STIMULATION OF THE FERRICYANIDE HILL REACTION AND COUPLED PHOTOPHOSPHORYLATION BY PHENAZINE METHASULPHATE

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Received 28 January 1972

1. Introduction

It has been found that the photophosphorylation rate of a reaction mixture containing phenazine methosulphate (PMS) and ferricyanide (FeCy) is lower than the photophosphorylation rate in the presence of PMS alone and only slightly higher than that associated with FeCy reduction. After reduction of the FeCy in the reaction mixture the phosphorylation rate increased up to the value observed with PMS alone. The interpretation of this result was that FeCy strongly inhibited phosphorylation catalyzed by PMS [1, 2]. It was suggested that a competition exists between the Hill oxidant (FeCy) and the photophosphorylation cofactor (PMS) for the electron, the oxidant draining electrons from the chain at a point which precedes the site of cofactor reduction.

In this paper we present data on simultaneous measurement of photophosphorylation, FeCy reduction and oxygen evolution which indicate that the interaction between PMS and FeCy in the electron carrier chain is somewhat more involved than one might have expected on basis of the earlier data.

2. Materials and methods

Chloroplasts were isolated from 2-weeks-old pea (*Pisum sativum*) seedlings by the West and Wiskich technique [3]. The plants were grown on vermiculite in a greenhouse with supplemental fluorescent lamp illumination.

Chlorophyll was determined according to Arnon [4]. The rate of photophosphorylation catalyzed by PMS was determined potentiometrically according to Nishimura et al. [5]. The FeCy reduction rate and coupled phosphorylation were simultaneously measured by a potentiometric technique proposed by Zabotin [6].

Briefly, this method consists of the following. During phosphorylation on the average 0.9 proton is absorbed from the reaction mixture per synthesized ATP molecule [5]. Hence $\Delta ATP = n_- / 0.9$, where ΔATP is the number of ATP molecules formed and n_- is the number of protons taken up from the reaction mixture. Reduction of a FeCy molecule, on the other hand, is accompanied by the liberation of a proton into the solution. Therefore $\Delta FeCy = n_+$, where n_+ is the number of protons produced in the reaction mixture.

The values of n_+ and n_- can be determined as follows. The net change in number of protons $\Delta H^+ = n_+ - n_-$. From the pH change and by titration the value of ΔH^+ can be determined. The number of protons liberated during a certain time (t) can be determined if a given amount of FeCy molecules (N) are introduced into the reaction mixture. The essence of the method is that down to very low FeCy concentrations the FeCy reduction rate is independent of FeCy concentration (zero order reaction). Conse-

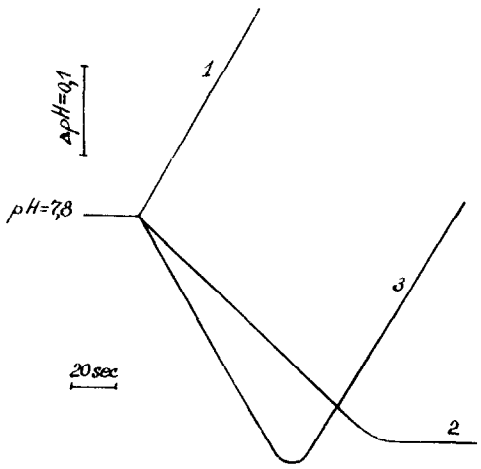

Fig. 1. Time course of pH changes in a chloroplasts suspension containing phosphorylation components and PMS (1), ferricyanide (2) and PMS + ferricyanide (3).

quently, termination of FeCy reduction is manifest in a break in the pH time-variation curve (e.g. curves 2 and 3 in fig. 1). The time, t, required for complete reduction of FeCy can be determined from the position of the break.

The FeCy reduction (Hill reaction), rate, therefore, is $N/t = n_+/t$ (N is the number of FeCy molecules introduced into the reaction mixture). Since $\Delta H^+/t$ is also known, one can now determine n_-/t and hence the photophosphorylation rate, $\Delta ATP/t = n_-/0.9t$.

The pH measurements were performed with an LPU-01 pH-meter. Oxygen evolution was measured in a separate chamber with a Clark-type oxygen electrode [7].

The chloroplast suspension was irradiated with 560–740 nm red light with a spectral distribution peak at 620 nm.

The total volume of the reaction mixture was 10 ml in the pH measurements. The mixture contained 0.4 M sucrose, 50 mM NaCl, 2 mM $MgCl_2$, 1 mM Na_2HPO_4, 1 mM ADP, 20 μM PMS, 0.1 mM $K_3Fe(CN)_6$. The experiments were carried out at room temp and pH 7.8. In the oxygen evolution experiments the suspension volume was 1.6 ml and 0.31 mM of ferricyanide were used.

3. Results

Typical curves of variation of pH with time observed on irradiating a chloroplast suspension containing the phosphorylation components and PMS (curve 1), ferricyanide (curve 2) and PMS + ferricyanide (curve 3) are shown in fig. 1.

Curve 3 is of particular interest. If on addition of PMS to a medium containing FeCy not all PMS-catalyzed photophosphorylation were inhibited [1, 2], one would expect the resultant curve to lie above curve 2 due to additional proton absorption. In the case of complete inhibition the two curves would coincide. However, the experiments show that in a medium containing PMS + FeCy the resultant pH curve (curve 3) lies below curve 2. This means that proton production is enhanced. Moreover, the time required for ferricyanide reduction decreases, i.e. the Hill reaction rate increases. After complete reduction of FeCy the variation of pH with time becomes the same as that observed for PMS-catalyzed phosphorylation alone.

The results of one set of such experiments and of measurements of the oxygen evolution rate are presented in table 1.

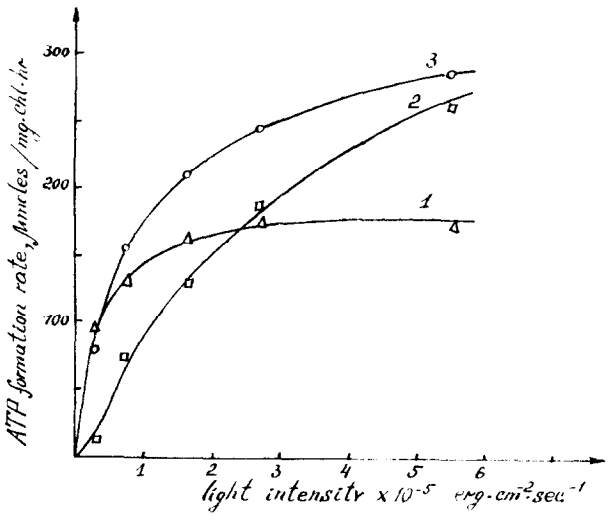

Fig. 2. Light intensity dependence of ATP formation in a chloroplast suspension containing ferricyanide (1), PMS (2) and ferricyanide + PMS (3).

Table 1
Effect of PMS on ferricyanide photoreduction, photophosphorylation and O_2 evolution.

Conditions	FeCy reduction (μmoles FeCy × mg chl^{-1} × hr^{-1})	Photophosphorylation (μmoles ATP × mg chl^{-1} × hr^{-1})	Average P/2e	O_2 evolution rate (relative units)
FeCy	174 ± 16	113 ± 12	1.31 ± 0.02	100 ± 3
FeCy + PMS	244 ± 9	151 ± 5	1.19 ± 0.03	140 ± 6
Ratio $\frac{\text{FeCy + PMS}}{\text{FeCy}}$	1.40	1.34		1.40

Red light, 2.6×10^5 erg × cm^{-2} × sec^{-1}. In FeCy reduction and photophosphorylation measurements the chlorophyll content was 195 μg in 10 ml. In the O_2 evolution measurements the chlorophyll content was 90 μg in 1.6 ml. The results are the average of 4 measurements.

It can be seen from the table that addition of PMS to a medium containing FeCy increases both the FeCy reduction and the oxygen evolution rates on the average by about 40%. A similar, although somewhat smaller, enhancement of the photophosphorylation rate is also observed. These data were obtained at light intensities of about 2.6×10^5 erg/cm^2 × sec which saturate the Hill reaction.

Plots of the photoreduction and photophosphorylation rates as functions of light intensity, obtained in another set of measurements are shown in figs. 2 and 3. The curves show that the stimulating effect elicited

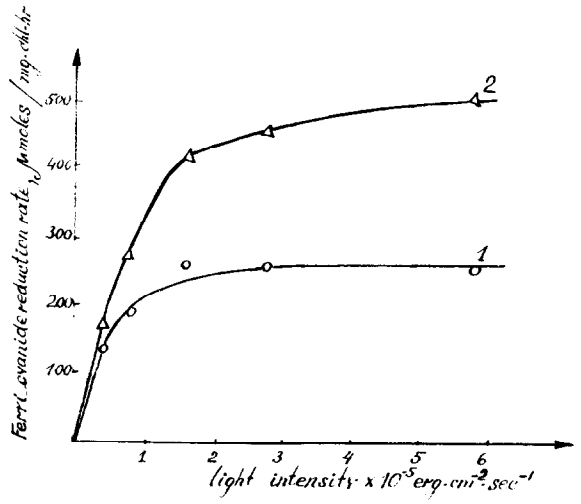

Fig. 3. Light intensity dependence of ferricyanide reduction without PMS (1) and with PMS (2).

by PMS is not observed at low light intensities, i.e. on the linear part of the ferricyanide photoreduction light curve.

4. Discussion

As figs. 2 and 3 show, the addition of PMS to the reaction mixture at low light intensities does not lead to an increase of the Hill reaction (ferricyanide photoreduction and oxygen liberation) or photophosphorylation rates. At high light intensities, however, which correspond to light saturation of FeCy photoreduction, an appreciable PMS-induced stimulation of both the Hill reaction and of photophosphorylation is observed. This type of dependence of the PMS stimulatory effect on light intensity shows that the effect is not due to the presence of PMS as such, but rather to its operation under conditions when light saturation of FeCy reduction sets in.

The fact that both the Hill reaction and photophosphorylation rates are stimulated in about the same proportion (P/2e remains equal to approx. 1.3) apparently signifies that PMS activates noncyclic photophosphorylation coupled to FeCy reduction, whereas FeCy inhibits cyclic photophosphorylation catalyzed by PMS.

The stimulating effect of PMS on the Hill reaction with FeCy and on coupled photophosphorylation observed in the present work may be explained by assuming that PMS, which in the presence of FeCy

does not function in cyclic electron transport, switches over to noncyclic electron transfer; at high light intensities it shunts a certain part of the electron transport chain which limits the Hill reaction rate. On the other hand, at low light intensities endogenous electron carriers suffice for electron transport and hence no stimulation of the Hill reaction by PMS is observed.

This explanation is in accordance with available data which show that PMS may indeed participate in noncyclic electron transfer [8–10]. Our data show that this transfer probably involves the shunting of some bottleneck in the chain, the result being enhancement of the Hill reaction and noncyclic photophosphorylation rates.

References

[1] M. Avron and A.T. Jagendorf, J. Biol. Chem. 234 (1959) 1315.
[2] A.T. Jagendorf, Brookhaven Symposia in Biology N 11 (1959) 236.
[3] K.B. West and J.T. Wiskich, Biochem. J. 109 (1969) 527.
[4] D.I. Arnon, Plant Physiol. 24 (1949) 1.
[5] M. Nishimura, T. Ito and B. Chance, Biochim. Biophys. Acta 59 (1962) 177.
[6] A.I. Zabotin, in: Metody Issledovania Fotofosforilirovania (Methods for Investigation of Photophosphorylation) (Pushchino, Moscow Region, 1970) p. 182.
[7] G.S. Grishina, L.N. Bell and G.S. Bukina, Fiziol. Rastenii 13 (1966) 737.
[8] M. Schwartz, Biochim. Biophys. Acta 131 (1967) 548.
[9] A.B. Rubin and T.E. Krendeleva, in: Fiziologia i Biokhimiya Zdorovovo i Bolnovo Rasteniya (Physiology and Biochemistry of Healthy and Diseased Plants) (Moscow University Press, 1970) p. 257.
[10] S. Izawa, Biochim. Biophys. Acta 223 (1970) 165.