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RESEARCH PAPER

Inter-species variation in the oligomeric states of the higher plant Calvin cycle enzymes glyceraldehyde-3-phosphate dehydrogenase and phosphoribulokinase

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Abstract

In darkened leaves the Calvin cycle enzymes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoribulokinase (PRK) form a regulatory multi-enzyme complex with the small chloroplast protein CP12. GAPDH also forms a high molecular weight regulatory mono-enzyme complex. Given that there are different reports as to the number and subunit composition of these complexes and that enzyme regulatory mechanisms are known to vary between species, it was reasoned that protein–protein interactions may also vary between species. Here, this variation is investigated. This study shows that two different tetramers of GAPDH (an A2B2 heterotetramer and an A4 homotetramer) have the capacity to form part of the PRK/GAPDH/CP12 complex. The role of the PRK/GAPDH/CP12 complex is not simply to regulate the 'non-regulatory' A4 GAPDH tetramer. This study also demonstrates that the abundance and nature of PRK/GAPDH/CP12 interactions are not equal in all species and that whilst NAD enhances complex formation in some species, this is not sufficient for complex formation in others. Furthermore, it is shown that the GAPDH mono-enzyme complex is more abundant as a 2(A2B2) complex, rather than the larger 4(A2B2) complex. This smaller complex is sensitive to cellular metabolites indicating that it is an important regulatory isoform of GAPDH. This comparative study has highlighted considerable heterogeneity in PRK and GAPDH protein interactions between closely related species and the possible underlying physiological basis for this is discussed.

Key words: Blue native PAGE, Calvin cycle, CP12, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoribulokinase (PRK), protein–protein interactions.

Introduction

The Calvin cycle is the primary pathway of CO_2 fixation and is driven by ATP and NADPH produced by photosynthetic electron transport. Regulation of the Calvin cycle must therefore be responsive to changes in the supply of products from electron transport and demand for carbon skeletons. As a result, many Calvin cycle enzyme activities are sensitive to changes in stromal pH, redox state (via thioredoxin), and metabolite and Mg^{2+} concentrations. Such mechanisms allow modulation of enzyme activities in response to changes in light intensity during the day and complete suppression of the Calvin cycle at night.

Protein–protein interactions provide an additional level of regulation over enzyme activity. In darkened leaves the Calvin cycle enzymes phosphoribulokinase (PRK, EC 2.7.1.19) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1.2.1.13) form a multi-enzyme complex with the small chloroplast protein CP12 (Wedel *et al.*, 1997; Wedel and Soll, 1998). A high molecular weight oligomeric form of GAPDH is also present (Scheibe *et al.*, 2002). The activities of the enzymes that form part of these complexes are inhibited when oligomerization occurs, indicating a regulatory role for these protein complexes. The formation and

Abbreviations: BN-PAGE, blue native PAGE; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PRK, phosphoribulokinase.

dissociation of the PRK/GAPDH/CP12 complex *in vivo* is also rapid and responds quantitatively to changes in light intensity, allowing swift activation of enzyme activities in response to light fluctuations (Howard *et al.*, 2008). Illumination of leaves, or reduction of isolated chloroplasts with thioredoxin or DTT, results in dissociation of the PRK/GAPDH/CP12 complex and promotes GAPDH and PRK activity (Wedel *et al.*, 1997). Conversely, incubation of partially purified PRK, GAPDH, and CP12 (Scheibe *et al.*, 2002) or recombinant expressed proteins (Marri *et al.*, 2005) in oxidizing conditions, in the presence of NAD, leads to protein aggregation and decreased enzyme activity. Activation of GAPDH also results in dissociation of the high molecular weight mono-enzyme complex *in vitro* and *in vivo* (Scagliarini *et al.*, 1993; Trost *et al.*, 1993; Baalmann *et al.*, 1994; Scheibe *et al.*, 2002).

The occurrence of such regulatory complexes appears widespread in higher plants. GAPDH multimeric forms have been reported in spinach, pea, and maize (Scagliarini *et al.*, 1993; Baalmann *et al.*, 1994). The PRK/GAPDH/CP12 complex has been shown to be present in spinach, pea, and maize (Wedel *et al.*, 1997; Wedel and Soll, 1998; Scheibe *et al.*, 2002), in a cyanobacterium (Tamoi *et al.*, 2005), and several algal species (Avilan *et al.*, 1997; Boggetto *et al.*, 2007; Oesterhelt *et al.*, 2007). Recently however, it was shown that regulation of PRK and GAPDH activity by CP12 and by redox state is not uniform across algal species (Maberly *et al.*, 2010). It is also known that the activities of GAPDH in darkened leaves of spinach and maize are different (Scagliarini *et al.*, 1993).

In addition to this, the number and subunit composition of PRK and GAPDH complexes has been the subject of debate. In the algae and higher plants studied PRK is present in one of two forms, either in an active ~80 kDa form (a homodimer) or aggregated as part of the complex with GAPDH and CP12. In higher plants there are two GAPDH subunits, GapA and GapB. The major difference between the GapA and GapB proteins is a C-terminal extension on the GapB peptide which confers thioredoxin-mediated redox regulatory capacity onto the GAPDH enzyme and plays an essential role in GAPDH protein–protein interactions (Baalmann *et al.*, 1996; Scheibe *et al.*, 1996; Fermani *et al.*, 2007). The predominant active form of GAPDH in higher plants is assumed to be a heterotetramer comprising two GapA and two GapB subunits (A2B2). In addition an A4 tetramer has been described in spinach chloroplast preparations representing 15–20% of the total GAPDH activity (Scagliarini *et al.*, 1998). This form of GAPDH has been termed 'non-regulatory' because of the absence of the C-terminal extension. It has been suggested that the PRK/GAPDH/CP12 complex allows regulation of this form of GAPDH (Trost *et al.*, 2006). The existence of this isoform in higher plants is contentious and alternative reports could find no evidence for the A4 tetramer in spinach (Scheibe *et al.*, 2002). Reports also indicate that the PRK/GAPDH/CP12 complex can involve an interaction solely with the A2B2 heterotetramer (Wedel *et al.*, 1997; Scheibe *et al.*, 2002; Howard *et al.*, 2008) and with both A4 and A2B2 tetramers (Clasper *et al.*, 1991). With respect to GAPDH mono-enzyme complexes, a 300-kDa aggregate has been reported in some spinach purifications (Scagliarini *et al.*, 1993, 1998; Baalmann *et al.*, 1994), but not in others (Trost *et al.*, 1993; Scheibe *et al.*, 2002). This complex is hypothesized to represent an intermediate step in GAPDH aggregation (Baalmann *et al.*, 1994).

In order to explore the extent of this potential variation, the oligomeric status and subunit composition of PRK and GAPDH protein complexes from nine species, from four plant families were investigated. To allow parallel analysis of protein complexes blue native PAGE (BN-PAGE) was used, because it provides higher resolution than conventional gel filtration chromatography approaches in conjunction with simultaneous analysis of protein complexes (Eubel *et al.*, 2005).

Materials and methods

Plant material

The plants used in this study were pea (*Pisum sativum* 'Onwards'), *Medicago* (*Medicago truncatula* 'Jemalong'), broad bean (*Vicia faba* 'The Sutton'), French bean (*Phaseolus vulgaris* 'Vilbel'), potato (*Solanum tuberosum* 'Desiree'), tomato (*Solanum lycopersicon* 'Gardener's Delight'), tobacco (*Nicotiana tabacum* 'Samson'), spinach (*Spinacea oleracea* 'Giant Winter'), and *Arabidopsis* (*Arabidopsis thaliana* 'Colombia'). All plants were grown in a controlled environment chamber with 16-h light/8-h dark and light levels of 115 μmol m^{-2} s^{-1} except potato, tomato, and *Medicago*, which were grown under glass in a controlled environment greenhouse (16-h photoperiod, 25–30 °C day/20 °C night with natural light supplemented with high-pressure sodium light bulbs, giving 600–1600 μmol m^{-2} s^{-1}). Pea and tobacco plants were grown under both conditions and no effect was observed on protein profiles of these species.

Protein purification

Procedures for the isolation of chloroplasts and stromal proteins were described in Howard *et al.* (2008), based on the procedure of Scheibe *et al.* (2002). Protein concentrations were determined using Bradford reagent (Sigma-Aldrich, UK) to ensure equal loading in each well.

BN-PAGE and western transfer

BN-PAGE, second-dimension Tricine SDS-PAGE and western transfer conditions were all previously described in Howard *et al.* (2008), based on the procedure of Schägger and von Jagow (1991) and Schägger *et al.* (1994). Equal protein loading and protein transfer to PVDF membranes was confirmed by staining membranes with Ponceau S (Sigma-Aldrich, UK). Gel images shown are representative of at least three different extractions, separated on different occasions.

Results

Inter-species variation in GAPDH and PRK protein complex profiles

To determine the interactions of GAPDH in the dark, both with itself and with PRK, stromal extracts were prepared from dark-adapted leaves of species from within the

Leguminosae (pea, *Medicago*, broad bean, French bean), the Solanaceae (potato, tomato, and tobacco), the Amaranthaceae (spinach), and the Brassicaceae (*Arabidopsis*). Native proteins were separated using BN-PAGE. Bands containing GAPDH or PRK were detected following western transfer and immunolocalization with polyclonal antibodies raised against GAPDH (holoenzyme containing both the A and B subunits) and PRK. The results indicate that GAPDH has a number of different oligomeric states in extracts of darkened leaves. Bands recognized by the GAPDH antibodies were detected at 150, 160, 300, 600, and 700 kDa (Fig. 1A). Antibodies raised against PRK detected bands at 70 kDa or 600 kDa indicating the presence of the dimeric active form of PRK and the presence of the PRK/GAPDH/CP12 complex, respectively (Fig. 1B).

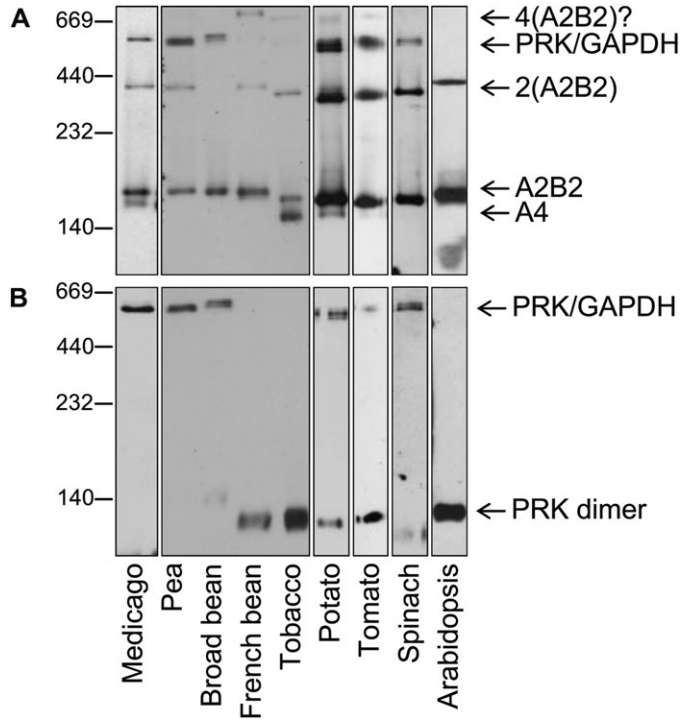

Fig. 1. PRK and GAPDH aggregates in darkened leaves visualized using BN-PAGE. Western blot of stromal protein complexes separated using BN-PAGE from darkened leaves of *Medicago*, pea, broad bean, French bean, tobacco, potato, tomato, spinach, and *Arabidopsis*. Immunoblots were probed with (A) GAPDH or (B) PRK polyclonal antibodies. Sixty micrograms of stromal protein were loaded per well. Sizes indicated are kDa.

In all species there was a band representing a GAPDH multimer at 160 kDa (Table 1, Fig. 1A). Second-dimension Tricine SDS-PAGE of lanes excised from the gels revealed that this band contained both GapA and GapB subunits of GAPDH and is therefore the previously identified A2B2 heterotetramer (Howard *et al.*, 2008; Fig. 2). Second-dimension Tricine SDS-PAGE of lanes excised from the gels revealed that the smallest of all the GAPDH bands, seen at ~150 kDa in *Medicago*, tobacco, and potato contained only the GapA GAPDH subunit (Fig. 2). From these data it is concluded that the smallest GAPDH complex represents an A4 GAPDH tetramer. The ability to detect this isoform in spinach was dependent upon illumination of the leaves prior to extraction (Fig. 2). Furthermore, whilst the A2B2 tetramer was present in all species, the A4 tetramer was present in some and absent in others. The A4 tetramer was also never the sole tetrameric form of GAPDH.

At ~600 kDa a band was identified that both PRK and GAPDH antibodies recognized (Fig. 1). This band corresponds to the PRK/GAPDH/CP12 complex. In pea, in dark-adapted leaves, PRK was present only as the high molecular weight form at 600 kDa. In broad bean, potato, tomato, and spinach PRK was present in the dark in both high molecular weight (600 kDa) and dimeric (70 kDa) forms. In French bean, tobacco, and *Arabidopsis* the PRK/GAPDH/CP12 complex was a minor component in stromal extracts from dark-adapted leaves.

Further GAPDH signals were evident at both 300 kDa and 700 kDa. Second-dimension SDS-PAGE of these samples revealed that these bands contained the A and B subunits of GAPDH. The GAPDH signal at 300 kDa was a feature common to all species and represents the 2(A2B2) GAPDH complex (Baalmann *et al.*, 1994). In broad bean

Table 1. Occurrence of PRK and GAPDH complexes isolated from darkened tissues of a range of higher plant species and separated using BN-PAGE

	Medicago	**Pea**[a]	**Broad bean**	**French bean**	**Tobacco**	**Potato**	**Tomato**	**Spinach**	***Arabidopsis***
PRK signal (kDa)									
600	•	•	•			•	•	•	
70			•	•	•	•	•	•	•
GAPDH signal (kDa)									
>700		•[b]		•	•	•	•	•	
600	•	•	•	•	•[b]	•	•	•	
300	•	•	•[b]	•	•	•	•	•	•
160	•	•	•	•	•	•	•	•	•
150	•				•	•		•[c]	

[a] Reported in Howard *et al.* (2008).
[b] Detected at low abundance following long exposure of the film.
[c] Detected only following illumination.

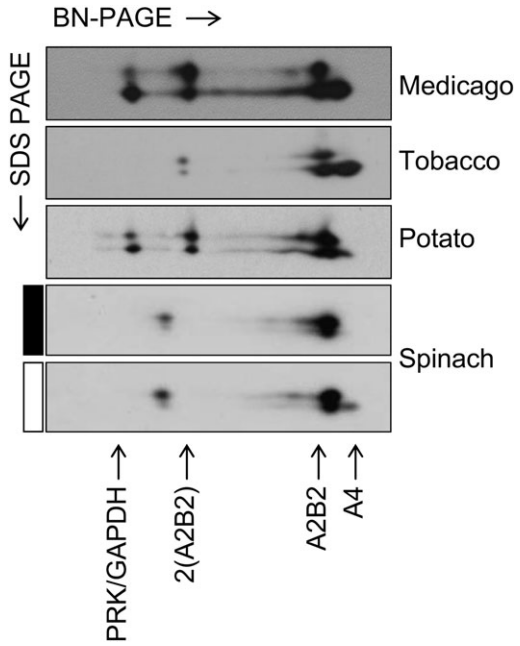

Fig. 2. Subunit composition of GAPDH complexes. Western blot of second-dimension Tricine SDS-PAGE of stromal proteins from darkened leaves of *Medicago*, tobacco, and potato. Stromal proteins from spinach were prepared from either darkened (black bar) or illuminated (white bar) leaves. Immunoblots were probed with GAPDH polyclonal antibodies.

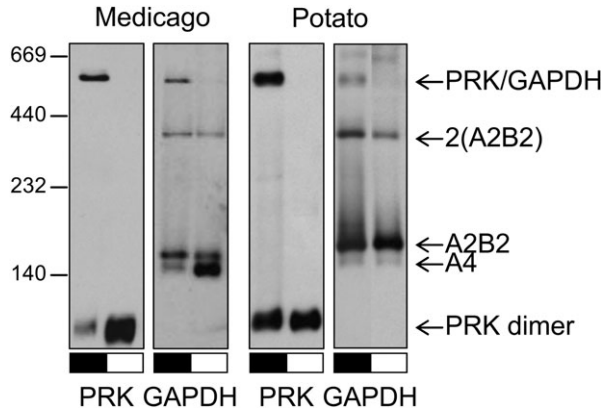

Fig. 3. Light/dark responses of A4 and A2B2 GAPDH tetramers. Western blot of stromal protein complexes separated using BN-PAGE from darkened (black bar) or illuminated (white bar) leaves of *Medicago* and potato. Immunoblots were probed with GAPDH or PRK polyclonal antibodies as indicated. Sizes indicated are kDa.

this complex was present at low abundance and was only detected following long exposure of the film (data not shown). The GAPDH signal seen at a molecular weight of ~700 kDa was only a minor component of the GAPDH profile, and was detectable in French bean, pea (following long exposure of the photographic film to the western blot), and all of the solanaceous species examined. It is likely that this signal represents the 2(A2B2) complex described by Scheibe *et al.* (2002).

Light/dark responses of A4 and A2B2 tetramers

Previously, it was shown using BN-PAGE that illumination of pea leaves results in the dissociation of the high molecular weight PRK/GAPDH/CP12 complex into a PRK dimer and an A2B2 GAPDH heterotetramer (Howard *et al.*, 2008). In order to further investigate the composition and light/dark response of the PRK/GAPDH/CP12 complex extracts from *Medicago* and potato plants were examined. Leaves were either dark adapted overnight, or dark adapted overnight followed by illumination for 30 min, 600 µmol m^{-2} s^{-1}. In extracts from darkened tissues of *Medicago* and potato both PRK and GAPDH are detectable in a high molecular weight aggregate (GAPDH is detectable at four different molecular weights, including both tetramers). Illumination of leaves prior to complex isolation has the same effect, namely the dissociation of the high molecular weight complex (Fig. 3). In *Medicago* however, the A4 GAPDH tetramer is strongly enhanced upon complex dissociation, whilst in potato the A2B2 GAPDH signal is enriched. It was also possible to detect an A4 tetramer in spinach on illumination (Fig. 2). These data indicate that the subunit composition of the pea and potato PRK/GAPDH/CP12 complex is different from the PRK/GAPDH/CP12 complex in spinach and *Medicago*.

PRK and GAPDH interactions in pea and tobacco

Pea provides an example of a species with a routinely detectable high molecular weight PRK/GAPDH signal. In contrast this was not observed in darkened tobacco leaves. Tobacco, however, demonstrates a strong A4 GAPDH tetramer, which is absent in pea. In order to test whether compounds within the tobacco leaves might disrupt the PRK/GAPDH/CP12 complex during the extraction procedure pea and tobacco leaves were mixed at the onset of extraction. In pea three GAPDH complexes are resolved (at 160, 300, and 600 kDa) and a high molecular weight PRK complex is visible. In tobacco three GAPDH complexes are also visible (at 150, 160, and 300 kDa) and a dimeric PRK signal is detectable (~80 kDa). The results indicate that when the protein complexes are purified simultaneously a combined enzyme aggregate profile is recovered (Supplementary Fig. S1 available at *JXB* online): two PRK complexes and six GAPDH complexes are resolved in the lane that comprises both pea and tobacco. PRK is present in both high molecular weight and dimeric forms and GAPDH signals are seen at 150 kDa, two at ~160 kDa, two at ~300 kDa, and one at 600 kDa. This demonstrates the absence of a factor in the tobacco leaves capable of causing dissociation of pea complexes. These data also demonstrate the resolving power of BN-PAGE as it can be seen that in the mixed sample it is possible to distinguish not only the A4 form of GAPDH from the A2B2 form, but remarkably, it is also possible to clearly distinguish the pea A2B2 GAPDH from the tobacco A2B2 GAPDH.

The effect of NAD on PRK and GAPDH interactions

NAD is known to promote PRK/GAPDH/CP12 complex formation (Wedel *et al.*, 1997; Scheibe *et al.*, 2002; Marri *et al.*, 2005, 2009). In order to test the effect of NAD on complex formation across species, stromal proteins from overnight dark-adapted leaves were incubated at 25 °C for 30 min either with or without 2.5 mM NAD, following the method of Scheibe *et al.* (2002). The results indicate that NAD did enhance formation of the PRK/GAPDH/CP12 complex in many species (Fig. 4). For example, long exposure of immunoblots from pea extracts revealed the presence of a small proportion of PRK in the dimeric state in darkened leaves. This signal was absent in samples incubated with NAD prior to electrophoresis. In species in which PRK had been seen in both dimeric and oligomeric forms in darkened leaves (potato and *Medicago*) incubation with NAD resulted in the loss of dimeric PRK. In these species there was also a reduction in the intensity of the tetrameric forms of GAPDH. In keeping with previous findings NAD had little or no effect in any species if DTT was also included in the incubation buffer or if stromal proteins were isolated from illuminated leaves (data not shown). NAD also had a noticeable effect on the migration behaviour of PRK and GAPDH in tobacco and *Arabidopsis* (Fig. 4) and a high molecular weight GAPDH and PRK signal was observed in tobacco, in conjunction with an almost complete loss of the low molecular weight forms of these enzymes.

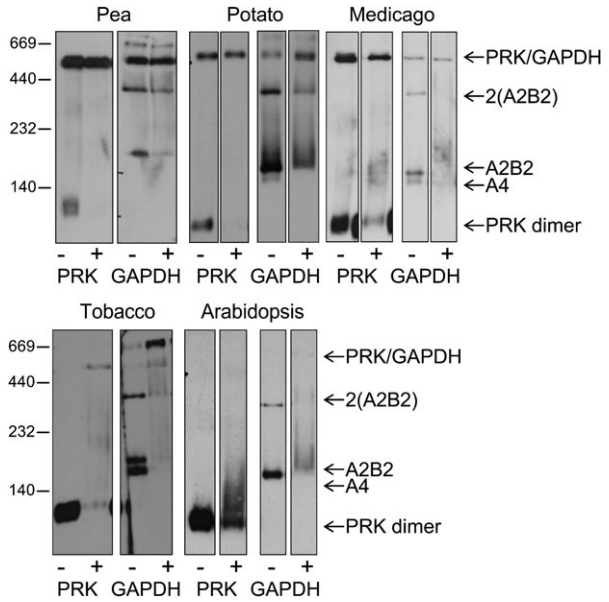

Fig. 4. The effect of NAD on PRK and GAPDH interactions. Western blot of stromal protein complexes incubated in the absence (–) or presence (+) of 140 μM NAD for 30 min prior to separation. Extracts are from darkened leaves of pea, *Medicago*, potato, tobacco, and *Arabidopsis*. Immunoblots were probed with PRK or GAPDH polyclonal antibodies as indicated. Sizes indicated are kDa.

Discussion

In this study considerable heterogeneity in the oligomeric forms of plastid GAPDH was observed, even between closely related species. This plasticity of interactions was exemplified by the variation in the GAPDH tetramer. The predominant form of active GAPDH in higher plant chloroplasts has been considered to be the heterotetrameric A2B2. In spinach an A4 tetramer, similar to that in algae, has also been detected, with some reports indicating that the A4 complex may constitute as much as 20% of total GAPDH activity (Scagliarini *et al.*, 1998). However, the A4 GAPDH tetramer has not been identified in all experiments (Scheibe *et al.*, 2002). The use of BN-PAGE in this study was instrumental in enabling the separation and identification of the two tetrameric forms. All of the plant species examined were shown to have an A2B2 heterotetramer. In extracts from *Medicago* and tobacco, a strong signal was obtained suggesting the presence of significant amounts of A4 GAPDH. An A4 tetramer was also observed in potato and spinach. In none of the species examined was the A4 tetramer the sole tetrameric form of GAPDH.

Whilst the functional significance of the A4 tetramer in higher plants is not known, it has been suggested that the role of the PRK/GAPDH/CP12 complex is to regulate this form of GAPDH (Trost *et al.*, 2006). Alternative reports, however, have indicated that it is the A2B2 form of GAPDH that is present within the PRK/GAPDH/CP12 complex (Wedel *et al.*, 1997; Scheibe *et al.*, 2002; Howard *et al.*, 2008). Here it is seen that in potato (a species with both A4 and A2B2 tetramers) the A2B2 GAPDH tetramer forms an oligomer with PRK, whereas in spinach and *Medicago* (like potato, species with both A4 and A2B2 tetramers) the A4 tetrameric form of GAPDH forms part of the PRK/GAPDH/CP12 complex. These data establish that different tetrameric forms of GAPDH have the capacity to form part of the PRK/GAPDH/CP12 complex. It would therefore appear unlikely that the sole function of the PRK/GAPDH/CP12 complex is to regulate A4 GAPDH.

One might ask instead, if the role of PRK/GAPDH/CP12 complex assembly is to regulate PRK activity? If this were the case, it would not matter which form of GAPDH interacted with PRK. Such a proposal for GAPDH in the regulation of PRK is consistent with the results of Tamoi *et al.* (2005) in which *Synechococcus* cells lacking CP12 have an increase in RuBP content. The authors conclude that the presence of an active dimeric form of PRK in the dark was sufficient to convert R5P into RuBP, with the effect that carbon flux into the oxidative pentose phosphate pathway (OPPP) in the dark was reduced in these cells. In *Synechococcus* therefore, the PRK/GAPDH/CP12 complex appears to regulate PRK (not GAPDH) activity and thereby separates the activities of the OPPP from the Calvin cycle during light/dark transitions. Under such a proposal the role of GAPDH and CP12 is to regulate PRK activity.

In many species, detection of a high molecular weight PRK/GAPDH/CP12 complex in extracts from dark-adapted leaves was easily reproducible. However, in tobacco and

Arabidopsis a high molecular weight band corresponding to the PRK/GAPDH/CP12 complex was only detected following pre-incubation with NAD. These data suggest that the requirements for formation of a stable PRK/GAPDH/CP12 complex and the abundance and ubiquity of the PRK/GAPDH/CP12 complex differs between species. These differences may in part be due to the stability of the interactions between the different constituent proteins. Experiments using recombinant expressed proteins have shown that oxidized CP12 and GAPDH from *Arabidopsis* interact with a dissociation constant of ~0.18 μM (Marri *et al.*, 2008) whilst in *Chlamydomonas reinhardtii* the dissociation constant is 450-fold lower, at ~0.4 nM (Graciet *et al.*, 2003). The interactions in *C. reinhardtii* are stronger than they are in recombinant *Arabidopsis* proteins. These differences are important as they have a bearing on complex formation (Lebreton *et al.*, 2003) and therefore the functional significance of the protein complexes *in planta*.

Of further interest was the observation that the well documented oligomeric form, 4(A2B2), of GAPDH appeared to be a minor component of GAPDH in all species examined in this study, with the exception of extracts from dark-adapted leaves of tobacco and French bean. It has been proposed that this 4(A2B2) form of GAPDH provides a pool of latent GAPDH activity dependent not only on reduction but also on activation by high 1,3-bisphophoglycerate concentrations, indicating a specific demand for increased activity of GAPDH in the Calvin cycle (Scheibe *et al.*, 2002). An additional less well documented multimer of GAPDH, the 2(A2B2) form, was present in all species. This 2(A2B2) complex has been proposed to be an intermediate stage in the aggregation of the GAPDH mono-enzyme complex (Baalmann *et al.*, 1994). Recently it was shown that a combination of reduced DTT together with either NADPH or ATP resulted in the complete loss of this form of GAPDH and a concomitant increase in the A2B2 tetramer in pea (Howard *et al.*, 2008). This provides some evidence that the 2(A2B2) GAPDH complex has a similar role to that of the large 4(A2B2) complex and in conditions of high light it is dissociated into the active A2B2 form. Therefore, whilst this multimer may represent an intermediate step in GAPDH oligomerization, it is likely that it is itself an important complex in the regulation of GAPDH.

This comparative study using BN-PAGE analysis has revealed heterogeneity in the oligomeric forms of PRK and plastid GAPDH within plant families and between closely related species. The relative importance of the protein complexes of GAPDH and PRK in regulating the rate of photosynthesis has not been determined but may reflect difference in the requirement and ability of the plant to respond rapidly to fluctuating environmental and metabolic conditions (Howard *et al.*, 2008). Although the species in this study were grown in similar environments the growth habit and life cycle of these plants are different and this may impact on the metabolism in the leaves. Little is known about the extent of variation in metabolic regulation that exists between species but it has been shown that the response of photosynthesis to short-term changes in the light environment varies between closely related higher plant species (Murchie and Horton, 1997). There is also some evidence that in natural vegetation the ability of photosynthesis to respond rapidly to changes in the light environment may be related to leaf longevity (Kursar and Coley, 1993). The only comparative study that provides evidence of variation between species in the regulation of PRK and GAPDH comes from analysis of a range of algae taken from different environments. This work showed that the extent of redox regulation of PRK and GAPDH was greater in freshwater algae than the marine species. It has been proposed that this is linked to the more dynamic nature of productive freshwaters, compared with the open oceans, where a more rapid light attenuation with depth and a more variable supply of CO_2 is evident (Maberly *et al.*, 2010). In order to gain a fuller understanding of the relative importance of these PRK and GAPDH complexes in regulating photosynthetic carbon assimilation in higher plants it will be necessary to expand the species studied and to include a combination of analysis of photosynthetic induction response curves together with rates of enzyme activation.

Supplementary data

Supplementary data are available at *JXB* online.

Supplementary Fig. S1. PRK and GAPDH interactions in pea and tobacco. Western blot of stromal protein complexes separated using BN-PAGE from darkened leaves of pea and tobacco, or pea and tobacco leaves mixed at the onset of the extraction procedure. Immunoblots were probed with GAPDH or PRK polyclonal antibodies as indicated. Sizes indicated are kDa.

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