THE RELATIONSHIP OF OXYGEN UPTAKE TO ELECTRON TRANSPORT IN PHOTOSYSTEM I
OF ISOLATED CHLOROPLASTS: THE ROLE OF SUPEROXIDE AND ASCORBATE

J.F. Allen and D.O. Hall, University of London, King's College,
Plant Sciences Department, 68 Half Moon Lane, London SE24 9JF.

Summary: It has recently been proposed that two oxygen molecules are
consumed per electron pair transferred through photosystem I of isolated
chloroplasts. This value $(O_2\downarrow/2e^- = 2)$ is contrary to an earlier assumption
that $O_2\downarrow/2e^- = 1$, and derives from a proposed interaction of ascorbate with
superoxide. Effects of superoxide dismutase and catalase on photosystem I-
mediated oxygen uptake are reported. These enzymes did not change the
corresponding rate of NADP photoreduction, suggesting that they affect only
the reactions which follow superoxide production. The results are consistent
with the proposed superoxide-ascorbate interaction, and hence also with the
value $O_2\downarrow/2e^- = 2$, some implications of which are discussed.

Oxygen uptake by isolated chloroplasts is a simple and commonly used

measurement of photosynthetic electron transport, and it is therefore important

to know the stoichiometry of oxygen molecules consumed to electron pairs

transferred (the $O_2\downarrow/2e^-$ ratio) for any chloroplast electron transport system

being studied.

In the Mehler reaction (1) oxygen uptake is a consequence of non-cyclic

photosynthetic electron transport from water to oxygen. With an added

autoxidisable electron acceptor such as methyl viologen, and in the absence

of catalase, the relationship of oxygen uptake to electron transfer is

$O_2\downarrow/2e^- = \frac{1}{2}$. The recent proposal that the superoxide anion (O_2^-) is involved

in this "pseudocyclic" reaction (2) has led to the conclusion that the

stimulatory effect of ascorbate on O_2 uptake in this system may be explained

by the interaction of ascorbate with superoxide, and hence that ascorbate

may increase the $O_2\downarrow/2e^-$ ratio from $\frac{1}{2}$ to a maximum of $1\frac{1}{2}$ (2,3).

Epel and Neumann have noted (2) that this putative oxidation of ascorbate

by superoxide may also be considered to be occurring where ascorbate is

present to provide a 'pool' of electrons for donation (via, for example,

DCPIP) to photosystem I. It follows from this that for such a photosystem I-

mediated oxygen uptake the $O_2\downarrow/2e^-$ ratio has a value of 2, while at present

Abbreviations: DCPIP; 2,6-dichlorophenolindophenol. MV; methyl viologen.
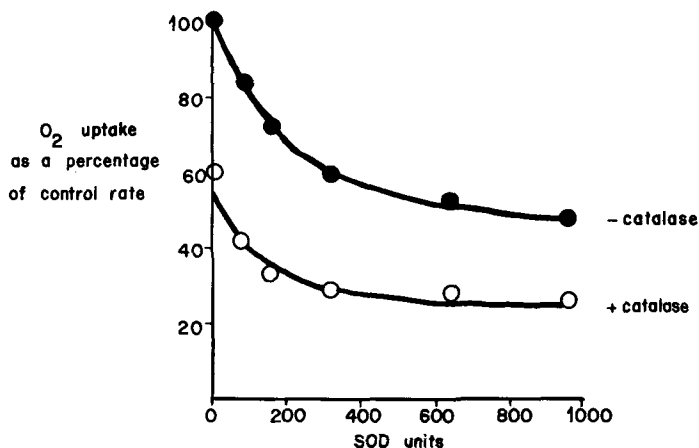SOD; superoxide dismutase. DCMU; 3,(3',4'- dichlorophenyl)-1,1-dimethyl urea.

579

Figure 1. The effect of superoxide dismutase (SOD) on oxygen uptake by photosystem I of isolated spinach chloroplasts. O—O; with catalase (4,000 units). ●—●; without catalase. The control rate was 130μmoles/mg chl/hr. Oxygen uptake was measured using an oxygen electrode (Rank Bros., Bottisham, Cambridge) of the type described by Delieu and Walker (5), which contained 0.1M sorbitol, 5mM $MgCl_2$, 20mM NaCl, 2mM EDTA, 50mM HEPES buffer pH 7.5. 50μM MV, 25μM DCPIP, 2mM ascorbate and chloroplasts equivalent to 100ug chlorophyll in a total volume of 2 ml. Chloroplasts (type C (6)) were isolated by a method previously described (2), with a subsequent treatment with tris buffer (pH 9.0) similar to that described by Haveman et al (7). Chlorophyll estimation, illumination of the reaction vessel, and SOD addition were as reported in ref. 2. SOD, isolated as bovine erythrocuprein was a gift from Dr. U. Weser, Tübingen.

the widely-assumed stoichiometry for this reaction is $O_2\downarrow/2e^- = 1$. The latter value derives from the conventional interpretation of oxygen uptake by photosystem I (e.g. ref. 4) which omits the participation of the superoxide ion.

Results and Discussion: The enzyme superoxide dismutase is thought to competitively replace the superoxide-ascorbate interaction with an oxygen-releasing superoxide dismutation (2,3). Figure 1 shows that a saturating concentration of superoxide dismutase (SOD) decreases to a half of the original value the rate of oxygen uptake which accompanies electron transport through photosystem I, using isolated spinach chloroplasts with ascorbate plus DCPIP as electron donor and MV as the autoxidisable electron acceptor. Catalase is also seen to halve the initial rate of oxygen uptake in this system. Addition of SOD also halves the catalase-affected rate, so that oxygen
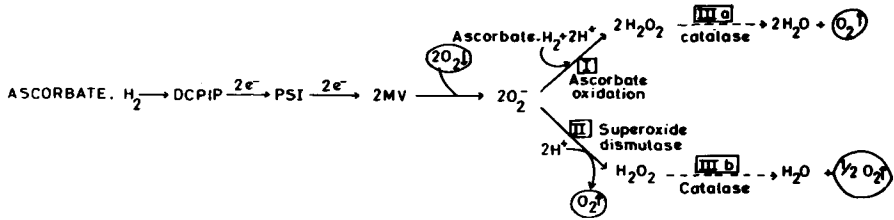
ADDITIONS	REACTIONS INVOLVED	O_2 molecules consumed per $2e^-$ transferred	PRODUCT (oxygen balance)
None	I	2	H_2O_2 $(2O_2\downarrow)$
SOD	II	1	H_2O_2 $(2O_2\downarrow + O_2\uparrow)$
Catalase	I + III a	1	H_2O $(2O_2\downarrow + O_2\uparrow)$
SOD + Catalase	II + III b	½	H_2O $(2O_2\downarrow + 1½O_2\uparrow)$

Figure 2. A scheme for oxygen uptake by photosystem I with reactions involving ascorbate (I), superoxide dismutase (II), and catalase (IIIa, IIIb).

uptake in the presence of both catalase and SOD proceeds at a quarter of the rate observed when neither enzyme is present.

The scheme outlined in figure 2 shows an explanation of these results. In the presence of neither SOD nor catalase the production of O_2^- is followed only by reaction I (ascorbate oxidation). Two oxygen molecules are therefore consumed per electron pair transferred through photosystem I ($O_2\downarrow/2e^- = 2$). In the presence of SOD alone, O_2^- production is followed by O_2^- dismutation (reaction II), releasing one oxygen molecule, and hence giving a net uptake of only one oxygen molecule per electron pair transferred ($O_2\downarrow/2e^- = 1$). This situation is stoichiometrically equivalent to the earlier interpretation, which did not require the addition of SOD, and in which oxygen was thought to be reduced directly to hydrogen peroxide. Catalase alone causes reaction I (ascorbate oxidation) to be followed by an oxygen-releasing dismutation of hydrogen peroxide (reaction IIIa) resulting, as for SOD alone, in a net stoichiometry of $O_2\downarrow/2e^- = 1$. In the presence of both catalase and SOD, both the oxygen-releasing reactions (II and IIIb) occur, giving a net stoichiometry of $O_2\downarrow/2e^- = \frac{1}{2}$. Thus the effects of SOD and catalase shown in
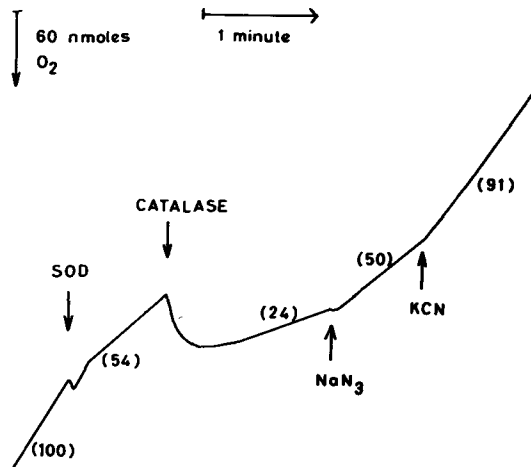
Figure 3. The effects of successive additions of SOD (800 units), catalase (4,000 units), azide (final concentration 10mM) and cyanide (final concentration 10mM) on oxygen uptake in the photosystem I reaction. The figures in brackets are the rates of oxygen uptake for the corresponding parts of the trace, as a percentage of the initial rate, which was 90μmoles/ mg chl/hr. The reaction conditions were those described in the legend to figure 1.

figure 1 can be understood as results only of their effects on the fate of

the oxygen molecules that are reduced to superoxide; it is not necessary to

assume that these enzymes have any effect on electron transport per se.

The results of table I support this conclusion by showing that the

rate of electron transport itself, measured as NADPH production by photosystem

I, is not significantly affected by SOD, by catalase, or by both enzymes

together. The effects on oxygen uptake of azide (an inhibitor of catalase)

and cyanide (an inhibitor of both catalase and SOD) are summarised in the

oxygen electrode trace reproduced in figure 3. This illustrates the

reversibility of the effects of SOD and catalase. Azide and cyanide did not

seem to affect NADPH production (table I) and did not significantly influence

oxygen uptake in the absence of the corresponding enzymes (results not shown).

The use of oxygen uptake as an absolute measurement of electron transport

is satisfactory only when all factors that may influence the $O_2\downarrow/2e^-$ ratio

are taken into account. These factors must now include: (i) the presence of

Table I. NADPH production by photosystem I. The reaction conditions were as described for figure 1, with the following exceptions. (i) Ferredoxin (20μM) and NADP+ (2mM) were present; (ii) MV was omitted; (iii) the chloroplasts had not been tris-treated; (iv) the following compounds were added only where indicated to give the stated final concentrations; DCMU 5μM, ascorbate 2mM, DCPIP 25μM, NaN3 10mM and KCN 10mM; SOD (800 units) and catalase (4,000 units) were also added. NADP photoreduction took place in the oxygen electrode vessel, which was illuminated for 6 minutes. The chloroplast suspension was then removed and centrifuged at 4,000 x g for 5 minutes. The A_{340} of the supernatant was measured; from this the corresponding A_{340} of a dark control was subtracted. Ferredoxin, isolated from Spirulina maxima (9) was a gift from Dr. K.K. Rao.

Additions	Rate of NADPH formation (umoles mg chl^{-1} hr^{-1})
(a) H$_2$O as electron donor	
None	137
DCMU	1
DCMU, ascorbate	1
(b) Ascorbate/DCPIP as electron donor (+ DCMU)	
None	40
SOD	41
SOD, catalase	37
SOD, catalase, NaN$_3$	39
SOD, catalase, NaN$_3$, KCN	33

a reducing agent such as ascorbate, which may be oxidised by superoxide (cysteine, glutathione and dithiothreitol also act in this way (10)); (ii) the effectiveness of such a reductant in competing with spontaneous superoxide dismutation; (iii) SOD activity; (iv) catalase activity; (v) inhibitors of either of these enzymes. Catalase activity is also masked by ethanol, since ethanol prevents the release of oxygen from hydrogen peroxide (1) and would therefore decrease the contributions of reactions IIIa and IIIb to the overall oxygen balance - see figure 2. (The presence of ethanol at a final concentration of 0.25% (V/V) in the experiment represented in figure 1 resulted in only a 20% decrease of oxygen uptake by catalase, and the SOD-affected oxygen uptake was decreased proportionately. This was also the case where DCMU had been added as 5μl of an ethanolic solution, giving the same final ethanol concentration. Results not shown).

Where oxygen uptake by photosystem I proceeds in the absence both of SOD and of catalase, the implicit assumption of a value of 1 for the $O_2\downarrow/2e^-$ ratio may lead to values for rates of electron transport that are over-estimated by a factor of two. This in turn may lead to calculations of values for the quantum requirement of photosystem I (11) (this is discussed by Epel and Neumann (2)) or for the stoichiometry of phosphorylation ($P/2e^-$) associated with photosystem I which are underestimated by the same factor. It is possible that recently published values of 0.5 - 0.6 (12,13) for the $P/2e^-$ ratio of photosystem I phosphorylation are subject to this error, and that these results may therefore reflect a value for the number of ATP mole-cules synthesized per electron pair transferred through photosystem I that is actually close to unity. This would be in agreement with the value proposed originally by Losada et al (14) (measuring NADP reduction) and more recently by Goffer and Neumann (15) (measuring O_2 uptake with diaminobenzidine as electron donor). The results of Strotmann and von Gosseln (4) may also reflect a $P/2e^-$ of one, even without the correction that they make for a non-phosphorylating rate of oxygen consumption. Ort and Izawa (16) report that in the electron transport system DCPIP/ascorbate $\rightarrow$ MV $\rightarrow$ O_2 the "true" $P/2e^-$ is 0.68, a value obtained by correcting an observed P/O_2 of 0.50 for only a 27% decrease of oxygen uptake by SOD. The results shown here (figures 1 and 3) suggest that a cyanide-reversible SOD-mediated halving of oxygen uptake may be demonstrated for the same electron transport system. It is therefore possible that a value of 0.68 for the $P/2e^-$ ratio may also be a significant underestimate.

It is likely that direct measurement of oxygen uptake is preferable to the use of correction factors, since the latter require assumptions additional to a value for the $O_2\downarrow/2e^-$ ratio. In any event the role of superoxide must be taken into account. It is clear that where the $O_2\downarrow/2e^-$ ratio is not known, oxygen uptake is justifiable only as a purely relative measure of electron transport.

References

 1. Mehler, A.H. (1951) Arch. Biochem. Biophys. 33, 65-77.
 2. Epel, B.L. and Neumann, J. (1973) Biochim. Biophys. Acta 325,
        520-529.
 3. Allen, J.F. and Hall, D.O. (1973) Biochem. Biophys. Res. Commun.
        52, 856-862.
 4. Strotmann, H. and von Gosseln, C. (1972) Z. Naturforsch. 27,
        445-455.
 5. Delieu, T. and Walker, D.A. (1972) New Phytol. 71, 201-225.
 6. Hall, D.O. (1972) Nature 235, 125-126.
 7. Havemann, J., Duysens, L.N.M., van der Geest, C.M. and van Gorkom, H.J.
        (1972) Biochim, Biophys. Acta. 283, 316-327.
 8. Weser, U., Bunnenberg, E., Cammack, R., Djerassi, C., Flohe, L.,
        Thomas, G. and Voelter, W. (1971) Biochim. Biophys. Acta 243,
        203-213.
 9. Hall, D.O., Rao, K.K. and Cammack, R. (1972) Biochem. Biophys. Res.
        Commun. 47, 798-802.
10. Marchant, R.H. (1974) Biochem. Soc. Trans. London (in press).
11. Avron, M. and Ben-Hayyim, G. (1969) in Progress in Photosynthesis
        Research (Metzner, H. ed.) Vol.3, pp 1185-1196, Tübingen.
12. Gould, J.M. and Izawa, S.   (1973) Biochim. Biophys. Acta 314,
        211-223.
13. Ort, D.R. and Izawa, S. (1973) Plant Physiol. 52, 595-600.
14. Losada, M., Whatley, F.R. and Arnon, D.I. (1961) Nature 190, 606-610.
15. Goffer, J. and Neumann, J. (1973) FEBS letters 36, 61-64.
16. Ort, D.R. and Izawa, S. (1974) Plant Physiol. (in press).