

Extraction and Purification of a Factor which Stimulates Silicomolybdate Reduction by Photosystem II of Spinach Chloroplasts

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Introduction*

As shown by Swanson, Thomson and Mudd (10), extraction of tobacco chloroplasts with acetone concentrations up to 30% in fresh or glutaraldehyde-fixed material removes some neutral lipid, acylated sterol glycoside, and monogalactosyl diglyceride, but the structural integrity of chloroplast membranes remains undisturbed. Likewise, the activity of most photosynthetic electron transport reactions is undiminished or even stimulated after a 30% acetone wash (Barr, unpublished results). The only exception may be silicomolybdate reduction by PS II in presence of DCMU (3), a reaction which normally gives good O₂ evolution rates for the first 30 seconds but stops thereafter. In this study, we have explored the possibility of loss of a factor from chloroplast membranes in presence of silicomolybdate. For this purpose, we used low acetone concentrations (1-2%) to extract and purify a factor from spinach chloroplasts, which, when added to the silicomolybdate assay, stimulates O₂ evolution up to 50%. The identity of the stimulator is also discussed. It may be a pteridine or an analog of folic acid (4).

Materials and Methods

Chloroplasts were made from market spinach in 0.4 sucrose—0.05M NaCl according to a modified method of Jagendorf and Avron (5). Chlorophyll was determined according to Arnon (1). Chloroplasts containing 1 mg chl/ml were osmotically shocked by suspending in water prior to an acetone wash (10 mg chl/50 ml 1 or 2% acetone in batches of 3-5). The washed chloroplasts were sedimented by centrifugation at 2,000x g for 10 min. The supernatant containing silicomolybdate stimulation factor was further purified by centrifugation at 7,500 x g. The clear yellow supernatant was used for further purification on columns or by TLC. Sometimes it was dialyzed overnight to remove the acetone, especially if used in silicomolybdate assays.

Purification of silicomolybdate stimulation factor was carried out in 3 ways: (1) on DEAE cellulose columns equilibrated with 5 mM phosphate buffer, pH 7; elution of yellow band with 0.5 M NaCl in 5mM phosphate buffer, (2) on

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*Abbreviations used frequently are:

DCMU—3-(3,4-dichlorophenyl)-1,1-dimethylurea;

PS II—photosystem II;

SM—silicomolybdate acid.

Merck aluminum oxide columns equilibrated with 2% acetone; elution of yellow band with 2% ammonium hydroxide after a 1% ammonium wash, (3) on PEI Cellulose F TLC plates developed in 8% ammonium hydroxide; yellow stimulator band has an R_F of 0.37 or higher depending on purity of starting material.

Silicomolybdate reduction by spinach chloroplasts in presence of DCMU was measured as O_2 evolution with a Clark-type oxygen electrode connected to a Yellow Springs Oxygen Monitor. Rates were recorded with a Sargent-Welch SRG recorder. Illumination (5×10^5 ergs/cm²-sec) was provided by a specially built light source using a General Electric CBA (120V) Quartzline projector lamp. Reaction mixtures are given in the legend of Fig. 2.

Folic acid (Sigma) was added to chloroplasts as alkaline solutions in water.

Results and Discussion

The silicomolybdate stimulation factor removed from chloroplasts by 1 or 2% acetone washes remains unidentified, although certain similarities between folic acid and the factor in various stages of purification are apparent from

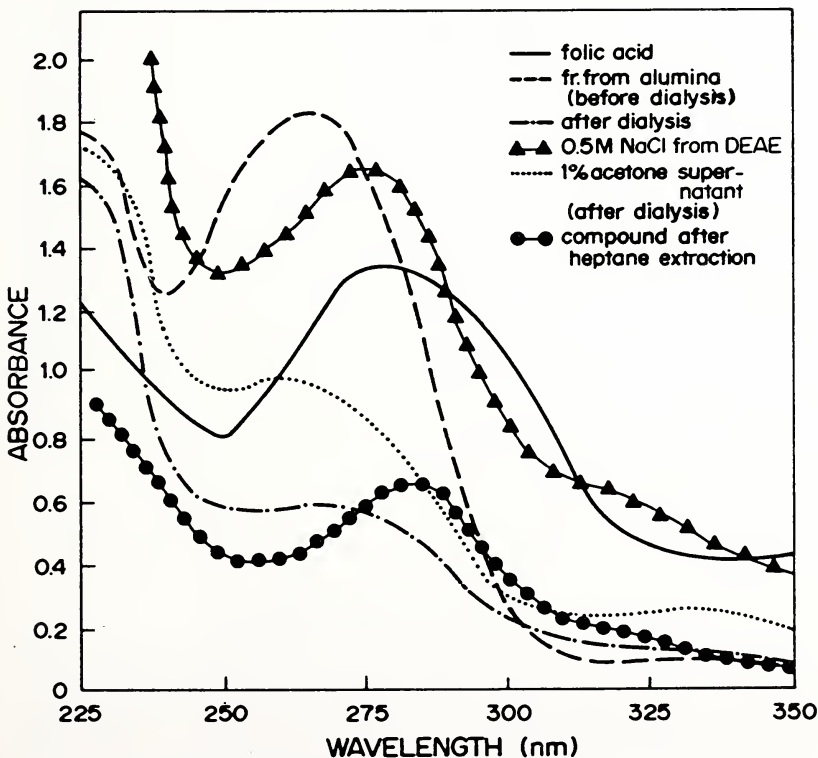


FIGURE 1. The Ultraviolet Absorption Spectra of the Silicomolybdate Stimulation Factor Isolated from Spinach Chloroplasts by 1 or 2% Acetone Extraction in Various Stages of Purification. The U.V. absorption spectrum of folic acid is included for comparison.

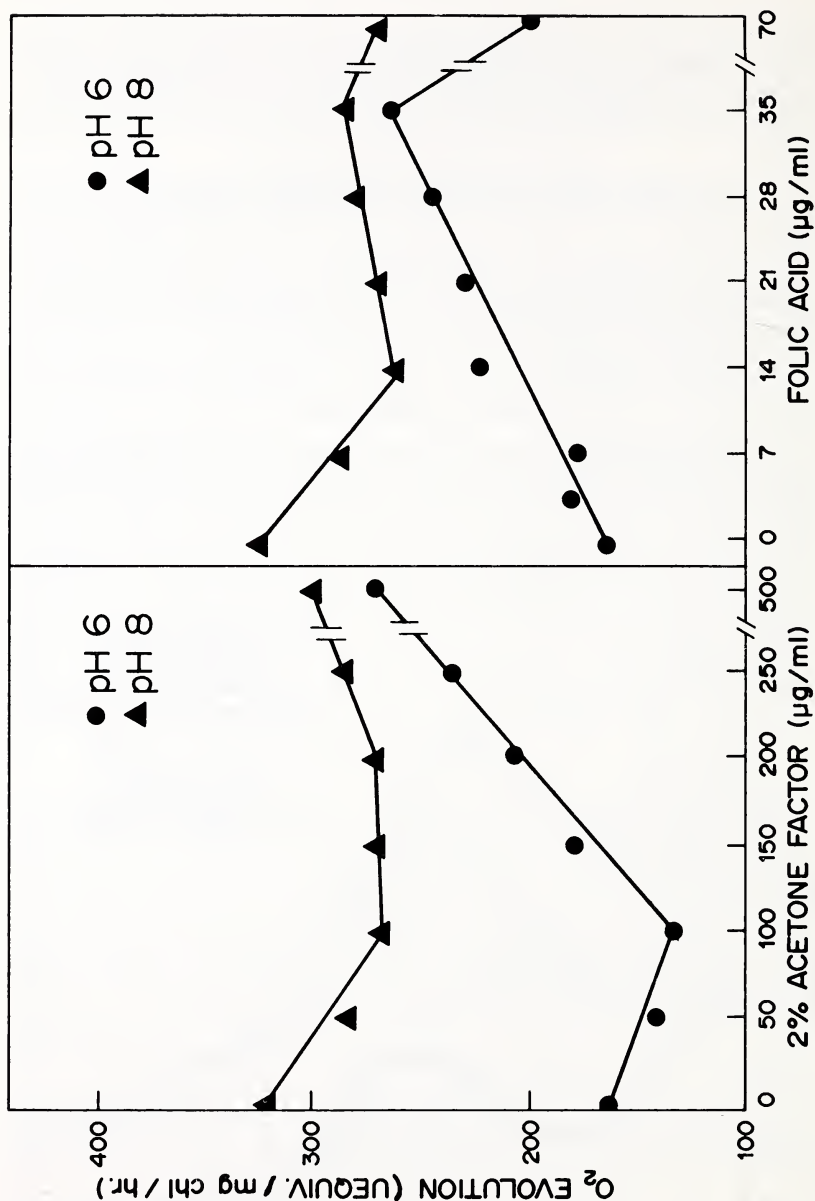


FIGURE 2. Stimulation of Silicomolybdate Reduction in Presence of DCMU at pH 6 by the Factor Isolated from 1 or 2% Acetone Extracts of Spinach Chloroplasts Compared to Stimulation by Folic Acid Under the Same Conditions. Silicomolybdate reduction assayed polarographically with a Clark-type electrode. Reaction mixture contained in 1.5 ml volume: chloroplasts (50 µg chlorophyll), buffer (25 mM Tris-Mes, pH 6 or 8), 2 mM $MgCl_2$ at pH 6 only, 2 mM NH_4Cl at pH 8 only, DCMU ($1.5 \mu M$), silicomolybdic acid (0.2 mg).

absorption spectra in the U.V. region of the spectrum (Fig. 1). The fraction which resembles folic acid the most comes from purification of 2% acetone extracts on a DEAE cellulose column and is eluted with 0.5 M NaCl. This factor shows an absorption maximum at about 280nm, a minimum at 250 nm, as does folic acid itself. However, the factor does not give blue fluorescence. The R_F on PEI Cellulose F TLC plates developed in 8% ammonium hydroxide is also different (0.37) versus 0.67 for folic acid). This difference may be due to insufficient purification of the factor or to breakdown during our isolation procedures, which require long periods of dialysis to remove residual acetone. However, stimulation of silicomolybdate reduction by PS II in presence of DCMU is shown by the impure 2% acetone factor after centrifugation at 7,500 x g and dialysis (Fig. 2), as well as by various purified forms (not shown). Better stimulation of the rate at pH 6 is given by the acetone factor than by folic acid itself (Fig. 2), although lower concentrations of folic acid are required for maximum stimulation (1:15) before inhibition of the rate is observed. At pH 8, the acetone factor and folic acid are slightly inhibitory.

Folic acid was first isolated from spinach leaves by Mitchell and co-workers (6,7). Folic acid and other pteridines stimulate photophosphorylation in spinach chloroplasts (8,9). However, this is the first report of its action on silicomolybdate reduction by PS II. The significance of stimulation of the forward electron transport reactions by folic acid and the isolated acetone factor may be related to the redox feedback control mechanism described earlier by Barr and Crane (2) in which substances which inhibit silicomolybdate reduction in presence of DCMU stimulate forward electron transport and photophosphorylation. Higher concentrations of folic acid or of the isolated factor than shown in Fig. 2 inhibit silicomolybdate reduction more than 75%, resulting in increased forward electron transport according to predictions.

In conclusion, it can be stated that a stimulator of silicomolybdate reduction has been isolated from dilute acetone extracts of spinach chloroplasts which resembles folic acid in certain aspects but not in others. Complete identification of the factor awaits further study.

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