1. Introduction

Photosystem II (PS II) of green plants oxidizes water and reduces plastoquinone (PQ). The reduction of 1 PQ molecule requires 2 consecutive photo-reactions of a PS II center and involves 2 bound PQ molecules. The 'primary' PQ molecule Q is reduced photo-chemically to the semiquinone state Q⁻ and it in turn reduces a 'secondary' PQ molecule R to the semiquinone state R⁻. When Q is photo-reduced a second time, it reduces R⁻ to the fully reduced plastoquinol state RN; protons are taken up in this process also, in a manner that is still unclear [1–3].

It was originally assumed that R was a permanent component of PS II. However, I have proposed that, except while semireduced, it exchanges rapidly (>50 s⁻¹) with free PQ(H) diffusing in the membrane [4]. By implying that its binding site is frequently left vacant, this view suggests a simple hypothesis for the mechanism by which a variety of compounds prevent PQ reduction, i.e., that the binding of PQ and of inhibitor (I) to PS II centers with vacant secondary PQ binding sites (PS IIᵥ) are competitive reactions:

\[
\text{PS IIᵥ + PQ} \rightleftharpoons \text{PS II · PQ} \tag{1}
\]

\[
\text{PS IIᵥ + I} \rightleftharpoons \text{PS II · I} \tag{2}
\]

Inhibitor binding prevents the secondary PQ binding and thereby also prevents its reduction. Conversely, secondary PQ binding prevents inhibitor binding [4].

An interesting consequence of this proposed mechanism of inhibitor action is that the redox state of PS II should affect inhibitor binding, as experimentally observed [4]. Results obtained with antimycin A and dibromothymoquinone (DBMIB) will further illustrate the phenomenon.

2. Methods

Broken chloroplasts were isolated, concentrated, and stored in the dark at 4°C [5]. Additions, e.g., of inhibitors, were made at the time of dilution, 30 s before each measurement. Absorbance changes induced by short (few µs) Xe-flashes were measured [5].

Flash-induced formation of PQH₂ was monitored at ~320 nm, the peak of the PQ → PQ⁻ difference spectrum [6–8]. Fig.1 illustrates this method and presents the absorbance changes induced by 3 consecutive flashes with long-term dark-adapted (~1 day) chloroplasts. Hardy any PS II centers of such chloro-
plasts contain PQ\(^{-}\) before the illumination. Each of the flashes initially induces an absorbance increase caused by a PQ\(^{-}\) formation. Only when the centers contain a pair of PO\(^{-}\) molecules (which requires 2 flashes since no PQ\(^{-}\) was present at the start) does a large and rapid absorbance decrease follow the absorbance increase [9,10]. This absorbance decrease is ascribed to the dismutation of PQ\(^{-}\) pairs and the formation of PQH\(_2\) according to the reaction [11,12]:

\[2\text{PQ}^{-} + 2\text{H}^+ \rightarrow \text{PQ} + \text{PQH}_2\]

The PS II inhibitor diuron greatly inhibits the absorbance increase by the second flash and also the postflash decrease [10] (fig. 1, ——). The residual changes in the presence of diuron probably result from PS I-associated reactions rather than PQ\(^{-}\).

3. Results and discussion

3.1. Inhibition of PQH\(_2\) formation by antimycin

Fig. 2 shows the effect of antimycin on PQH\(_2\) formation. The measurements, some of which are reproduced in the insert of fig. 2, monitor PQH\(_2\) formation as PQ\(^{-}\) decay after the second flash of a flash pair (see section 2). They show that high concentrations of antimycin inhibit PS II much like diuron (cf. fig. 1). However, its efficacy greatly diminishes as the interval between the 2 flashes increases from 3–200 ms.

These results can be understood by considering both the conversions (1) and (2) discussed earlier and the electron transfer between primary PQ (Q) and secondary PQ:

\[\text{PS II}(\text{Q}^-) \cdot \text{PQ} \rightarrow \text{PS II}(\text{Q}) \cdot \text{PQ}^-\]  
(3)

The centers can be in 4 different states before the first flash: (a) contain secondary PQ; (b) have a vacant binding site; (c) contain inhibitor; or (d) contain PQ\(^{-}\) (because of insufficient dark-adaptation, cf. [10]). However, only the first 3 states are relevant. Those centers that contain PQ\(^{-}\) will form PQH\(_2\) after the first, not the second, flash; therefore, they can be ignored for the interpretation of fig. 2.

In the centers that start with PQ at their binding site, conversion (3) will occur after the first flash and be largely complete within 3 ms [14–16]. Almost complete conversion is also evident by noting that, in the absence of inhibitor, further increases in the flash interval cause little increase in PQH\(_2\) formation (a small slow phase, however, is always observed, also in [14–16]). This conversion (3), in conjunction with conversion (1), will cause a decrease in the concentration of centers with vacant binding sites, which in turn drives conversion (2) to the left. The centers that are thus freed of inhibitor subsequently bind PQ, reduce it by conversion (3), and form PQH\(_2\) after the final flash of the flash pair in a normal manner.

Although phenomena identical to the antimycin action are observed with other inhibitors of PQH\(_2\) formation, the ~25 s\(^{-1}\) release rate of antimycin (derived from fig. 2) is unusually high. It is ~25-times faster than that of o-phenanthroline [17] and ~250-times faster than that of diuron [18].

3.2. Inhibition of PQH\(_2\) formation by DBMIB

Under many conditions, DBMIB appears to selectively inhibit PQH\(_2\) oxidation by the cytochrome
Flash-induced PQH₂ formation at 0 μM, 0.6 μM and 2 μM DBMIB, as function of dark time (>30 ms) between pre-flash and measuring flash. Measurements as for fig.2.

Chloroplasts dark-adapted for 5–8 h at 4°C (pH 7.5). The decrease with time at 0 μM is due to PQ⁻ reoxidation by e⁻ backflow to the PS II donor side (it is inhibited by artificial donor).

If semireduced DBMIB (I⁻) is restrained (like PQ⁻) from leaving the reaction center, then conversion (4) drives conversion (2) further to the right, and the number of centers containing inhibitor tends to increase. This process competes with reactions (3) and (1), which tend to cause decreased inhibitor binding. Thus, the increased DBMIB binding that we obtain as a net result simply means that the semiquinone/quinone potential of (bound) DBMIB is higher than that of (bound) PQ.

An actual signal under fig.3 conditions [2 μM DBMIB added, 10 s interval between pre-flash (first flash) and final flash (second flash)] is shown in fig.4 (insert). In contrast to what is seen when such non-reducible inhibitors as diuron and antimycin are used, it is not the production of PQ⁻ by the second flash that is abolished, but only its rapid reoxidation. Since slow decay of the semiquinone signal still occurs it indicates that either PQ⁻ or semireduced DBMIB, or both, are slowly converted (the extinction coefficient
Fig. 5. Summary of the conversions of the acceptor complex of PS II caused by light (hν) and by binding and release of PQ and inhibitor (I). The several possible states are tagged with numbers. In the antimycin experiments, the centers started predominantly in state 3. After photo-conversion to state 7, we observed a dark-conversion to state 4. We assume the conversion pathway to be 7 → 6 → 5 → 4. In the DBMIB experiments, the centers started predominantly in state 1. After photo-conversion to state 5, they initially reached state 4; subsequently, however, a dark-conversion to state 8 occurred. We assume the pathway of this conversion to be 4 → 5 → 6 → 7 → 8.

of semireduced DBMIB is unknown). Kinetics that are similar to those of the semiquinone decay, i.e., ~30 ms (first) half-time, were detected for the reoxidation of primary PQ- by measuring the PQ- yield of a third flash as a function of the dark time after the second flash (fig. 4, main panel). This primary PQ- reoxidation did not — by a (conceivable) expulsion of semi-reduced DBMIB followed by binding and semireduction of PQ — form secondary PQ2: the third flash produced very little PQH2 (monitored as rapid PQ- decay) (not shown). After considering this evidence, we conclude that primary PQ- reacts with semireduced DBMIB to produce the quinol form of DBMIB. This species subsequently should be released, i.e., replaced by, mostly, PQ. If the quinol form of DBMIB were not released, irreversible inhibition would result, which is not observed [20].

These interpretations of the DBMIB effects and of the effects of non-reducible inhibitors are summarized in fig. 5.

4. Concluding remarks

A brief discussion of antimycin effects seems justified. In mitochondria and in several species of bacteria, antimycin specifically binds at the cytochrome b-c complex and inhibits its function [21], most likely by preventing the reoxidation of cytochrome b pairs by ubiquinone [22]. In contrast, in chloroplasts, the cytochrome b6-f complex appears relatively insensitive to antimycin [23]. However, low antimycin concentrations may inhibit the specific step of endogenous cyclic flow [24, 25], i.e., PQ reduction by ferredoxin [26], which is catalyzed by an unidentified enzyme. At relatively high antimycin concentrations (>1 per chlorophyll), PQ-mediated electron flow between PS II and PS I also is affected [27]. As shown above, the reduction of PQ by PS II is one of the steps that is weakly sensitive to antimycin. Thus, the results suggest that antimycin, like DBMIB and many other inhibitors of quinone-mediated electron flow [4], is a general quinone-antagonist that, with varying efficacy, competes with quinone for binding at the active sites of several quinone-converting enzymes.

It seems likely that the competition between quinone and inhibitors at other quinone-converting enzymes is also redox-state dependent. A direct parallel of the inhibitor—PS II interaction is the binding of ubiquinone reduction inhibitors at reaction centers of photosynthetic bacteria [28, 29]. Antimycin binding to the cytochrome b-c complexes of mitochondria is a more interesting, still unanalyzed, analogy; this binding also was affected by the reduction state of the enzyme [21].

Acknowledgements

This research was supported in part by NSF grant PCM77-20526 and by USDA (Competitive Research Grant Office) grant 5901-0410-8-0179-0. I thank Dr R. Radmer for a critical reading of the manuscript.

References