A REQUIREMENT FOR ADP AND PHOSPHATE IN THE INHIBITION OF PHOTOSYSTEM 1 ELECTRON TRANSPORT BY SALICYLALDOXIME

David MILTON and K.G. RIENITS
School of Biochemistry, University of New South Wales, P.O. Box 1, Kensington 2033 N.S.W., Australia

Received 28 February 1972

1. Introduction

The current view of the light reaction of photosynthesis is of two photoacts within an electron transport pathway from H_2O to NADP [1]; artificial electron donors and acceptors interact at various points in this pathway.

In addition, there is evidence for alternate paths in the PS1 region: plastocyanin and cytochrome *f* function in parallel [2] perhaps as part of different paths; Schwartz [3] has distinguished 2 paths of electron donation from PMS to PS1, one of them phosphorylating. The latter may be identified with the electron transport path in cyclic phosphorylation [4]. Nishimura [5] has distinguished a cyclic path around PS1 in red algae. Two pathways for cyclic phosphorylation have been distinguished on the basis of γ-irradiation sensitivity [6].

The following presents data consistent with the hypothesis that the presence of ADP and P_i determines a preferred pathway for the donation of electrons from $PMSH_2$ to PS1.

Abbreviations:

PS	: Photosystem;
P_i	: Inorganic (ortho)phosphate;
PMS, $PMSH_2$	: *N*-methyl phenazonium methosulphate and reduced form;
DCI, $DCIH_2$	: 2,6 dichloroindophenol, and reduced form;
TMPD, $TMPDH_2$	: *N, N, N', N'* tetramethyl-*p*-phenylene diamine and reduced form.

2. Materials and methods

2.1. *Chloroplasts*

All operations were carried out at or near 0°. Broad bean leaves (approx. 5 g) were ground in a mortar and pestle with approx. 5 × 30 ml of 0.4 M sucrose containing 0.05 M Na phosphate pH 8.0 and 0.01 M KCl. The mixture was strained through a layer of Miracloth and centrifuged at 1,000 *g* for 90 sec. The supernatant was centrifuged at 2,000 *g*/10 min, and the green pellet resuspended in 0.4 M sucrose containing 0.02 M tricine–NaOH pH 8.0 and 0.01 M KCl. Chloroplasts were sedimented at 2,000 *g*/10 min and suspended in approx. 5 ml of the above suspending solution. Chlorophyll was determined by the method of Vernon [7].

2.2. *Photoreduction of methyl red*

Reactions were carried out at room temp in Thunberg type cuvettes, evacuated and flushed 3 times with N_2 before tipping. Methyl red reduction was followed by changes in absorbance at 430 nm measured in a Unicam SP800 spectrophotometer fitted with an external recorder. The reaction was commenced by tipping the contents of a side arm into the main body and measuring absorbance. Absorbance was measured after 30 sec sequential illuminations with a tungsten lamp and methyl red reduction calculated using a molar absorption coefficient of 1.34×10^4 at 430 nm. The body of the cuvette contained in a final volume of 3 ml (in μmoles) glucose (350), tricine-NaOH pH 8.0 (200), $MgCl_2$ (20), glucose oxidase 0.2 mg; and where indicated ADP (4) and

Table 1
Comparison of the effect of salicylaldoxime on rates of methyl red reduction using various electron donors involving only PS1.

Additions to reaction mixture	Rate of methyl red reduction (μmoles hr^{-1} (mg chlorophyll)$^{-1}$)			
	$PMSH_2$ (25 μM)	$PMSH_2$ (250 μM)	$DCIH_2$ (200 μM)	$TMPDH_2$ (200 μM)
None (control)	3.3	5.2	3.6	5.2
ADP, P_i	3.7	5.7	4.0	5.6
Salicylaldoxime	3.8	4.5	2.8	1.8
ADP, P_i, salicylaldoxime	1.8	5.6	2.9	2.1

Conditions as in Materials and methods. Chlorophyll concentration 26.6 μg/ml; light intensity 20 klux.

Table 2
Comparison of effect of salicylaldoxime on methyl red photoreduction by 25 μM $PMSH_2$ (PS1) and water (PS1 + 2).

Additions to reaction mixture	Rate of methyl red reduction (μmoles hr^{-1} (mg chlorophyll)$^{-1}$)	
	$PMSH_2$ (25 μM)	H_2O
None (control)	2.3	9.0
Salicylaldoxime	2.4	1.0
Salicylaldoxime, ADP, P_i	1.4	0.9
Salicylaldoxime, ADP, P_i, NH_4^+	2.3	0.6

Conditions as in Materials and methods. Chlorophyll concentrations 36.1 μg/ml; light intensity 20 klux.

salicylaldoxime (30). When reactions involving only PS1 were studied, DCMU (0.03) and Na ascorbate (16.5) were added with either PMS (0.075 or 0.75), DCI (0.6) or TMPD (0.6). The side arm held chloroplast suspension containing the indicated amount of chlorophyll, and where indicated Na phosphate pH 8.0 (30). When used NH_4Cl (15) was distributed between the main body and side arm such that the chloroplasts were pre-incubated with the final NH_4^+ concentration.

3. Results

The data of table 1 indicate that PS1 mediated reduction of methyl red from 25 μM $PMSH_2$ differed from that of 250 μM $PMSH_2$ and also from that from $DCIH_2$ and $TMPDH_2$. Electron flow from $DCIH_2$ and $TMPDH_2$ to methyl red was inhibited in the presence or absence of ADP and P_i. The reduction of methyl red mediated by 250 μM $PMSH_2$ was unaffected by salicylaldoxime whereas 25 μM $PMSH_2$ mediated a reduction of methyl red which required the presence of ADP and P_i in order to become sensitive to the inhibitory action of salicylaldoxime. Photoreduction of methyl red by water involving both photosystems 1 and 2 was sensitive to salicylaldoxine whether ADP and P_i were present or not (table 2). Further experiments indicated that the 25 μM $PMSH_2$-mediated methyl red reduction required ADP and P_i together: only marginal salicylaldoxime sensitivity was conferred by adding ADP or P_i separately.

Table 2 also indicates another unusual property of the sensitivity to salicylaldoxime with 25 μM $PMSH_2$. The inhibition by salicylaldoxime in the presence of ADP and P_i was relieved by 5 mM NH_4^+, in contrast to the salicylaldoxime inhibition operating when water served as the electron donor for methyl red reduction. In addition chloroplasts which had been extracted with 0.75 mM EDTA had very much less salicylaldoxime sensitivity conferred by ADP and P_i when using 25 μM PMS as electron donor, although the salicylaldoxime sensitivity of the water mediated methyl red reduction was unaffected (table 3).

The selective inhibition of electron transport from 25 μM $PMSH_2$ in the presence of ADP and P_i was apparent only at chlorophyll concentrations above 20–25 μg/ml (table 4). This was found to be a light intensity effect (table 5).

Table 3
Effect of EDTA extraction on salicylaldoxime inhibition.

Additions to reaction mixture	Rate of methyl red reduction (μmoles hr^{-1} (mg chlorophyll)$^{-1}$)	
	Broken chloroplasts	EDTA extracted chloroplasts
H_2O as donor (PS1 + 2)		
None (control)	5.53	7.29
Salicylaldoxime	2.25	2.6
25 μM $PMSH_2$ as donor (PS1)		
None (control)	5.4	4.56
Salicylaldoxime, ADP, P_i	2.55	3.55

Conditions as in Materials and methods. Chloroplasts extracted by the method of McCarty [8]. Broken chloroplasts prepared by substituting 10 mM NaCl for EDTA in this procedure. Chlorophyll concentration 23 μg/ml for PS1 + 2 and 30 μg/ml for PS1, light intensity 20 klux.

Table 4
Effect of chlorophyll concentration on inhibitory effect of salicylaldoxime with ADP and P_i.

Chlorophyll (μg/ml)	Rate of methyl red reduction (μmoles hr^{-1} (mg chlorophyll)$^{-1}$)	
	Control	Salicylaldoxime, ADP, P_i added
8.4	16.5	19.7
16.7	8.4	10.6
20.8	6.2	3.4
25.0	4.9	2.9

Conditions as in Materials and methods, with 25 μM PMS as electron donor. Light intensity 20 klux.

4. Discussion

The sensitivity to salicylaldoxime of electron flow from low concentrations of $PMSH_2$ to methyl red differed from the other donor systems studied in its requirement for ADP and P_i and its relief by NH_4^+ and EDTA extraction of the chloroplasts. These observations are consistent with the suggestions that electrons from low concentrations of $PMSH_2$ may enter the electron transport system via more than one path, one of them phosphorylating [3]; and that electrons from concentrations of $PMSH_2$ higher than those supporting phosphorylation [9] enter the electron transport sequence at a point after the phosphorylation site [4].

Our experiments indicate that in the presence of ADP and P_i electrons from low concentrations of $PMSH_2$ may be constrained to follow a salicylaldoxime sensitive pathway.

ADP and P_i may induce a conformational change [10] which could give rise to the preferred salicylaldoxime sensitive path. Both NH_4^+ and EDTA treatment uncouple chloroplasts and diminish the light scattering changes observed when chloroplasts are illuminated, and prevent the development of a high energy state [11]. The findings of Ryrie and Jagendorf [12] suggest that a conformational change may occur in the phosphorylating protein (chloroplast coupling factor, CF_1) during ATP formation. We did not detect any phosphorylation in the PMS–methyl red system although it is known that phosphorylation accompanies noncyclic flow through PS1 [13]; however, a phosphorylation associated with the low rates of electron flow in our PMS–methyl red system may have been below the limits of detection.

Stuart [14] proposed two salicylaldoxime sensitive sites: one near PS1 in a phosphorylating side-path and another nearer PS2. Both sites are effectively inhibited at 10 mM salicylaldoxime, the concentration used in these experiments. The first of these sites could be concerned with the inhibition of $PMSH_2$ mediated methyl red reduction, and the second accounting for the inhibition of electron flow from H_2O to methyl red. Electrons from $DCIH_2$ and $TMPDH_2$ have access to a salicylaldoxime sensitive site in the absence of ADP and P_i (perhaps the second site mentioned above) while electrons from high concentrations of $PMSH_2$ enter the electron transport chain after the salicylaldoxime sensitive site(s).

The inhibition is more evident at subsaturating light intensities; this may indicate that at high enough intensities, the rate of electron flow through the salicylaldoxime insensitive path is great enough to compensate for the blockage of the sensitive path; or that at very high intensities, an electron transport step past the convergence point of the sensitive and insensitive pathways may become rate limiting, so that any inhibition prior to this point is not detected.

Table 5
Effect of light intensity on inhibitory effect of salicylaldoxime, ADP and P_i on methyl red reduction with 25 μM $PMSH_2$.

Experiment number	Chlorophyll concentration (μg/ml)	Additions to reaction mixture	Rate of methyl red reduction (μmoles hr^{-1} (mg chlorophyll)$^{-1}$)		
			1.75 klux	20 klux	72 klux
1	17.5	None (control)	7.3	8.1	–
		Salicylaldoxime	5.6	8.4	–
		ADP, P_i			
2	24.3	None (control)	–	4.8	6.3
		Salicylaldoxime	–	2.7	6.4
		ADP, P_i			
	30.4	None (control)	–	4.4	4.1
		Salicylaldoxime	–	3.2	2.0
		ADP, P_i			

Conditions as in Materials and methods.

References

[1] N.I. Bishop, Ann. Rev. Biochem. 40 (1971) 197.
[2] B. Kok and H.J. Rurainski, Biochim. Biophys. Acta 94 (1965) 588.
[3] M. Schwartz, Biochim. Biophys. Acta 131 (1967) 548.
[4] L.P. Vernon and M. Avron, Ann. Rev. Biochem. 34 (1965) 269.
[5] M. Nishimura, Biochim. Biophys. Acta 153 (1968) 838.
[6] A. Goffeau and J.M. Bove, Biochim. Biophys. Acta 102 (1965) 103.
[7] L.P. Vernon, Anal. Chem. 32 (1970) 1144.
[8] R.E. McCarty, in: Methods in Enzymology, Vol. 23 part A, ed. A. San Pietro (Academic Press, New York, 1971) p. 251.
[9] A.T. Jagendorf and M. Avron, J. Biol. Chem. 231 (1958) 277.
[10] L. Packer, Biochim. Biophys. Acta 75 (1963) 12.
[11] G. Hind and A.T. Jagendorf, J. Biol. Chem. 240 (1965) 3202.
[12] I.J. Ryrie and A.T. Jagendorf, J. Biol. Chem. 246 (1971) 3771.
[13] N. Shavit and V. Shoshan, FEBS Letters 14 (1971) 265.
[14] T.S. Stuart, Planta 96 (1971) 81.