PHOTOSYNTHETIC CONTROL IN BROKEN SPINACH CHLOROPLASTS

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1. Introduction

West and Wiskich [1] showed that the rate of photosynthetic electron transport in class 1 pea chloroplasts was regulated by the presence or absence of the intermediates required for photophosphorylation. By analogy with respiratory control in mitochondria [2] they termed this regulation photosynthetic control.

West and Wiskich [1] showed that in the presence of magnesium and phosphate the initial rate of electron transport was stimulated by addition of ADP. After conversion of all of the ADP to ATP the rate of electron transport decreased to a rate lower than the initial rate (i.e. to the controlled rate). The ratio of the fast phosphorylating rate to the slow controlled rate was termed the photosynthetic control (PC) ratio.

Kraayenhof [3] obtained similar results with class 1 chloroplasts.

West and Wiskich [1] showed that the PC ratio was decreased by exposing their whole chloroplasts to increasing periods of sonic oscillation and therefore concluded that photosynthetic control is dependent on the structural integrity of the chloroplasts. However, the loss of control in their experiments was accompanied by a decrease in the ADP/O ratio.

Earlier workers using different measurement techniques had shown that the rate of electron transport was higher under phosphorylating that under non-phosphorylating conditions [4] and that the rate of electron transport decreased when all the ADP was phosphorylated [5].

We have reinvestigated photosynthetic control, in chloroplasts broken by osmotic shock. Broken chloroplasts retain the ability to catalyse photophosphorylation with an ADP/O ratio of one and show photosynthetic control ratios greater than two.

2. Methods

Broken washed spinach chloroplasts ($P_1 S_1$) were isolated essentially as described by Whatley and Arnon [4]. 'Whole' chloroplasts were isolated essentially as described by Horton and Hall [6]. The grinding medium consisted of 0.35 M NaCl, 0.04 M TES (*N*-tris(hydroxymethyl)-methyl-2-aminoethane sulphonic acid), 1 mM $CaCl_2$ and 1 mM EDTA adjusted to pH 7.3 with KOH. The resuspending medium consisted of 0.2 M sucrose, 0.03 M tricine buffer (*N*-tris (hydroxymethyl) methylglycine) and 5 mM $CaCl_2$ adjusted to pH 7.3 with KOH. These chloroplasts retain their structural organisation while losing their outer membranes. The chlorophyll concentration was measured by the method of Arnon [7]. Electron transport was measured as an oxygen uptake in the presence of methyl viologen. The rates of electron transport and photophosphorylation were found to be independent of methyl viologen concentration between 2 and 100 μM, the range of concentrations which were used in these experiments. The autoxidation of methyl viologen, reduced during photosynthetic electron transport, results in the formation of hydrogen peroxide [8] and the net uptake of 1 atom of oxygen per two electrons transported. Photophosphorylation was monitored by changes in pH, associated with ATP synthesis [9], in reaction mixtures with lower buffer concentration than those routinely used.

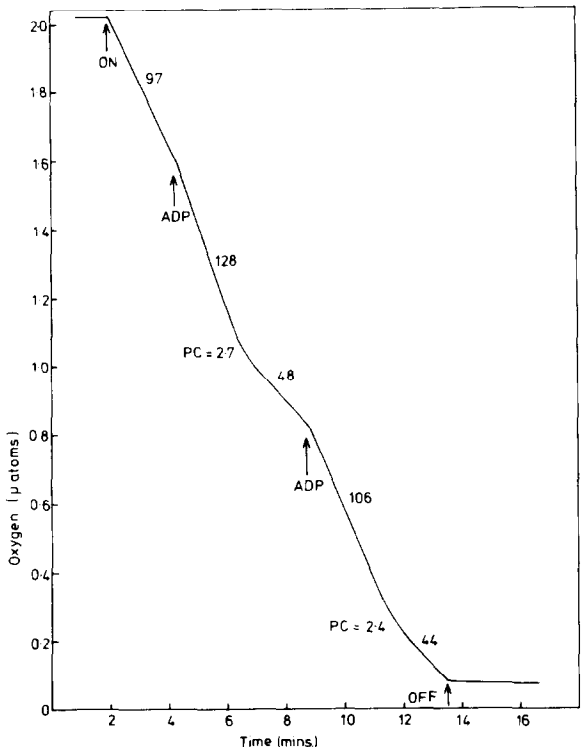

Fig. 1. Photosynthetic control with broken chloroplasts. The reaction mixture contained, in a total volume of 3 ml: broken chloroplasts equivalent to 116 μg chlorophyll: 150 μmoles tricine buffer (pH 8.4); 600 μmoles sucrose; 60 μmoles KCl; 6 μmoles $MgCl_2$; 3 μmoles sodium azide; 0.15 μmole methyl viologen and 30 μmoles potassium phosphate (pH 8.2). 0.5 μmole ADP (pH 8.2) was added as indicated. The rate of oxygen uptake in μatoms oxygen/mg chlorophyll/hr and photosynthetic control (PC) ratios are shown.

Simultaneous measurements of oxygen and pH changes were made in a Rank oxygen electrode cell fitted with a combined glass electrode through the lid of the reaction vessel. The reaction mixture was maintained at a temperature of 20° and was illuminated by a slide projector (300 W) through a heat filter and a filter transmitting light between 540 nm and 740 nm.

ADP and ATP were obtained from Boehringer Corporation Ltd., TES was obtained from Calbiochem Ltd. and methyl viologen from British Drug Houses Ltd.

Table 1
Photosynthetic control ratios obtained with 'whole' and broken chloroplasts.

Expt.	ADP additions	Rate of ET (μatoms O_2/mg Chl/hr)			Photosynthetic Control ratio
		Initial	Phos.	Controlled	
'Whole chloroplasts'					
A	1st	125	172	58	3.0
	2nd	–	154	46	3.4
B	1st	125	167	63	2.7
	2nd	–	134	58	2.3
'Broken chloroplasts'					
C	1st	97	128	48	2.7
	2nd	–	106	44	2.4
D	1st	93	110	57	1.9
	2nd	–	97	57	1.7
E	1st	101	123	62	2.0
	2nd	–	106	57	1.9

The reaction mixtures contained, in a total volume of 3 ml: 'whole' chloroplasts equivalent to 122 μg chlorophyll (expts. A and B), or broken chloroplasts equivalent to 116 μg chlorophyll (expts. C, D and E); 150 μmoles tricine buffer (pH 8.4), 600 μmoles sucrose (except expt. E); 60 μmoles KCl; 6 μmoles $MgCl_2$; 3 μmoles sodium azide; 0.15 μmole methyl viologen and 30 μmoles potassium phosphate (pH 8.2). ADP (pH 8.2) was added as follows: Expts. A and C, 0.5 μmole and Expts. B, D and E, 0.25 μmole.

3. Results and discussion

Fig. 1 shows oxygen uptake by illuminated broken washed chloroplasts using methyl viologen as electron carrier for pseudocyclic electron transport. Oxygen uptake in the presence of magnesium and phosphate is stimulated by addition of ADP. When all the ADP has been phosphorylated the rate of oxygen uptake decreases to a slow controlled rate. Simultaneous measurement of photophosphorylation indicated that changes in the rate of electron transport coincided with the onset and completion of phosphorylation. The controlled rate of electron transport can be restimulated to the phosphorylating rate by addition of more ADP or of an uncoupler. Similar results are obtained using 'whole' chloroplasts. Table 1 compares the rates of electron transport, the effect of

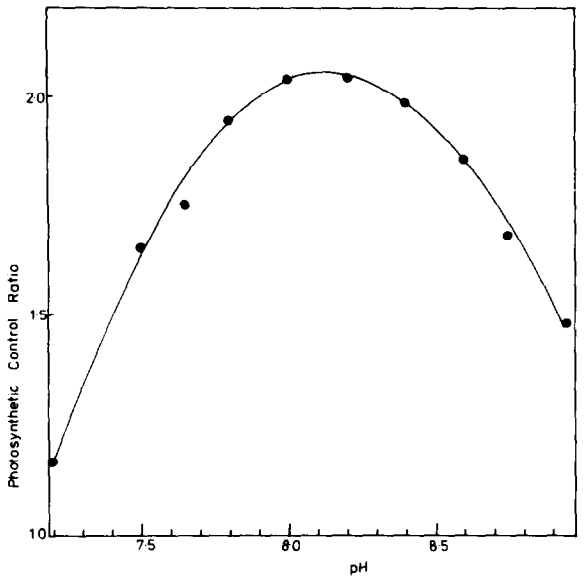

Fig. 2. The effect of pH on the photosynthetic control ratio of broken chloroplasts. The reaction mixtures contained, in a total volume of 3 ml: broken chloroplasts equivalent to 200 μg chlorophyll; 150 μmoles tricine buffer at the indicated pH; 150 μmoles KCl; 6 μmoles $MgCl_2$; 6 μmoles sodium azide; 6 μmoles methyl viologen; 10 μmoles potassium phosphate (pH 8.2) and 0.4 μmole ADP.

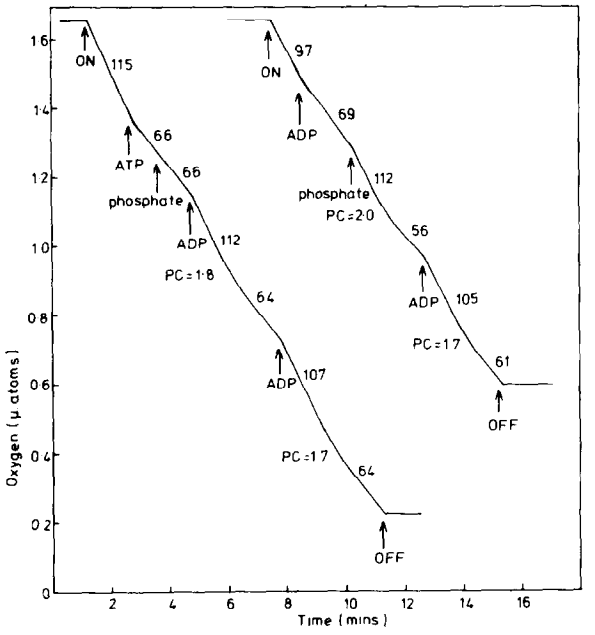

Fig. 3. The effect of ADP and ATP on the initial rate of electron transport by broken chloroplasts in the absence of phosphate. The reaction mixtures contained, in a total volume of 3 ml: broken chloroplasts equivalent to 100 μg chlorophyll; 150 μmoles tricine buffer (pH 8.4); 150 μmoles KCl; 3 μmoles $MgCl_2$; 6 μmoles sodium azide; 0.3 μmole methyl viologen. 0.2 μmole ADP, 0.3 μmole ATP and 2 μmoles potassium phosphate (pH 8.2) were added as indicated. The rate of oxygen uptake in μatoms oxygen/mg chlorophyll/hr and the photosynthetic control (PC) ratios are shown.

the addition of ADP and the PC ratios of 'whole' and broken chloroplasts.

The PC ratio for 'whole' chloroplasts varies between 2.3 and 3.4 while the values for broken chloroplasts are slightly lower, varying between 1.7 and 2.7. The values obtained also vary with the variety of spinach used and the time of year.

These results obtained with broken chloroplast fragments and 'whole' chloroplasts, which have lost their outer membranes while retaining their lamellar structure, are very similar to those reported by West and Wiskich [1] using chloroplasts with apparently intact outer membranes. They reported PC values between 2.2 and 3.0 for comparable experiments.

Fig. 2 shows the effect of pH on the PC ratios obtained with broken chloroplasts. The ratios were highest at pH 8.0–8.2 falling at both higher and lower pH. The decrease in Pc ratio at lower pH is accompanied by a decrease in electron transport rates, while at higher pH electron transport and phosphorylation rates increase to a maximum at pH 8.5. West and Wiskich [1] carried out the majority of their experiments at pH 7.2 obtaining lower PC ratios at pH 8.0 and above; it is not clear whether this is a difference between pea and spinach chloroplasts or between class 1 and other types of chloroplast preparations.

Fig. 3 shows the effect of ADP and ATP on the basal rate of electron transport in the presence of magnesium but absence of phosphate, confirming reports of ADP and ATP inhibition by other workers [see 10]. Both ADP and ATP inhibit the rate of electron transport. The inhibition is relieved by addition of phosphate in the presence of ADP or by the addition of ADP and phosphate in the presence of ATP. After phosphorylation of all the ADP the rate of electron transport again decreases to the controlled rate. The ADP or ATP inhibited rate is essentially the same as the controlled rate, suggesting that the

controlled rate results from the presence of adenine nucleotides under conditions where phosphorylation cannot occur.

The results presented here show that control of electron transport by photophosphorylation in chloroplasts is a property of the grana membranes and not of the gross structure of the chloroplasts.

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