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REVIEW ARTICLE

Energy Coupling in Chloroplasts

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Scope

Water is split by chloroplasts in the light; oxygen is evolved and electrons are transferred to a suitable acceptor such as NADP by way of a chain of electron or hydrogen carriers. This process is essentially a reversal of electron transport in mitochondria, where molecular oxygen oxidizes a reductant such as NADH. As in mitochondria, ATP is formed during electron transport in chloroplasts.

As far as we know, the major purpose of chloroplast NADPH and ATP production is to drive assimilation of CO_2. The reactions of the Calvin cycle involved in the synthesis of a simple sugar consume ATP and NADPH at a ratio of 1.5[1]. The synthesis of starch and disaccharide causes some small rise in this ratio. In a large group of plants (C_4-plants) photosynthesis appears to proceed according to the Hatch–Slack–Kortschack pathway, which needs ATP and NADPH at a ratio of 2.5 [2].

Unfortunately, and in contrast to the situation for mitochondria, even after 20 years of work, there is still considerable controversy as to the "correct" number of ATP molecules synthesized during the transfer of two electrons in chloroplasts. At a recent congress, four different groups reported three different ATP/2e ratios, which were 1, 1.33, or 2, respectively[3–6].

Obviously, if there is a fixed stoichiometry of ATP formation in relation to electron transport, i.e., if coupling is "tight," the questions to be asked are dictated by whatever ATP/2e ratio is accepted to be "true." If the coupling ratio or ATP/2e ratio is 2 in a Calvin-type plant, there is the question of how the surplus ATP is expended to permit photosynthesis to proceed. If it is 2 in a C_4-plant or if it is less than 1.5, the question arises as to how the gap between ATP production during

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TABLE I. ATP imbalance created during electron transport from water to NADP[a]

Photosynthesis	ATP/NADPH requirement	Assumed ATP/2e ratio of NADP reduction (ATP imbalance in μmol/mg chl/hr)		
		1.0	1.33	2.0
Via Calvin cycle	1.5	−150	−50	+150
Via Hatch-Slack-Kortschak-pathway	2.5	−450	−350	−150

[a] The rate of CO_2 reduction is assumed to be 150/μmol/mg chlorophyll/hr and coupling ratios are as indicated. Minus signs show ATP deficiency, plus signs ATP surplus.

electron transport to NADP and consumption is closed. Table I indicates the magnitude of the ATP imbalance when the rate of photosynthesis is 150 μmol CO_2 reduction/mg chlorophyll/hr, and only ATP is considered which is made during electron transport to NADP. Assumptions are that coupling is tight and coupling ratios are as reported. It is apparent that the ATP imbalance may assume large proportions.

As an alternative to tight coupling, there might be no fixed stoichiometry at all. If it is assumed that the maximal coupling ratio possible in chloroplasts is 2, a decrease in the coupling efficiency could result in a situation where production of ATP and NADPH in a Calvin-type plant is geared to, and matches, consumption. This is obviously the situation in a leaf. The problem of an existing ATP gap cannot be solved by flexible coupling, which might even worsen it, as will be discussed.

Assuming high rates of ATP formation, which are supported by cyclic electron flow as observed in vitro in the presence of unphysiological cofactors [7, 8], it is widely thought and even documented in textbooks that cyclic photophosphorylation fills ATP gaps left by electron transport to NADP in vivo. However, it must be emphasized that there is little experimental justification for such an assumption. Rather, there is evidence that reduced carriers of the electron transport chain of chloroplasts can react with oxygen [9–16] and do, in fact, prefer in vivo oxygen for affinity reasons before another, oxidized, carrier of a cyclic electron transfer pathway can be reduced [17].

Most of the work done on energy conservation in chloroplasts has been performed with organelles which, during isolation, lost their envelopes and with them the soluble components such as stroma enzymes, ions, etc. The environment of the thylakoid membrane systems constituting such "naked," "broken," or "leaky" chloroplasts is altered when compared with intact chloroplasts. The latter, which are more

difficult to isolate, have special permeability characteristics. They do not, for instance, permit ready entry of added adenylates or hydrophilic electron acceptors such as ferricyanide or NADP [18–22] and have a low permeability for cations [23, 24].

This paper deals mainly with the problem of ATP availability in intact and functional chloroplasts. As background, it considers, in a somewhat simplified manner, available information on energy conservation in broken chloroplasts devoid of stroma. For a more detailed account of present knowledge on energy coupling, the reader is urged to consult the excellent recent reviews of Hind and McCarty [25], Trebst [26], and Trebst and Hauska [27] as well as previous literature cited there.

Background

When broken chloroplasts are illuminated, protons are taken up from the medium [28]. They are released on darkening. The pH of the intrathylakoid space drops by about 3 pH units during illumination [29–33]. Four protons have been measured to be taken up during the transfer of two electrons to a suitable acceptor [34–37, cf. 26]. There is experimental evidence for the existence of two energy conserving sites in the electron transport chain [36, 38–42]. Each of them was reported to contribute to ATP synthesis by thylakoid membranes with an ATP/2*e* ratio of about 0.6 [39, 40]. In addition to proton translocation, electron transport induces a potential difference across thylakoid membranes whose magnitude is not yet well established and may vary considerably with conditions. Values as high as 100 mV have been reported for the steady state [43, 44]. According to the chemosmotic theory of Mitchell [45], both the proton gradient and the membrane potential, which constitute an electrochemical energy reservoir, provide the driving force for phosphorylation. Convincing proof that a proton gradient can indeed support ATP formation has been provided by Jagendorf and Uribe [46]. While photophosphorylation of chloroplasts requires light, which drives electron and ion transport, they demonstrated that ATP is also formed in the dark if a sufficiently large proton gradient is set up across thylakoid membranes by first allowing vesicular thylakoid systems to take up protons in a medium of low pH and subsequently raising the pH in the presence of ADP and phosphate. In Mitchell's hypothesis, H^+ movement is the primary act of energy conservation. Alternative hypotheses [47, 48] make the formation of a high-energy intermediate or a high-energy conformational state of the membranes responsible for ATP formation. While the acid/base shift experiment does not permit a distinction between these possibilities, it clearly shows that the proton gradient must be able to equilibrate with high-energy states, if they really exist. That this equilibration would be a very rapid process is indicated

by the high uncoupling activity of a variety of compounds whose sole common property it is to increase the ion permeability of biomembranes [49, 50]. Thus the following considerations apply to whichever molecular mechanism ATP synthesis follows.

The magnitude of the electrochemical energy gradient established across thylakoid membranes in the light, when compared with the intrachloroplast phosphorylation potential (ATP)/(ADP)(P_i), gives some indication of the maximum amount of ATP which can be formed during electron transport. Kraayenhof reported a phosphorylation potential in broken chloroplasts* of about 30,000 under saturating light [51]. This means that depending on the accepted $\Delta G_0'$ value of ATP synthesis (between 6.9 and 8.4 kcal/mol [52]) light can produce ATP of a ΔG value which may be between 13 and 15.5 kcal/mol or, expressed in voltage terms according to $\Delta G = F\Delta E$, between 560 and 675 mV. Since the electrochemical energy gradient is made up of a pH difference of 150–210 mV and a membrane potential of perhaps 100 mV giving a total of about 300 mV, it is clear from a comparison with the ATP potential of chloroplasts (ca. 600 mV) that at least two protons must cooperate to synthesize one molecule of ATP. As four protons are transferred during the transport of two electrons, maximally two ATP molecules can be formed during reduction of one NADP. There is no need to assume, from such a consideration, that this maximal possibility is realized by the system. In mitochondria, ATP formation has been shown to be accompanied by the translocation of two protons per ATP [42].

Maximal Coupling

Unfortunately, three rather different H^+/ATP ratios have been obtained experimentally for thylakoid membranes. They were 4[53], 3[34, 54] and 2[35] implying maximal ATP/2*e* ratios of 1, 1.33, and 2. It has been suggested that the smaller H^+ flux giving rise to the low H^+/ATP ratio and, consequently, to the high ATP/2*e* ratio represented an artificial situation and was actually caused by rate-limiting fluxes of counter ions [34, 53].

However, the idea of a fixed stoichiometry of energy conservation does not appear to be a very useful concept in relation to the "real" availability of ATP and reducing equivalents. It is important to note that, no matter which of the proposed mechanisms of ATP synthesis is preferred, the experimental observation of ATP synthesis in a proton gradient links the permeability characteristics of thylakoid membranes to the efficiency of phosphorylation. Since biological membranes which are

* He assumed the chloroplasts to be intact, but their reported properties clearly show that they no longer had functional envelopes.

completely impermeable to small ions do not exist, leakage flows must occur which will compete with phosphorylation. It follows that the idea of a fixed stoichiometry between phosphorylation and electron or proton flow applies strictly in a chemical sense only when the molecular mechanism of ATP synthesis by a reversible ATPase is considered. It is not a useful concept for a consideration of the "real" availability of ATP in chloroplasts in relation to reducing equivalents for photosynthetic use.

When reduction of a suitable electron acceptor and ATP formation during illumination of broken chloroplasts are measured in the same sample, ATP/2*e* ratios can be computed. Reported values are widely scattered in the literature and range from near zero up to 1.7. The latter ATP/2*e* ratio has been observed by Reeves and Hall [55]. Values higher than 1 were reported by a number of workers [56–63]. It was recognized early that the treatment of chloroplasts drastically influences coupling. Coupling ratios are lowered especially when thylakoids are exposed to hypotonic stress [64] or to salt stress [65]. To obtain "maximal" coupling, which, as will be shown, is not necessarily identical with "real" coupling, corrections have been applied to experimental values. They are based on the assumption that a basal or nonphosphorylating electron flow must be substracted from the total electron flow to get the "phosphorylating" electron flow, which can then be compared with ATP formation [55, 62].

The validity of this assumption deserves some comment. There is little doubt that corrections are necessary when membranes have been damaged and their permeability characteristics are altered, as is often the case with broken chloroplasts. It is not difficult to predict that this would increase leakage flows of ions and decrease the efficiency of phosphorylation. There is, on the other hand, no evidence to show that chloroplasts in vivo possess a nonphosphorylating electron transport pathway which must be corrected for. Rather the reported characteristics of light-dependent oxaloacetate reduction by intact chloroplasts, which does not need ATP, contradicts such as possibility [66]. Even in the absence of a nonphosphorylating electron transport pathway and even in well-preserved thylakoid systems, small leakage flows of ions will occur. Because of competition with phosphorylation, they will increase in magnitude if phosphorylation is in some way curtailed. It follows that "optimal" ATP/2*e* ratios can be measured only in intact membrane systems and under conditions which place no restrictions on phosphorylation. Nonrestrictive conditions are certain to be met under rate-limiting light as long as rates of phosphorylation are proportional to light intensities. Under such conditions no corrections for nonphosphorylating electron flow should be necessary, and experimental values should approximate maximal ATP/2*e* ratios. These ratios are bound to decrease when leakage flows are increased in relation to phosphorylation.

Only a few measurements made under clearly nonrestrictive con-

ditions are available. Usually electron transport and phosphorylation were measured under high-intensity illumination. Under these conditions electron flow is limited by the capacity of the electron transport chain, by a low intrathylakoid pH, and possibily to some extent also by kinetic restrictions governing reduction of added electron acceptors. There may even be restrictions on phosphorylation. Electron transport observed in the absence of phosphorylation [62] or in the presence of ATP [55] was deducted from total electron transport under the assumption that this is a constant nonphosphorylating electron transport. The inference in such a procedure is that coupling is tight. As a matter of fact, electron transport in well-preserved thylakoid membranes was shown to be subject to photosynthetic control [67], a term analogous to respiratory control in mitochondria. Electron transport became slow, as substrates of phosphorylation were exhausted, and increased, by a factor of up to 6[68], on readdition of substrates. The intrathylakoid proton concentration has been suggested to be the factor controlling electron flow [53, 69]. When "nonphosphorylating" electron flow was subtracted from the electron transport rate observed in the presence of ADP and phosphate, ATP/2*e* ratios of 2 were obtained [55, 62].

However, it is very unlikely that the nonphosphorylating electron transport, which is permitted by leakage flows of ions across the thylakoid membranes, remains nonphosphorylating when ADP and phosphate are added. In the presence of these reagents phosphorylation is facilitated and lowers the proton gradient [34] thereby stimulating electron transport. It appears that the correction dictated by the assumption of a constant nonphosphorylating pathway is too large and gives rise to ATP/2*e* ratios which are too high. Indeed, there are instances where this correction leads to ATP/2*e* ratios much higher than 2[55, 62]; this is in conflict with thermodynamics as considered above. Siggel, Schröder, and Rumberg [6] have recently tried to obtain the "true" ATP/2*e* ratio by subtracting from the overall electron transport a basal rate which was not directly measured but calculated according to the concept of rate control. Even under widely varying conditions they arrived at a constant ATP/2*e* ratio of 1. This contrasts with the experimental data of Reeves and Hall and others [55, 56-62], who directly measured ratios up to 1.7 without applying corrections.

The possibility that cyclic electron transport supporting cyclic phosphorylation is superimposed, under high-intensity illumination, on noncyclic electron transport, which alone is measurable, must also be considered. It has largely been discounted by Reeves and Hall [55]. There is also the possibility that the high electron pressure produced by high-intensity illumination forces electrons to secondary acceptors, such as oxygen, present in the system. Contributions of cyclic phosphorylation and of ATP production during electron transport to oxygen would tend to increase observed ATP/2*e* ratios.

Coupling in Intact Chloroplasts

Ideally, and in contrast to the conditions of most measurements, coupling ratios should be determined in unperturbed systems and under rate-limiting light intensities which ensure that electrons are trapped only by the added electron acceptor. Also, phosphorylation potentials are kept low under low light intensities in the presence of ATP-consuming substrates. Light intensities should meet the condition of proportionality between electron flow, phosphorylation, and light. The "light-lag" of phosphorylation, which becomes efficient only after a low threshold value of incident intensity has been exceeded [35], must be avoided.

The closest approximation to an unperturbed system are fully functional chloroplasts capable of photoreducing CO_2 at rates corresponding to the rates observed in vivo. Unfortunately, in view of the permeability characteristics of intact chloroplasts, direct measurements of ATP/2*e* ratios are not possible. It has been observed that a sudden reduction in light intensity did not result in a decrease in the stationary level of NADPH, but caused a drastic drop in the ATP levels of chloroplasts engaged in CO_2 reduction under rate-limiting light. This implies a limitation of CO_2 reduction by ATP rather than by NADPH and is in disagreement with ATP/2*e* ratios of and above 1.5 [69].

Further information on coupling in intact chloroplasts has come from a comparison between the quantum requirements of the reduction of oxaloacetate, phosphoglycerate, CO_2, and glycerate [66]. Table II shows

TABLE II. Consumption of ATP and reducing equivalents in different chloroplast reactions

Substrate reduced	Number of ATP and reducing equivalents required for the reduction of 1 molecule substrate		ATP/2*e* ratio of reduction
	ATP	Reducing equivalents	
Oxaloacetate	0	2	0
3-Phosphoglycerate	1	2	1
CO_2	3	4	1.5
Glycerate	2	2	2

consumption of ATP and reducing equivalents and ATP/2*e* ratios of consumption in these reductive chloroplast reactions. Four quanta of red light are required to reduce one molecule of phosphoglycerate. This is in agreement with current assumptions on electron transfer and the role of two photosystems in photosynthesis [26, 71]. It is in disagreement with

tight coupling between phosphorylation and electron transport and ATP/2*e* ratios considerably higher than 1. This should result in ATP accumulation in chloroplasts during reduction of phosphoglycerate and suppress electron flow by photosynthetic control, thereby increasing quantum requirements. Such an effect is not apparent during the reduction of phosphoglycerate under rate-limiting light. It is easily observed when quantum requirements of oxaloacetate reduction are determined as a function of incident light energy [66]. At low light intensities (below 1 n-Einstein/cm^2/sec), the quantum requirements of oxaloacetate and of phosphoglycerate reduction are equal. They remained essentially unchanged as long as light was still rate limiting, when phosphoglycerate was the substrate, or when oxaloacetate reduction by uncoupled chloroplasts was measured. In coupled chloroplasts the quantum yield of oxaloacetate reduction decreased considerably with increasing light intensities, indicating significant photosynthetic control. However, at high light intensities phosphoglycerate reduction also appears to be under photosynthetic control. This was shown by stimulation of phosphoglycerate reduction in chloroplasts of the C_4-plant *Atriplex spongiosa* on addition of pyruvate, which was, under consumption of ATP, converted in the chloroplasts to phosphoenolpyruvate [72]. This observation can be explained in different ways. It may suggest that ATP/2*e* ratios in intact chloroplasts exceed 1. Another possible interpretation is that there is more than one light-driven reaction supplying ATP in chloroplasts. At high light intensities both would be activated producing high ATP levels, which suppress electron flow to phosphoglycerate by photosynthetic control.

Energetically, CO_2 reduction and glycerate reduction differ from phosphoglycerate reduction in their higher ATP consumption, as can be seen from the ATP/2*e* requirements listed in Table II. Glycerate is first phosphorylated by intact chloroplasts, and the resulting phosphoglycerate is then reduced [73]. The capability of spinach chloroplasts to perform this reaction is remarkable since the ATP/2*e* ratio of 2 which is necessary for reduction will rarely have to be maintained in vivo by the electron transport chain of a C_3-plant such as spinach. All reactions shown in Table II lead to oxygen evolution. Under optimal conditions the quantum requirement for the production of one molecule of oxygen was found to be 8 during phosphoglycerate reduction [66, 70], 10–12 during CO_2 reduction [70, 74], and, at an appropriate pH, 12–14 during glycerate reduction [66].

Since light was strictly rate-limiting, it is possible to relate the different quantum requirements for the reduction of phosphoglycerate, CO_2, and glycerate to the different ATP requirements of these reactions. This, of course, implies the existence of more than one light-driven reaction which can produce ATP in chloroplasts. Coupling ratios can be calculated from observed quantum requirements under the assumption

TABLE III. Comparison between observed quantum requirements of different chloroplast reactions with quantum requirements expected, when coupling ratios are 1, 1.2, 1.33, 1.5 and 2, respectively

Substrate	ATP/2*e* stoichiometry	Observed quantum requirement of oxygen evolution during reduction of substrate	Expected quantum requirements when coupling ratios are:				
			1.0	1.2	1.33	1.5	2
Phosphoglycerate	1	8	8	(8)[a]	(8)	(8)	(8)
CO_2	1.5	10-12	12	10	9	8	(8)
Glycerate	2	12-13	16	13.3	12	10.6	8

[a] Numbers in brackets are expected quantum requirements at very low light intensities; they should rise with increasing light intensities because of photosynthetic control.

that the quantum yield of ATP formation in the different reactions is identical. The basis of such calculations is simple. If the coupling ratio were 1, it follows from the quantum requirement of phosphoglycerate-dependent oxygen evolution of 8 that the quantum requirement of CO_2 reduction, which needs 50% more ATP, should be 12, and that of glycerate reduction, which requires twice as much ATP, should be 16. Table III compares quantum requirements expected from different coupling ratios with observed quantum requirements. The values suggest that coupling ratios were between 1 and 1.2 during CO_2 reduction and between 1.2 and 1.4 [66] during glycerate reduction.

A more direct approach to the measurement of coupling ratios in intact chloroplasts is facilitated by the observation that the malate shuttle permits oxidation of extrachloroplast NADH by intact chloroplasts even though the chloroplast envelope is impermeable for NADH [75]. Necessary conditions are that there is a reducible substrate in the chloroplasts and a redox gradient between extra- and intrachloroplast space. It was found that during phosphoglycerate reduction in rate-limiting light reducing equivalents were shuttled from external NADH into the chloroplasts [66]. Since the ATP/2*e* stoichiometry of phosphoglycerate reduction is 1, this clearly shows that there was a surplus of ATP inside the chloroplasts. Together with imported reducing equivalents it facilitated reduction of phosphoglycerate in addition to that indicated by oxygen evolution. A comparison of the rate of phosphoglycerate reduction with the rate of oxidation of external NADH permitted calculation of the maximal coupling ratio during phosphoglycerate reduction. It was in approximate agreement with the calculations listed in Table III between 1.1 and 1.4.

It should be emphasized that these measurements were performed with functional chloroplasts under low-intensity illumination, i.e., under conditions of a low phosphorylation potential, which should reveal the "real" coupling efficiency of the electron transport chain. However, there are also observations which suggest higher coupling ratios in intact chloroplasts. They were made at light intensities close to or higher than saturation of CO_2 reduction and may have to be interpreted with caution. Werdan et al. [76], Forti and Rosa [77], and Kagawa and Hatch [72] reported that partial uncoupling of intact chloroplasts by amines increased rates of phosphoglycerate and CO_2 reduction. Also, low concentrations of the uncoupler *m*-carbonylcyanide phenylhydrazone (CCCP) inhibited CO_2 fixation without affecting phosphoglycerate reduction [76]. These effects might be considered to be in conflict with coupling ratios much below 2. Werdan et al. [76] explained the amine-induced stimulation of both the reduction of phosphoglycerate and CO_2 by stimulation of electron flow through a lowering of the proton gradient or, in other words, by relief of photosynthetic control.

The CCCP effect appeared to be very different from that caused by the amines in that the light-induced alkalization of the chloroplast stroma [33, 78] was abolished by CCCP. This preferentially inactivated CO_2 reduction which is much more pH sensitive than phosphoglycerate reduction [76]. If this interpretation is correct, the results do not give information on ATP/2*e* ratios.

Reduction of Oxygen During CO_2 Assimilation

If insufficient ATP is made for CO_2 or glycerate reduction during electron transport to NADP, an additional photoreaction is needed. ATP could also be provided by cyclic electron flow or by electron transport to oxygen [7, 16, 79–81]. A number of recent observations suggest that electron transport to oxygen plays a role in functional intact chloroplasts and in leaves. Mass spectrometric oxygen exchange measurements revealed considerable oxygen uptake during CO_2-dependent oxygen evolution of intact chloroplasts [74]. Only a part of it could be accounted for by glycolate formation during CO_2 reduction. The oxygen reducing reaction was largely absent in chloroplasts photoreducing phosphoglycerate. It should be recalled that the latter reaction requires less ATP than CO_2 reduction does. The quantum yield of CO_2 reduction by well-washed chloroplasts was very low. It was dramatically increased by the addition of catalase, indicating that H_2O_2 is formed during CO_2 reduction. Chloroplasts scarcely responded to catalase while photoreducing phosphoglycerate. Under these conditions they produced much less H_2O_2 than during reduction of CO_2. The results indicate that functional chloroplasts, which are capable of high rates of photosynthesis, reduce oxygen to H_2O_2 during CO_2 reduction but not during reduction of phosphoglycerate. That oxygen reduction by broken chloroplasts is coupled to ATP formation was shown long ago by Forti and Jagendorf [82]. Light-dependent H_2O_2 production has also been seen in intact cells [83], but has been interpreted to represent a "leak" of electrons to oxygen.

Photosystem I is mainly responsible for oxygen reduction in chloroplasts [11–13]. This reaction also proceeds in leaves [17]. In the absence of electron acceptors such as CO_2 or oxygen, chlorophyll fluorescence of illuminated leaves is high and this indicates reduction of the electron transport chain [87]. It is rapidly quenched by oxygen to an even lower level than by CO_2 [85]. This is caused both by oxidation of the electron transport chain and by energy-dependent fluorescence lowering. Fluorescence changes in leaves are accompanied by light-scattering changes [17, 85]. These are caused by changes in size and conformational state of the thylakoid system, which in the light are a function of the pH gradient and of the ion distribution across the

thylakoid membranes [86]. The magnitude of these gradients in vivo is related to the phosphorylation potential [87]. Thus scattering of low-intensity 535-nm light can be used to monitor the energy state of chloroplasts in vivo [17, 88]. Under nitrogen and high-intensity far-red light, which excites photosystem I, light scattering by leaves is high, indicating a large pH gradient and, in consequence, a high phosphorylation potential [17]. Since electron acceptors are absent, this is presumably maintained by photosystem I–dependent cyclic electron flow. Admission of low levels of oxygen is sufficient to lower light scattering drastically to levels that are also seen in the complete absence of far-red light. The half-maximal scattering decrease was seen at 0.1% oxygen in the gas phase. The sensitivity of the reaction indicates that oxygen competes successfully with the cyclic system for electrons [17, 89]. Oxygen was not a good competitor when compared with CO_2. In isolated chloroplasts, oxygen is reduced only after NADP is no longer available [16, 90]. It appears from such measurements that in leaves cyclic electron transport can exist and supply ATP for photosynthesis only when the reaction reducing oxygen is largely saturated. Its saturation characteristics are presently unknown.

Flexibility of Coupling

Intact chloroplasts are capable of reducing oxaloacetate, whose reduction does not require ATP, even in the absence of uncouplers [66, 70]. Light-saturated rates of oxaloacetate reduction by fully coupled chloroplasts often were higher than 100 μmol/mg chlorophyll/hr. This reaction is under photosynthetic control as shown by a considerable decrease of the quantum yield of oxaloacetate-dependent oxygen evolution with increasing light intensities. The extent of photosynthetic control varied considerably in different chloroplast preparations. Uncoupling stimulated the rate of oxaloacetate reduction by a factor ranging from 1.5 to almost 10. Surprisingly, chloroplasts which exhibited little uncoupler-induced stimulation of oxaloacetate reduction were highly efficient in photoreducing CO_2. Since electron transport to NADP is linked to proton uptake and phosphorylation, which in turn can control the rate of electron transport, high rates of oxaloacetate reduction by fully functional chloroplasts give evidence that leakage flows of protons can assume massive proportions even in obviously undamaged chloroplasts. These results are difficult to reconcile with assumptions that coupling between phosphorylation and electron transport is tight. The evidence rather is that it is flexible. While there may be little competition between leakage flows and phosphorylation as long as the phosphorylation potential is low and ADP is available, such competition is bound to decrease the efficiency of phosphorylation

when the phosphorylation potential inside the chloroplasts becomes high. Under these conditions electron transport will be accompanied by less efficient phosphorylation. Preexisting ATP gaps for CO_2 reduction will be widened (cf. p. 158). Flexibility of coupling may be able to help explain differences in reported coupling ratios. Such differences may reflect not only differences in experimental design, but also physiological variations in thylakoid membrane permeability. Thus it seems that the highest reported ATP/2*e* ratios reflect the properties of the ATP synthetase in a largely ion-impermeable membrane. However, the situation appears to be different in intact chloroplasts, where leak conductivity for ions may be considerable.

Concluding Remarks

In view of the permeability characteristics of thylakoid membranes, it appears unlikely that an ATP/2*e* ratio as high as 2 can be realized by the chloroplast system. Rather under most circumstances even "good" ATP/2*e* ratios seem to be below 1.5. Flexibility of coupling decreases coupling ratios under conditions which increase nonphosphorylating leakage flows of protons across thylakoid membranes. Such conditions are a shortage of ATP-consuming substrate or high light intensities. Both are physiological conditions. It is possible that flexible coupling is not only a passive consequence of the ion conductivity of thylakoids but is regulated also by a special mechanism.

It may be argued that ATP/2*e* ratios below 1.5 as calculated for intact chloroplasts already reflect damage to these isolated organelles and that in vivo optimal coupling ratios are higher and, in fact, sufficient to satisfy ATP requirements of CO_2 reduction in C_3-plants. Such an argument is difficult to counter. Even if it were valid, it would bypass the physiological problem. Isolated chloroplasts perform photosynthesis at rates comparable to those supported by leaves. By this criterion they have not suffered damage. In spite of ATP/2*e* ratios not much higher than 1, they can produce enough ATP for photosynthetic CO_2 reduction. The mechanism permitting them to do so probably will exist also in leaves. It presumably would not exist there, if it had no function.

In combination, flexibility of coupling and subsidiary ATP production by electron flow to oxygen (when NADP is in the reduced state), and by cyclic electron flow (when the oxygen-reducing reaction is saturated), allow for maximal metabolic flexibility of chloroplasts. In the one extreme, chloroplasts can synthesize ATP without having to use NADPH for substrate reduction. In the other, they permit substrate reduction without having to consume ATP. The price for metabolic flexibility is loss of energy. However, waste of energy occurs only when it is available in excess. Energy is lost at high phosphorylation potentials by a decrease

in the efficiency of coupling only when ATP-consuming substrate is either lacking or light is abundant. Similarly, reductant is wasted by electron transport to oxygen only when NADPH levels are high and NADPH is unavailable as electron acceptor.

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