REGULATION OF ELECTRON TRANSPORT IN PHOTOSYNTHESIS

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Photophosphorylation coupled to NADP photoreduction in isolated chloroplasts results in the production of one ATP for each NADP reduced (Arnon et al., 1958). However, Kandler (1957) and others have pointed out that each mole of CO_2 fixed by the Calvin-Bassham cycle requires three moles of ATP and two moles of NADPH. Additional ATP may be generated by either ferredoxin dependent cyclic photophosphorylation (Tagawa et al., 1963) or pseudocyclic phosphorylations requiring ferredoxin (Forti and Jagendorf, 1961) or phosphodoxin (Black et al., 1963). These observations suggest that there must be some regulation of the relative output of ATP and NADPH. Tagawa et al. (1963) suggest that the availability of NADP as an electron acceptor would serve as a physiological regulator between cyclic and noncyclic photophosphorylation. The experiments reported in this paper demonstrate that compounds found in boiled spinach extracts selectively inhibit ferredoxin-NADP reductase causing inhibition of NADP photoreduction and the associated phosphorylation. However, these same extracts either stimulate or at least show no inhibition of other photophosphorylation reactions. These results suggest the possibility of a control mechanism operative in the photosynthetic electron transport system which directs electrons from NADP reduction to a cyclic or pseudocyclic production of ATP.

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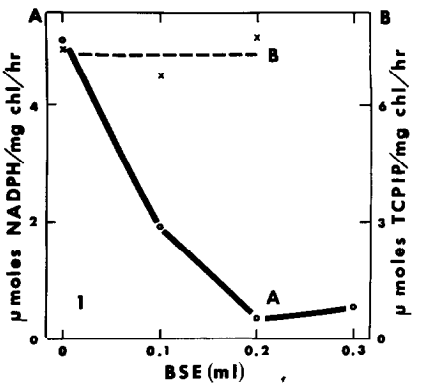
MATERIALS AND METHODS

Transhydrogenase (ferredoxin-NADP reductase) and ferredoxin were isolated by acetone fractionation of crude spinach homogenates according to San Pietro and Lang (1958). These proteins were separated on a DEAE cellulose column following the procedure of Shin et al. (1963). Ferredoxin was eluted from the column, dialyzed and used at this state of purity. The transhydrogenase was further purified by fractionation between 40 and 65% saturation with ammonium sulfate (Shin et al., 1963). Boiled spinach extracts (BSE) were prepared by homogenizing spinach leaves in distilled water with a Waring blender. The homogenate was boiled for ten minutes, cooled, filtered through glass wool and centrifuged. The supernatant fluid (BSE) was characterized by having two absorbance peaks in the ultraviolet (A_{260nm} = 24, A_{325nm} = 15.8) Chloroplast preparations used included the "grana" preparations of San Pietro and Lang (1958), the Cls chloroplast fragments of Whatley et al. (1959) and chloroplasts prepared according to the procedure of Jagendorf and Avron (1958) using the Tris, sucrose, NaCl buffer medium.

NADP photoreduction was followed directly by measuring the increase in absorbance at 340nm against a blank of identical composition except NADP was omitted (cf San Pietro and Lang, 1958). The trichlorophenolindophenol (TCPIP) Hill reaction was measured by noting the absorbance change at 625nm against a blank cuvette of identical composition except lacking TCPIP. The transhydrogenase reaction with NAD as acceptor was measured at pH 8.0 according to the procedure of Keister et al. (1960). Photophosphorylation was followed by measuring the decrease in inorganic phosphate in the reaction mixtures by the method of Taussky and Shorr (1953). All reactions were carried out aerobically at pH 8.0 - 8.2. Protein concentrations were estimated by the procedure of Lowry et al. (1951) and chlorophyll by the procedure of Arnon (1949).

RESULTS AND DISCUSSION

Figure 1 shows that BSE contains a compound(s) which strongly inhibits



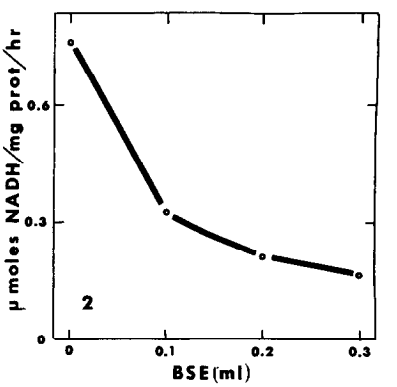


Figure 1. Curve A. Effect of BSE on NADP photoreduction. The reaction mixtures contained in a final volume of 3 ml: chloroplasts (Jagendorf and Avron, 1958) containing 155 ug of chlorophyll, partially purified ferredoxin equivalent to 0.132 mg of protein, 200 umoles of Tris-HCl (pH 8.0), 0.4 umoles of NADP and BSE as indicated. The reactions were carried out at room temperature in white light (1400 ft. can.). Curve B. Effect of BSE on the TCPIP Hill reaction. The reaction mixtures contained in a final volume of 3 ml: chloroplasts (San Pietro and Lang, 1958) equivalent to 75 ug chlorophyll, 200 umoles Tris-HCl (pH 8.0), 0.19 umoles of TCPIP and BSE as indicated. The reactions were carried out at 15° in white light (400 ft. can.).

Figure 2. Effect of BSE on transhydrogenase activity. The reaction mixtures contained in a final volume of 3 ml: partially purified transhydrogenase equivalent to 2.59 mg of protein, an excess of isocitric dehydrogenase, BSE as indicated and the following in umoles: Tris-HCl (pH 8.0), 150; sodium isocitrate, 10; $MgCl_2$, 10; NADP, 0.1; NAD, 1.0. The reactions were run at room temperature.

photoreduction of NADP (curve A) but not the photoreduction of TCPIP (curve B). In terms of the current formulation of electron transport in photosynthesis (cf Vernon and Avron, 1965) these results suggest that a reaction associated with photosystem I is selectively inhibited. This view is strengthened by the observation (Figure 2) that the transhydrogenase activity of ferredoxin-NADP reductase is strongly inhibited by comparable levels of BSE suggesting that the inhibitor sensitive site is ferredoxin-NADP reductase. A further correlation between the inhibition of transhydrogenase activity and the inhibition of NADP photoreduction is observed when BSE is treated with charcoal. The inhibitor of both the reactions appears to be adsorbed by charcoal since the unadsorbed components of BSE inhibit neither NADP photo-

reduction nor transhydrogenase activity.

Photophosphorylation coupled to NADP reduction is also inhibited by BSE (Figure 3, curve A), but the degree of inhibition is less than that observed in the case of NADP photoreduction. This disparity might be explained on the basis of another observation. Although electron transfer to NADP is strongly inhibited by BSE (Figure 1, curve A), in the absence of added NADP BSE strongly stimulates the rate of endogenous photophosphorylation (Figure 3, curve B).

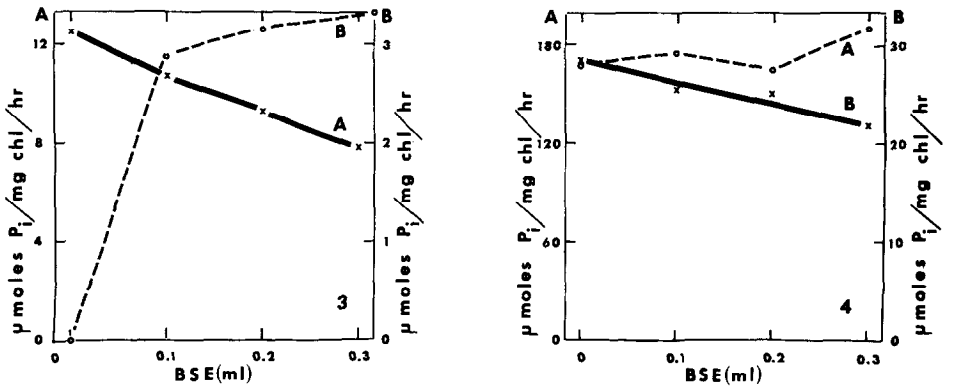


Figure 3. Curve A. Effect of BSE on photophosphorylation coupled to NADP photoreduction. Curve B. Effect of BSE on photophosphorylation catalyzed by low levels of ferredoxin. The reaction mixtures contained in a final volume of 3 ml: chloroplasts (Jagendorf and Avron, 1958) equivalent to 439 ug of chlorophyll, partially purified ferredoxin equivalent to 0.26 mg of protein, BSE as indicated and the following in umoles: K_2HPO_4, 5; $MgCl_2$, 10; ADP, 8; NaCl, 88; Tris-HCl (pH 8.0), 40. In addition reaction mixtures of curve A (but not curve B) contained 10 umoles of NADP. The reactions were run at room temperature in white light (1400 ft. can.).

Figure 4. Curve A. Effect of BSE on PMS catalyzed photophosphorylation. The reaction mixtures contained in a final volume of 3 ml: chloroplasts (Jagendorf and Avron, 1958) equivalent to 103 ug chlorophyll, BSE as indicated, and the following in umoles: ascorbate, 15; PMS, 0.1; ADP, 8; K_2HPO_4, 5; $MgCl_2$, 10; NaCl, 88; Tris-HCl (pH 8.0), 40. Reactions were run at room temperature in white light (1400 ft. can.).

Curve B. Effect of BSE on photophosphorylation catalyzed by high levels of ferredoxin. The reaction mixtures contained in 3 ml: chloroplasts (Whatley et al., 1959) equivalent to 140 ug chlorophyll, partially purified ferredoxin equivalent to 1.66 mg of protein, BSE as indicated and the following in umoles: $MgCl_2$, 6; ADP, 10.5; K_2HPO_4, 10.5; Tris-HCl (pH 8.2), 90. The reactions were carried out at 15° in low intensity red light.

This latter effect is presumably due to the presence of phosphodoxin in BSE (Black et al., 1963). Hence in Figure 3, curve A addition of BSE results in

decrease in electron transport to NADP with a corresponding decrease in phosphorylation, but this effect is partly reversed by a stimulation by phosphodoxin of a pseudocyclic production of ATP.

Figure 4 shows that BSE has very little effect on photophosphorylation reactions catalyzed by phenazine methosulfate (PMS)(curve A) or relatively high levels of ferredoxin (curve B). At low levels of ferredoxin (Figure 3, curve B) BSE stimulated the rate of phosphorylation. This suggests that although the site of inhibition by BSE is a reaction associated with photosystem I, the inhibitor sensitive reaction is not involved in photophosphorylation catalyzed by ferredoxin or PMS. As a working hypothesis it is suggested that compounds in BSE modulate the activity of ferredoxin-NADP reductase diverting electron flow into pathways which produce ATP but not NADPH. The relative production of ATP and NADPH are thus regulated to conform to the requirements for synthetic events in the cell. A corollary to this hypothesis is that the concentration of the inhibitor itself reflects the relative amounts of ATP and NADPH.

Efforts are currently being made to identify the inhibitor found in BSE. Using the transhydrogenase activity of ferredoxin-NADP reductase as a model reaction, experiments, which will be reported elsewhere, suggest that the inhibitor is an organic acid. This conclusion is based in part on the observations that the inhibitor has a low molecular weight, anionic properties and is destroyed by ashing.

Over forty organic compounds have been tested for inhibitory activity in addition to those reported by Keister et al. (1960). Several of those tested inhibited the transhydrogenase activity. They include: pyruvate, α-ketoglutarate, ascorbate, methylglyoxal, diacetyl, and glyoxal. A common feature of all these compounds is that they possess vicinal carbonyl groups. Every compound tested that has this grouping has been found to have some effect on the enzyme activity, e.g. oxalate stimulates the reaction. Two compounds, formaldehyde and acetaldehyde, also act as inhibitors at high concentrations,

perhaps by virtue of the ability of two molecules to simulate the vicinal carbonyl configuration.

These findings are consistent with the hypothesis that ferredoxin-NADP reductase, located at a potential branch point in electron transfer reactions, plays a key role in the regulation of electron flow in photosynthesis. By control of the activity of this enzyme the relative output of ATP and NADPH is controlled.

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