Conformational Selection and Folding-upon-binding of Intrinsically Disordered Protein CP12 Regulate Photosynthetic Enzymes Assembly*†‡

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Background: In the dark CP12 is oxidized and regulates photosynthetic GAPDH.
Results: The disordered C terminus of oxidized CP12 gets ordered when bound to GAPDH.
Conclusion: Transient complexes between GAPDH and selected conformations of CP12 evolve into a stable binary complex in which CP12 blocks GAPDH catalytic sites.
Significance: Disordered proteins can bind structured partners through a synergistic combination of conformational selection and folding-upon-binding.

Carbon assimilation in plants is regulated by the reduction of specific protein disulphides by light and their re-oxidation in the dark. The redox switch CP12 is an intrinsically disordered protein that can form two disulphide bridges. In the dark oxidized CP12 forms an inactive supramolecular complex with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoribulokinase, two enzymes of the carbon assimilation cycle. Here we show that binding of CP12 to GAPDH, the first step of ternary complex formation, follows an integrated mechanism that combines conformational selection with induced folding steps. Initially, a CP12 conformation characterized by a circular structural motif including the C-terminal disulphide is selected by GAPDH. Subsequently, the induced folding of the flexible C-terminal tail of CP12 in the active site of GAPDH stabilizes the binary complex. Formation of several hydrogen bonds compensates the entropic cost of CP12 fixation and terminates the interaction mechanism that contributes to carbon assimilation control.

In oxygen phototrophs a complex regulatory system based on thioredoxins ensures that carbon fixation is active during the day and nil during the night (1). In the light, chloroplast thioredoxins are reduced by photosystem I via ferredoxin and ferredoxin:thioredoxin reductase (2) and impose a reduced state to their targets (protein disulfides). In the dark, re-oxidation of protein disulfides to disulphides is allowed (1).

Besides other proteins and regulatory systems, coordinated light/dark regulation involves the participation of (i) glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which catalyzes the only reducing step of the Calvin-Benson cycle of carbon assimilation (3), (ii) phosphoribulokinase, which consumes a third of the ATP required for CO2 fixation into sugars, and (iii) the scaffold protein CP12, an ubiquitous regulatory protein of oxygenic photosynthetic organisms that contains, with few exceptions, two cleavable disulphide bridges (4–9).

GAPDH of land plants includes two plastidic isoforms named A4 and A8B8 (3). The former is a stable, constitutively active tetramer, whereas the latter can form A4B4 and A8B8 oligomers in the presence of NAD(H) (3, 5, 9). Thioredoxin f specifically reduced a disulphide bridge in the C-terminal extension (CTE)2 of subunits B, thereby relieving the inhibition of enzyme activity caused by oxidized CTE (10). The CTE is homologous to the C-terminal half of CP12 (4), and A4-GAPDH may bind CP12 in a similar way, as A4B4>GAPDH does bind its own CTE (11). In land plants only, the autonomous regulation of AB-GAPDH co-exists with the regulation of A4-GAPDH by CP12 (3, 5), ubiquitous in oxygen phototrophs.

The three proteins A4>GAPDH, phosphoribulokinase, and CP12 form a supramolecular complex where the activity of both GAPDH and phosphoribulokinase is inhibited (4–6, 12). The CP12-assembled complex, stored in the dark in chloroplasts, is rapidly dissociated by the onset of light (13). Because both GAPDH and phosphoribulokinase activities are inhibited in the complex but are fully recovered upon dissociation (10),

* This work was supported by the Italian Ministry of University and Research (MIUR, projects FIRB and PRIN 2008). This work was also supported by Très Grand Equipement de Recherche (TGER) Resonance Magnétique Nucléaire (RMN) à Très Haut Champs (THC) FR3050.
† This article contains supplemental Tables S1–S3, Figs. S1–S7, and Video S1.
‡ The atomic coordinates and structure factors (codes 3QV1 and 3RVD) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).
¶ NMR structural models and chemical shift assignments have been deposited in the Protein Data Bank and Biological Magnetic Resonance Data Bank under accession codes 2U9J and 17926, respectively.
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CP12 could effectively contribute to the modulation of the Calvin-Benson cycle under the natural variable light/dark regime (13). The thioredoxin-dependent CP12 may, therefore, work as a light-sensitive redox switch in chloroplasts and was recently shown by antisense technology to be required for normal growth and development in transgenic tobacco plants (14). Moreover, in the cyanobacterium Synechococcus elongatus PCC7942, growth of CP12-knock out mutants was inhibited under light/dark cycle but not in continuous light (7). Interestingly, CP12 is also coded by the ultrasmall genome of cyanophages infecting marine cyanobacteria (e.g. Synechococcus), where CP12-inhibition of the Calvin-Benson cycle may favor NADPH production (essential for phage replication) by the oxidative pentose phosphate pathway (15).

Differently from what was recently demonstrated by the x-ray structure of A4-GAPDH-CP12 complex from S. elongatus (16), Arabidopsis thaliana GAPDH (isoform A4) can bind two CP12 monomers per GAPDH tetramer (17). Formation of the GAPDH-CP12 binary complex requires, as a seemingly compulsory condition, that NAD or NADH are bound to the dehydrogenase (12) and that C-terminal cysteines of CP12 are oxidized into a disulfide (4, 17, 18).

Only after formation of the binary CP12-GAPDH complex, interaction with dimeric phosphoribulokinase leads to stabilization of an inactive ternary complex made by two GAPDH tetramers and two phosphoribulokinase dimers linked together by four CP12 monomers (17). The stability of the ternary complex is controlled by thioredoxins, which can reduce CP12 disulfides (10). Also, nucleotides (NAD(P)H, ATP) and the GAPDH substrate 1,3-bisphosphoglycerate can dissociate the ternary complex leading to enzyme reactivation (4, 6, 7, 12).

CP12 is a small protein of about 80 amino acids. It is intrinsically disordered both in the green alga Chlamydomonas reinhardtii (6) and in the higher plant A. thaliana (17, 8) and predicted disordered in other phototrophs (e.g. Synechococcus) (8). Intrinsically disordered proteins (IDPs) constitute a significant fraction of eukaryotic proteomes, including plants (19, 20), and often play a role as scaffolds in the assembly of supramolecular complexes (21, 22). At odds with most IDPs, an uncommon property of most CP12 is the presence of four conserved cysteines able to form two consecutive disulfide bridges (4, 6, 8, 17). Although formation of disulfides increases the overall degree of order in terms of α-helix content, even fully oxidized CP12 appears largely devoid of secondary structures in circular dichroism spectra (6, 8).

Intrinsically disordered proteins (such as CP12) may bind to structured partners (like GAPDH) in different ways (23, 24). In a model based on conformational selection (25), association with a structured partner occurs when a single conformation of the disordered protein, appreciably populated in solution, is selected by the target and stabilized into the complex. In such a case, the structure of the IDP in the complex corresponds to one of the several accessible conformational states that are present in the free energy landscape (26, 27). Alternatively, the final structure of the IDP in the complex is only reached through a multistep folding process that takes place upon binding (23). The structure of the IDP in the complex often becomes markedly different from native unbound states (28–30). The induced folding can be initiated by the recognition of preformed structural elements in the IDP (Molecular Recognition Features (31)) by the structured partner (32). Although examples of both types of mechanisms are known (27–30), their combination may also be active in real systems (23). A synergistic model has been proposed to reconcile both hypotheses (24).

With the aim of unraveling the interaction mechanism between the intrinsically disordered protein CP12 and its structured partner GAPDH, here we describe the NMR structures of the C-terminal region of free-oxidized CP12 in solution and the crystal structure of the GAPDH-CP12 binary complex from A. thaliana at 2.0 Å. Its formation is the first crucial step toward the assembly of the GAPDH-CP12-phosphoribulokinase ternary complex.

Molecular interactions between CP12 and GAPDH in the Arabidopsis complex were found very similar to those observed in the Synechococcus complex (16), indicative of a high degree of conservation in this regulatory system (4). On the other hand, although CP12 bound to A4-GAPDH(NAD) (this work) was found in the same cleft occupied by CTE in A2B2-GAPDH-NADP (11), the two structures diverged completely in terms of folding and molecular interactions.

Leveraging on the NMR structural ensemble of the C-terminal part of CP12, the crystal structure of the binary complex, and molecular dynamics (MD) simulations, we develop a model of binding that starts from the intrinsically disordered structure of CP12. The interaction model between CP12 and GAPDH, proposed in the following, presents features consistent with a synergistic mechanism including both conformational selection and induced folding steps. Additional complexity is added by the redox-regulation of the system.

**EXPERIMENTAL PROCEDURES**

**Protein Preparations**—Recombinant CP12 (isoform 2, TAIR data base: At3g26410) and A4-GAPDH (TAIR data base: At3g26650) from A. thaliana were purified as described (12). After His-tag removal (12), CP12 was treated overnight with 20 mM oxidized DTT to ensure disulfide formation. Purified CP12 and A4-GAPDH were quantified by UV-absorbance (12) and stored at −20 °C in 25 mM potassium phosphate, pH 7.5.

For crystallization trials, preformed binary complex (10 mg ml⁻¹) was prepared by mixing A4-GAPDH and CP12 in a 1:2 ratio plus 1 mM NAD in 25 mM potassium phosphate, pH 7.5. For NMR experiments, labeled CP12 was prepared in M9 medium containing 1 glt⁻¹ ⁵¹NH₄Cl with or without 5 glt⁻¹ D-[¹³C₆]glucose (17). The His tag was not removed.

**Crystallization, Data Collection, and Processing**—GAPDH-CP12 complex crystals were produced both by crystallization of the preformed complex in solution and by soaking crystals of recombinant A4-GAPDH (33) in a CP12 solution. The vapor diffusion method was used to screen several crystallization conditions at 277 or 293 K. A drop of 2-μl complex solution plus 2 μl of reservoir solution was equilibrated against 750 μl of reservoir solution. The best diffracting crystals were obtained in hanging drop at 277 K with 15% (w/v) PEG 4000, 0.1 M MES, pH 6.5, 0.6 M NaCl, 1 mM NAD as reservoir solution.
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For soaking experiments, crystals of recombinant A4-GAPDH (33) were transferred in a 5-µl drop containing 2.4 M ammonium sulfate, 0.1 M sodium citrate, 1 mM NAD, and 3.125 mg·ml⁻¹ CP12. The drops were equilibrated against 750 µl of 2.4 M ammonium sulfate, 0.1 M sodium citrate for 4 days at 293 K.

Data from co-crystallized and soaked complex crystals were collected at European Synchrotron Radiation Facility (Grenoble) at beam lines ID14-3 and ID14-1, respectively at 100 K. Co-crystallized crystals diffracted to a maximum resolution of 1.98 Å, and data were processed with IMOSFLM (34), POINTLESS, and SCALA (35). Soaked crystals diffracted to a maximum resolution of 2.7 Å, and data were processed with DENZO and scaled with SCALEPACK (36). Space groups, unit cell parameters, and diffraction data statistics for both structures are reported in Table 1.

Structure Determination and Refinement—Both structures were solved by molecular replacement (MR) by the program AMORE (37) using the structure of A. thaliana A4-GAPDH (33) as the search model. The MR procedures gave unequivocally two and three solutions for co-crystallized and soaked complexes, respectively. Refinement was performed with REFMAC 5.2.005 (38) (co-crystallization) or CNS 1.3 (39) (soaking) and manual rebuilding with Coot (40).

CP12, NAD, and sulfate ions were inserted after few refinement cycles in the electron density regions not occupied by GAPDH chains. In the final stages of refinement and model building, water molecules were added. Stereochemical quality of the models was checked with PROCHECK (41). Ramachandran plots show that 99% (co-crystallization) and 97.8% (soaking) of residues lie in the most favored plus additional allowed regions. Only 0.5% of residues are in disallowed regions for both structures. Refinement statistics are reported in Table 1.

NMR Analysis—NMR samples (non labeled or uniformly ¹⁵N- or ¹⁵C,¹⁵N-labeled CP12) contained about 1 mM protein in NMR buffer (25 mM potassium phosphate, 95% H₂O, 5% D₂O, 0.02% NaN₃) at pH 7.0. The binary complex was made in vitro by mixing 0.2 mM U,¹⁵N-labeled CP12 with 0.5 mM A₄-GAPDH and 2.7 mM NAD in NMR buffer. NMR experiments were recorded at 20 °C on Bruker Avance 400, Avance 600, and Avance 800 spectrometers and on an Agilent vnmrs 800 spectrometer.

Backbone resonance assignments were obtained from a series of standard heteronuclear experiments. NMR distance restraints were extracted from three-dimensional ¹H,¹⁵N NOESY-HSQC and two-dimensional NOESY with 60- and 150-ms mixing times.

NMR data were processed and analyzed using NMRPipe (42) and NMRView (43). All proton dimensions were referenced to ¹H/X frequency ratios of the zero point. Homonuclear NOE assignment was made manually. The resulting distance restraints, 40 backbone dihedral predictions from TALOS+ (44), and 2 hydrogen bonds extracted from a long-range three-dimensional BEST-TROSY-HNCO (45) were used as input for CNS (39). The 20 structures with the lowest total energies were selected. Refinement statistics are reported in Table 2. The stereochemical quality of the models was checked with PROCHECK (41). Residues in most favored regions and in additional allowed regions represent 81.7 and 18.3%, respectively.

Molecular Dynamics—All-atom molecular dynamics simulations were carried out with AMBER (46). AMBER 99 force field parameters (47) were employed. Water molecules were described by the TIP3P model. An equilibration protocol consisting of three individual steps was applied resulting in an unconstrained well tempered NPT ensemble (isothermal-isobaric ensemble, moles (N), pressure (P), and temperature (T) are conserved).

The Cartesian coordinates of CP12 are taken from the crystal structure. The structure is relaxed (minimized with SANDER using steepest descent algorithm) to remove the constraint imposed by the binding with A₄-GAPDH. The explicit net charge of CP12 is neutralized adding Na⁺ counterions at positions of high negative electric potential, then the protein is solvated with explicit water. An 8 Å radius buffer of TIP3P water is put around CP12 in each direction. To allow the water box to relax before running molecular dynamics, about 5000 steps of steepest descent minimization were performed with SANDER. In the first 1000 steps CP12 was restrained to its original position using a force constant of 5 kcal·mol⁻¹·Å⁻², then the constraint is released to relax the entire system. Particle Mesh Ewald summation was used throughout (cutoff radius of 10 Å for the direct space sum). H-atoms were frozen with the SHAKE algorithm (48), and a time step of 2 fs was applied in all equilibration runs. After successfully minimization, the system was heated from 0 to 300 K. To avoid wild fluctuations in the solute, a weak restraint (5 kcal·mol⁻¹·Å⁻²) on CP12 atoms was applied in an NVT ensemble (canonical ensemble, moles (N), volume (V), and temperature (T) are conserved) and temperature coupling according to Langevin for 100 ps. Constant pressure was then applied, so that the density of water could relax. Temperature was 300 K. Safe removal of the restraints on CP12 was possible. A further 400 ps of equilibration in NPT ensemble resembling laboratory conditions were carried out. Finally a production run of 50 ns was performed. Snapshot structures were saved into individual trajectory files every 25,000 time steps, i.e. every 2 ps of molecular dynamics.

RESULTS

GAPDH-bound CP12 Contains a Circular Structural Motif—A. thaliana CP12 (isofrom 2) is an intrinsically disordered protein with little secondary structure also in the oxidized state where two intramolecular disulfide bridges are present (17, 8). In line with the disorder, all attempts to grow crystals of CP12 failed. A different crystallographic approach exploited the interaction of CP12 with GAPDH. It is reasoned that CP12 might undergo a disorder-to-order transition upon binding to a structured protein. GAPDH-assisted crystallization of CP12 was attempted both by setting-up co-crystallization trials with preformed GAPDH-CP12 complex in solution and by soaking A₄-GAPDH(NAD) crystals within a solution of oxidized CP12. The crystal structure of recombinant GAPDH (isofrom A₄), in complex with NAD, was recently solved at 2.6 Å resolution (33). Both approaches, co-crystallization and soaking, produced x-ray diffracting crystals amenable to structural studies. Two structures of the GAPDH-CP12 binary complex were refined at
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TABLE 1
Data collection and refinement statistics for A4-GAPDH/CP12 complex structures

|                        | Co-crystallized complex | Soaked complex |
|------------------------|-------------------------|----------------|
| **Data collection**    |                         |                |
| Space group            | C222                    | 1222           |
| Cell dimensions a, b, c (Å) | 142.7, 246.0, 139.0     | 153.2, 188.8, 312.2 |
| a, b, c (Å)            | 90.0, 90.0, 90.0        | 90.0, 90.0, 90.0 |
| Resolution (Å)         | 34.2 (1.98) (2.08-1.98) | 95.3 (2.70) (2.80-2.70) |
| R(sym)                 | 0.139 (0.716)           | 0.098 (0.587)  |
| I/σ                    | 5.9 (1.3)               | 16.7 (2.1)     |
| Completeness (%)       | 99.2 (98.2)             | 96.6 (84.0)    |
| Redundancy             | 3.3 (2.2)               | 11.3 (7.9)     |
| **Refinement**         |                         |                |
| Resolution (Å)         | 34.2-2.0                | 94.6-2.7       |
| No. reflections        | 153,928                 | 118,509        |
| Rwork/Rfree            | 0.225/0.272             | 0.247/0.319    |
| No. atoms              |                         |                |
| Protein                | 15,827                  | 26,538         |
| Ligand/ion             | 299                     | 580            |
| Water                  | 191                     | 556            |
| B-Factors              |                         |                |
| Protein                | 32.4                    | 63.9           |
| Ligand/ion             | 25.7                    | 63.4           |
| Water                  | 21.2                    | 43.4           |
| Root mean square deviations |               |                |
| Bond lengths (Å)       | 0.016                   | 0.009          |
| Bond angles (°)        | 1.74                    | 1.40           |

2.0 Å (co-crystallized complex) and 2.7 Å (soaked complex) (Table 1). The following description of the GAPDH-CP12 binary complex is mostly based on the higher resolution data produced by co-crystallization. The conclusions are fully supported also by the data obtained from soaked crystals.

In the co-crystallized complex structure, the asymmetric unit contained one tetramer and one dimer of GAPDH that generated a second tetramer by symmetry. For simplicity, the different subunits in the model (all A subunits with identical sequence) have different labels (chains ABCD, Fig. 1a; and chains-EF, not shown). Additional electron density was associated to NAD molecules (one bound to each GAPDH subunit) and to CP12 (one for each pair of GAPDH subunits; supplemental Fig. S1). Depending on the cleft, only the last 19–21 residues of CP12 could be modeled (chains G, H, and I), indicating that the N-terminal region of CP12 is largely disordered within the crystal. This conclusion is consistent with the GAPDH-CP12 complex structure from *S. elongatus* where only 23 C-terminal residues were placed in the electron density map, but the exactness of CP12 in the crystals was confirmed by mass spectroscopy (16).

Two CP12 molecules were bound to each tetramer of GAPDH. CP12 occupied an eccentric position within the cleft (Fig. 1b) roughly similar to the position of CTE in the structure of spinach A2B2-GAPDH complexed with NADP (11). The negatively charged C-terminal part of CP12 fits in a positively charged binding site of GAPDH, whose surface potential mainly depends on Arg-77 and Arg-191 belonging to different subunits (Fig. 1c). Although GAPDH clefts contained two symmetrical binding sites, only one was occupied by CP12, consistent with the stoichiometry of the binary complex in solution (17). Half-occupation of CP12 binding sites was also observed in GAPDH crystals soaked with excess CP12 (supplemental Fig. S2). In both co-crystallized and CP12-soaked complexes the two CP12s were either on the same side of the tetramer (e.g. symmetric to axis-P) or on opposite sides (e.g. symmetric to axis-Q, supplemental Fig. S2), suggesting that the type of CP12 occupation was influenced by the crystallographic environment.

Differently, in the structure of the binary complex from *S. elongatus* both symmetrical positions of the GAPDH cleft were occupied by CP12, forming an hetero-octamer in which four CP12 molecules are bound to tetrameric GAPDH (16). The full occupation of GAPDH clefts by CP12 in the cyanobacterial complex was probably favored by the presence of small amino acids at the CP12-C12 interface (Ala-67, Ala-68, Leu-71). In Arabidopsis CP12, the replacement of these residues by more bulky side chains (Thr-70, Asn-71, Arg-74) might prevent double occupation of the GAPDH cleft. Beside this difference in the binary complex stoichiometry, the overall complex structures of *Synechococcus* and *Arabidopsis* are quite similar, and their superposition gave root mean square deviation values ranging between 0.8 and 1.1 Å over about 1400 Cα atoms of CP12 and GAPDH and between 0.8 and 1.3 Å over 22 Cα atoms of CP12 alone. As previously observed (16), the overall structure of the CTE in spinach A2B2-GAPDH(NADP) (11) was instead completely different and not superimposable with CP12 in either *Synechococcus* or *Arabidopsis* complexes.

Apart from the initial (Asp-58–Pro-59) and the final C-terminal residues (Asn-78) of CP12, the backbones of all other residues were unambiguously positioned and displayed the same structure (Fig. 1, d and e). The influence of CP12 binding on the overall tetrameric structure of GAPDH was limited (supplemental Fig. S3a). The model of the C-terminal fragment of CP12 includes an eight-residue amphipathic α-helix-C (for the C terminus, from Pro-59 to Asp-66) followed by a seven-residue loop-C (Asn-67–Cys-73) and by a five-residue C-terminal tail (C-tail, Arg-74–Asn-78) (Fig. 1, d and e). Loop-C consists of two consecutive β-turns (Asn-67–Thr-70 and Thr-70–Cys-73) stabilized by hydrogen bonds (Fig. 1f). Cys-73 establishes a disulfide bridge with Cys-64 (belonging to α-helix-C). The α-helix-C, loop-C, and the disulfide bridge constitute the circular structural motif of CP12. The C-tail has no apparent secondary structure.

Side-chain-mediated interconnections between different portions of CP12 reinforce the structure (Fig. 1g). The α-helix-C is capped by the side chain of Asn-67, the first residue of loop-C (supplemental Table S1a). The first two residues of the C-tail (Arg-74 and Thr-75) are hydrogen-bonded with loop-C. The last three residues of the C-tail are completely free from interactions with other residues of CP12 (Fig. 1g).

*C-tail of CP12 Blocks GAPDH Catalytic Sites*—Interactions between GAPDH and the two portions of CP12 entail mainly H-bonds. Their number differs depending on the portion of CP12 considered. Similarly to other chains, the well resolved chain G of co-crystallized CP12 makes 13 short-range interactions with GAPDH.

There are four interactions between the circular structural motif and GAPDH. The α-helix-C (CP12-chain G) binds to GAPDH (chain D) via a single hydrogen bond with Arg-191. Glu-69 (in the first β-turn of loop-C and Arg-77 (GAPDH) form a salt bridge (Fig. 2a). Arg-191 and Arg-77 have a role in...
the positioning of CP12 within GAPDH deep clefts (Fig. 1b).

The second β-turn of loop-C forms two additional H-bonds with GAPDH (Fig. 2a); one of them, which involves the side chains of Glu-72 of CP12 and Thr-33 of GAPDH (chain A), is the only link between CP12 and the distal GAPDH subunit of the cleft (Fig. 2a). Moreover, the carboxyl group of Glu-72 approaches to the adenine ribose hydroxyl groups of NAD bound to the distal subunit (distance Glu-72.OE2-NAD335.O2B 3.5 Å). All other interactions between CP12 and GAPDH involve the vicinal subunit (GAPDH-chain D for CP12-chain G; supplemental Table S1b).

There are nine H-bonds between the terminal C-tail of CP12 (chain G) and GAPDH. Two of them are fully conserved in all nine CP12 chains of either co-crystallized and CP12-soaked complexes; the main-chain carbonyl of CP12-Arg-74 is H-bonded with the side chain of GAPDH-His-190, and the side-chain hydroxyl of CP12-Tyr-76 is H-bonded with NAD (Fig. 2b, supplemental Table S1b). Residues Tyr-76 and Glu-72 represent two of the six highly conserved residues of the CP12 C-terminal region from oxygenic photosynthetic organisms. Both amino acids seem to have a specific function in complex formation in presence of NAD (16). Tyr-76 contributes to cofactor recognition by interacting with its backbone phosphate group; at the same time Glu-72 prevents NADP binding to GAPDH by steric hindrance and electrostatic repulsion between its carboxylic group and the 2’-phosphate of the pyridine nucleotide. The competition between Glu-72 and the 2’-phosphate of NADP(H) explains why the GAPDH-CP12 complex does form in the presence of NAD(H) but not NADP(H) (12). Other interactions involving the C-tail vary in the different CP12 chains and always engage GAPDH residues of the catalytic sites (Fig. 2, b and c, and supplemental Table S1b).

Each GAPDH subunit contains one Ps and one Pi site (Fig. 2c). When A4-GAPDH crystals were grown in the presence of ammonium sulfate, each P-site hosted a sulfate ion, mimicking the binding of the phosphate groups of the substrate (33). In the co-crystallized complex (crystals grown in the absence of

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**FIGURE 1. The structure of CP12 bound to GAPDH.** a, two CP12 molecules (purple, chains G and H) are bound to GAPDH (chains ABCD; co-crystallization). Each CP12 is inserted in a cleft between two GAPDH subunits. b, shown is the top view of the cleft formed by GAPDH chain A and D. Four arginines, two per GAPDH subunit, delimit the cleft. CP12 chain G interacts with Arg-191 and Arg-77 of GAPDH-chain D. Arg-191 and Arg-77 of GAPDH chain A delimit a symmetrical binding site for CP12. c, shown is the same view as in panel -b, with colors representing the electrostatic surface potential calculated by GRASP (54). The potential of CP12 (mesh) is mainly negative (red), and the GAPDH left is largely positive (blue). d, shown is the amino acid sequence of the portion of CP12 detected in the crystals. e, shown is superimposition of 3 and 6 CP12 chains from co-crystallization and soaking. The α-helix-C (red), loop-C (gray), and the disulfide (S atoms of Cys-64 and Cys-73 in yellow) form the circular structural motif. The last five residues are in extended conformation and form the C-tail (blue). f, loop-C is made of two consecutive β-turns. The backbone of CP12-chain G plus T70 side chain are shown. Each β-turn (Asn-67–Thr-70 and Thr-70–Cys-73) is stabilized by two H-bonds. These H-bonds, found in most CP12 chains, were shorter than 3.1 Å, except for N67.O-T70.N, which was 3.5 Å. g, the circular structure is reinforced by several intramolecular H-bonds. Only side chains involved in interactions are shown. Although the first two residues of the C-tail are H-bonded with the circular structural motif, the last three are completely free to interact with GAPDH. Interactions are listed in supplemental Table S1.
ammonium sulfate), these pockets are entirely occupied by CP12 in two of four GAPDH subunits (GAPDH chains C and D; Fig. 2c; see also Fig. 1, a and b). The backbone of CP12 interacted with the side chains of the charged residues forming the Ps sites (Asp-181, Arg-231, Arg-195) and with NAD via the side chain of Tyr-76 (Fig. 2c). The last two residues of CP12 formed a variety of interactions with hydroxylated residues of the Pi site (Thr-150, Thr-208; Fig. 2c) and in some chains also with catalytic Cys-149 (supplemental Table S1b). The interactions of CP12 within P-sites engage the same residues that stabilize sulfates in CP12-free GAPDH (33).

Occupation of P-sites by CP12 was also observed in the binary complex obtained by soaking (supplemental Fig. S3b). Because these crystals were grown in the presence of ammonium sulfate, this finding demonstrates that CP12 can compete with and displace co-crystallized sulfate ions. P-sites of the opposite subunits that are not involved in CP12 binding were normally occupied by sulfate ions (supplemental Fig. S3b).

Major interactions between CP12 and GAPDH were conserved in both Arabidopsis and Synechococcus complexes (16) (supplemental Table S2). CP12 residues Glu-72 and Tyr-76 (Arabidopsis numbering) are engaged in the most relevant interactions and conserved in both species. Arg-74 in Arabidopsis CP12 is replaced by leucine in Synechococcus (supplemental Fig. S4a), but the main chain carbonyl group of both residues is hydrogen-bonded to a homologous GAPDH histidine (His-190 in Arabidopsis, His-195 in Synechococcus; supplemental Table S2). Also the position of CP12 and GAPDH interacting partners, including the coenzyme, is similar in both structures (supplemental Fig. S4b). Although the side chain orientation of Arg-191 in Arabidopsis GAPDH diverged from the homologous Arg196 in Synechococcus, the corresponding H-bond with CP12 glutamate involves main chain atoms and is fully conserved (supplemental Table S2).

**NMR Confirms CP12-GAPDH Interaction in Solution**—The NMR spectrum (1H,15N HSQC) of CP12 in solution showed limited dispersion of signals. The chemical shifts of many backbone amide protons fell in the random coil region (8-8.5 ppm, Fig. 3a). The backbone amide protons of only 44 over 74 non-proline residues gave detectable peaks. All missing signals belonged to the central part of the protein, between Val-10 and Ala-48. A short nine-residue N-terminal peptide (Ala-1-Asp-9) and a long 30-residue C-terminal peptide (Arg-49-Asn-78) were assigned (Fig. 3b).

In NMR experiments conducted with 15N-labeled CP12 and unlabeled GAPDH(NAD), all CP12 signals from residues Asp-55 to Asn-78 disappeared. They include all resolved residues in binary complex crystals (Fig. 3, a and b, and Fig. 1d). This result strongly suggests direct interactions or close proximity of this portion of CP12 with GAPDH, which is large enough (145 kDa) to broaden the interacting CP12 signals below the detection limit. Residues more distant from the C terminus of CP12, e.g. Ser-46, retained sufficient mobility and were still detectable. The results are consistent with what is expected if the entire C-terminal portion of CP12 is inserted in the deep cleft delimited by two GAPDH subunits. No new signals from the central part of CP12 appeared when the binary complex was formed.

**Free CP12 Contains Circular Structural Motif**—The structure of the C-terminal part of oxidized CP12 was modeled on the basis of NMR data (Table 2; Fig. 4, a and b). Twenty low energy models were selected. They all contain the α-helix-C encompassing residues Pro-59 to Asp-66, helix-capped by Asn-67. This portion of CP12 is superimposable in all NMR and x-ray structures (Fig. 4d).

Solution heteronuclear 15N NOE experiments indicated that the residues of CP12 circular motif have slow dynamics in the binary complex (Fig. 4c). In most NMR models residues of loop-C (Asn-67-Cys-73) have torsion angles typical of β-turns, and the loop-C adopted a helicoidal structure formed by two consecutive β-turns (Fig. 4e). However, the position of the loop with respect to the α-helix-C can vary in the different models (Fig. 4f).
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Signals of red residues were little or not affected by GAPDH. Signals that disappeared under conditions of complex formation with GAPDH. The central part of the protein was almost completely NMR silent.

CP12 were little affected by GAPDH (GAPDH (145 kDa). Assigned residues that do not belong to the C terminus of CP12 were little affected by GAPDH (red spots), both samples containing 2.7 mM NAD. Oxidized CP12 showed limited dispersion of signals, and many residues gave no detectable resonances. Assignment of the C-terminal portion of CP12 (Arg-49–Asn-78) was complete. In the presence of unlabeled GAPDH (red spots), all signals belonging to the C terminus of CP12 (from Asp-55 to Asn-78; blue labels) disappeared, indicating direct interaction with or close proximity to GAPDH (145 kDa). Assigned residues that do not belong to the C terminus of CP12 were little affected by GAPDH (red labels). s.c., side chain; Rs.c., arginine side chain. b, in the primary structure of CP12, assigned residues are underlined. The central part of the protein was almost completely NMR silent. Blue residues gave signals that disappeared under conditions of complex formation with GAPDH. Signals of red residues were little or not affected by GAPDH.

TABLE 2
NMR and refinement statistics for CP12 structures

| CP12                              |
|-----------------------------------|
| NMR distance and dihedral constraints |
| Distance constraints              |
| Total NOE                         | 165 |
| Intra-residue                     | 69  |
| Inter-residue                     |     |
| Sequential ([i−j] = 1)            | 66  |
| Medium range ([i−j] < 4)          | 29  |
| Long range ([i−j] > 5)            | 1   |
| Intermolecular                    | 0   |
| Hydrogen bonds                    | 2   |
| Total dihedral angle restraints   | 40  |
| ∆φ                               | 20  |
| Ψ                                | 20  |

Structure statistics

Violations (mean and S.D.) 0.54 ± 0.59
Dihedral angle constraints (°) 1.08 ± 1.06
Deviations from idealized geometry
Bond lengths (Å) 0.0060 ± 0.00091
Bond angles (°) 0.8429 ± 0.0524
Improper (°) 0.8814 ± 0.1057
Average pairwise root mean square deviation (Å) Residues: 59–66/59–70/59–73
Heavy 0.855/1.284/1.966
Backbone 0.216/0.754/1.286

The last five residues forming the C-tail of CP12 in solution are predicted dynamic (TALOS+, Fig. 4a) and display low values of heteronuclear 15N NOE (Fig. 4c) and limited homo-
nuclear 1H,1H NOE signals (Fig. 4b). These features indicate high structural dynamism.

The ensemble of 20 CP12 models obtained by NMR was structurally clustered. The two major structural clusters (N1 and N2) including 11 and 4 models, respectively, were neatly separated and represented alternative structures of CP12 C terminus in solution. The nine crystallographic CP12 models (three from co-crystallization, six from soaking) formed another homogeneous cluster (C1) that mapped closely to cluster N1 in the principal-component analysis (Fig. 5a). In cluster N1, both the α-helix-C and the first β-turn of loop-C (i.e. from Pro-59 to Thr-70) were nicely superimposed with crystallographic CP12 structures. From Thr-70 to Asn-78, the structure of clusters N1 and C1 diverged completely (Fig. 5b). Except for the α-helix-C, all other NMR clusters diverged from crystallographic cluster C1 for the entire CP12 C-terminal structure.

Exploring Region of Cluster C1 by Molecular Dynamics—Starting from CP12 crystallographic structure, we carried out MD simulations of the last 21 residues of CP12 in explicit water for 50 ns to explore the conformational space around cluster C1. Projection of the trajectory in the conformational landscape shows that MD models occupy a wide region of the map, overlapping with both crystallographic cluster C1 and NMR cluster N1 (Fig. 5a). The B-factors from the MD trajectory (supplemental Fig. S5a) are in agreement with NMR data and identify a region of 15 residues with slow dynamics (Pro-9–Arg-74) that corresponds to the circular structural motif (α-helix-C/loop-C/disulfide). The C-tail is highly dynamical, also in accordance with the NMR data (Fig. 4, a, b, and c).

In longer simulations in implicit water for 100 ns, the trajectories provide further atomistic details (supplemental Video S1). In the Pro-59/Asp-66 region, α-helix-C temporally breaks down in two 310 helices due to the conformational restraint of the Cys-64 residue, which is involved in the sulfur-bridge (supplemental Fig. S5). The conformation of the remaining part of CP12 was mostly turn, as in the NMR models. Asn-71–Cys-73 and Arg-74–Tyr-76 tend to form short 310 helices before or after the disulfide or, alternatively, to give a short-lived α-helix. The disulfide limits the dynamics of Cys-73 and hinders the formation of a well defined structure. In 1 of 20 NMR models, a short α-helix was found in this region (supplemental Fig. S5a). Populations of the H-bonds in percentages are given in supplemental Table S3. The top ranking hydrogen bonds stabilize the α-helix-C and β-turns/310 helices of loop-C. No stable H-bonds involve residues of the C-tail.

MD and NMR Concur—Both NMR and MD indicated that the last 20 amino acids of free, oxidized CP12 can be divided into two regions. The first region is characterized by slow dynamics and corresponds to the circular structural motif (α-helix-C/loop-C/disulfide). The second region is a faster dynamics region that corresponds to the C-tail.

The “slow” circular motif (Pro-59–Cys-73) of CP12 populates two minima (Fig. 6, a–d, and supplemental Fig. S5). In the free energy surface obtained from MD simulations, the white dots represent the crystallographic structure. The most populated MD conformation, representing the absolute minimum, is similar to the conformation of the circular structural motif observed in the crystals (cluster C1) and in cluster N1 of NMR.
FIGURE 5. The structural ensemble of the C-terminal portion of oxidized CP12 in solution. a, TALOS+ analysis of CP12 C terminus is shown. The torsion angles of residues Ser-57–Arg-74 were predicted (green bars). This region corresponds to the circular structural motif of crystallized binary complexes. The last four residues of the C-tail (Thr-75–Asn-78) were predicted to be dynamic (blue). b, 165 homonuclear (n) NOE signals were recorded and classified as intraresidue: \( i = j \) red, sequential; \( |i - j| = 1 \) (gray), medium range; \( 1 < |i - j| < 5 \) (blue); long range, \( |i - j| > 4 \) (purple). The majority of NOE signals involve residues of the \( \alpha \)-helix C (Leu-60–Asn-67). c, heteronuclear (he) NOE values demonstrate the relative rigidity of the circular structural motif (Leu-60–Cys-73) in contrast with the C-tail (Arg-74–Asn-78). d, superimposition of 20 models of CP12 C terminus (Asp-58–Asn-78) was calculated from NMR data. The color code is the same as in Fig. 1: \( \alpha \)-helix C (red), loop-C (gray), C-tail (blue). Disulfides are in stick representation with sulfur atoms in yellow. The figure also includes the crystallographic structure of CP12 chain G (purple). The structural alignment was based on the \( \alpha \)-helix C (Pro-59–Asp-66), present in all 20 NMR models and crystallographic CP12. e, the aligned loops-C of the 20 NMR showed a helicoidal structure formed by two \( \beta \)-turns (Asn-67–Thr-70; Thr-70–Cys-73). The position of the \( \mathrm{C\alpha} \) of crucial residues is indicated. Crystallized loop-C could not be aligned with NMR loops-C. f, loop-C-based alignment of the 20 NMR models shows that the angle of insertion of loop-C with \( \alpha \)-helix C varies substantially in the different models.

FIGURE 4. The structural ensemble of the C-terminal portion of oxidized CP12 in solution. a, TALOS+ analysis of CP12 C terminus is shown. The torsion angles of residues Ser-57–Arg-74 were predicted (green bars). This region corresponds to the circular structural motif of crystallized binary complexes. The last four residues of the C-tail (Thr-75–Asn-78) were predicted to be dynamic (blue). b, 165 homonuclear (n) NOE signals were recorded and classified as intraresidue: \( i = j \) red, sequential; \( |i - j| = 1 \) (gray), medium range; \( 1 < |i - j| < 5 \) (blue); long range, \( |i - j| > 4 \) (purple). The majority of NOE signals involve residues of the \( \alpha \)-helix C (Leu-60–Asn-67). c, heteronuclear (he) NOE values demonstrate the relative rigidity of the circular structural motif (Leu-60–Cys-73) in contrast with the C-tail (Arg-74–Asn-78). d, superimposition of 20 models of CP12 C terminus (Asp-58–Asn-78) was calculated from NMR data. The color code is the same as in Fig. 1: \( \alpha \)-helix C (red), loop-C (gray), C-tail (blue). Disulfides are in stick representation with sulfur atoms in yellow. The figure also includes the crystallographic structure of CP12 chain G (purple). The structural alignment was based on the \( \alpha \)-helix C (Pro-59–Asp-66), present in all 20 NMR models and crystallographic CP12. e, the aligned loops-C of the 20 NMR showed a helicoidal structure formed by two \( \beta \)-turns (Asn-67–Thr-70; Thr-70–Cys-73). The position of the \( \mathrm{C\alpha} \) of crucial residues is indicated. Crystallized loop-C could not be aligned with NMR loops-C. f, loop-C-based alignment of the 20 NMR models shows that the angle of insertion of loop-C with \( \alpha \)-helix C varies substantially in the different models.

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models (cluster N1). The conformer in which the $\alpha$-helix breaks down, and the loop adopts different conformations (Fig. 6c), is $\sim 2$ kcal mol$^{-1}$ higher in energy (Fig. 6d). The energy landscape of C-tail is broader, and the conformation of the crystallized C-tail is $\sim 4$ kcal mol$^{-1}$ higher in energy than the most populated conformations in solution (Fig. 6e).

**DISCUSSION**

CP12s are intrinsically disordered proteins with some propensity to fold under proper conditions, such as oxidation (6, 17) or low pH (8). At the NMR, the solution structure ensemble of oxidized CP12 from *A. thaliana* was found to contain a region of local order in its C-terminal part. An $\alpha$-helix-C and a helicoidal loop-C made of two consecutive $\beta$-turns are interconnected by a disulfide bridge and few H-bonds to create a circular structural motif. The $\alpha$-helix-C and the helicoidal loop-C are relatively rigid, whereas the circular structural motif is not. At the helix/loop interface, Asn-67 and the disulfide allow articulation of loop-C with respect to the $\alpha$-helix-C. In free CP12 in solution, one conformation of the circular structural motif was more common than others (cluster N1 including 11 models; Fig. 5a) and probably represents an energy minimum of the CP12 structural ensemble. The flexible C-tail that immediately follows the circular structural motif was disordered in all models. The N-terminal part that precedes the circular structural motif was almost entirely NMR silent. The dynamics of CP12 N-terminal part did not, however, impede stabilization of the GAPDH-CP12 complex.

In crystallized GAPDH-CP12 binary complexes, CP12 also displayed a circular structural motif encompassing the same $\alpha$-helix-C, a loop-C made of two consecutive $\beta$-turns, and the disulfide. However, the two $\beta$-turns were differently articulated...
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at the level of Thr-70 (Fig. 5b), the residue that ends the first β-turn and initiates the second. The crystallized loop-C (Fig. 1f) was, therefore, not helicoidal as that of CP12 solution structures (Fig. 4e). Despite this difference, the connection between loop-C and α-helix-C (via the joint residue Asn-67) is similar in both the crystallized binary complex and in cluster N1 of CP12 solution structures (Fig. 5b). All other NMR alternative structures are different in this respect. The extended conformation of the C-tail embedded in the GAPDH was never found in solution.

The circular structural motif α-helix-C/loop-C/disulfide is likely to play a major role in the process of binding, in agreement with PONDR VL-XT (49) analysis of CP12 (supplemental Fig. S6). Short ordered sequences within large unstructured regions are often involved in binding interactions (49). These molecular recognition features (31) often include amphipathic α-helices (23) and fold either before or after binding (50). PONDR VL-XT predicts a short segment of nine residues corresponding to amphipathic α-helix-C as the only ordered element of the entire CP12, strongly suggesting a role in binding. The disorder of the rest of CP12 in solution is experimentally supported also by its ability to diffuse into GAPDH crystals, allowing formation of binary complexes by soaking.

We predict that an encounter complex forms by interaction of GAPDH with oxidized CP12 in cluster-N1 conformation (one of the possible conformations of the NMR landscape; Fig. 5, a and b). Although subject to rapid movements that can even break the α-helix-C, the circular structural motif of cluster N1 is the most stable conformation of the MD landscape (Fig. 6, a and b). In the encounter complex, the α-helix-C and the first β-turn of loop-C lodge in a GAPDH cavity delimited by positive side chains (Arg-74, Arg-191; Fig. 1, b and c). Few (and variable) interactions stabilize this binding; often one single interaction linking the α-helix-C to GAPDH (Arg-191) and an ionic bond between the side chains of Glu-69 (CP12) and Arg-77 (GAPDH). The essential role of these two GAPDH arginines in CP12 binding was proved in the orthologous Chlamydomonas system (51, 52) and confirmed by the crystallographic structure of the binary complex from S. elongatus (16). Therefore, the GAPDH-CP12 encounter complex is the result of a preliminary, albeit essential, conformational selection performed by GAPDH on the CP12 structural ensemble. Our data do not support the recently proposed model of CP12 folding upon binding to GAPDH (16), although induced folding steps are indeed involved in the stabilization of the encounter complex.

Evolution of the encounter complex into the final stable structure of the crystallized binary complex implies a major rearrangement of the rest of CP12, whereas the general folding of the enzyme is not influenced. The possibility of CP12 Glu-72 to form H-bonds with different GAPDH residues induces the second β-turn to flip in the GAPDH cavity (Fig. 5b), thus adopting the conformation observed in the crystallized binary complex.

The flip of the second β-turn starts the zippering process that drives the C-tail to fit into the GAPDH groove that leads to the substrate-phosphate binding sites (Fig. 2c). The crystal structure of the binary complex suggests that the energy of CP12 binding to GAPDH (enthalpic term) must crucially depend on the C-tail, which is anchored to GAPDH by up to 12 H-bonds (supplemental Table S1b). In this view, the inability of reduced CP12 to bind GAPDH (12) is simply a consequence of the destabilization of this structural motif in the absence of the disulfide bridge. Without the initial recognition of the circular structural motif, the binding of the C-tail is hindered by its dynamism. Although the capability of reduced CP12 to bind GAPDH was reported in C. reinhardtii (53), we could not confirm the same type of binding in the Arabidopsis system.3

The previously measured negative entropic term of 5 kcal·mol⁻¹ at room temperature for the CP12-GAPDH interaction (17) can be attributed to the stiffening of the C-tails in the GAPDH-CP12 complex. The enthalpic counterpart is represented by the great number of short-distance interactions that are formed between the C-tail of CP12 and GAPDH, which can supply the energy necessary for the binding (−15 kcal·mol⁻¹; Ref. 17). We propose that the interaction of CP12 with GAPDH follows a synergistic mechanism (24) that combines conformational selection, triggered by CP12 oxidation, and folding upon binding.

Conformational selection is important for the initial recognition event when GAPDH samples the CP12 conformational ensemble and fishes out the oxidized molecules with the preformed circular structural motif in apt conformation. Folding upon binding occurs only in a second step. The flip of the second β-turn helps the flexible C-tail of the CP12 molecule recognized by GAPDH to slip into the catalytic site where it assumes an ordered, extended conformation. The MD show that in solution this extended conformation of the C-tail is not usual. The calculations show that for the Arg-74–Asn-78 segment the energy cost is −4 kcal/mol for folding upon binding (Fig. 6e). The process allows going from the more stable conformation of the tail in water to that of the bound state. Pictorially, oxidation baits the fishing rod of CP12 with the circular structural motif α-helix-C/loop-C/disulfide, and upon catching its GAPDH-prey, the terminal part of the rod stiffens.

Acknowledgments—We thank the European Synchrotron Radiation Facility (Grenoble, France) for access to ID14 beam lines. We thank Ewen Lescop (ICSN, UPR CNRS 2301, Gif sur Yvette, France), Nathalie Sibille, Guy Lippens, Isabelle Landrieu (UIGSF, UMR CNRS/Université Lille 1: 8576, Villeneuve d’Ascq, France), Pierre Gans, Adrien Favier, Bernard Brutcher (IBS, UMR CNRS/CEA/Université Grenoble1 5075, Grenoble, France) for helpful discussions. The 600- and 800-MHz NMR facilities used in this study were funded by the Région Nord–Pas de Calais (France), FEDER, Ministère de la Recherche, the CNRS, the Université Lille1-Science et Technologies, and the Institut Pasteur de Lille.

REFERENCES

1. Buchanan, B. B., and Balmer, Y. (2005) Redox regulation. A broadening horizon. Annu. Rev. Plant Biol. 56, 187–220
2. Dai, S., Friemann, R., Glauser, D. A., Bourquin, F., Manieri, W., Schürmann, P., and Eklund, H. (2007) Structural snapshots along the reaction pathway of ferredoxin-thioredoxin reductase. Nature 448, 92–96
3. Trost, P., Fermani, S., Marli, L., Zaffagnini, M., Falini, G., Scaglari, S., L. Marri, unpublished information.

3 L. Marri, unpublished information.
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Pupillo, P., and Sparla, F. (2006) Thioredoxin-dependent regulation of photosynthetic glyceraldehyde-3-phosphate dehydrogenase. Autonomous vs. CP12-dependent mechanisms. Photosynth. Res. 89, 263–275

4. Wedel, N., and Soll, J. (1998) Evolutionary conserved light regulation of Calvin cycle activity by NAPD-mediated reversible phosphorylubolization/CP12/glyceraldehyde-3-phosphate-dehydrogenase complex dissociation. Proc. Natl. Acad. Sci. U.S.A. 95, 9699–9704

5. Scheibe, R., Wedel, N., Vetter, S., Emmerlich, V., and Sauermann, S. M. (2002) Co-existence of two regulatory NADP-glyceraldehyde 3-phosphate dehydrogenase complexes in higher plant chloroplasts. Eur. J. Biochem. 269, 5617–5624

6. Graciet, E., Lans, P., Wedel, N., Lebreton, S., Samadaro, J. M., and Gontero, B. (2003) The small protein CP12. A protein linker for supramolecular complex assembly. Biochemistry 42, 8163–8170

7. Tamoi, M., Miyazaki, T., Fukumizo, T., and Shigeoka, S. (2005) The Calvin cycle in cyanobacteria is regulated by CP12 via NAD(H)/NADP(H) ratio under light/dark conditions. Plant J. 42, 504–513

8. Marri, L., Paresani, A., Valero, C., Lamba, D., Pupillo, P., Trost, P., and Sparla, F. (2010) In vitro characterization of Arabidopsis CP12 isoforms reveals common biochemical and molecular properties. J. Plant Physiol. 167, 939–950

9. Howard, T. P., Lloyd, J. C., and Raines, C. A. (2011) Interspecies variation in the oligomeric states of the higher plant Calvin cycle enzymes glyceraldehyde-3-phosphate dehydrogenase and phosphoribulokinase. J. Exp. Bot. 62, 3799–3805

10. Marri, L., Paresani, A., Collin, V., Issakidis-Bourguet, E., Lemaire, S. D., Pupillo, P., Sparla, F., Miginiac-Maslow, M., and Trost, P. (2009) Prompt and easy activation by specific thioredoxins of Calvin cycle enzymes of Arabidopsis thaliana associated in the GAPDH-CP12/PRK supramolecular complex. Mol. Plant 2, 259–269

11. Fermani, S., Sparla, F., Falini, G., Martelli, P. L., Casadio, R., Pupillo, P., Rapantoni, A., and Trost, P. (2007) Molecular mechanism of thioredoxin regulation in photosynthetic A2B2-glyceraldehyde-3-phosphate dehydrogenase. Proc. Natl. Acad. Sci. U.S.A. 104, 11109–11114

12. Marri, L., Trost, P., Pupillo, P., and Sparla, F. (2005) Reconstitution and properties of the recombinant glyceraldehyde-3-phosphate dehydrogenase/CP12/phosphoribulokinase supramolecular complex of Arabidopsis. Plant Physiol. 139, 1433–1443

13. Howard, T. P., Metodiev, M., Lloyd, J. C., and Raines, C. A. (2008) Thioredoxin-mediated reversible dissociation of a stromal multiprotein complex in response to changes in light availability. Proc. Natl. Acad. Sci. U.S.A. 105, 4066–4061

14. Howard, T. P., Fryer, M. J., Singh, P., Metodiev, M., Lytvchenko, A., Obata, T., Fernie, A. R., Kruger, N. J., Quick, W. P., Lloyd, J. C., and Raines, C. A. (2011) Antisense suppression of the small chloroplast protein CP12 in tobacco alters carbon partitioning and severely restricts growth. Plant Physiol. 157, 620–631

15. Thompson, L. R., Zeng, Q., Kelly, L., Huang, K. H., Singer, A. U., Stubbe, J., and Chisholm, S. W. (2011) Phage auxiliary metabolic genes and the redox properties of the recombinant glyceraldehyde-3-phosphate dehydrogenase complexes in higher plant chloroplasts. J. Mol. Biol. 410, 1015–1026

16. Tompa, P., and Fuxreiter, M. (2008) Fuzzy complexes. Polymorphism and structural disorder in protein-protein interactions. Trends Biochem. Sci. 33, 2–8

17. Wright, P. E., and Dyson, H. J. (2009) Linking folding and binding. Curr. Opin. Struct. Biol. 19, 31–38

18. Espinoza-Fonseca L. M. (2009) Reconciling binding mechanisms of intrinsically disordered proteins. Biochem. Biophys. Res. Commun. 382, 479–482

19. Boehr, D. D., Nussinov, R., and Wright, P. E. (2009) The role of dynamic conformational ensembles in biomolecular recognition. Nat. Chem. Biol. 5, 789–796

20. Dyson, H. J., and Wright, P. E. (2005) Intrinsically unstructured proteins and their functions. Nat. Rev. Mol. Cell Biol. 6, 197–208

21. Kjaergaard, M., Teilmann, K., and Poulsen, F. M. (2010) Conformational selection in the molten globule state of the nuclear coactivator binding domain of CBP. Proc. Natl. Acad. Sci. U.S.A. 107, 12535–12540

22. Sugase, K., Dyson, H. J., and Wright P. E. (2007) Mechanism of coupled folding and binding of an intrinsically disordered protein. Nature 447, 1021–1025

23. Wang, T., Darwin, K. H., and Li, H. (2010) Binding-induced folding of prokaryotic ubiquitin-like protein on the Mycobacterium proteasomal ATPase targets substrates for degradation. Nat. Struct. Mol. Biol. 17, 1352–1357

24. Lee, C. W., Martinez-Yamout, M. A., Dyson, H. J., and Wright, P. E. (2010) Structure of the p53 transactivation domain in complex with the nuclear receptor coactivator binding domain of CBP. Biochemistry 49, 9964–9971

25. Mohan, A., Oldfield, C. J., Radiovijac, P., Vacie, V., Cortese, M. S., Dunker, A. K., and Uversky, V. N. (2008) Analysis of molecular recognition features (MoRFs), J. Mol. Biol. 362, 1043–1059

26. Fermani, S., Sparla, F., Marri, L., Thomiger, A., Pupillo, P., Falini, G., and Trost, P. (2010) The crystal structure of photosynthetic glyceraldehyde-3-phosphate dehydrogenase (isofrom A) from Arabidopsis thaliana in complex with NAD. Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 66, 621–626

27. Leslie, A. G. W. (1992) Recent changes to the MOSFLM package for processing film and image plate data. Joint CCP4 + EGF-HMACB Newsletter on Protein Crystallography 26, 27–33

28. Evans, P. (2006) Scaling and assessment of data quality. Acta Crystallogr. D Biol. Crystallogr. 62, 72–82

29. Otwinowsky, Z., and Minor, W. (1997) Processing of X-ray diffraction data collected in oscillation mode. Methods Enzymol. 276, 307–326

30. Navaza, J. (1994) AmoRe. An automated package for molecular replacement. Acta Crystallogr. A 50, 157–163

31. Vagin, A. A., Steinke, S., Lepeshov, A. A., Potterton, L., McNicholas, S., Long, F., and Murshudov, G. N. (2004) REFMAC5 dictionary: organization of prior chemical knowledge and guidelines for its use. Acta Crystallogr. D Biol. Crystallogr. 60, 2184–2195

32. Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Immink, G., Karplus, P., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Crystallography and NMR system (CNS). A new software system for macromolecular structure determination. Acta Crystallogr. D Biol. Crystallogr. 54, 905–921

33. Emsley, P., and Cowtan, K. (2004) Coot. Model-building tools for molecular graphics. Acta Crystallogr. D Biol. Crystallogr. 60, 2126–2132

34. Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1993) PROCHECK: a program to check the stereochemical quality of
protein structures. J. Appl. Crystallogr. 26, 283–291
42. Delaglio, F., Grzesiek, S., Vuister, G. W., Zhu, G., Pfeifer, J., and Bax, A. (1995) NMRPipe. A multidimensional spectral processing system based on UNIX pipes. J. Biomol. NMR 6, 277–293
43. Johnson, B. A., and Blevins, R. A. (1994) NMRView. A computer program for the visualization and analysis of NMR data. J. Biomol. NMR 4, 603–614
44. Shen, Y., Delaglio, F., Cornilescu, G., and Bax, A. (2009) TALOS+. A hybrid method for predicting protein backbone torsion angles from NMR chemical shifts. J. Biomol. NMR 44, 213–223
45. Favier, A., and Brutscher, B. (2011) Recovering lost magnetization. Polarization enhancement in biomolecular NMR. J. Biomol. NMR 49, 9–15
46. Case, D. A., Cheatham, T. E., 3rd, Darden, T., Gohlke, H., Luo, R., Merz, K. M., Jr, Onufriev, A., Simmerling, C., Wang, B., and Woods, R. J. (2005) The Amber biomolecular simulation programs. J. Comput. Chem. 26, 1668–1688
47. Cornell, W. D., Cieplak, P., Bayly, C. I., Gould, I. R., Merz, K. M., Ferguson, D. M., Spellmeyer, D. C., Fox, T., Caldwell, J. W., and Kollman, P. A. (1995) A second generation force field for the simulation of proteins, nucleic acids, and organic molecules. J. Am. Chem. Soc. 117, 5179–5197
48. van Gunsteren, W. F., and Berendsen, H. J. C. (1997) Algorithms for macromolecular dynamics and constraint dynamics. Mol. Physiol. 34, 1311–1327
49. Garner, E., Romero, P., Dunker, A. K., Brown, C., and Obradovic, Z. (1999) Predicting binding regions within disordered proteins. Genome Inform. Ser. Workshop Genome Inform. 10, 41–50
50. Oldfield, C. J., Cheng, Y., Cortese, M. S., Romero, P., Uversky, V. N., and Dunker, A. K. (2005) Coupled folding and binding with α-helix-forming molecular recognition elements. Biochemistry 44, 12454–12470
51. Graciet, E., Mulliert, G., Lebreton, S., and Gontero, B. (2004) Involvement of two positively charged residues of Chlamydomonas reinhardtii glycer- aldehyde-3-phosphate dehydrogenase. Eur. J. Biochem. 271, 4737–4744
52. Erales, J., Mekhalfi, M., Woudstra, M., and Gontero, B. (2011) Molecular mechanism of NADPH-glyceraldehyde-3-phosphate dehydrogenase regulation through the C terminus of CP12 in Chlamydomonas reinhardtii. Biochemistry 50, 2881–2888
53. Erales, J., Lignon, S., and Gontero, B. (2009) CP12 from Chlamydomonas reinhardtii, a permanent specific “chaperone-like” protein of glyceraldehyde-3-phosphate dehydrogenase. Eur. J. Biochem. 284, 12735–12744
54. Nicholls, A., Sharp, K. A., and Honig, B. (1991) Protein folding and association. Insights from the interfacial and thermodynamic properties of hydrocarbons. Proteins: Struct. Funct. Genet. 11, 281–296
55. Frishman, D., and Argos, P. (1995) Knowledge-based protein secondary structure assignment. Proteins 23, 566–579