Oxygen reduction and optimum production of ATP in photosynthesis

THE accepted pathway of CO_2 fixation in plant photosynthesis requires that the photosynthetic light reactions produce ATP and reduced pyridine nucleotide (NADPH) in the molar ratio 3:2 (ref. 1). Early studies of photosynthetic phosphorylation suggested that non-cyclic electron transport could produce only equimolar amounts of ATP and NADPH, and the source of the extra ATP was presumed to be cyclic electron flow[2]. The view that the non-cyclic system is by itself able to produce twice as much ATP as NADPH has been expressed[3,4], and removes the need for the *in vivo* operation of a cyclic electron flow which can be demonstrated *in vitro* only in artificial conditions[5]. An inflexible ATP–NADPH ratio of 2:1 for the products of the light reactions would, however, result in a feedback inhibition of electron transport, with ADP concentration as the limiting factor. One way of achieving flexibility in the relative production of ATP and NADPH would be for a low

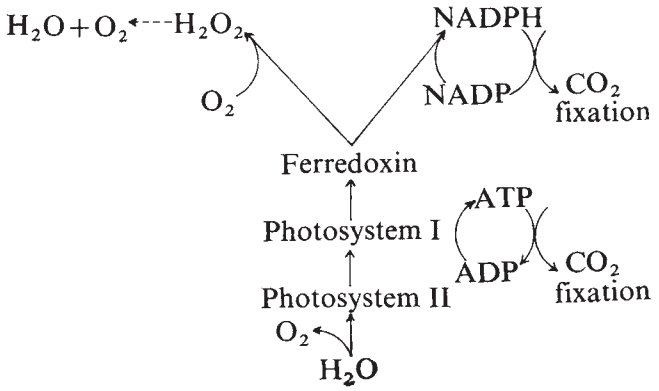

Fig. 1 The combined reduction of oxygen and of NADP by non-cyclic electron transport. The reaction of ferredoxin with oxygen makes possible the synthesis of ATP without the production of NADPH. Stoichiometries are discussed in the text and are not implied in the diagram.

absolute stoichiometry of non-cyclic photophosphorylation (corresponding perhaps to a 1 : 1 ratio) to be accompanied by an ancillary reaction producing ATP but not NADPH. Such an ancillary reaction would be especially important in situations where ATP might be required for phosphorylations additional to those of the reductive pentose phosphate pathway.

Figure 1 outlines a mechanism by which an optimum balance of ATP and NADPH could be maintained by non-cyclic electron transport in photosynthesis. Here the ancillary reaction is assumed to be oxygen-reducing electron transport, and the mechanism, therefore, relies on a "pseudocyclic" contribution to overall ATP synthesis, as suggested previously by Heber[6]. The novel feature of this scheme is a competition for reduced ferredoxin by NADP and oxygen. Production of too little ATP would inhibit the dark regeneration of NADP, and ferredoxin would then be oxidised only by oxygen. With oxygen as the effective electron acceptor, ATP but not NADPH would be produced, and a higher concentration of ATP would be restored. Excess ATP would lead to pyridine nucleotide being present predominantly in the oxidised form, and thus to electrons being diverted from oxygen to NADP. In this way the active non-cyclic electron transport chain would continually meet the requirements of CO_2 fixation, and would be able to respond to any changes in the metabolic demands made on it by synthesis of carbohydrate, protein or lipid.

Ferredoxin is an effective mediator of photosynthetic oxygen uptake by isolated chloroplasts[7], a reaction which accompanies non-cyclic (in this case also termed "pseudocyclic") photophosphorylation[8]. It is also the penultimate component of the photosynthetic electron transport chain, and as such is essential for the reduction of pyridine nucleotide in photosynthesis[9]. The hypothesis represented in Fig. 1 requires ferredoxin to perform both of these functions simultaneously, a situation which is demonstrated experimentally in Fig. 2. In the absence of both NADP and catalase, the rate of photosynthetic oxygen uptake by isolated chloroplasts is a function of ferredoxin concentration (curve *a*), and this oxygen uptake is abolished by addition of catalase (curve *b*). The photosynthetic oxygen-consuming reaction can, therefore, be written as the reversed dismutation of hydrogen peroxide:

$$H_2O+\tfrac{1}{2}O_2 \rightarrow H_2O_2$$

This describes non-cyclic transfer of one electron pair from water to oxygen. In the presence of NADP a catalase-insensitive evolution of oxygen should occur as transfer of one electron pair from water to NADP results in the reaction:

$$H_2O+NADP^+ \rightarrow NADPH+H^++\tfrac{1}{2}O_2$$

In this situation, however, catalase stimulates observed oxygen evolution (curves *c* and *d*), and the degree of this stimulation increases with increasing ferredoxin concentration. This stimulatory effect of catalase on net oxygen evolution can be considered to be no more than an inhibitory effect of catalase on a "background" oxygen uptake. If, of each pair of electrons from water, an average of *n* electrons are transferred to oxygen and $2-n$ to NADP, then the overall reaction occurring in the

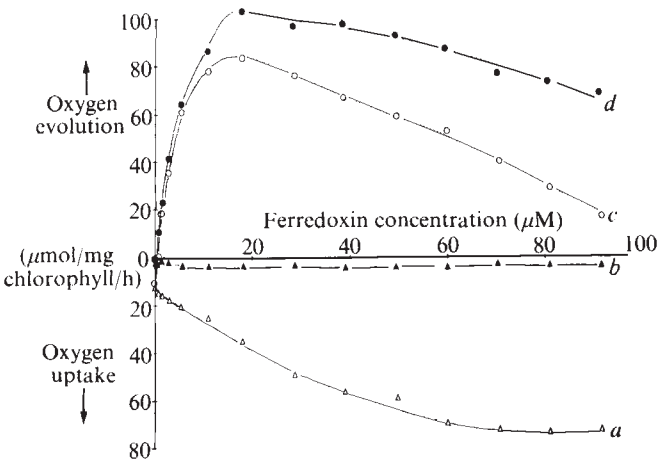

Fig. 2 The effects of catalase on ferredoxin-mediated uptake and overall evolution of oxygen by illuminated spinach chloroplasts. For curves *c* and *d* oxygen evolution was supported by the presence of NADP (2 mM). Curves *b* and *d* represent rates obtained in the presence of 8×10^3 U catalase (Boehringer). For curves *a* and *c* sodium azide (2 mM) was present as an inhibitor of any endogenous catalase.

Oxygen exchange was measured in a Rank oxygen electrode, with illumination by two 300-W slide projectors. Broken, washed chloroplasts had been isolated from spinach by a method described previously[14], and the reaction vessel contained sorbitol (0.1 M), $MgCl_2$ (5 mM), NaCl (20 mM), EDTA (2 mM), HEPES (*p*H 7.5, 50 mM), NH_4Cl (5 mM) and chloroplasts (100 μg of chlorophyll) in a final volume of 2 ml. Ferredoxin, isolated from *Spirulina maxima* by the method of Hall *et al.*[15], was added as a 1.4-mM solution. Substantially similar results have been obtained with a number of plant-type ferredoxins (unpublished work). The extent of stimulation of net oxygen evolution by catalase is apparently unaffected both by omission of NH_4Cl and by addition (in the absence of NH_4Cl) of ATP or ADP with phosphate (these results also unpublished).

absence of catalase activity (curve *c*) becomes

$$H_2O+\frac{n}{4}O_2+[(2-n)/2]NADP^+ \rightarrow \frac{n}{2}H_2O_2+$$
$$+[(2-n)/2](NADPH+H^+)+[(2-n)/4]O_2$$

and the ratio of oxygen molecules evolved to electron pairs transferred is given by

$$(O_2/2e^-)_{-cat} = [(2-n)/4]-(n/4) = [(1-n)/2]$$

In the presence of catalase, however, the compensatory reaction

$$\frac{n}{2}H_2O_2 \rightarrow \frac{n}{4}H_2O+\frac{n}{4}O_2$$

also occurs (curve *d*), and so the corresponding ratio is merely

$$(O_2/2e^-)_{+cat} = [(2-n)/4]$$

For any absolute rate of electron transport which is unaffected by catalase, the ratio r of the observed rates of net oxygen evolution in the presence (v_{+cat}) and absence (v_{-cat}) of catalase will be given by

$$r = v_{+cat}/v_{-cat} = (O_2/2e^-)_{+cat}/(O_2/2e^-)_{-cat}$$
$$= (2-n)/[2(1-n)]$$

and thus n ($0 \leqslant n \leqslant 2$) can be calculated from the relationship

$$n = [1/(1-2r)]+1$$

In Fig. 3*a* this measure of the contribution of the oxygen-reducing reaction to total electron flux has been calculated

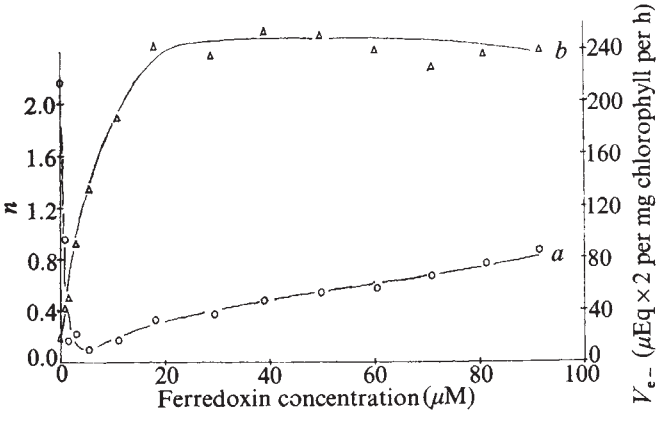

Fig. 3 *a*, n is the average number of electrons which go to oxygen, per pair transferred through the photosynthetic chain. *b*, v_{e^-} is the rate of electron transport in μEq ×2 per mg chlorophyll per h, units comparable with similar units involving μatoms oxygen exchanged or μmol NADP reduced. Values for each variable (n and v_{e^-}) were calculated as described in the text from the data in Fig. 2, with individual values corresponding to observed rates of oxygen evolution at each ferredoxin concentration.

from the data in Fig. 2, and is plotted against the same scale of ferredoxin concentration. As v_{-cat} is related to the absolute rate of electron transport (v_{e^-}) as follows

$$v_{-cat}/v_{e^-} = (1-n)/2$$

the absolute rate of electron flux can also be calculated. The dependence of electron transport *per se* on ferredoxin concentration is shown in Fig. 3*b*. As in the case of net oxygen evolution (Fig. 2), a saturated rate of electron transport is achieved at a ferredoxin concentration of 15–20 μM. Unlike net oxygen evolution, however, the rate of electron transport does not significantly decrease with higher ferredoxin concentrations. The decline in the observed rate of oxygen evolution with high ferredoxin concentrations (Fig. 2) presumably reflects an increasing displacement of NADP by oxygen as the terminal electron acceptor (Fig. 3*a*). At these saturating ferredoxin concentrations of >15 μM $n \geqslant 0.3$, and so not less than 15% of total electron transport is supported by reduction of oxygen.

These results question Arnon's statement[8] that in photosynthesis reduction of oxygen cannot occur simultaneously with reduction of NADP. Using mass spectrometry Egneus *et al.*[10] have identified an uptake of $^{18}O_2$ by CO_2-fixing chloroplasts in a reaction apparently unrelated to glycolate synthesis. This provides independent support for a competition of oxygen with NADP for electrons from the photosynthetic chain. Whitehouse *et al.*[11] have suggested that a significant rate of reduction of oxygen occurs in the presence of added FMN even where the artificial electron acceptor ferricyanide is used instead of NADP and ferredoxin.

The importance to photosynthesis of electron flow to oxygen has also been suggested by Heber, whose detailed measurements of the quantum requirements of various photoreductions support a contribution of oxygen-reducing electron transport to overall ATP synthesis[6], and point to a flexibility in the stoichiometry of photosynthetic phosphorylation and NADP reduction[12]. The work of Patterson and Myers[13] with *Anacystis nidulans* indicates that an *in vivo* photosynthetic production of hydrogen peroxide may indeed occur.

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1 Gregory, R. P. F., *Biochemistry of Photosynthesis*, (Wiley, London, 1971).
2 Arnon, D. I., Whatley, F. R., and Allen, M. B., *Science*, **127**, 1026-1034 (1958).
3 West, K. R., and Wiskich, J. T., *Biochim. biophys. Acta*, **292**, 197-205 (1973).
4 Reeves, S. G., and Hall, D. O., *Biochim. biophys. Acta*, **314**, 66-78 (1973).
5 Simonis, W., and Urbach, W., *A. Rev. Pl. Physiol.*, **24**, 89-114 (1973).
6 Heber, U., *Biochim. biophys. Acta*, **305**, 140-152 (1973).
7 Telfer, A., Cammack, R., and Evans, M. C. W., *FEBS Lett.*, **10**, 21-24 (1970).
8 Arnon, D. I., Tsujimoto, H. Y., and McSwain, B. D., *Nature*, **214**, 562-566 (1967).
9 Buchanan, B. B., and Arnon, D. I., *Adv. Enzymol.*, **33**, 119-176 (1970).
10 Egneus, H., Heber, U., and Matthieson, U., *Hoppe Seylers Z. physiol. Chem.*, **355**, 1190 (1974).
11 Whitehouse, D. G., Ludwig, L. J., and Walker, D. G., *J. exp. Bot.*, **73**, 772-791 (1971).
12 Heber, U., and Kirk, M. R., *Biochim. biophys. Acta*, **376**, 136-150 (1975).
13 Patterson, C. O. P., and Myers, J., *Pl. Physiol., Lancaster*, **51**, 104-109 (1973).
14 Allen, J. F., and Hall, D. O., *Biochem. biophys. Res. Commun.*, **52**, 856-862 (1973).
15 Hall, D. O., Rao, K. K., and Cammack, R., *Biochem. biophys. Res. Commun.*, **47**, 798-802 (1972).

Agonism and antagonism of γ-aminobutyric acid

EVIDENCE that (+)bicuculline (BIC) is a competitive antagonist of the central inhibitory transmitter γ-aminobutyric acid (GABA) is based[1] mainly on apparent similarities between the molecular structures of BIC, GABA and agonists of GABA. Until recently, there has not been any explanation of the differing potencies of the agonists, or why BIC is an antagonist instead.

In 1970 it was noted[1] that the N and lactone C–C=O in BIC could be isosteric with the N and carboxylate COO of an extended (that is fully *trans*) GABA molecule (zwitterion at physiological *p*H) and its equivalent (O–C=N) in the agonist muscimol. Alternatively[2], a partially folded GABA molecule could have its N and COO congruent with the N and lactone