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STUDIES ON THE ENZYME SYSTEMS INVOLVED IN ELECTRON AND ENERGY TRANSFER IN ISOLATED CHLOROPLASTS

I. EFFECT OF ENDOGENOUS PHOSPHATE ON THE PHOTOPHOSPHORYLATION COUPLED WITH NONCYCLIC ELECTRON TRANSPORT IN INTACT CHLOROPLASTS

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SUMMARY

1. It was found that the P/2*e* ratio was independent of the degree of electron transport stimulation in the presence of ADP and P_i and exceeded 1.0 in the preparations with slight (30 %) as well as with high (80 %) stimulation.

2. Chloroplast preparations having a low content of endogenous P_i showed higher stimulation than those with higher contents.

3. Illumination of the chloroplasts in the presence of ADP and electron acceptor led to a decrease of endogenous P_i content that resulted in an increase of electron transport stimulation in the presence of exogenous P_i.

4. Electron transport in the absence of exogenous P_i was inhibited by both exogenous ADP and ATP.

5. It appears that the electron transport in the absence of exogenous P_i is coupled to phosphorylation, which occurs because isolated chloroplasts contain endogenous P_i. Stimulation of the electron transport by the addition of ADP and P_i seems to be caused by acceleration of the existing electron transport pathway, and not from the initiation of a new one.

INTRODUCTION

Addition of ADP and P_i increase the electron flow in spinach chloroplasts[1]. The acceleration of electron transport in chloroplasts due to the addition of ADP and P_i reported by various authors varies from 15 % (see ref. 2) to 70–400 % (see refs. 1, 3–5).

DEL CAMPO *et al.*[6] postulate that the stimulation of electron flow in the presence of ADP and P_i results from the noncyclic electron transport coupled as a whole with phosphorylation with a P/2*e* ratio = 1.0. Addition of ADP and P_i only increases the rate of electron transport by the pathway already existing in chloroplasts.

Abbreviation: Tricine, *N*-Tris(hydroxymethyl)methylglycine.

On the other hand, IZAWA AND GOOD[7] consider the stimulation of electron flow by ADP and P_i as due to phosphorylating electron transport in chloroplasts. Electron flow in the absence of exogenous ADP and P_i ("basal electron transport") is not coupled with phosphorylation. According to these authors the stoicheiometry of noncyclic phosphorylation is expressed by the $P/\Delta 2e$ ratio $= 2.0$ which considers exclusively the electron transport brought into action when ADP and P_i are added.

It is probable that considerable electron transport occurs in chloroplasts even in the absence of the exogenous P_i and ADP. The effect of endogenous P_i has not so far been studied.

In this paper the yield of photophosphorylation and the increment of electron transport stimulation in the presence of ADP and P_i correlated with the level of endogenous P_i are presented. A preliminary account of this investigation has been reported[8–10].

MATERIALS AND METHODS

Chloroplasts were prepared from freshly harvested spinach leaves by the method of JENSEN AND BASSHAM[11]. The solution for the isolation of chloroplasts contained no phosphate, the buffer Tricine (*N*-tris(hydroxymethyl)methylglycine) being used.

Chlorophyll was measured by the method of ARNON[12].

The reactions were performed under air in Warburg manometer vessels at 19°. White light from a bank of 500 W lamps provided illumination of 50 000 lux at the bottoms of the vessels.

Ferricyanide was estimated by measurement of absorbance at 420 nm.

ATP formation was measured by the increase in labelled organic phosphate determined by the method of NIELSEN AND LEHNINGER[13], as modified by AVRON[14]. The ATPase activity and the exchange of P_i–ATP were determined according to the procedure of KRAAYENHOF *et al.*[15].

To establish the amount of endogenous P_i in the chloroplast preparation (1 mg chlorophyll), it was extracted for 2 min in a Potter homogenizer with 10 % trichloroacetic acid. The protein was removed by centrifugation. The phosphate in the supernatant was determined by the method of CHEN *et al.*[16].

To remove endogenous P_i the chloroplast preparation (50–70 μg of chlorophyll) was incubated in the light in a mixture that included 50 mM Tricine–NaOH buffer (pH 8), 5 mM $MgCl_2$, 34 mM NaCl, 2 mM $K_3Fe(CN)_6$ and 2 mM ADP.

Reagents. The reagents used were commercial preparations: ADP, Sigma and Calbiochem; ATP, Reanal; Tricine, Calbiochem.

RESULTS

Table I shows both the ATPase and P_i–ATP exchange activities in chloroplast preparations. Regarding the increment of their activity the chloroplasts should be considered as Class I spinach chloroplasts[15,17]. The percentage of intact chloroplasts as judged by a light microscopic examination of the isolated suspension used for experiments was at least 85 %

The chloroplasts, whose $P/2e$ ratio is shown in Table II, were characterized by a varying value for the electron transport stimulation in the presence of ADP and P_i. In the chloroplast preparations the stimulation amounted to 12–90 % (Table II, see

TABLE I

ACTIVITY OF ATPASE AND P_i–ATP EXCHANGE IN ISOLATED SPINACH CHLOROPLASTS

The reaction mixture contained in 3 ml: 50 mM Tricine–NaOH buffer (pH 8), 5 mM $MgCl_2$, 35 mM NaCl. The reaction was initiated by 3 mM ATP. The content of chlorophyll was 80–100 μg. The reaction mixture used to measure the activity of the P_i–ATP exchange contained 5 mM $^{32}P_i$ (80000–120000 counts/min per 3 ml mixture).

Expt.	*(A) ATPase activity (nmoles/mg chlorophyll per min)*	*(B) P_i–ATP exchange (nmoles/mg chlorophyll per min)*
1	346	60
2	407	40
3	515	58
4	200	34
5	486	70

TABLE II

THE EFFECT OF THE STIMULATION OF ELECTRON TRANSPORT FLOW BY P_i AND ADP ON $P/2e$ AND $P/\Delta 2e$ RATIOS

The reaction mixture contained in 3 ml: 50 mM Tricine–NaOH buffer (pH 8), 5 mM $MgCl_2$, 1.5 mM $^{32}P_i$ (90000–120000 counts/min per 3 ml reaction mixture), 35 mM NaCl, 1 mM $K_3Fe(CN)_6$, 3 mM ADP. Reaction time 3 min.

Expt.	*$K_3Fe(CN)_6$ reduction**		*ATP**	*Stimulation (%)*	*P/2e*	*P/Δ2e*
	$-P_i$	$+P_i$				
1	250	414	245	65	1.18	3.30
2	160	305	163	90	1.06	2.24
3	218	348	200	60	1.16	3.06
4	263	355	206	36	1.25	5.70
5	304	408	250	33	1.04	4.80
6	287	365	194	27	1.06	5.00

* μmoles/mg chlorophyll per h.

TABLE III

THE EFFECT OF ENDOGENOUS P_i CONTENT ON THE STIMULATION OF ELECTRON TRANSPORT IN THE PRESENCE OF P_i AND ADP

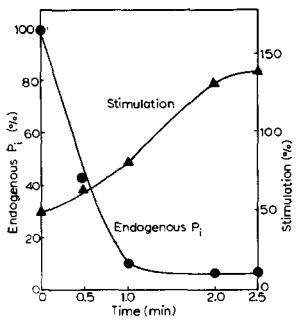
Experimental conditions as in Table II. $^{32}P_i$ was omitted from the reaction mixture. Increment of the electron transport stimulation evaluated according to the formula: $[A-B)/B] \times 100\%$, where A is reduction of $K_3Fe(CN)_6$ in the presence of P_i and ADP, and B, reduction of $K_3Fe(CN)_6$ in the absence of P_i and ADP.

Expt.	*Endogenous P_i (nmoles/mg chlorophyll)*	*$K_3Fe(CN)_6$ reduction (μmoles/mg chlorophyll per h)*		*Stimulation (%)*
		$-P_i$	$+P_i$	
1	750	320	358	12
2	880	326	370	13
3	320	510	755	48
4	460	205	292	43
5	124	405	754	86
6	220	290	500	72

also Table III). The $P/2e$ ratio was independent of the electron transport stimulation in the presence of ADP and P_i. Slight stimulation raised the $P/2e$ ratio above 1.0; at a much higher stimulation the phosphorylation yield reflected by the $P/2e$ ratio also exceeded 1.0 (Table II). Regarding varying stimulation of the electron transport in the presence of ADP and P_i, the $P/\Delta 2e$ ratio was subject to variation. The $P/\Delta 2e$ ratio even reached 5.0 at a low stimulation of electron transport, resulting from the addition of ADP and P_i (Table II).

A considerable amount of electron transport occurring in the absence of exogenous ADP and P_i as well as a varying increase in the electron transport stimulation resulting from these two factors was dependent on the presence of endogenous P_i in the preparation of chloroplasts. As shown in Table III, the quantity of endogenous P_i varied in different chloroplast preparations. There was a relationship between the quantity of endogenous P_i and the degree of electron transport stimulation. In chloroplasts containing a small content of endogenous P_i the electron flow was more susceptible to stimulation induced by these factors than in chloroplasts containing a markedly higher content of endogenous P_i.

Fig. 1 shows that incubation of chloroplasts in the presence of ferricyanide and



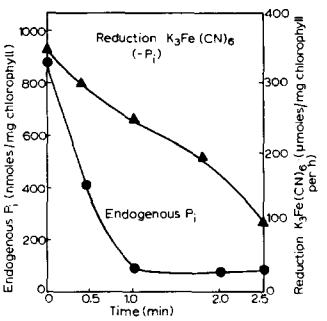


Fig. 1. Effect of illumination of chloroplasts in the presence of ferricyanide and ADP on the content of endogenous P_i, and the level of the electron transport stimulation in the presence of P_i. Conditions of incubation specified in MATERIALS AND METHODS. Content of endogenous P_i in the control 620 nmoles/mg of chlorophyll.

Fig. 2. Effect of illumination of chloroplasts in the presence of ferricyanide and ADP on the content of endogenous P_i and the electron transport in the absence of P_i. Conditions of the incubation specified in MATERIALS AND METHODS.

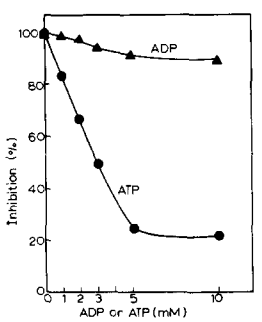


Fig. 3. Effect of ADP and ATP on the electron transport in the absence of exogenous P_i. Experimental conditions as in Table II.

ADP led to the depletion of endogenous P_i. The incubation of chloroplasts, carried out for 2 min in the light in the presence of ferricyanide and ADP, resulted in the depletion of nearly 95 % of the endogenous P_i. Depletion of endogenous P_i from chloroplasts was observed simultaneously with the increased electron transport stimulation in the presence of exogenous P_i. Incubation of chloroplasts carried out in the presence of ferricyanide and ADP leading to the release of endogenous P_i resulted in a decrease in electron transport in the absence of exogenous P_i (Fig. 2). The results presented in Figs. 1 and 2 suggest the coupling of the electron transport to photophorsphoylation in the absence of exogenous P_i. This observation is supported by the effect of ADP and ATP on the electron flow in the absence of exogenous P_i (Fig. 3).

DISCUSSION

Considerable electron transport coming into existence in chloroplasts in the absence of exogenous ADP and P_i makes it difficult to estimate the yield of noncyclic phosphorylation. The presence of these factors affects the electron transport acceleration whose magnitude, however, was not as great as that of the electron transport in mitochondria[18]. The photosynthetic electron transport exhibited a considerable instability even in the chloroplast preparation obtained by the same methods. The value of the electron transport stimulation ranged from 12 to 90 % (Tables II and III). The $P/2e$ ratio was independent of the increment of the electron transport stimulation which existed in the presence of ADP and P_i. It appears that the increment of stimulation cannot be regarded as a measure of chloroplast coupling. Chloroplast preparations having a very small stimulation showed a $P/2e$ ratio as high as chloroplast preparations that had a high electron transport stimulation in the presence of ADP and P_i (Table II). In mitochondria no close correspondence is observed between the P/O ratio and the respiratory control; hence LEHNINGER[19] postulates respiratory control as an appropriate measure in place of the P/O ratio. The results presented suggest that there is no such relationship for chloroplasts.

Instability of the electron transport stimulation in the presence of ADP and P_i, independently of the $P/2e$ ratio, often results in very high variation of the $P/\Delta 2e$ ratio. It seems that, in spite of the point of view of IZAWA AND GOOD[7], the $P/\Delta 2e$ ratio cannot reflect the stoicheiometry of noncyclic phosphorylation. Calculation of the yield of phosphorylation with the $P/\Delta 2e$ ratio taken into account would be possible only in the condition of the existence of two independent noncyclic electron transports.

The results of this work seem to show that there exists a correspondence of electron transport with the presence of endogenous P_i in chloroplast preparations. Considerable differences in content of endogenous P_i were also observed in individual chloroplast preparations. The content of endogenous P_i was related to the level of electron transport stimulation in the presence of exogenous P_i and ADP. Incubation of chloroplasts in the presence of an electron acceptor and ADP resulted in the rapid depletion of endogenous P_i (Figs. 1 and 2). Decrease in the level of P_i was attended by a decreasing electron transport rate, that resulted simultaneously in an increased electron transport stimulation in the presence of exogenous P_i. Similarly in mitochondria the 2–3 min incubation with a respiratory substrate and P_i trap resulted in depletion of endogenous P_i. The depletion of P_i caused inhibition of respiration[20,21].

The correspondence observed in this work between the quantity of chloroplastic

endogenous P_i on one hand and the level of electron transport stimulation on the other supports the postulate of DEL CAMPO *et al.*[6] of the existence of one kind of non-cyclic electron transport. It is likely that the electron transport in the absence of exogenous P_i is coupled with phosphorylation because (i) it is dependent on the presence of chloroplastic endogenous P_i (Fig. 2); (ii) it is subject to a notable inhibition in the presence of ADP (see ref. 2) and ATP (Fig. 3); and (iii) it is inhibited by energy transfer inhibitors[22,23].

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