INHIBITION OF SPINACH-LEAF PHOSPHOFRUCTOKINASE BY 2-PHOSPHOGLYCOLLATE

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1. Introduction

A considerable amount of the CO_2 fixed during photosynthesis in green plants is utilised for the synthesis of starch in chloroplasts. The possibility that the subsequent degradation of this starch might involve formation of triose phosphates which can be easily exported to the cytoplasm [1] was recently strengthened by the detection, in this laboratory, of phosphofructokinase activity in chloroplasts [2]. Experiments described in this communication have confirmed that phosphoglycollate, a proposed intermediate of photorespiration, inhibits the activity of spinach-leaf phosphofructokinase. Strong inhibition of the chloroplast enzyme may significantly hinder the degradation of chloroplast starch during daylight hours.

2. Materials and methods

Spinach was purchased locally. All biochemicals and auxiliary enzymes were obtained from Boehringer, Mannheim, except dithioerythritol was purchased from Merck, Darmstadt.

A crude preparation of spinach-leaf phosphofructokinase was obtained by homogenising 50 g leaves in 100 ml extraction solution (5 mM $MgCl_2$, 5 mM dithioerythritol and 1 mM EDTA, pH 7.7), centrifuging at 18 000 × *g* for 25 min and dialysing a sample of the supernatant for 3 h against 1 litre of dialysis solution (5 mM imidazole–HCl, 1 mM $MgCl_2$, 1 mM dithioerythritol and 0.5 mM EDTA, pH 7.7). Chloroplast phosphofructokinase was partially purified by fractionating the crude extract obtained after centrifugation with saturated ammonium sulphate (pH 8); protein precipitated between 49 and 61% saturation was collected at the centrifuge (18 000 × *g*, 25 min), dissolved in 3 ml extraction solution and dialysed as described above. This preparation was diluted 4-fold with extraction solution and ethanol added dropwise to a final concentration of 21%. Precipitated protein obtained by centrifugation at 22 000 × *g* for 10 min was dissolved in 1.5 ml extraction solution and dialysed as above. All operations were at 2–4°C. Preparations obtained in this manner were free of phosphoglycollate phosphatase (fig.2); specific activities were around 0.06 μmol fructose-1,6-P_2 formed per min per mg protein, representing an approximately 18-fold increase over that in the crude extract (fig.2).

Reaction mixtures for the determination of phosphofructokinase activity contained, in a final volume of 1 ml, 50 μmol imidazole–HCl buffer, pH 7.7, 2.5 μmol $MgCl_2$, 2.5 μmol dithioerythritol, 0.25 μmol D-fructose-6-P, 0.08 μmol NADH, 1 unit aldolase, 12 units triose-P isomerase, 1 unit α-glycero-P dehydrogenase, and enzyme preparation. The reaction was started with 0.5 μmol ATP and the change in extinction at 340 nm followed spectrophotometrically. Protein was determined by the biuret procedure after precipitation with trichloroacetic acid and washing with acetone.

3. Results

The inhibition of spinach chloroplast phosphofructokinase by phosphoglycollate is shown in fig.1. Fifty-percent inhibition was caused by 50 μM phosphoglycollate while almost complete inhibition resulted when the concentration was increased to 160 μM. Enzyme

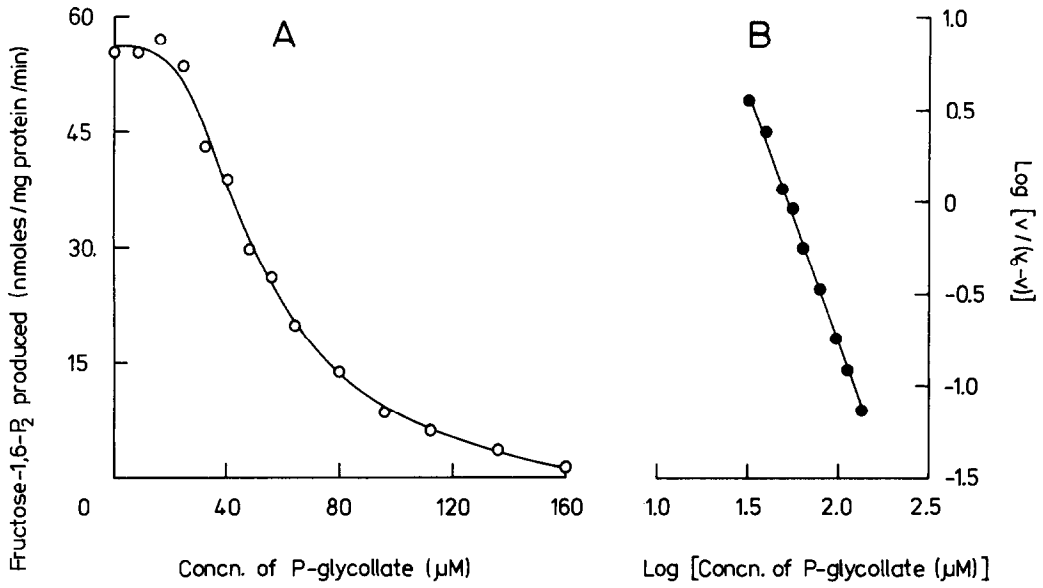

Fig.1. Inhibition of spinach chloroplast phosphofructokinase by phosphoglycollate. Enzyme activity was determined as described under methods, with phosphoglycollate added as shown. A, saturation curve; B, Hill plot. v_0 is the activity in the absence of phosphoglycollate, v is the activity with the different concentrations of phosphoglycollate.

activity responded to inhibitor concentration in a sigmoid fashion (fig.1A) and when the results were replotted according to the Hill equation (fig.1B) a Hill coefficient of 2.7 was revealed. In a control experiment. 1 mM glycollate neither significantly affected the enzyme activity nor relieved the inhibition caused by 0.2 mM phosphoglycollate.

Further control experiments are shown in fig.2. In experiment a, the inhibition of phosphofructokinase activity in a crude extract from spinach leaves by 0.2 mM phosphoglycollate was apparent for no longer than 1 min; presumably the inhibitor was rapidly hydrolysed by the specific phosphoglycollate phosphatase known to be present in leaves [3]. When the

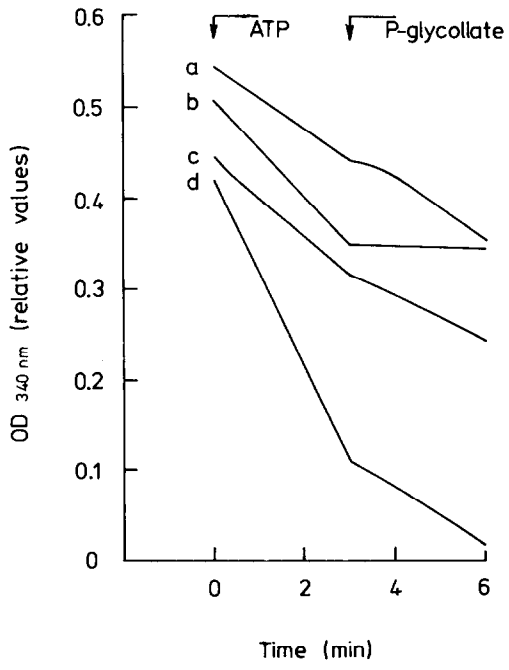

Fig.2. Effect of 0.2 mM phosphoglycollate on the activities of various phosphofructokinase preparations. Enzyme activity was determined as described under methods; the relative changes in extinction at 340 nm are shown before and after addition of phosphoglycollate at 3 min. a, 0.1 ml (containing 0.6 mg protein) of crude preparation from spinach leaves; b, 0.05 ml (containing 0.06 mg protein) of partially purified spinach chloroplast phosphofructokinase; c, 5 μg dialysed rabbit muscle phosphofructokinase; d, mixture of b + c.

phosphoglycollate concentration was increased to 1 mM (not shown) the phosphofructokinase activity was completely inhibited for 3 min (although no inhibition remained after 7 min), implying that both the chloroplast and cytoplasmic enzymes are susceptible to inhibition by phosphoglycollate. In experiments b, c and d (fig.2) a comparison is made between the sensitivity of spinach chloroplast and rabbit muscle phosphofructokinase to phosphoglycollate. Activity of the chloroplast enzyme was 98% inhibited by 0.2 mM phosphoglycollate (experiment b), but only 41% of the activity of the rabbit muscle enzyme was inhibited (experiment c). When the two enzymes were assayed together in the one reaction mixture (experiment d) the amount of activity inhibited by phosphoglycollate was that predicted on the basis that each enzyme responded to the inhibitor as when assayed separately. Clearly, the chloroplast enzyme was considerably more sensitive to the inhibitor. These experiments also show that any phosphoglycollate inhibition of the auxiliary enzymes used to assay phosphofructokinase activity was unlikely to have contributed significantly to the results shown in fig.1, although some interference cannot be ruled out since phosphoglycollate is reported to inhibit triose-P isomerase [4]. Comparison of experiments a and b indicates that the partially purified chloroplast phosphofructokinase was free of phosphoglycollate phosphatase.

4. Discussion

The strong inhibition of chloroplast phosphofructokinase by phosphoglycollate is reminiscent of the similarly effective inhibitions of pea-seed phosphofructokinase by phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate [5]. All four of these compounds possess a phosphoryl group and a carboxyl group. It may be more than coincidence that proposal was quite recently made for the inhibition phosphoglycollate inhibition of chloroplast phosphofructokinase lies in the same range of values (2.3 to 2.9) found earlier for the inhibitors of the pea-seed enzyme. Phosphoenolpyruvate also inhibits chloroplast phosphofructokinase [2]; a general property of plant phosphofructokinase may be that 3-phosphoglycerate, 2-phosphoglycerate, phosphoenolpyruvate and phosphoglycollate all bind with high affinity to the same site on the enzyme. The idea that the first three of these inhibitors bind to the one site was previously expressed for the pea-seed enzyme [5] and a similar proposal was quite recently made for the inhibition of rabbit muscle phosphofructokinase by 3-phosphoglycerate and phosphoenolpyruvate [6].

The 2C-acid glycollate is believed to be the substrate for the apparently wasteful process of photorespiration in which photosynthetically fixed CO_2 is subsequently released as CO_2. One proposed reaction leading to glycollate formation involves the oxidative cleavage of ribulose-1,5-P_2 to one molecule each of 3-phosphoglycerate and phosphoglycollate [7]. Phosphoglycollate has been detected in *Chlorellla* [8,9], isolated chloroplasts [10] and tobacco leaves [11] during or shortly after photosynthesis. If oxidation of ribulose-1,5-P_2 is the sole source of phosphoglycollate in photosynthetic tissues, then the compound would be formed mainly during illumination. In this case, phosphoglycollate inhibition of chloroplast phosphofructokinase could constitute an ideal regulatory mechanism for the prevention of chloroplast starch degradation during its synthesis. A pertinent question is whether chloroplast ADP-glucose pyrophosphorylase, the enzyme with a major regulatory role in starch biosynthesis, is activated by phosphoglycollate; this enzyme is activated by 3-phosphoglycerate and phosphoenolpyruvate [12] which inhibit phosphofructokinase [2,5].

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