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'State 3 – State 4 transition' and phosphate potential in 'Class I' spinach chloroplasts

In a recent report of WEST AND WISKICH[1] it is clearly demonstrated that carefully isolated pea chloroplasts show 'photosynthetic control' comparable with the respiratory control of intact liver mitochondria, the classical parameter for integrity of these organelles. Appreciable photosynthetic-control ratios were only observed[1] with the so-called 'Class I' chloroplasts[2], containing the outer envelope membrane and the stroma phase. Although chloroplasts or chloroplast fragments lacking the stroma phase show up to a 3-fold stimulation of electron transport under conditions of phosphorylation, no control is observed when the ADP is exhausted. 'Class I' chloroplasts, therefore, seem to present a phosphorylating system with properties comparable to those of phosphorylating mitochondria.

On the assumption that the 'State 3–State 4 transition' in these chloroplasts reflects the moment that the phosphorylation reaction comes into equilibrium (*cf.* ref. 3), it is of interest to determine the photosynthetic phosphorylation potential and to compare this with the values recently reported for the oxidative phosphorylation potential in mitochondria[3–5].

'Class I' spinach chloroplasts were prepared by a procedure based on recent reports[6–8]. Young leaves (10 g) of spinach grown in a glasshouse were chilled directly after harvesting, washed and gently crushed in a cold mortar containing 30 ml isolation medium (300 mM sucrose, 30 mM tris(hydroxymethyl)methylaminoethanesulphonic acid (pH 7.2) and 3 mM $MgCl_2$) using a ribbed spindle-shaped Teflon roller. The slurry was filtered through 4 layers of perlon net (mesh width, 56 μ) and briefly centrifuged. The centrifuge was accelerated to 3000 $\times$ g_{max} and decelerated by hand, the total centrifugation time being 1.5 min. The loose pellet was homogenized with 1 ml of isolation medium by putting the centrifuge tubes on a Vortex mixer and by sucking up in a pipette. The suspension usually contained about 1.5 mg chlorophyll per ml. Under the phase-contrast microscope, it appeared to be a fairly homogeneous preparation of refractive 'Class I' chloroplasts[2,6].

Electron-transport and phosphorylation activities were measured in a small thermostated cuvette (volume 1.2 ml) supplied with a Clark oxygen electrode, a micro pH electrode (type GM 23B, Electronic Instruments), to follow ADP consumption under conditions of cyclic phosphorylation, and a fluorescence device (Eppendorf) for measurement of $NADP^+$ reduction. The fluorescence scale was calibrated under the experimental conditions used by additions of known amounts of NADH. The contents of the cuvette were vigorously stirred by a Teflon-coated magnetic stirrer and were illuminated by a strong light source (a 24 V, 150-W halogen lamp). A red filter (Schott RG1) and a 6-cm water layer were placed in the light path, and the photomultiplier was shielded by a narrow-band filter (440–460 nm). AMP, ADP and ATP were enzymically determined[9,10] in a double-beam spectrophotometer, and inorganic phosphate was determined colorimetrically[11].

Fig. 1 demonstrates the 'State 3–State 4 transition' of $NADP^+$ reduction and O_2 production, concomitant with the exhaustion of most of the added ADP. The ADP/NADPH and ADP/O ratios were about 0.8 in this experiment. The photo-

synthetic control ratio was about 2; in other experiments it was usually between 1.5 and 2.5. The phosphate potential was calculated according to the formula[3–5]:

$$\Delta G' = \Delta G_0' + 1.36 \log [ATP]/[ADP]\,[P_i]$$

where $\Delta G'$ is the phosphate potential in kcal/mole, and $\Delta G_0'$ the standard free-energy change when [ATP], [ADP] and [P_i] are 1 M. It was, in this experiment, 15.53

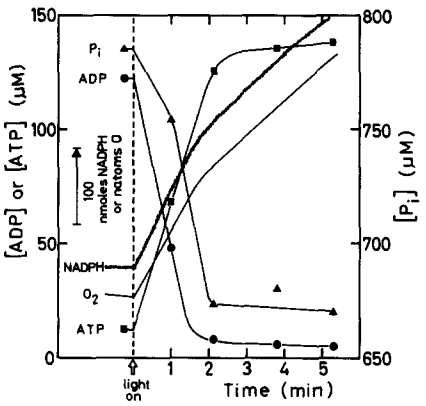

Fig. 1. 'State 3–State 4 transition' in $NADP^+$ reduction and O_2 production of illuminated 'Class I' spinach chloroplasts. The incubation medium (1.2 ml) contained 250 mM sucrose, 20 mM tris-(hydroxymethyl)methylaminoethanesulphonic acid (pH 7.8), 3 mM $MgCl_2$, 1 mM $NADP^+$, a saturating amount of a ferredoxin preparation[12], 1 μg oligomycin, ADP and P_i as indicated and chloroplasts containing 97 μg chlorophyll. Before the addition of chloroplasts and ferredoxin, the medium was made anaerobic by flushing with oxygen-free nitrogen. The reaction temperature was 25°. The final pH was 7.60. Samples were taken at different times and the reaction stopped with $HClO_4$ to 3.5%. AMP, ADP, ATP and P_i were determined[9–11] in the neutralized supernatants. In no case was AMP detected.

kcal/mole (at 5.3 min). Oligomycin was present in the incubation medium to avoid any contribution to the potential of possible mitochondrial contamination. Omission of the antibiotic, however, did not influence the results.

Table I shows that the potential is independent of the initial concentrations of

TABLE I

THE EFFECT OF DIFFERENT INITIAL CONCENTRATIONS OF ATP, ADP AND P_i ON THE PHOSPHATE POTENTIAL

Conditions as in Fig. 1. $\Delta G_0'$ was calculated from refs. 13 and 14. In the case of high ATP concentrations a small correction for ATP hydrolysis in the acid extraction was necessary.

Expt. No.	*Initial concn. (μM)*			*Final concn. (μM)*			$\frac{[ATP]}{[ADP]\,[P_i]}$ (M^{-1})	*pH*	$\Delta G_0'$ *(kcal/mole)*	$\Delta G'$ *(kcal/mole)*
	ATP	*ADP*	P_i	*ATP*	*ADP*	P_i				
1	12	122	785	138	5	670	41 194	7.60	9.25	15.53
2	995	138	775	1150	46	667	37 481	7.60	9.25	15.47
3	4150	212	840	4214	158	789	33 803	7.80	9.40	15.56
4	12	104	1821	140	2	1720	40 698	7.60	9.25	15.51
5	1672	176	4140	1819	27	4011	16 796	7.80	9.40	15.15
6	4158	121	1690	4210	71	1620	36 602	7.80	9.40	15.61

TABLE II

THE PHOTOPHOSPHORYLATION POTENTIAL WITH DIFFERENT ELECTRON-TRANSPORT SYSTEMS

Incubation conditions as in Fig. 1. In the case of cyclic phosphorylation, the moment of ADP exhaustion was determined by sensitive pH recording, and the medium was aerobic.

Electron-transport system	$\Delta G'$ (*kcal/mole*)
Non-cyclic	
$NADP^+$ (1 mM) + ferredoxin	15.46
$K_3Fe(CN)_6$ (1 mM)	15.45
Cyclic	
Pyocyanine (5 μM)	15.55
Phenazine methosulphate (5 μM)	15.57

ATP, ADP and P_i. The influence of different electron-accepting systems is shown in Table II. It is clear that the phosphate potential is the same with non-cyclic and cyclic electron transport.

In 9 out of the 10 experiments cited in Tables I and II, the phosphate potential was found to lie between 15.45 and 15.61 kcal/mole. This is very similar to the value found for mitochondrial oxidative phosphorylation, *i.e.*, 15.8 kcal/mole[3,5].

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