NUCLEOTIDES EFFECT ON THE DECAY KINETICS OF THE 520 nm ABSORBANCE CHANGE IN TIGHTLY COUPLED CHLOROPLASTS

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SUMMARY : The faster dark decay of the 520 nm absorbance change in phosphorylating conditions can be achieved with the same efficiciency by addition of ATP alone.

The substitution of 1,N6 etheno ADP (εADP) to ADP or of 1,N6 etheno ATP (εATP) to ATP are absolutely ineffective on the 520 nm absorbance change dark decay in spite of the good phosphorylation of εADP into εATP.

The conclusion is that the faster dark decay of the 520 nm absorbance change is not linked directly with phosphorylation but is the consequence of the interaction of newly formed or added ATP with coupling factor.

INTRODUCTION

It has been shown by Rumberg and Siggel (1) that the dark decay of the 520 nm absorbance change following a single flash was accelerated by suspending chloroplasts in a complete phosphorylating medium. Removing of the coupling factor (CF_1) led to a complete disappearance of the photophosphorylation and the slow phase of the 520 nm absorbance change. Progressive reincorporation of the purified CF_1 restored stepwise these both processes (2). Using groups of short flashes technics (3) we show in the present communication that, in tightly coupled chloroplasts, the acceleration of the dark decay of the 520 nm absorbance change by phosphorylating conditions is the result of the interaction of the newly synthesized ATP with the membranes and is also observed by addition of the ATP only.

MATERIAL AND METHODS

Spinach chloroplasts were prepared according to standard technics (4-5).

Chloroplasts were suspended in a medium 10 mM tricine pH 8.2, 5 mM

List of abbreviations :
PMS : phenazine methosulfate
BSA : bovine serum albumin

$MgCO_3$, 0.4 M sucrose, 0.8 % BSA, 50 µM PMS and eventually ADP, ATP or Pi as indicated.

Chloroplasts were illuminated in a 10 x 10 mm 2.5 ml cell, jacketted at 5°C. Illumination was supplied alternatively through two horizontal light guides by two xenon flashes (General Radio Stroboslar) perpendicular to the measuring beam. The exciting light passed through a calflex (Balzers) filter and a Schott RC 630-3 mm filter. Saturating exciting flashes (flash duration 2 µs) were given in groups of six, at the cadence of one flash each 2ms. The repetition rate of the flashes groups was 0.07 s^{-1}. The measuring beam came through a Baush and Lomb monochromator, crossed the cuvette and was led by a light guide to a EMI 9558 B photomultiplier protected by a Schott BG 18-3 mm filter. 10 signals were averaged using a Didac 800 multichannel analyzer (6).

The 520 nm absorbance change extent was practically saturated after the fifth flash. The ratio of the amplitude of the 520 nm absorbance change after the sixth flash over the first one varied between 2 and 3 according to the preparations of chloroplasts. For each experiment, the decay kinetics of the absorption change at 520 nm were decomposed into a rapide and a slow exponential phase, the initial amplitude of this last one being related to the ability of those chloroplasts to synthesize ATP (2). Acceleration of the dark decay of the 520 nm absorbance change was measured by the decrease of the amplitude of this slow phase.

RESULTS

Figure 1 shows the effect of ATP addition on the decay rate of 520 nm absorbance change after a group of short flashes. Thus the decrease of the slow phase extent usually obtained in phosphorylating conditions is also observed with ATP aone.

Table 1 shows that the efficiency of ATP alone on the slow phase of

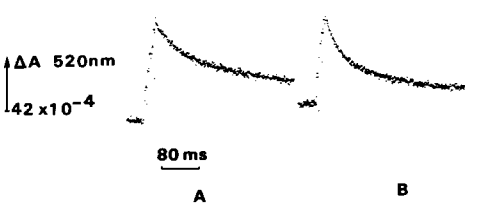

Fig. 1 Time course of the absorbance change at 520 nm after excitation with short 6 flashes groups
A - control decay rate without ATP
B - decay rate after addition of 2 mM ATP

	ΔA 520 nm x 10^{-4}
control	40
+ ADP	40
+ ADP +Pi	28
+ ATP	25
control	37
+ ADP + Pi	28
+ ATP	21
Control	59
+ Pi	53
+ Pi + ADP	39
+ ATP	39

Table 1 - Effect of phophorylating medium or ATP alone on the slow phase amplitude of the 520 nm absorbance change after excitation with short flashes groups.

In the medium given in the methods are added when indicated ADP 0.4 mM and Pi 0.8 mM or ATP 0.4 mM.

	ΔA 520 nm x 10^{-4}
Control	39
+ Pi	38
+ Pi + ADP	27
+ ATP	24
+ ATP + PEP +pyruvate kinase	24

Table 2 - Effect of phosphorylating medium or ATP incubated with an ATP regenerating system on the slow phase amplitude of the 520 nm absorbance change.

Concentrations of nucleotides or Pi as indicated in Table 1.

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the 520 nm absorbance change is of the same order as that of phosphorylating medium.

The effect of ATP cannot be caused by contamination of commercial ATP by small amounts of ADP :

- when ATP was incubated before use with an ATP regenerating system, phospho-enol pyruvate (PEP) + pyruvate kinase, and was indeed ADP free, results were identical (Table 2).

- ADP needs to be effective the presence of a high concentration of inorganic phosphate (Table 3) whereas ATP is active without Pi addition.
- ATP is effective at a fairly low concentration when contaminating amounts of ADP-Pi are too low to be active (Table 4).

The presence of Mg^{++} is necessary for observation of these effects (Table 5).

	ΔA 520 nm x 10^{-4}
control	63
control + 1000 nmole ADP	61
" + " + 10 nmole Pi	60
" + " + 100 " "	56
" + " + 250 " "	55
" + " + 500 " "	51
" + " + 750 " "	41
" + " + 1000 " "	37
" + 1000 nmole ATP	32

Table 3 - Effect of increasing amounts of Pi in the presence of a definitive amount of ADP on the 520 nm absorbance change slow rate amplitude.
The amounts of nucleotides or Pi indicated were added in the 2.5 ml cuvette.

ΔA 520 nm x 10^{-4}		
control 41	control..... 38	control 45
+ 750 nmole Pi 38	+ 750 nmole Pi 34	
+ 750 nmole Pi + 1 nmole ADP 35	+ 750 nmole Pi + 1 nmole ATP* 32	+ 1 nmole ATP* 41
+ 750 nmole Pi + 11 nmole ADP 30	+ 750 nmole Pi + 11 nmole ATP 28	+ 11 nmole ATP 36
+ 750 nmole Pi + 50 nmole ADP 27	+ 750 nmole Pi + 50 nmole ATP 24	+ 50 nmole ATP 31
+ 750 nmole Pi + 100 nmole ADP 25	+ 750 nmole Pi + 100 nmole ATP 21	+ 100 nmole ATP 30

* in these two batches ATP was added after incubation in the ATP regenerating system (PEP + Pyruvate kinase)

Table 4 - Effect of increasing amounts of nucleotides on the 520 nm absorbance change slow rate amplitude.
Amounts of Pi, ADP or ATP were added successively in the same 2.5 ml cuvette.

	ΔA 520 nm x 10^{-4}
control	41
+ ATP	40
+ ATP + KCl	40
+ ATP + KCl + $MgCl_2$	30
control	56
+ KCl	56
+ KCl + ATP	56
+ KCl + $CaCl_2$ + ATP	47
+ KCl + $CaCl_2$ + $MgCl_2$ + ATP	20

Table 5 - Effect of cations on the slow phase amplitude of the 520 nm absorbance change.
In the medium given in the methods in which $MgCO_3$ is omitted are added when indicated : KCl, $CaCl_2$, $MgCl_2$ 10 mM, ATP 0.16 mM.

	ΔA 520 nm x 10^{-4}
control*	54
control + εATP	53
" + " + Pi	56
" + " + " + εADP	58
" + " + " + " + ADP	47
" + " + " + " + " + ATP	44
control + ATP	42
control*	38
control + εATP	36
" + " + εADP	36
" + " + " + Pi	36
" + " + " + " + ADP	26
control + ATP	28

Table 6 - Effect of various nucleotides on the 520 nm slow phase absorbance change.

* In these two experiments the reagents indicated are added successively in the same batch : εATP, εADP, ATP, ADP 0.4 mM, Pi 0.8 mM. The same chloroplasts tested in continuous photophosphorylation with PMS have synthezized 1065 µmoles ATP. h^{-1}.mg $chlorophyll^{-1}$ and 815 µmoles εATP.h^{-1}.mg $chlorophyll^{-1}$.

Besides the role of the third phosphate of ATP, the structure of adenine moiety is very important.

With the adenine analog, 1,N6 etheno adenine neither the nucleotide diphosphate (εADP) in the presence of inorganic phosphate nor the nucleotide triphosphate (εATP) are active (Table 6). The faster decay of the 520 nm absorbance change dark decay can hardly be directly linked to phosphorylation reactions : though εADP is phosphorylated almost at the same speed as ADP, it is in fact ineffective on the 520 nm absorbance change kinetics.

DISCUSSION

It is often admitted that the 520 nm absorbance change is proportional to the membrane potential and that it is decaying parallelly to the decrease of this potential by ions transport through the membrane. ATP synthesis is directly linked to an efflux of protons which is so initiated in tightly coupled chloroplasts by phosphorylating conditions and lead to an acceleration of the membrane potential dissipation and of the 520 nm absorbance change decay.

The results of our experiments are not consistent with this interpretation. In our conditions phosphorylation reactions are not directly involved in the acceleration of the 520 nm absorbance change dark decay, but newly formed ATP molecules modify the membrane properties and so the kinetics of the 520 nm signal decay. As we have seen that, after addition of immunoglobuline against the coupling factor, ATP does not lead to an acceleration of the 520 nm absorbance change dark decay, it seems that those effects we observed need the newly formed or added ATP to act on coupling factor. That kind of action of added or newly formed ATP on the 520 nm absorbance change kinetics is very close to the enhancement of the proton uptake by ATP described by Mac Carty with chloroplast suspensions (7). In this last case coupling factor is also involved.

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