosynthesis of chlorophylls by reaction with the thiol groups of enzymes of δ-aminolevulinic acid and protochlorophyllide synthesis. It was furthermore established that the central magnesium atom of tile chlorophyll molecule can be substituted by a heavy metal atom (Hg, Cd, Cu, Ni, Zn and Pb). Pigment molecules thus substituted cannot harvest solar radiation effectively any more[163].

The multiprotein complex photosystem II is localized in the thylakoid membrane. From numerous studies follows that a wide range of toxic metals exert a negative influence on electron transport, particularly in the region around PS II [164–167].

However, two key enzymes of photosynthetic CO_2 fixation appear to be the chief target of the effect of toxic metals: ribulose-l,5-bisphosphate

carboxylase/oxygenase (RuBisCO) in the case of the so-called C3-plants (e.g. wheat, other cereals, spinach) and phosphoenolpyruvate carboxylase (PEPC) in C4-plants (e.g. maize, sugar cane), equally *in vivo* and *in vitro*. Furthermore, high Cd^{2+} concentrations can also lead to irreversible dissociation of small and large subunits of RuBisCO, with the consequence of total inhibition of the enzyme[168,169].

Higher plants developed several mechanisms to protect themselves against the effect of toxic metals[170,171]:

1. Immobilization, a mechanism which is chiefly active in root systems, where metal toxic ions can bind to cell walls and some extracellular saccharides[172–174].

2. Formation of phytochelatins (plant metallothioneins, (γ-glutamylcysteinyl)$_n$glycine, n = 2 to 11), i.e., specific polypeptides which, due to the presence of SH groups in their structure, are able to chelate toxic ions and thus keep them from circulating in cytosol[175]. Phytochelatins are synthesized from glutathione. In the presence of toxic metal ions their synthesis begins within a few minutes and proceeds as long as the metal ions are available[176]. The production of phytochelatins is a widespread mechanism of toxic metal detoxication in higher plants[177–179].

3. Compartmentation, involving transport of toxic ions into a vacuole, which again hinders their free circulation in the cytosol.

4. Production of stress proteins, discussed above (Chapter 2.3.).

2.6. Phytoremediation

The widespread contamination of the environment caused by human activities over many centuries now makes it essential to choose among leaving the main problems and degraded environment to the following generations, carrying out enormous decontamination programs, or looking for alternative decontamination pathways. Plants are not yet being used, to any large degree, to remove organic and inorganic pollutants from the environment, although there are numerous possibilities in this respect. Plants are able to concentrate metals in their roots and shoots in levels far higher than in either soil or water which surrounds them[133]. The importance of metal accumulation by plants for decontamination of the environment is now coming to be appreciated, and a new technology called phytoremediation is evolving[180]. This is a new and promising approach to the difficult problem of remediating heavy-metal polluted soil and water. Plants capable of hyperaccumulating metals are essential if phytoremediation is to succeed in environmental and economic terms. These are

so-called metal hyperaccumulators or hyperaccumulator plants, of which about 400 taxa from 35 families of angiosperms have been described so far[181]. Thus, in addition to the interesting question of the biochemical mechanism of metal tolerance, metal hyperaccumulators are attracting increasing attention because of their potential application in decontamination of the environment[182].

Phytoremediation, although still in its infancy, may one day become an established environmental clean-up technology[180], as it is also an aesthetically pleasing technology and achieves high public acceptability.

3. SYMBOLS

Chl	chlorophyll
CP	chlorophyll-protein
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea (Diuron)
GSH	glutathione
HSE	heat-shock promotor element
HSF	heat-shock transcriptional factor
HSP	heat-shock protein
LHC	light-harvesting complex
LHC2	light-harvesting complex of PS2
MW	molecular weight
OEC	oxygen-evolving complex
PQ	plastoquinone
PS	photosystem
PS1	photosystem 1
PS2	photosystem 2
RTA	radiation target analysis

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