REVIEWS

Too much of a good thing: light can be bad for photosynthesis

James Barber and Bertil Andersson

Even though light is the ultimate substrate for photosynthetic energy conversion, it can also harm plants. This toxicity is targeted to the water-splitting photosystem II and leads to damage and degradation of the reaction centre D_1-polypeptide. The degradation of this very important protein appears to be a direct consequence of photosystem II chemistry involving highly oxidizing radicals and toxic oxygen species. The frequency of this damage is relatively low under normal conditions but becomes a significant problem for the plant with increasing light intensity, especially when combined with other environmental stress factors. However, the plant survives this photoinhibition through an efficient repair system which involves an autoproteolytic activity of the photosystem II complex, D_1-polypeptide synthesis and reassembly of active complexes.

THE ULTIMATE SOURCE of energy for maintaining the biosphere on our planet is sunlight which is converted to chemical energy by the process of photosynthesis. It is therefore surprising that light can also be bad for photosynthesis. The efficiency of photosynthesis can be significantly reduced when plants are exposed to high light levels, particularly under adverse environmental conditions[1]. Indeed there is evidence that too much light can affect plant growth and in the case of agricultural crops lead to reduction in yields. This 'stress' condition is known as photoinhibition and its recognition can be traced back to Ewart[2], who reported as long ago as 1896[3] that

> Leaves exposed, attached to the parent plant, to full sunlight till 4 PM show no assimilation, though living, green, and with normal chlorophyll grains. After 2 h. in diffuse light still no assimilation, but the next day the same preparations show, if living, a weak but distinct power of assimilation, and fresh preparations made from the same leaves now show a fairly active power of assimilation.

Today we know that photoinhibition is common to all photosynthetic organisms which evolve oxygen and that the primary target for light damage is photosystem II. Since photosystem II catalyses the fundamental water-splitting reaction, light must inhibit one of the key steps in the process of photosynthetic energy conversion. The photosystem II complex, which is embedded in the chloroplast thylakoid membrane and contains at least 23 different subunits[4], plays a central role in photosynthetic electron transport. It collaborates with the cytochrome *b/f*, photosystem I and the ATP synthase complexes to bring about oxidation of water, reduction of $NADP^+$ and the production of ATP[5]. Therefore, the overall consequence to the plant of light-induced damage to photosystem II is a loss of photosynthetic capacity. To survive and flourish, the plant has developed a complicated and costly repair mechanism involving the sacrifice and resynthesis of one of its most essential proteins: the D_1-polypeptide[6,7].

Understanding the molecular processes giving rise to the vulnerability of photosystem II to sunlight and its subsequent repair is a key area of present-day photosynthesis research. Recent progress has, to a great extent, relied on detailed understanding of the functional and structural properties of photosystem II. This knowledge was greatly aided by the concept of a close analogy between photosystem II and the reaction centre of non-oxygenic photosynthetic purple bacteria, and benefited particularly from the crystallization of the latter[8] for which the 1988 Nobel Prize for Chemistry was awarded. In this review we focus particularly on recent biochemical studies with the firm belief that the *in vitro* approach is contributing greatly to our understanding of photoinhibition *in vivo*.

Photosystem II – a delicate chemical machine with inherent weaknesses

The creation of photosystem II and a water-splitting system driven by sunlight represents the 'big bang' in evolution since it guaranteed the biosphere an unlimited supply of 'fuel' for the conversion of gaseous carbon dioxide to organic material. Prior to this major step, photosynthetic organisms relied on hydrogen donors, such as H_2S and organic acids, which were in limited supply. Also of great importance was that the appearance of photosystem II gave rise to the build-up of oxygen in our atmosphere and thus paved the way for the development of animal life. There is, therefore, no doubt that photosystem II is the 'engine of life'.

We know now that the reaction centre of photosystem II is composed of two polypeptides known as D_1 and D_2. These probably form a heterodimer[8–10], very similar in structure to the L/M protein heterodimer in the photosynthetic purple bacteria[8]. Moreover, there is a close analogy between the non-oxygenic and oxygenic systems in that they both use pheophytin and quinone (Q_A and Q_B) molecules as their primary and secondary electron acceptors. They also use chlorophyll as their primary

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electron donor but the oxidizing potential generated in photosystem II is greater than 1.0 V, while in the purple bacterial system the potential is about 0.5 V. It is this significant and remarkable difference which allows photosystem II, through its electron donor reactions involving a tyrosine radical and a manganese cluster (Fig. 1), to split water and bring about the release of molecular oxygen[11].

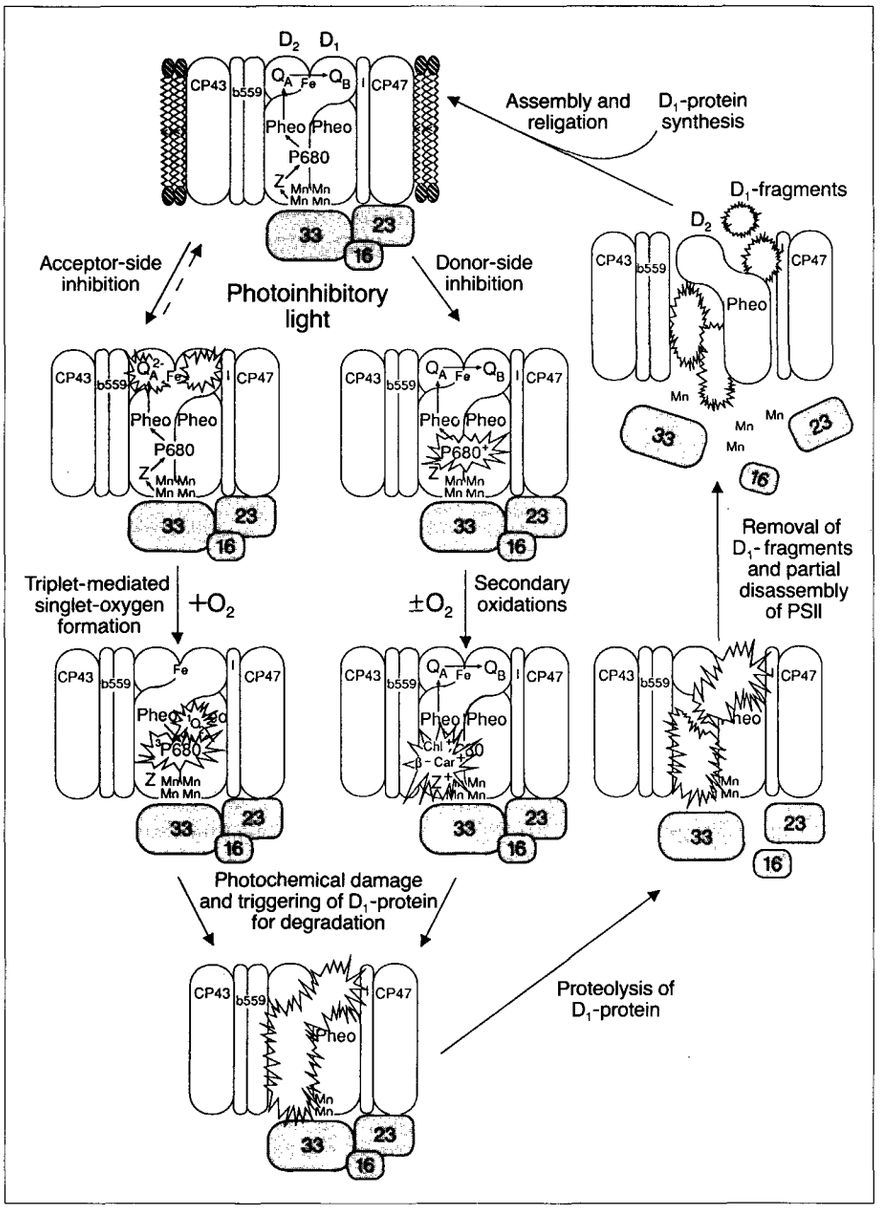

Figure 1

The series of events which occur in photosystem II that give rise to donor- and acceptor-side photoinhibition. A similar scheme can be found in Ref. 12. The detrimental photochemical processes involving oxidized P680 or P680 triplet state are believed to trigger the proteolytic degradation of the D_1-polypeptide and the disassembly of the photosystem II complex. The repair process involves the resynthesis of the D_1-polypeptide and reassembly of all the components of the complex in order to re-establish full functional activity. For simplicity, the diagram of the photosystem II complex has been restricted to a limited number of polypeptide components. P680 is the primary electron donor comprised of chlorophyll *a*; Pheo is pheophytin *a*, the primary electron acceptor; Q_A and Q_B are plastoquinone molecules which act as secondary electron acceptors; Z is a tyrosine residue 161 on the D_1-polypeptide, acting as an electron donor to P680; Fe, non-haem iron; chl, accessory chlorophyll, absorbing at 670 nm; β-car, β-carotene; cyt b_{559}, cytochrome b_{559}; CP43 and CP47 are light-harvesting chlorophyll *a* binding proteins of 43 and 47 kDa, respectively; the extrinsic proteins marked 33, 23 and 16 kDa are involved in stabilizing the water-splitting reaction which is thought to involve a cluster of four manganese atoms; D_1- and D_2-polypeptides are the reaction centre polypeptides that bind P680, Pheo, Fe, Q_A and Q_B and form a heterodimer thought to be similar to the L and M subunits of purple bacteria; ^{3}P680, P680 triplet; $^1O_2$, singlet oxygen.

It is quite surprising that such a crucial photochemical machine has weak points and needs to be repaired from time to time. It seems that a penalty is paid in performing light-driven water-splitting which can manifest itself as frequent damage to the photosystem II reaction centre. One problem is that light as a substrate is difficult to control, so that primary photochemistry of photosystem II goes on even when other reactions are limiting. Another problem is that photosystem II produces molecular oxygen as a byproduct and oxygen can easily form highly toxic species when pigments are excited by light or redox reactions are occurring. Still another problem is the fact that intermediates with very high oxidizing potentials (>1.0 V) are created within the reaction centre. In fact, these are the most oxidizing potentials found in any biological system!

The multifunctional but sacrificial D_1-polypeptide

A considerable amount of research on photoinhibition has been focused on the rapid and light-dependent turnover of the 32 kDa D_1-polypeptide[6,7]. This turnover can easily be illustrated by supplying a leaf with radioactive amino acids in the light. The main protein that becomes visible in a subsequent analysis by electrophoresis and autoradiography is the D_1-polypeptide, with a half-time for the turnover which can be as short as 60 minutes[12]. This polypeptide is encoded in the chloroplast by the *psbA* gene and it has been known for many years that the mRNA produced by this gene is present in greater abundance than the transcript of any other chloroplast gene[13,14].

In addition to the property of rapid turnover, the D_1-polypeptide is remarkable in that it harbours most of the functionally important sites involved in the photosystem II electron transfer reactions (Fig. 1). Not only does D_1 bind the secondary quinone acceptor Q_B but it also contains the binding sites for the primary electron donor and acceptor, P680 and pheophytin (Pheo), respectively[8,10]. Recently it has also been shown to contain a tyrosine residue at position 161, denoted as Z (see Fig. 1 and Fig. 3) which acts as a redox intermediate between the manganese cluster involved in water oxidation and the $P680^+$/$Pheo^-$ radical pair state[11]. There

is also increasing evidence that other amino acid residues of the D_1-polypeptide act as ligands for the binding of the manganese cluster[4,11]. There is no other protein within photosystem II that carries such a host of functional responsibilities. Nevertheless, in the light it undergoes a continuous cycle of degradation and resynthesis which must have dramatic implications for the functional organization of all the reaction centre components. It is noteworthy that this cycle is induced by UV as well as visible light[15] but is is not known what the relationship is between the two since UV damage is less specific[16].

Acceptor- and donor-side mediated photodamage

The obvious question is, which step in the sequential photosystem II electron transfer processes is blocked during light stress and what damage triggers the degradation of the D_1-polypeptide? The answer is not simple since it can depend on events occurring either on the oxidizing or reducing side of the primary reactions (Fig. 1)[4,12].

It was suggested initially that the main target for photoinhibition involves events at the binding site of the secondary quinone acceptor Q_B[1,6,12], but more recent *in vitro* studies suggest that the Q_B site has an initiating rather than an active role in the chemical events leading to photodamage of photosystem II. The Q_B binding site is located on the D_1-polypeptide and strongly binds semiplastoquinone-9 but has a lower affinity for the fully reduced form of the quinone. Therefore under high light conditions, if the donor side of photosystem II is efficient, the plastoquinone pool in the thylakoid lipid matrix can become fully reduced and the operational frequency of the Q_B site will be significantly lowered. However, the primary photochemical processes will continue to occur. Under these light stress conditions it is possible for the bound quinone acceptor Q_A not only to be reduced to its normal semiquinone state but also to an abnormal, doubly reduced, protonated state, Q_AH_2 (Refs 4 and 17), which subsequently leaves its binding site on the D_2-polypeptide[18]. When these events occur, the reaction centre is restricted to primary charge separation leading to the formation of the radical pair, $P680^+/Pheo^-$. If charge recombination occurs then the P680 chlorophyll triplet state will be generated and indeed this species has recently been detected by

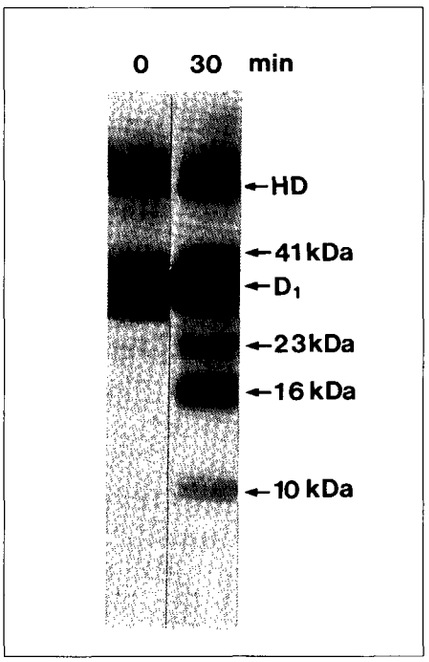

Figure 2
The appearance of breakdown products of the D_1-polypeptide detected by immunoblotting after exposing isolated photosystem II cores to strong illumination for 30 min. HD is the heterodimer of D_1- and D_2-polypeptides, 23 kDa and 16 kDa (and also 10 kDa) are breakdown fragments of the monomeric D_1-polypeptide, while the 41 kDa band is thought to be the breakdown product of the D_1-polypeptide still bound to the D_2-polypeptide.

EPR spectrometry in isolated thylakoid membranes after they had been photoinactivated in the absence of oxygen[18]. In aerobic conditions the chlorophyll triplet rapidly reacts with oxygen leading to the formation of singlet oxygen and studies using isolated photosystem II reaction centres have shown that this very reactive and toxic species leads to preferential destruction of the chlorophyll molecules of P680 (Ref. 19) followed by D_1-polypeptide degradation[18,20]. Under anaerobic conditions photosystem II is photoinactivated, but not irreversibly damaged, since recovery from the inhibited state is achieved simply by reoxidizing the quinone acceptors without degradation and resynthesis of the D_1-polypeptide[21,22].

However, there is more than one way to skin a cat and another scenario that leads to photoinhibition is when the donation of electrons to the photosystem II reaction centre does not keep pace with electron withdrawal. Such conditions could occur under low as well as high light intensities, in contrast to the acceptor-side mechanisms outlined above which would normally require high light levels to reduce the plastoquinone pool fully. If this occurs then the lifetime of the highly oxidizing species $P680^+$ will increase. This species is potentially very dangerous since it has sufficient oxidizing potential to extract electrons from its surroundings and cause damage to its molecular neighbourhood. For example, using isolated photosystem II reaction centres it has been shown that the photoaccumulation of $P680^+$ leads to the oxidation and destruction of an accessory chlorophyll, known as chl670, and of β-carotene[19,23]. As a consequence of this photodamage, the D_1-polypeptide was degraded and specific breakdown fragments appeared[20]. Related studies using thylakoids, treated so as to inhibit water-splitting, also showed a greater susceptibility to photoinhibition and D_1-polypeptide degradation[24,25]. Moreover, deletion of the gene in the cyanobacterium *Synechocystis* sp. PCC 6803, coding the 33 kDa protein that regulates the electron donation to P680 (see Fig. 1), resulted in a higher susceptibility to photoinhibition *in vivo*[26]. Of particular mechanistic importance is that donor-side photoinhibition and associated D_1-polypeptide degradation can occur under anaerobic conditions[20,25] and is therefore distinct from the acceptor-side initiated mechanism which is dependent on the presence of oxygen (see Fig. 1).

D_1-polypeptide turnover and an autoproteolytic activity of photosystem II

As a consequence of irreversible photodamaging events within the photosystem II reaction centre, the plant can regain photosynthetic function in two ways. It can either set aside the inactivated photosystem II, synthesize all its 23 subunits again and reassemble a completely new functional complex, or remove and replace only the damaged protein(s) and re-use all other subunits. Clearly the latter must be the most economic strategy and is, indeed, the mechanism that the plant favours. Under 'normal' photoinhibitory stress the D_1-polypeptide is the sacrificial subunit[6] although under severe conditions the D_2-polypeptide is also degraded to some extent[27,28].

Recently, considerable understanding of the events leading to the degradation of the D_1-polypeptide have emerged from studies of photoinhibition using systems ranging from isolated thylakoid

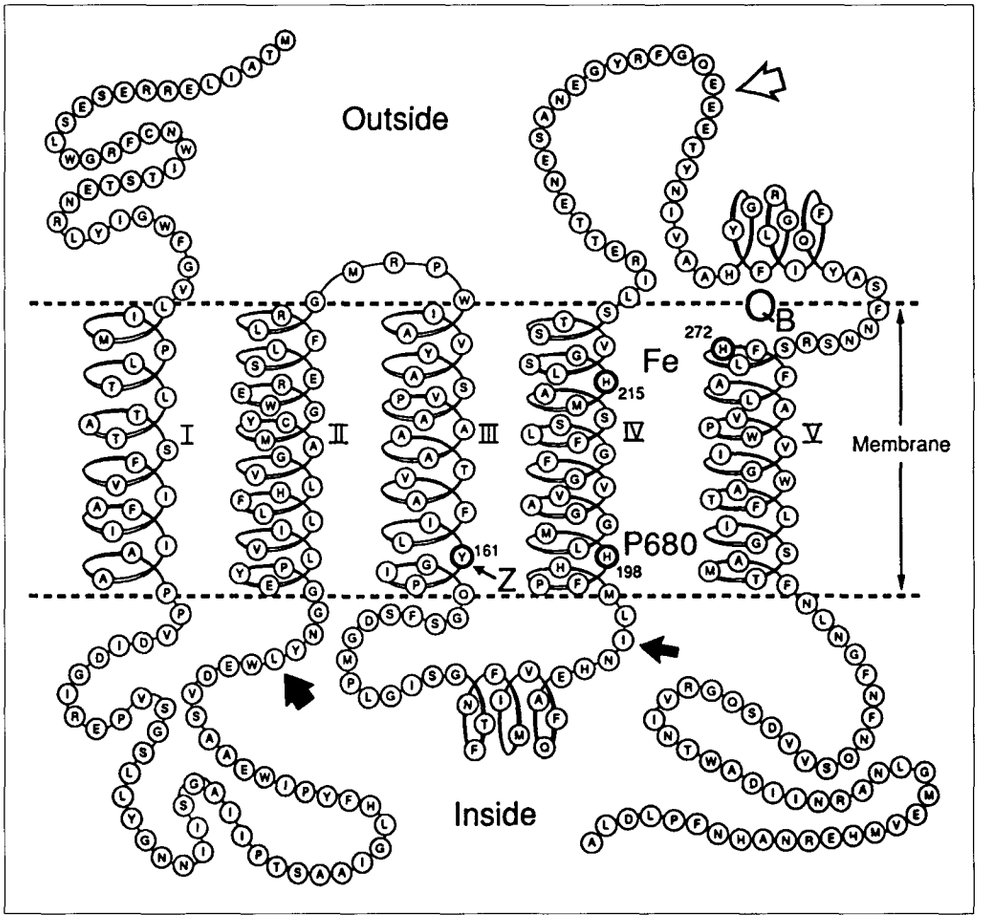

Figure 3

A model of the folding of the D_1-polypeptide based on the amino acid sequence of spinach and on consideration of hydropathy and analogy with the structure of the L subunit of the reaction centre of purple photosynthetic bacteria[8–10]. The model identifies: tyrosine 161 (Z) that functions as the donor to oxidized P680; histidine 198 probably binds the chlorophyll of P680, and histidines 215 and 272 are thought to form ligands to the non-haem iron.

membranes to various photosystem II preparations. Photoinhibitory treatment of isolated thylakoids resulted in degradation of the D_1-polypeptide at 20°C but not at 4°C (Ref. 29). Interestingly, the degradation could be observed in the latter sample in complete darkness when transferred to 20°C. This experiment showed that light is necessary to trigger the degradation but that the D_1-polypeptide cleavage itself does not require light. It was therefore concluded that the degradation is due to a membrane-bound endoproteolytic activity. Further photoinhibition studies with isolated photosystem II complexes including purified reaction centres (see Fig. 2) showed quite unexpectedly high levels of D_1-polypeptide degradation and concomitant appearance of breakdown fragments[20,30]. Moreover, the degradation could be specifically blocked by serine-type protease inhibitors[31,32]. Therefore it can be concluded that the 'D_1-protease' is an integral part of photosystem II, adding yet another functional property to this complex.

Experimentally the site of proteolysis on the D_1-polypeptide has been difficult to determine and is at present a matter of debate. Until recently it was generally believed that the primary cleavage occurs in the exposed peptide loop on the outer surface of the membrane linking putative transmembrane helices IV and V[33,34] (see Fig. 3). Support for this proposal stems partly from the close location of the cleavage site to the Q_B binding pocket on the D_1-polypeptide and the fact that proteolysis does not occur when Q_B is replaced by herbicides, such as diuron and atrazine[7,12,34]. Recently, however, conflicting data have emerged[35], suggesting that proteolytic digestion occurs on the donor side of the D_1-polypeptide in the region linking transmembrane helices I and II (see Fig. 3). The degradation of the D_1-polypeptide must involve more than one proteolytic process for its total removal from the membrane but it has yet to be established which is the initial cleavage site. Alternatively, the preferred site of cleavage could depend on whether the degradation is triggered by a donor- or acceptor-side event.

D_1-polypeptide degradation and secondary changes to photosystem II

Clearly the degradation of the D_1-polypeptide must induce secondary changes to the complicated machinery of photosystem II (Fig. 1). Indeed recent subfractionation studies have shown that concomitant with D_1-degradation there is a release of Mn atoms and extrinsic proteins involved in the water-splitting process into the thylakoid lumen[4] (see Fig. 1). A lateral migration of the various photosystem II units from the appressed to the non-appressed regions of the stacked thylakoid membrane has also been seen by several workers (see Refs 4 and 12 for review). This is of significance for the repair process since the synthesis of the new D_1-polypeptide is restricted to the non-appressed regions to which the ribosomes have access while functional photosystem II is located in the shielded, appressed thylakoid regions. It is envisaged that following insertion of a precursor D_1-polypeptide, reassembled complexes remigrate to the appressed region where they regain full functional activity after the incorporation and activation of cofactors, including the religation of the Mn cluster. The migration seems to involve a transient post-translational palmitoylation of the D_1-polypeptide which may direct the photosystem II complex back to the appressed thylakoid regions[7] and indicates that the repair after photoinhibition does not only involve synthesis of new D_1-polypeptides.

Molecular protection against light stress and photoinhibition

Fortunately, under most circumstances, the molecular repair process

can cope with the toxic nature of the photosystem II photochemistry. But the repair process is costly in terms of cellular energy and inevitably has its limitations. The plant cannot turn off the sunlight but it has developed several molecular strategies to acclimate and protect itself. These can be classified into two categories: those associated with the intake and delivery of light to the photosystem II reaction centre and those related directly to the functioning of the reaction centre itself. In the former case various strategies are used ranging from long- and short-term adjustments in the size of the pool of light-harvesting antenna proteins through gene regulation and reversible protein phosphorylation[5] to non-photochemical quenching of excitations by mechanisms involving pH gradients[36] and carotenoid interconversions[37].

In addition, there seem to be several self-protecting mechanisms in the photosystem II reaction centre itself. There are two β-carotenes within the D_1/D_2 heterodimer which might quench chlorophyll triplets and scavenge for toxic oxygen species. However, because of the very high oxidizing potential of $P680^+$ it may be impossible to place either of these carotenes close to the primary donor since they would then be vulnerable to rapid oxidation and destruction[23]. This may be the reason why it is possible to observe significant levels of P680 triplet in purified photosystem II reaction centres[38] and isolated thylakoids[18] at room temperature.

Other protective mechanisms involve redox cycles which short-circuit the acceptor and donor side of the reaction centre and therefore reduce the possibility of photoaccumulating highly oxidizing species and also compete with the deleterious recombination reactions involving the formation of the P680 triplet state. Probably the most important of these cycles involves cytochrome *b*559 which is intimately associated with the D_1/D_2 heterodimer and has been suggested to catalyse electron flow from reduced quinones to $P680^+$ (Refs 20 and 39).

Can we create plants that are more resistant to light?

It is important to realise that it is not only the light intensity that determines the severity of photoinhibition but also the superimposition of other environmental factors such as temperature, metabolic state, nutrient, water and carbon dioxide supply[1]. Of particular importance is the combination of light and cold stress frequently observed in many natural habitats. The nature of this synergism is probably at the level of protection and repair rather than in the actual photoinactivation process. For example, membrane diffusional processes between appressed and non-appressed regions of the thylakoids associated with the turnover of the D_1-polypeptide[7] will be significantly slowed at low temperatures. Some plants, however, acclimatize to low temperatures and as a consequence show a greater resistance to photoinhibition[40]. Moreover, different sensitivities to photoinhibition are observed within and between plant species. Such adaptation and variation give credibility to the future use of the techniques of molecular biology to create new plant varieties more resistant to light stress. This genetic engineering will probably have to be directed towards the protection and repair systems rather than to the photosystem II reaction centre itself, since the primary damage seems to be an unavoidable consequence of the chemistry of the water-splitting process.

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Phosphorylase: a biological transducer

Michelle F. Browner and Robert J. Fletterick

A transducer is a device that receives energy from one system and transmits it, often in a different form, to another. Glycogen phosphorylase receives information from the cell or organism in the form of metabolic signals. The energy associated with the binding of these ligand signals is integrated and transmitted at an atomic level, allowing precise adjustment of the enzymatic activity. Understanding this elegant allosteric control has required several different approaches, but the structural requirements of allostery are being defined.

GLYCOGEN PHOSPHORYLASE is an essential enzyme which, in mammals, provides the fuel needed to sustain life between feeding. Phosphorylase senses the metabolic state of the cell or organism, and responds by liberating glucose from stored glycogen as needed. Phosphorylase is studied both for its central role in an important biological process and also as a model for allosteric behavior in proteins.

The activation and inhibition of phosphorylases are cell, tissue and organism specific. In mammals, phosphorylase must be switched from its dormant state to the active form in order to liberate glucose 1-phosphate (Glc-1-P) from glycogen, with the help of inorganic phosphate[1]. Inside muscle cells, an increase in the products of ATP hydrolysis, principally AMP, signals the enzyme to commence digesting glycogen. Activation of phosphorylase by AMP can be competitively inhibited by metabolic indicators of ample energy, such as glucose 6-phosphate (Glc-6-P) and ATP.

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The muscle enzyme is activated by both AMP and covalent phosphorylation, but of the two, covalent phosphorylation is dominant. Generally, the needs of the whole organism, however, supersede those of the individual cell. Thus, phosphorylases which are responsible for global glucose regulation, such as the yeast and mammalian liver enzymes, are primarily activated by phosphorylation. In mammalian phosphorylases, Ser14 is the substrate for phosphorylase kinase. The phosphorylated state of the enzyme is controlled by extracellular signals, such as epinephrine (adrenaline), which act through the cAMP-dependent protein kinase cascade to turn on phosphorylase kinase. Activation by phosphorylation is reversed by the reciprocal action of protein phosphatase, PP1[2].

Ultimately, phosphorylase is controlled by glucose. When glucose is bound to the phosphorylated enzyme,