Modulation, via Protein-Protein Interactions, of Glyceraldehyde-3-phosphate Dehydrogenase Activity through Redox Phosphoribulokinase Regulation*

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The activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) embedded in the phosphoribulokinase (PRK)-GAPDH-CP12 complex was increased 2–3-fold by reducing agents. This occurred by interaction with PRK as the cysteiny1 sulfhydrals (4 SH/subunit) of GAPDH within the complex were unchanged whatever the redox state of the complex. But isolated GAPDH was not activated. Alkylation plus mass spectrometry also showed that PRK had one disulfide bridge and three SH groups per monomer in the active oxidized complex. Reduction disrupted this disulfide bridge to give 2 more SH groups and a much more active enzyme. We assessed the kinetics and dynamics of the interactions between PRK and GAPDH/CP12 using biosensors to measure complex formation in real time. The apparent equilibrium binding constant for GAPDH/CP12 and PRK was 14 ± 1.6 nM for oxidized PRK and 62 ± 10 nM for reduced PRK. These interactions were neither pH- nor temperature-dependent. Thus, the dynamics of PRK-GAPDH-CP12 complex formation and GAPDH activity are modulated by the redox state of PRK.

Phosphoribulokinase (PRK)¹ (EC 2.7.1.19) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (EC 1.2.1.13) form multienzyme complexes. Several multienzyme complexes with different compositions have been isolated from chloroplasts (1–21). The PRK-GAPDH core complex is linked to photosynthesis, as these two enzymes are part of the Benson-Calvin cycle and use ATP and NADPH produced by the primary reactions of photosynthesis. PRK catalyzes the ATP-dependent phosphorylation of ribulose 5-phosphate to form ribulose 1,5-bisphosphate, the CO₂ acceptor in photosynthetic organisms, and GAPDH catalyzes the reversible reduction and dephosphorylation of 1,3-bisphosphoglycerate (BPGA) to glyceraldehyde 3-phosphate using NADPH.

We have purified a complex from the green alga Chlamydomonas reinhardii (10) that is made up of two dimeric PRK and two tetrameric GAPDH. The protein CP12 (20–22) was found recently to be associated with this supramolecular edifice (23). The two enzymes, PRK and GAPDH, may each be obtained in a free independent state. When they are not associated with each other they form dimers (PRK) or tetramers (GAPDH). CP12 is tightly associated with GAPDH (23). The complex can be dissociated by harsh reduction and reversed by oxidizing conditions, because the oxidized partners can spontaneously reform a complex in vitro that is quite similar to the native state (10). In only a few cases has it been possible to assemble particles from their separate parts in vitro that resemble the native complexes (24, 25). The association of these two enzymes also gives rise to new regulatory properties. PRK and GAPDH within the complex are regulated by NADP(H) rather than by NAD(H), whereas the independent stable enzymes are not (26). Oxidized PRK may be active when associated with GAPDH or when dissociated from the complex upon dilution (14). We have also shown that the complex may exist under mild reducing conditions (12) even if it is dissociated by severe reducing conditions (DTT concentrations up to 20 mM). But it dissociates faster upon dilution, as reduction weakens the complex.

Whereas plant enzymes are heterotetrameric (A4B4), algal GAPDHs are homotetrameric and made up of only A subunits (27, 28). The B subunit has a C-terminal extension that contains two cysteine residues believed to be involved in the regulation of the enzyme (29–31). Nonetheless, studies on crude extracts of Chlamydomonas indicate that the algal enzyme that lacks these 28 amino acid residues can be activated reductively by light (32). We have studied the regulation of this enzyme upon reduction or oxidation in its isolated state or within the PRK-GAPDH-CP12 complex to see if this reductive light activation is explained by the interaction of GAPDH with its protein partners. The same study was performed on the other partner, PRK. In previous studies (14) we have fully characterized the activity of isolated PRK and PRK in the complex. PRK in the so-called oxidized complex was active as mentioned above, but this result has been disputed (33), as the cysteinyl sulfhydryls of PRK in the complex have never been directly tested. We have therefore used mass spectrometry coupled with protein chemistry to analyze the cysteinyl sulfhydryl contents of PRK, GAPDH, and CP12 in the complex, whatever its redox state.

Finally, we have used a biosensor to study the interaction between the two enzymes depending on their redox states, as cellular redox signaling contributes to the control of the Benson-Calvin cycle and many other physiological processes (34). The qualitative and quantitative aspects of these interactions have been analyzed.

EXPERIMENTAL PROCEDURES

Materials—Oxidized glutathione, thioredoxin, iodoacetamide, ATP, ribose 5-phosphate, 3-phosphoglyceric acid, and phosphoribose isomerase were from Sigma, NADPH was from Calbiochem, and other reagents were supplied by Roche Molecular Biochemicals. BIAcore 2000
Interaction between PRK and GAPDH

system, CMS sensors, HBS-EP buffer (10 mM Hepes, 150 mM NaCl, 3.4 mM EDTA, 2% surfactant 0.005% (v/v), pH 7.4), and the amine coupling kit (N-hydroxysuccinimide, N-ethyl-N’-(3-diethylaminopropyl)-carbodiimide, ethanolamine hydrochloride) were from BIAcore AB (Uppsala, Sweden).

Purification of GAPDH-CP12/PRK and Isolated Forms from C. reinhardtii—The complex GAPDH-CP12/PRK from C. reinhardtii (WM3) cells, which had been microaerobically purified to apparent homogeneity as previously described (10). The isolated enzymes (GAPDH/CP12 and PRK) were obtained by reducing the purified bi-enzyme complex with 20 mM DTT for 1 h. This mixture was used onto a DEAE-trisacryl column equilibrated in buffer supplemented with 5 mM DTT (9, 23). The resulting purified enzymes were stored at −80°C in 10% aqueous glycerol.

Activity Measurements—PRK and GAPDH activities were determined using a Pye Unicam UV2 spectrophotometer (6, 23). The enzymes were reduced by incubating them for 30 min with 20 mM dithiothreitol or 10 μM reduced thioredoxin at 30°C. GAPDH was oxidized with 5 mM oxidized glutathione (5 mM cysteine or 2 mM diamide), or 10 μM oxidized thioredoxin at 4°C, with or without 5 mM BPOA or 5 mM NADP(H). Aliquots were withdrawn at intervals and activity was measured using NADPH as cofactor. PRK was also oxidized with the same oxidizing agents but protection experiments were carried out using its substrates (5 mM ribose 5-phosphate or 5 mM ATP). All experimental data were fitted to theoretical curves using Sigma plot 3.0. Protein concentrations were determined by the Bradford method (35).

Native Electrophoresis and Immunoblotting Experiments—Native PAGE was performed on 4–15% minigels using the Phastsystem apparatus from Amersham Biosciences. Proteins separated by PAGE were transferred to nitrocellulose filters (0.45 μm, Schleicher and Schuell) by passive diffusion for 24 h. The filters were then immunoblotted with a rabbit antiserum against spinach PRK. Antibody binding was revealed using alkaline phosphatase (36).

Mass Spectrometry Analysis of Free Cysteine Residues—The free SH groups in PRK and GAPDH, both isolated or in the complex, were quantified by alkylation of the free cysteine residues with iodoacetamide prepared as in Ref. 37. The enzymes were incubated with reducing or oxidizing agents or left untreated. They were alkylated with 100 mM iodoacetamide for 1 h at room temperature in the dark and analyzed by mass spectrometry MALDI-TOF (Voyager DE Pro mass spectrometer from Applied Biosystems). PRK and GAPDH were analyzed using sinapinic acid (3,5-dimethoxy-4-hydroxyphenylacetic acid) as matrix. Samples for analysis were desalted on C18 tip tips (Millipore) and eluted in 50% acetonitrile, 0.1% trifluoroacetic acid and 50% water, 0.1% trifluoroacetic acid.

Sulfhydryl Coupling—PRK or GAPDH that had been treated with reducing or oxidizing agents were coupled to the sensor chips using the amine coupling kit and the automated immobilization application wizard included in the BIAcore software to give a coupling level of around 100 resonance units. One flow cell of each CMS sensor chip served as negative control and was used to subtract the bulk refractive index.

Interaction Cycles—We studied the interaction of GAPDH with flow cells coupled to the various PRK in SPR experiments at 20 μL/min in HBS running buffer plus 0.1 mM NAD. In each interaction run, a given concentration was injected for 180 s to record the association phase to the flow cells, after which buffer was injected for 180 s to record the dissociation phase. Last, 10 μL of 10 mM glycine, pH 3, were injected to regenerate the flow cells. The whole process was automated, and we performed multiple interaction runs with different GAPDH concentrations. We checked that there was no mass transfer. The sensorgrams were processed by subtracting the corresponding reference flow cell sensorgram to remove contributions because of baseline drift, bulk, and nonspecific interactions. The reverse experiments were performed by immobilizing GAPDH and injecting different concentrations of PRK.

Theory—The analyte in the injected sample interacts with ligand immobilized on the sensor surface to give the association phase, which continues until a steady state is reached and association and dissociation rates are in equilibrium. The analyte begins to dissociate as soon as injection is stopped and replaced by buffer. The association and dissociation phases can be used to derive kinetic constants. Assuming pseudo-first order kinetics,

\[ \frac{dR}{dt} = k_a C + k_d R_t \]

where \( \frac{dR}{dt} \) is the rate of change of the SPR signal, \( k_a \) is the association rate constant, \( k_d \) the dissociation rate constant, \( C \) is the analyte concentration, \( R_t \) the maximum analyte binding capacity in response units (RU), and \( R_t \) is the signal at time \( t \).

The rate of dissociation can be expressed as the following.

\[ \frac{dR}{dt} = -k_d R_t \]

At equilibrium, association and dissociation rates are equal and the equilibrium-dissociating constant (\( K_d \)) is given by the following.

\[ K_d = \frac{k_d}{k_a} \]

Global fittings of the exponential curves (sensorgrams) giving both the \( k_a \) and \( k_d \) values were performed using Biacore evaluation 3.1.

RESULTS

Titration of Sulfhydryl Groups within the PRK-GAPDH-CP12 Complex—PRK in the complex was alkylated using iodoacetamide. The Chlamydomonas PRK monomer contained five cysteine residues (SH) but only three molecules of iodoacetamide bound to each PRK monomer within the oxidized complex (mass increment of 168.34 Da). This increase indicated that there was one disulfide bridge and three free thiol groups (SH) per monomer. The PRK released by dissociating the complex, the so-called metastable PRK, also had one disulfide bridge. All five cysteine residues in the PRK monomer were free when the complex was reduced with 20 mM DTT for 30 min, as shown by the mass increment of 274.36 Da (Fig. 1).

GAPDH and CP12 in the oxidized complex were also alkylated simultaneously. The four cysteine residues per subunit of GAPDH (mass increment of 222.7 Da, indicating the binding of four molecules of iodoacetamide) were all free, but no molecules of iodoacetamide were bound to CP12. As CP12 contains four cysteine residues, this indicated that CP12 has two disulfide bridges when it is present in the oxidized complex. Two populations of CP12 were found after the complex had been reduced with 20 mM DTT for 1 h. The CP12 in one was fully reduced (with 4 SH per monomer titrated with iodoacetamide), whereas the CP12 in the other was half-reduced (with 2 SH per monomer titrated with iodoacetamide). The fully reduced CP12 was only obtained after drastic dilution of the complex (100-fold) followed by reduction with 20 mM DTT for 1 h.

GAPDH Activity in the Reduced Complex—The enzyme activity of PRK in the oxidized complex was greatly enhanced by reduction of its disulfide bridge with DTT. No direct effect of the reducing agent could be detected as the GAPDH in the so-called oxidized complex already had 4 SH groups per subunit. Nonetheless, there was a reproducible 2- or 3-fold activation of the NADPH-dependent activity of GAPDH after reduction of the complex with DTT or reduced thioredoxins (from 40 to 100 units/mg). The NADH-dependent activity of the complex was also measured as chloroplast GAPDH is specific for both NADH and NADPH. This activity decreased from 40 to 17 units/mg, so that the ratio of NADPH/NADH activities increased from 1 to 6 upon reduction. This ratio was constant at 6 for isolated GAPDH (26) (Fig. 2).

GAPDH was no longer activated by reducing agent if the complex had first been dissociated by dilution. On the other hand, in vitro reconstitution of this complex (9, 10) using dissociated PRK and GAPDH restored the capacity of GAPDH to become activated (data not shown).

Treatment of the Complex and Isolated Enzymes with Oxidants—We also treated the reduced complex with oxidant as the activities of PRK and GAPDH within the complex were
modulated by reducing agents. The GAPDH activity decreased by one-half as soon as the reducing agent was removed, whereas the PRK activity remained constant and stable throughout the experiment. The GAPDH activity was lost as soon as the PRK-GAPDH interactions were weakened. This indeed happened when the reducing agent was removed by dilution on a desalting column. The oxidant GSSG had no further effect on the GAPDH or PRK activities of the complex. However, GAPDH activity was slightly reduced (20%) by a smaller oxidant like cystine, whereas the activity of the PRK in the complex was drastically decreased. The loss of PRK activity was reversible as it was restored by adding DTT, but that of GAPDH was not.

We used native electrophoresis followed by immunoblotting with anti-PRK antibody (Fig. 3) to examine the difference between the effect of cystine and GSSG on PRK activity within the complex. The cystine appeared to be responsible for the dissociation of the complex and thus led to oxidation of dissociated PRK, whereas GSSG treatment left the complex intact. Both 5 mM ATP and, to a lesser extent, 5 mM ribulose 5-phosphate protected PRK from oxidation by cystine (data not shown).

Isolated GAPDH and PRK were also treated with oxidant. All PRK activity was lost by incubation with GSSG or cystine. DTT restored almost full activity (85%), as expected. Treatment of isolated GAPDH with 5 mM GSSG resulted in a 50% decrease in activity (Fig. 4) but adding DTT (up to 25 mM) did not restore any activity. Reduction also failed to restore the activity of GAPDH incubated with 5 mM cystine. This irreversible decrease in GAPDH activity was not linked to simple denaturation of the enzyme, as the fluorescence spectrum underwent no red shift (data not shown). Interestingly, the substrate BPGA prevented the loss of activity because of oxidation, but NADP(H) did not (Fig. 4). Oxidized thioredoxin had no effect on isolated GAPDH activity, unlike GSSG, cystine, or diamide.

The activity of GAPDH within the complex was less severely inhibited by incubation with cystine than was that of the isolated enzyme, although cystine disrupted the complex. This could be because of these two forms of GAPDH having different conformations, as indicated elsewhere (26). We therefore compared the kinetic parameters of the two forms to test this possibility. Kinetic experiments were carried out with a saturating concentration of NADPH and the concentration of BPGA was varied (Fig. 5).

The form released from the complex displayed Michaelis-Menten kinetics unlike the isolated form, which displayed positive co-operativity (23). The data were fitted to a hyperbola to estimate the catalytic constant (kcat) and the Km for BPGA. The kcat was 650 ± 10 s⁻¹ and the Km was 262 ± 11 μM. These parameters were compared with those of the isolated enzyme (Table 1).

**Interaction Analysis of PRK and GAPDH/CP12 Using SPR**

We then studied the interaction of PRK with GAPDH using...
biosensor technology to determine whether the redox state of PRK altered complex formation.

GAPDH and PRK can both be covalently coupled to the CM5 sensorchip, but the enzymes were each immobilized under different conditions. Amine coupling is based on favorable electrostatic attraction between negative charges of the dextran carboxylic groups and positive charges on the immobilized ligand. Thus, PRK (pI 5.68) was covalently bound using HBS-EP buffer containing 10 mM acetate, pH 4.8 (immobilization buffer A), whereas GAPDH (pI 8.6) was covalently bound using HBS-EP buffer, pH 7.4. We studied the stability of the kinase under these conditions by preincubating the enzyme in immobilization buffer A for up to 40 min before measuring its activity. No activity was lost during the time of the Biosensor experiment.

Reduced PRK was immobilized and measurements at different GAPDH concentrations were performed (Fig. 6). These curves were fitted to a model equivalent to the Langmuir model for adsorption to a surface (Equation 1). The average apparent equilibrium dissociation constant was 62 ± 10 nM.

We also studied the interaction of oxidized PRK with different amounts of GAPDH. GAPDH from rabbit muscle was also injected onto oxidized PRK to check specificity. The responses at equilibrium (R_eq) for Chlamydomonas and rabbit muscle GAPDH for their interactions with immobilized oxidized PRK are reported as a function of their concentrations (Fig. 7). The data were fitted to the following hyperbola function.

\[ R_{eq} = \frac{R_{max} \times [\text{GAPDH}]}{K_d + [\text{GAPDH}]} \]  

(Eq. 5)

The average apparent equilibrium dissociation constant was 14 ± 1.6 nM for algal GAPDH and 628 ± 1.3 nM for rabbit muscle GAPDH.

Experiments were also carried out at 25 and 37 °C and at pH 7 or 8 to determine whether temperature or pH affected the interaction between GAPDH and PRK. No difference was observed. The same dissociation constants were obtained whether or not GAPDH had been treated with GSSG or DTT.

A range of PRK concentrations were tested with immobilized GAPDH to obtain an average apparent equilibrium dissociation constant. The same apparent equilibrium dissociation constants were obtained, as when PRK was immobilized. Only when PRK was reduced did we find a higher apparent equilibrium dissociation constant (62 ± 10 nM).

**DISCUSSION**

Many enzymes belonging to the Benson-Calvin cycle are regulated by dark-light transitions via thioredoxins in vivo (38) (or dithiothreitol in vitro). In particular, thioredoxin (39, 40) reduces the disulfide between Cys16 and Cys55 of inactive oxidized spinach PRK (41, 42).

We have shown (14) that oxidized PRK from *C. reinhardtii* may have some activity when it is associated with GAPDH, contrary to common belief, but no direct evidence of the regulatory disulfide bridge between Cys16 and Cys55 present in oxidized PRK, was given. We have now used alkylation of the so-called oxidized complex to show that there is one disulfide bridge in the PRK monomer. The activity of PRK is greatly increased when it is reduced, indicating that the Cys16–Cys55 bond is the target.

Another troubling aspect of the activity of oxidized PRK concerns the P-loop. This contains residues that interact with the β- and γ-phosphoryl of Mg2+–ATP (43). This loop should *a priori* be free to move for efficient catalysis. Some data for bacterial PRK (33, 44) suggest that the Cys16–Cys55 disulfide bridge found only in the eukaryotic enzymes could immobilize the P-loop, thereby preventing catalytic turnover. However, it is worthwhile to pinpoint that there are insertions and deletions in the plant and algal enzymes, such as an insertion of 15 residues after the P-loop of the eukaryotic enzyme that may modify its mobility even in the presence of the Cys16–Cys55 disulfide bridge. These sulphydryl groups are also not essential for catalysis, as a Cys16–Cys55 double mutant of spinach PRK still has some activity (42, 45). The conformational constraint imposed by this bond is more likely to be responsible for the decreased PRK activity than the formation of the disulfide bridge itself (45). Thus, although the bacterial PRK structure is a useful model, it may not be wise to extrapolate data obtained with it to eukaryotic PRKs, especially the eukaryotic enzyme in a multienzyme complex. Our results indicate that the conformational constraint near the P-loop could be attenuated when the PRK is within the complex, allowing oxidized PRK to be active. The interaction of PRK with GAPDH modifies the conformation of this enzyme (46) and hence its kinetic properties (12, 14).

There is now considerable published evidence from NMR studies that supramolecular edifices (protein-protein or DNA-protein complexes) appear to be flexible (47, 48). The entropic cost of the decrease in conformational freedom must be offset, to some extent, by preserving the flexibility of other regions. These reports support the assertion made above by the authors, but only structural data will clarify this point. Our direct evaluation of the redox status of the regulatory cysteine residues now clearly shows that oxidized PRK may be catalytically active. This activity is, however, lower than the activity of the released metastable form, as the PRK-GAPDH association hampers the overall flexibility of these enzymes. The released
PRK with decreased conformational constraint around the P-loop, because of a memory effect, and with greater overall flexibility is thus a better catalyst than the enzyme in the complex.

Whereas each PRK monomer in the oxidized complex has one disulfide bridge, all the cysteinyl sulfhydryl groups of GAPDH are free and CP12 has two disulfide bridges. We found five cysteinyl sulfhydryl groups per PRK subunit by alkylation after reduction of the oxidized complex. The GAPDH content of thiol groups obviously does not change, but two or four thiol groups become titrated per CP12 monomer. Reduction of the PRK disulfide bridge is followed by an increase in enzyme activity, as mentioned above.

Chlamydomonas GAPDH is an A4 homotetramer; the regulatory cysteine residues are thus absent (28, 31). Nonetheless, incubation of a crude extract with DTT increased its enzyme activity 3-fold (32). Somewhat surprisingly, we saw the same increase in NADPH-dependent activity for GAPDH when it was part of the complex, but not for the isolated enzyme. We therefore proposed (49) that this modulation had a physiological role, as the NADPH concentrations are increased in the light. Our present results also show that reducing the complex leads to a decrease in the use of NADH.

As titration of the thiol groups in GAPDH in the complex reveals 4 SH groups whatever the redox state of the complex, the modulation of GAPDH activity is not linked to disulfide reduction. Our present finding also indicates that these effects are linked to heterologous interactions within the complex as...

**TABLE I**

| BPGA | Hill coefficient | $k_{cat}$ |
|------|------------------|----------|
| Isolated GAPDH$^a$ | $K_{0.5} = 151 \pm 13 \mu M$ | $1.5 \pm 0.2$ | $223 \pm 9 \text{ s}^{-1}$ |
| Released GAPDH | $K_m = 262 \pm 11 \mu M$ | $650 \pm 10 \text{ s}^{-1}$ |

$^a$ The kinetic parameters of the isolated GAPDH were from Ref. 23.

**FIG. 5.** Steady-state kinetics of GAPDH within the complex. The bi-enzyme complex was placed in the reaction mixture containing 0.25 mM NADPH with BPGA concentrations of 0–1.8 mM and the appearance of product was monitored. The experimental points were fitted to a hyperbola. The bi-enzyme complex concentration in the assay cuvette was 0.65 nM.

**FIG. 6.** Plasmon resonance monitoring of GAPDH interaction with PRK surface. Net sensorgrams (after subtracting the bulk refractive index) were obtained with immobilized reduced PRK using 0.14 μM (1), 0.29 μM (2), 0.44 μM (3), and 0.58 μM (4) reduced GAPDH as analyte. The beginning of the association phase and the beginning of the dissociation phase were marked with a and d, respectively. The experimental data were analyzed using global fitting assuming a 1:1 interaction with the Biacore evaluation 3.1.
there is no longer an effect once these interactions are weakened or broken. This supports the idea mentioned above that new properties may emerge as a consequence of conformation change within multienzyme complexes. It also shows that the regulation of an enzyme like PRK may modulate the regulation of another (GAPDH) via a “domino-like” effect.

We have tested the effect of oxidizing agents on PRK and GAPDH activities, as oxidized thioredoxin causes the oxidation of the light-reduced enzymes. Treatment with oxidants caused the activity of reduced PRK isolated or released from the complex (e.g. using cystine) to decrease. This effect was reversed by reduction, as expected. The situation is quite different for GAPDH, as the loss of activity by the isolated enzyme that follows incubation with oxidized glutathione is very likely because of a modification of the active site cysteine (Cys\textsuperscript{196}), as it is well documented for other GAPDHs (50). This is also supported by protection experiments using BPGA and by the fact that oxidized thioredoxins have no effect on GAPDH activity. The inactivation is not reversed by a reducing agent. It has been suggested that the oxidation of glycolytic enzymes may introduce an element of strain resulting from the formation of a disulfide bond, which then causes an irreversible conformational change, perhaps with displacement of an essential residue from the active site. GAPDH is not significantly inactivated by oxidized glutathione even after dissociation of the complex, like PRK. This may be because the cysteinyl sulphydryl in this molecule is less exposed than is that in the isolated GAPDH. We checked this by measuring the enzyme kinetics of the isolated GAPDH and of the released form from the complex. The differences observed with the pseudo-affinity constants for BPGA clearly support the assertion made above. Thus, GAPDH retains the conformation it had within the complex (imprinting) even after the physical separation of PRK and GAPDH, so that the sulhydryl group of the Cys\textsuperscript{196} is poorly accessible to oxidized glutathione. This corroborates the imprinting effect previously reported (12, 13, 26).

Finally, the complex we have purified is a suitable model with which to study protein-protein interactions, but most of the information on it stems from in vitro reconstitution experiments. This report, for the first time, describes the use of Biosensor technology to determine the dynamics of these interactions between PRK and the GAPDH-CP12 subcomplex under different redox states. The dissociation constants between the PRK and GAPDH-CP12 subcomplex are rather low (nM range), with the lowest value (14 ± 1.6 nM) being obtained with oxidized PRK. The probable conformational change caused by the chemical modification of GAPDH by oxidation has no influence on the binding of GAPDH to PRK and vice versa. The SPR results indicate that these enzymes (PRK and GAPDH/CP12) may reconstitute the complex under reducing conditions with a low dissociation constant (62 ± 10 nM) even if complex formation is sensitive to the redox state of PRK. Our results also suggest that this complex may form at pH 7, under oxidizing conditions (dark conditions) or at pH 8, under mild reducing conditions (light conditions).

To conclude, thiol/disulfide exchange influences the state of activation of chloroplast PRK, in its isolated form and when it is part of a complex. On the other hand, the modulation of PRK activity influences the state of activation of GAPDH via protein-protein interactions, at least in the alga C. reinhardtii. A new mode of light regulation could emerge that involves a “domino effect” and this could well apply to enzymes that are not direct targets of light.

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