THE INHIBITION OF PHOTOSYNTHESIS BY OXYGEN

II. THE EFFECT OF OXYGEN ON GLYCERALDEHYDE PHOSPHATE DEHYDROGENASE FROM CHLOROPLASTS

By J. S. TURNER,* J. F. TURNER,† K. D. SHORTMAN,‡ and JUDITH E. KING†

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Summary

(i) An extract containing glyceraldehyde phosphate dehydrogenases was prepared from the chloroplasts of silver beet. Diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN) functioned as cofactors for the enzyme activity.

(ii) The activity of the glyceraldehyde phosphate dehydrogenases was a function of the concentration of added cysteine. The enzymes were inhibited by low concentrations of iodoacetate and *p*-chloromercuribenzoate.

(iii) The reaction catalysed by both the DPN- and TPN-linked dehydrogenase (in the presence of an appropriate concentration of cysteine) was inhibited by oxygen. The rates in nitrogen and air were not significantly different in most experiments, but there was always a marked inhibition in pure oxygen.

(iv) The possible significance of these observations on the inhibition of photosynthesis by oxygen is discussed.

I. INTRODUCTION

In Part I of this series (J. S. Turner, Todd, and Brittain 1956) the primary data on the inhibition of photosynthesis by oxygen were assembled. The strongly marked inhibition is produced rapidly and equally rapidly reversed. At high CO_2 concentration the inhibition caused by 20 per cent. oxygen is not significant; maximal inhibition is caused by pure oxygen. The inhibitory effect is found in all the green plants tested and applies both to CO_2 uptake and oxygen output. It has not been demonstrated for the Hill reaction (Brittain, unpublished data), but has been found to apply to CO_2-fixation by whole plastids (Arnon, Allen, and Whatley 1954).

The inhibition is not due to the effect of oxygen on the dark respiration, but the means by which oxygen inhibits real photosynthesis has not yet been established. In Part III of this series the conditions under which the effect is realized will be described and several different explanatory hypotheses discussed. In the present paper we summarize results which suggest that oxygen may act on photosynthesis in the same way as does iodoacetate—causing an inhibition of the whole process

*Botany School, University of Melbourne.

†Plant Physiology Unit, Division of Food Preservation and Transport, C.S.I.R.O., Botany School, University of Sydney.

‡Plant Physiology Unit, Division of Food Preservation and Transport, C.S.I.R.O., Botany School, University of Sydney; present address: Biochemistry School, University of Sydney.

by reacting with the sulphydryl-containing enzyme glyceraldehyde phosphate dehydrogenase (GPD).

The same mechanism has been proposed as one explanation of the Pasteur effect in plant respiration. During studies on sucrose synthesis, pea seed extracts were obtained which possessed a complete glycolytic system (J. F. Turner 1954, 1957). The glycolytic activity was higher under anaerobic than under aerobic conditions, and it was suggested that oxygen inhibited the glycolysis by inactivating one of the glycolytic enzymes (J. F. Turner and Mapson 1958). Subsequent investigations (Hatch and J. F. Turner, unpublished data) have shown that the decreased rate of glycolysis *in vitro* was due to inhibition of GPD by oxygen through oxidation of sulphydryl groups of the enzyme. It is not yet known whether such a mechanism operates *in vivo* in cells possessing a Pasteur effect (see J. S. Turner 1958).

Glyceraldehyde phosphate dehydrogenases, reacting with the two cofactors diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN) occur in green leaves (Arnon 1952; Gibbs 1952). It will now be shown that the reaction catalysed by these enzymes obtained from chloroplasts is inhibited by high oxygen concentrations, and it is suggested that this is one possible mechanism for the inhibition of photosynthesis by oxygen.

II. Materials and Methods

(*a*) *Substrates*

Fructose 1,6-diphosphate was prepared by the method of Neuberg and Lustig (1942). DPN and TPN were obtained from Pabst Laboratories, Wisconsin, U.S.A.

(*b*) *Preparation of Chloroplast Extract*

Chloroplasts were isolated from the laminae of leaves of silver beet (*Beta vulgaris* L. var. *cicla* Moq.) by the method of Arnon, Allen, and Whatley (1956). The chloroplast extract was prepared by suspending washed chloroplasts in water and centrifuging as described by Whatley *et al.* (1956). These authors have reported that chloroplast extracts contain GPD.

(*c*) *Assay of GPD Activity*

GPD (both DPN- and TPN-linked) was assayed substantially as described by Gibbs (1955). Where effects of gas phase were not being investigated the following components were placed in a spectrophotometer cuvette: 100 μmoles 2-amino-2- hydroxymethylpropane-1,3-diol (Tris)-HCl buffer, pH 8·45, 51 μmoles sodium arsenate, 12 μmoles cysteine, 60 μmoles sodium fluoride, 1·41 μmoles fructose 1,6-diphosphate, 0·3 ml chloroplast extract. The reaction was initiated by adding 0·2 μmole DPN (or TPN). The total volume of the enzymic digest was 3 ml. The reaction was followed by measuring the change in absorbance at 340 mμ in a Beckman model DU spectrophotometer.

In studying the effects of the gas phase on the enzyme activity, enzymic digests of the composition described previously (except that a lower concentration of cysteine was used) were placed in Thunberg tubes, the DPN (or TPN) being placed

in the stopper. The tubes were evacuated on a water-pump and refilled with the appropriate gas. This process was repeated twice and the tubes were allowed to stand at room temperature with occasional gentle agitation. After 1 hr the reaction was started by tipping the pyridine nucleotide from the stopper. At the appropriate reaction time, the tubes were opened and the absorbance at 340 mμ of the contents measured immediately.

(*d*) *Assay of Aldolase Activity*

Aldolase was assayed by the method of Stumpf (1948). The enzymic digests contained, in a total volume of 4 ml, 100 μmoles Tris-HCl buffer, pH 8·45, 20 μmoles fructose 1,6-diphosphate, 250 μmoles potassium cyanide, 1 ml chloroplast extract. The triose phosphates formed were decomposed with 1N NaOH at room temperature and the inorganic phosphate released determined by the method of Allen (1940). The effects of gas phase on aldolase activity were studied using Thunberg tubes as described for the assay of GPD; the reaction was started by tipping chloroplast extract from the stopper.

III. Results

Preliminary experiments showed that glucose 6-phosphate dehydrogenase did not interfere with the assay of GPD in the chloroplast extract. Neither DPN nor TPN was reduced in the presence of 10 mM iodoacetate which inhibits GPD but not glucose 6-phosphate dehydrogenase (Gibbs 1955). There was no significant reduction of the nucleotides in the absence of added fructose 1,6-diphosphate indicating that oxidation of cysteine was not interfering in the assay of GPD. No significant change in absorbance at 340 mμ was observed when the enzymic digests were incubated without added DPN or TPN.

(*a*) *Aldolase Activity*

As the chloroplast extracts contained aldolase, fructose 1,6-diphosphate could be used as substrate for GPD. There was no difference in enzyme activity when digests of the composition described for the assay of aldolase were incubated under oxygen and nitrogen. Any effect of oxygen on the GPD assay was therefore not due to an effect on aldolase but on GPD.

(*b*) *GPD Activity*

(i) *Effect of Cysteine.*—In the absence of added cysteine, there was no DPN-linked GPD activity in the chloroplast extracts in air. With some chloroplast preparations there was a small TPN-linked GPD activity without added cysteine, but the addition of an optimal amount of cysteine increased this rate by at least 10 times.

The effect of cysteine concentration on DPN-linked GPD activity is shown in Table 1. The critical concentration of cysteine lay between 0·4 and 0·04 mM. Cysteine concentration had a similar effect on the activity of TPN-linked GPD (see Table 1).

(ii) *Inhibition of GPD by Iodoacetate and* p-*Chloromercuribenzoate.*—GPD activity was very sensitive to iodoacetate. Both the DPN- and TPN-linked enzymes

were completely inhibited by 10 mM iodoacetate. The DPN-linked system was inhibited by 98 and 40 per cent. by 1 mM and 0·1 mM iodoacetate respectively,

TABLE 1

EFFECT OF CYSTEINE CONCENTRATION ON DPN- AND TPN-LINKED GLYCERALDEHYDE PHOSPHATE DEHYDROGENASE (GPD)

Enzymic digests were of the composition described for assay of GPD (see text)

Coupling	Cysteine Concn. (mM)	Incubation Time (min)	Increase in Absorbance at 340 mμ
DPN-linked	4	54	0·219
	2	54·5	0·213
	0·4	55	0·222
	0·04	48	0·016
	0·004	49	0·011
	0	49·5	0·001
TPN-linked	4	32	0·146
	0·4	32·5	0·138
	0	33	0·007

and the TPN-linked system by 100 and 66 per cent. *p*-Chloromercuribenzoate (0·3 mM) completely inhibited both DPN- and TPN-linked GPD; with 0·1 mM

TABLE 2

EFFECT OF OXYGEN AND NITROGEN ON DPN- AND TPN-LINKED GLYCERALDEHYDE PHOSPHATE DEHYDROGENASE (GPD)

Enzymic digests were of composition described for assay of GPD (see text)

Coupling	Cysteine Concn. (mM)	Incubation Time (min)	Increase in Absorbance at 340 mμ	
			Nitrogen	Oxygen
DPN-linked	0·4	15	0·330	0·013
	0·2	23	0·252	0·044
	0·1	30	0·023	0·051
TPN-linked	0·4	30	0·237	0·179
	0·2	30	0·226	0·083
	0·1	30	0·017	0·012

p-chloromercuribenzoate, the TPN-linked enzyme was unaffected and the DPN-system was inhibited by approx. 50 per cent. These experiments were carried out in the presence of 0·4 mM cysteine.

(iii) *Effect of Aerobic and Anaerobic Conditions on GPD Activity.*—In experiments designed to show a difference in GPD activity under aerobic and anaerobic conditions, a concentration of cysteine was chosen which would barely sustain maximum GPD activity in air. In preliminary experiments, it was found that no consistent difference could be demonstrated between the reaction rate in air and nitrogen. In subsequent experiments oxygen was used instead of air.

The effect of incubation in oxygen and nitrogen on DPN-linked GPD activity in the chloroplast extracts is shown in Table 2. Depending on the cysteine level GPD activity could be inhibited by over 90 per cent. in an atmosphere of oxygen compared with anaerobic conditions. The maximum effect was shown when the final concentration of cysteine was 0·4 mM. The rates of GPD activity in oxygen were barely significant. At the lowest concentration of cysteine used (0·1 mM) GPD activity under nitrogen was very small.

Table 2 also shows the effect of oxygen and nitrogen on TPN-linked GPD activity. The effects were, in general, similar to those described for the DPN-linked GPD. The maximum effect was obtained in the presence of 0·2 mM cysteine and here the inhibition by oxygen was in excess of 60 per cent.; in other experiments inhibitions of over 90 per cent. were obtained. The inhibition by oxygen with 0·4 mM cysteine was not as effective. Enzyme activity with low cysteine concentration was again barely significant in both nitrogen and oxygen.

IV. Discussion

It is known that the glyceraldehyde phosphate dehydrogenases from higher plants (like those of yeast and animal tissues) are affected by sulphydryl reagents (Gibbs 1952). In the present work this has been confirmed, GPD activity in chloroplast extracts being highly sensitive to iodoacetate and *p*-chloromercuribenzoate. The direct effects on these enzymes of aerobic and anaerobic conditions have not previously been studied, although Lipmann (1933) proposed that the Pasteur effect in yeast was due to the reversible oxidation of sulphydryl groups in dehydrogenases concerned in glycolysis. Engelhardt and Sakov (1943) have also suggested that the redox potential determines the activity of phosphohexokinase.

The present work has shown that the GPD activity of chloroplast extracts, (both DPN- and TPN-linked), may be inhibited by high oxygen concentration. This is probably due to the oxidation of sulphydryl groups of the enzyme protein, but the exact nature of the mechanism has not been established. The results obtained may, of course, be due to a direct effect of oxygen (or iodoacetate), not on the enzyme protein but on a soluble sulphydryl compound present *in vivo* and corresponding to the cysteine which forms a part of the enzyme digest in our experiments.

Iodoacetate and iodoacetamide inhibit the respiration of green cells (Arnon 1952; Hölzer 1954) and are presumed to act on GPD in so doing. It has, however, long been known that the oxygen output in photosynthesis is even more sensitive to these reagents than is respiration (Kohn 1935; Hölzer 1954). Stepka (in Calvin *et al.* 1951) showed that iodoacetamide also inhibited the uptake of $^{14}CO_2$ by 90 per

cent. Arnon, Allen, and Whatley (1956) have shown that CO_2-fixation in green plastids is inhibited by iodoacetamide, arsenite, and *p*-chloromercuribenzoate. The Hill reaction is not inhibited by these sulphydryl reagents. Arnon *et al.* therefore argue that the sulphydryl compound 6-thioctic acid does not take part in photolysis and that sulphydryl compounds are concerned not with the early but with later phases of photosynthesis.

All these observations lend support to the "assumption that the next step in photosynthesis after the formation of 3-phosphoglyceric acid is its reduction to triose by an iodoacetamide-sensitive hydrogenase. To a certain extent these observations also make it plausible that the H donor in this reduction is a pyridine

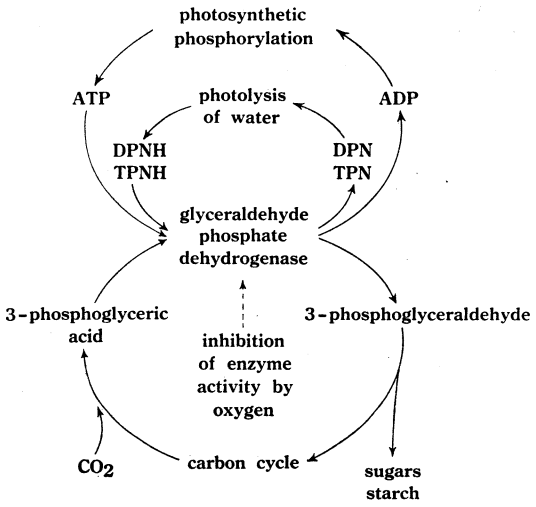

Fig. 1.—Suggested scheme for inhibition of photosynthesis by oxygen.

nucleotide and that ATP must be supplied to make the reduction possible" (Rabinowitch 1956). Such a scheme for photosynthesis (based largely on recent work in Arnon's school) is shown in Figure 1. The possible role of oxygen as an inhibitor, suggested by the results of the present investigation, is indicated. If GPD acted as a pacemaker in photosynthesis (e.g. at high light intensity and high CO_2 concentration) then inhibition of the enzyme by oxygen could inhibit photosynthesis (i) by depressing the rate of re-oxidation of reduced pyridine nucleotides derived from the photolysis of water, so that this process and oxygen evolution would slow down; (ii) by causing a block at the 3-phosphoglyceric acid level which could interfere with the operation of the carbon cycle.

Further work with plastids is in hand to test the hypothesis; meanwhile it may be pointed out that the effects of both oxygen and iodoacetate are greatest at saturating light, and that oxygen inhibits both photosynthesis (at high CO_2 concentration) and GPD only at very high oxygen concentrations.

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