A single chloroplast protein with latent activities of both NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase and phosphoribulokinase

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An algal chloroplast protein possessing latent NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase and latent phosphoribulokinase activities has been purified. Both activities were stimulated by incubation with dithiothreitol and NADPH. The protein had subunit composition 8G6R and on activation depolymerized to discrete NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase (4G) and phosphoribulokinase (2R). Similar depolymerization promoted by reduced thioredoxin could account for light-dependent activations of phosphoribulokinase and NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase observed in algae and higher plants.

Alga *Glyceraldehyde-3-phosphate dehydrogenase* (Scenedesmus obliquus) *Light-activated enzyme* *Phosphoribulokinase* *Thioredoxin*

1. INTRODUCTION

A regulatable form of G3PDH (D-enzyme) has been isolated from the green alga, *Scenedesmus obliquus*. This hexadecameric enzyme is characterized by high NADH-dependent activity [1]. Incubation with effector molecules induces NADPH-dependent G3PDH activity associated with depolymerization to a tetramer [2,3].

Recently two forms of PRK have also been purified from extracts of the alga [4]. A low-M_r form is active whilst a high-M_r form has latent activity, promoted by incubation with dithiothreitol. Activation by dithiothreitol also promotes depolymerization. Latent PRK, like regulatable G3PDH, was lost during DEAE-cellulose chromatography but this could be prevented by the inclusion of NAD in the buffers [5].

We now report that the regulatable G3PDH and the latent PRK are the same protein oligomer. On incubation with dithiothreitol this depolymerizes to give active PRK and NADPH-dependent G3PDH.

2. MATERIALS AND METHODS

The high-M_r regulatable form of G3PDH was prepared from heterotrophically grown *S. obliquus* [1]. During the DEAE-cellulose chromatography step the buffers contained 0.13 mM NAD to prevent depolymerization [5]. PRK and G3PDH activities were assayed as described [1,4].

3. RESULTS AND DISCUSSION

The regulatable G3PDH was also found to possess latent PRK activity, expressed on incubation with dithiothreitol. Further purification by hydroxyapatite chromatography (fig.1) failed to

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Abbreviations: G3PDH, glyceraldehyde-3-phosphate dehydrogenase; PRK, phosphoribulokinase; PAGE, polyacrylamide gel electrophoresis

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separate the PRK and G3PDH activities which were eluted together, associated with a single homogeneous protein as judged by PAGE. Incubation of the protein with dithiothreitol and NADPH promoted increases in the activities of both PRK and NADPH-dependent G3PDH and a decrease in the G3PDH activity linked to NADH (fig.2). The specific activities prior to incubation were 30 and 3 U/mg for G3PDH linked to NADH and NADPH, respectively, and zero for PRK. Specific activities following incubation became 19 and 50 U/mg for G3PDH linked to NADH and NADPH, respectively, and 93 U/mg for PRK.

The protein oligomer was shown by SDS-PAGE to contain two polypeptides with apparent M_r of 39000 and 42000. The latent form of PRK has been reported to be a hetero-oligomer of M_r 470000, composed of two subunits X and Y of M_r 39000 and 42000, respectively, and apparent subunit composition 8X4Y [4]. In contrast the regulatory form of G3PDH from the alga was thought to be a homo-hexadecamer of M_r 550000 [1]. As both activities have now been shown to be associated with a single protein it was necessary to resolve this discrepancy in M_r. Sedimentation equilibrium determination gave a value of 560000. This implies a subunit composition of 8G6R, where G is the subunit with G3PDH activity (M_r 39000) and R that with PRK activity (M_r 42000). This assignment of activities is consistent with the small, active form of PRK having an M_r of 83000 and migrating on SDS-PAGE as a single band with apparent M_r of 42000 [4]. The M_r of 470000 previously reported may represent an 8G4R species formed by the loss of a PRK dimer. All recent determinations of M_r have given values which correspond to an 8G6R composition.

The changes in M_r now considered to accompany the dithiothreitol-mediated stimulation of NADPH-dependent G3PDH and PRK activities are shown in fig.3. Ultracentrifugation has shown that the change in co-enzyme dependence of G3PDH activity promoted by incubation of the large M_r form of the enzyme ($s_{20,w}$ = 14.2 S) with dithiothreitol and NADPH is accompanied by depolymerization yielding two boundaries with sedimentation coefficients of 7.4 S and 4.4 S [3]. It is now apparent that the active form of PRK is responsible for the 4.4 S boundary rather than an inactive dimer of the G subunit as previously suggested. The 7.4 S boundary is that of the NADPH-dependent G3PDH. No reversal of this depolymerization occurred either on storage or dialysis of the sample following activation by

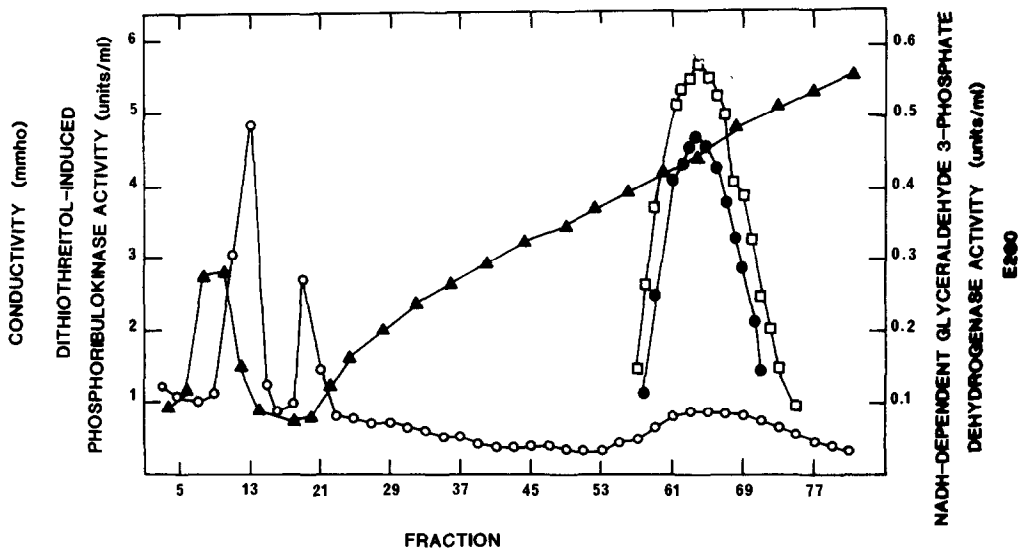

Fig.1. Co-purification of G3PDH and PRK. D-enzyme [1] was chromatographed on hydroxyapatite (1.6 × 28 cm). Elution was by a 1 l linear phosphate gradient (50–200 mM, pH 7.2) in 10% glycerol and 0.5 ml/l mercaptoethanol. The eluted fractions (5 ml) were assayed for PRK (—□—), NADH-dependent G3PDH (—●—) activities, E_{280} (—○—) and conductivity (—▲—).

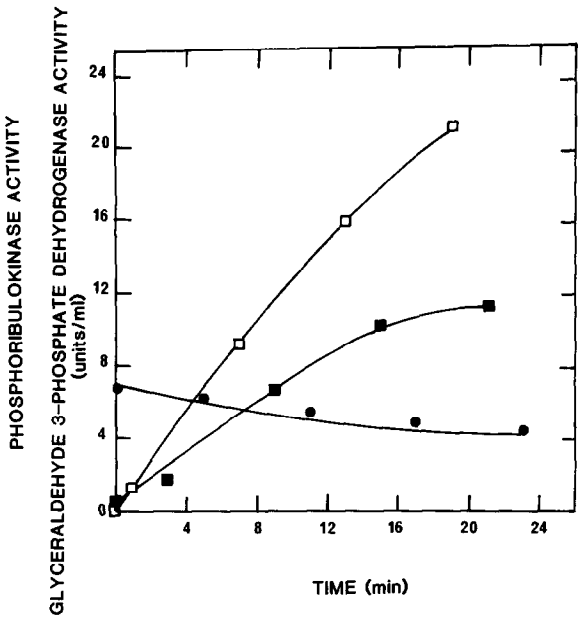

Fig.2. Co-activation of NADPH-dependent G3PDH and PRK. Purified enzyme from hydroxyapatite (44 μg, 1.3 units NADH-dependent G3PDH) was pre-incubated at 30°C with 20 mM dithiothreitol and 1 mM NADPH in 200 μl of 0.1 M Tris-HCl, pH 7.5. Aliquots (10 μl) were taken at specified times and their PRK (—□—), NADPH-dependent G3PDH (—■—) and NADH-dependent G3PDH (—●—) activities determined.

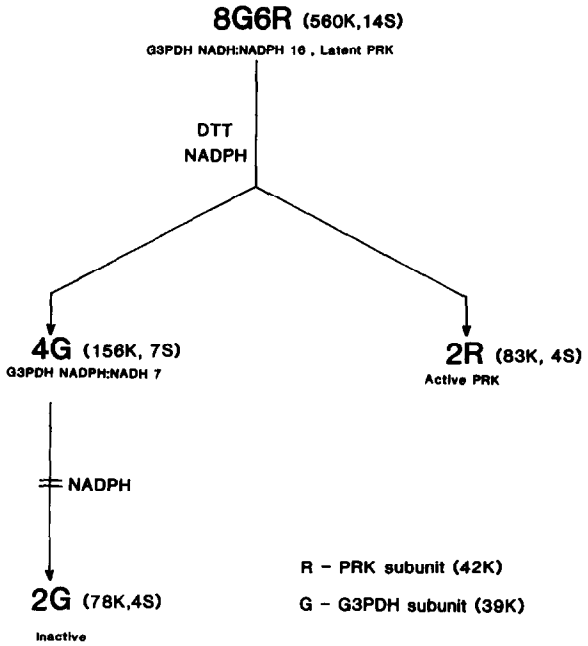

Fig.3. Molecular mass changes accompanying the dithiothreitol-promoted activation of PRK and NADPH-dependent G3PDH.

NADPH and dithiothreitol [3]. However, depolymerization promoted by 1,3-bisphosphoglycerate could be reversed by overnight standing [2], during which time the 1,3-bisphosphoglycerate decomposed.

Prolonged incubation with dithiothreitol in the absence of nucleotides resulted in the complete loss of G3PDH activity and an exclusively 4.6 S sedimenting form [3]. The present findings indicate this slowly sedimenting boundary to be due to a mixture of the active form of PRK and the inactive dimeric form of G3PDH. These two proteins would be indistinguishable in the ultracentrifuge. Similar depolymerization would account for the light-dependent activation of PRK and NADPH-dependent G3PDH observed in vivo [6,7]. In this case, photoreduced thioredoxin rather than dithiothreitol would be the reductant. Thioredoxin stimulates the activation of PRK on incubation of the latent form with low concentrations of dithiothreitol [4]. The concentration of nucleotides in the chloroplast would be more than adequate to prevent further depolymerization of the NADPH-dependent G3PDH to the inactive dimer [8]. Although thiol-promoted depolymerization in vitro could not be reversed, it is possible that in vivo depolymerization promoted by reduced thioredoxin would be more specific and is likely to be reversible. Reduction by dithiothreitol in vitro may have caused over-reduction of the enzyme.

There is evidence that a similar association of PRK with G3PDH occurs in higher plant chloroplasts. Many workers have reported high-M_r forms of G3PDH in extracts of plant leaves [9–12], whilst PRK has been partially purified in a high-M_r form from spinach [13]. Significantly this co-chromatographed on both DEAE-cellulose and Bio-gel with a regulatory G3PDH of M_r 600000 [10].

Reversible, pyridine nucleotide-induced changes in the aggregation of G3PDHs from chloroplasts are a general phenomenon [14]. NADP promotes depolymerization to tetramers. These are present in *Sinapis alba* as separable isoenzyme forms, a

heterotetramer (39 and 42 kDa subunits) and a homotetramer (39 kDa subunits) [15]. The polypeptide composition of the aggregated enzyme from *Sn. alba* resembles that of the heterotetramer. The major difference is quantitative with the aggregated enzyme containing more of the 42 kDa polypeptide [16]. NAD-promoted re-aggregation of the tetramers is dependent upon a binding fraction, the major component of which was shown by SDS-PAGE to be a 42 kDa polypeptide [16]. This polypeptide, co-migrating on SDS-PAGE with the larger subunit of the heterotetramer, is a major polypeptide in extracts of *Sn. alba* and is devoid of G3PDH activity. In view of our findings with the algal enzymes we would suggest that the binding fraction in *Sn. alba* is the active form of PRK and the aggregated form of G3PDH, like the algal enzyme, should possess latent PRK activity.

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