THE EFFECT OF ADENOSINE-5′-TRIPHOSPHATE ON OXYGEN EVOLUTION, FLUORESCENCE EMISSION AND THE EMERSON ENHANCEMENT EFFECT

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1. Introduction

A new hypothesis concerning the distribution of absorbed light energy between the two photosystems in chloroplasts has been proposed [1]. This hypothesis asserts that control of the distribution of light energy is dependent on the state of phosphorylation of the light-harvesting chlorophyll protein. The LHCP is phosphorylated by a protein kinase which requires light, reduced plastoquinone, ATP and Mg^{2+} for its activation. When the LHCP is phosphorylated it results in the diversion of a greater proportion of absorbed light energy to photosystem I with the reverse change occurring when the protein is dephosphorylated. The dephosphorylation process is catalyzed by a phosphatase which also requires magnesium [2]. An important feature of this model is that it includes a mechanism which is capable of sensing the relative distribution of light energy between the two photosystems, i.e., the redox state of plastoquinone. The latter will tend to be more oxidized and will thus not activate the kinase when photosystem I is receiving the bulk of the absorbed light energy but will be in a more reduced state and will activate the kinase when photosystem II is receiving most of the absorbed light energy.

Here, we investigate the effect of ATP on chloroplasts which are supplied with an electron acceptor and which are evolving oxygen. Previous observations on broken chloroplasts were chiefly done in the absence of added electron acceptors except that the results in [1] also showed that ferricyanide and methyl viologen inhibited the activation of the kinase. It was our objective to demonstrate that ATP could change fluorescence emission and light energy distribution in chloroplasts which were evolving oxygen.

Abbreviations: ATP, adenosine 5′-triphosphate; LHCP, light harvesting chlorophyll protein; MES, 2(N-morpholino) ethane sulfonic acid; tricine, N-tris (hydroxymethyl) methyl glycine; ADP, adenosine 5′-diphosphate; NADP, nicotine adenine dinucleotide phosphate

2. Methods

Broken chloroplasts were isolated from greenhouse-grown spinach leaves by homogenizing them in a Polytron in an ice-cold medium containing 0.33 M sorbitol, 0.2 mM $MgCl_2$ and 20 mM MES (pH 6.5). The homogenate was filtered through 8 layers of cheesecloth and one layer of cotton wool before being centrifuged at 2000 × *g* for 90 s. The resultant pellet was resuspended in an ice-cold medium containing 30 mM NaCl and 20 mM tricine (pH 8.2) and left for 3 min to permit osmotic rupture of the chloroplasts. The suspension was then centrifuged at 2000 × *g* for 90 s and the pellet resuspended in an identical medium. The chlorophyll content of this medium was measured as in [3] and adjusted to 0.1 mg/ml.

The relative rate of oxygen evolution was monitored using the modulated oxygen polarographic technique in [4]. The chloroplasts were enriched with a saturating amount of ferredoxin (Sigma Chemical Co., type III) and observed in a bathing medium containing 30 mM NaCl, 10 mM $MgCl_2$, 30 mM tricine (pH 8.2) and 2 mM NADP. The chloroplasts were illuminated by a modulated 640 nm light (mean intensity 5.4 $\mu E \cdot M^{-2} \cdot S^{-1}$) to which was added an unmodulated 700 nm light (intensity 20 $\mu E \cdot M^{-2} \cdot S^{-1}$) for enhancement measurements. The enhancement effect was taken as the modulated oxygen signal in the presence of both lights to the modulated oxygen signal in

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the absence of the 700 nm light. The two lights were shone down separate arms of a trifurcated optic light guide while the third arm was used to lead fluorescence emitted by the chloroplasts to a photomultiplier tube (type 9659B, EMI). The modulated fluorescence emission at 720 nm was monitored with an HR-8 lock-in amplifier (Princeton Applied Research). All experiments were done at 22 ± 1°C.

3. Results and discussion

The results of two experiments are shown in fig.1a–c. In one experiment (open symbols) a sample of chloroplasts was observed for almost 50 min in a medium which lacked ATP. It can be seen that after the first 10 min the parameters observed, i.e., the relative rate of oxygen evolution (1a), the fluorescence emission (1b) and the enhancement ratio (1c) scarcely varied in value. In the second experiment denoted by the closed symbols another sample of chloroplasts was exposed to the same medium until 9 min had elapsed when 0.15 mM ATP was added. While this change in the medium did not appear to affect the rate of oxygen evolution it did cause the fluorescence emission and the enhancement ratio to fall over the next 28 min.

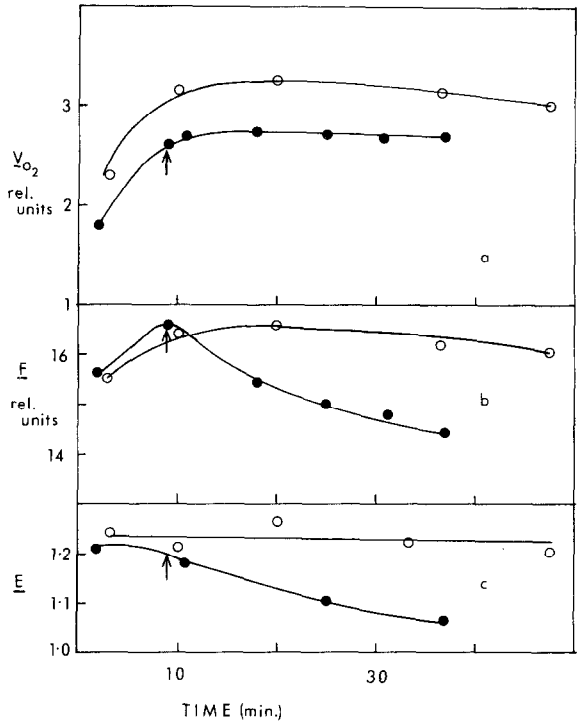

Fig.1. (a) The relative rate of oxygen evolution (V) is plotted against the time of 640 nm illumination for 2 samples of chloroplasts immersed in a bathing medium containing 30 mM NaCl, 10 mM $MgCl_2$, 30 mM tricine (pH 8.2) and 2 mM NADP. At the time indicated by the arrow (9 min), 0.15 mM ATP was added to the bathing medium of the chloroplasts whose oxygen evolution is indicated by the solid symbols. The corresponding results for fluorescence emission (F) and the enhancement ratio (E) are shown in (b) and (c), respectively.

The above experiments were also performed in a medium containing only 1 mM $MgCl_2$ instead of the 10 mM $MgCl_2$ used above. In this situation the enhancement ratio was unity at all times, i.e., the addition of unmodulated 700 nm light did not affect the modulated signal. When ATP (0.15 nm) was added it had no effect at all. In another experiment again similar to that in fig.1, nigericin (0.1 μg/ml) was present in the medium and ADP (0.15 mM) was added to test whether it could mimic the effect of ATP. It was found that ADP had no effect on either fluorescence or the enhancement ratio.

The behaviour of the fluorescence signal in these experiments is essentially similar to that in [1,5,6] although the extent of the fluorescence decrease (12%) and the rate of change are both smaller. We have found that ATP lowers the value of the enhancement ratio simultaneously with fluorescence. Since the enhancement ratio measured here is large when there is a surplus of excitation energy going to photosystem II, the changes in enhancement ratio observed here are consistent with the low temperature fluorescence measurements [6]. There are thus strong grounds for believing that we have shown that ATP can have the same effects on excitation energy distribution in chloroplasts which are reducing NADP as had been shown with chloroplasts which were not provided with an electron acceptor.

The reversibility of the ATP effect was tested in an experiment in which the chloroplasts were exposed to a solution lacking ATP initially. When ATP was added both fluorescence emission and the enhancement ratio decreased but when the original solution was restored neither the fluorescence nor the enhancement ratio returned to their original values although they did remain constant. This differs from [6] where a reversible effect on fluorescence was found.

There is no significant change in the rate of oxygen evolution when ATP is added to the medium (fig.1a). Thus although the imbalance in energy sup-

ply in favour of photosystem II is reduced by the addition of ATP this does not lead to an increase in oxygen yield. If the extra energy reaching photosystem I is not being used to speed up non-cyclic electron transport it presumably will be used to drive some other process and the most likely alternative is cyclic electron transport as suggested in [7]. This suggestion has the attractive feature that cyclic electron transport will tend to keep the plastoquinone pool in a reduced state and the kinase enzyme activated. If the extra energy going to photosystem I was used to speed up non-cyclic electron transport it would tend to oxidize the plastoquinone pool which would inactivate the kinase and possibly result in a greater energy supply for photosystem II.

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