Memory and Imprinting in Multienzyme Complexes

EVIDENCE FOR INFORMATION TRANSFER FROM GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE TO PHOSPHORIBULOKINASE UNDER REDUCED STATE IN CHLAMYDOMONAS REINHARDTII*

Sandra Lebreton and Brigitte Gontero‡

From the Institut Jacques Monod, CNRS-Universités Paris VI-VII, 2 place Jussieu, 75 251 Paris Cedex 05, France

(Received for publication, December 9, 1998, and in revised form, February 22, 1999)

The phosphoribulokinase, when it is in a reduced state in a bi-enzyme complex, is more active than when it is oxidized. This complex dissociates upon dilution to give a metastable reduced form of phosphoribulokinase, which differs from the stable form isolated beside the complex. The kinetic parameters of the reduced stable phosphoribulokinase and those of the complex are very similar, unlike those of the metastable form. Although the kinetic mechanism of the reduced stable form is ordered, with ribulose-5-phosphate binding first, ATP binds first to the phosphoribulokinase in the complex and to the metastable form. Therefore, phosphoribulokinase bears an imprint from glyceraldehyde-3-phosphate dehydrogenase after disruption of the complex. Dissociation of phosphoribulokinase from the complex also enhances its flexibility. The imprinting and greater flexibility result in the catalytic constant of dissociated phosphoribulokinase being 10-fold higher than that of the enzyme in the complex.

Imprinting corresponds to stabilization-destabilization energies resulting from conformation changes generated by protein-protein interactions. The energy stored within the metastable phosphoribulokinase is mainly used to decrease the energy barrier to catalysis.

It is widely accepted that there is macromolecular crowding in most living cells (1–5), so that protein-protein interactions are favored in vivo. The purification of multienzyme complexes from many organisms and organelles (mitochondria, chloroplasts, etc.), forming parts of various metabolic pathways (6–12) over the past decade, has provided further evidence of this.

We recently purified a two-enzyme complex made up of two molecules of dimeric phosphoribulokinase (EC.2.7.1.19) (PRK)¹ and two molecules of tetrameric glyceraldehyde-3-phosphate dehydrogenase (EC.1.2.1.13) from the chloroplast of Chlamydomonas reinhardii cells (13). These enzymes are involved in the so-called Benson-Calvin cycle, responsible for the assimilation of carbon dioxide (14–15). Phosphoribulokinase catalyzes the ATP-dependent phosphorylation of ribulose 5-phosphate to ribulose 1,5-bisphosphate, the CO₂ acceptor. Glyceraldehyde-3-phosphate dehydrogenase in this cycle catalyzes the conversion of 1,3-bisphosphoglycerate into glyceraldehyde 3-phosphate with NAD(P)H as a cofactor.

The Benson-Calvin cycle is regulated by light via several mechanisms, including pH, the magnesium and metabolite concentrations, and the reduction of target enzymes by thioredoxins (16–20). The chloroplast enzymes, fructose-1,6-bisphosphatase, sedoheptulose-1,7-bisphosphatase, glyceraldehyde-3-phosphate dehydrogenase, and phosphoribulokinase are all suitable for studying how a signal (light) can act as a regulator (21–23). In prokaryotic photosynthetic organisms, light regulates the PRK (dimer of 40 kDa) via positive allosteric activator NADH, whose intracellular concentration is light-dependent. AMP is also a negative effector (25). By contrast, light regulates eukaryotic PRK (dimer of 40 kDa) via breaking disulfide bridges (26–27). This is done by small proteins, thioredoxins (20, 28–29), that have been reduced by electron flow from photosystem I (20, 28–30). The sulphydryl/disulfide exchange reactions between the target enzyme and thioredoxin activate the enzyme. The nonphysiological reducing agent, dithiothreitol, can mimic this effect in vitro.

Contrary to a common belief, oxidized phosphoribulokinase may have quite significant activity when associated with glyceraldehyde-3-phosphate dehydrogenase or when dissociated from this enzyme upon dilution of the complex (31), so it may acquire new kinetic properties due to the conformation changes resulting from protein-protein interactions.

There is little doubt that chloroplast phosphoribulokinase becomes reduced by the thioredoxin system (26–27, 32–33) in the light. It has also been shown in vitro that reducing agents such as dithiothreitol or reduced thioredoxin cause the dissociation of the bi-enzyme complex (13). Therefore, one may wonder whether there exists a reduced bi-enzyme complex that may have kinetic properties different from either its oxidized state or to the “free” oxidized and reduced forms of PRK. This study was therefore carried out to look for a reduced bi-enzyme complex and compare its properties. We have examined the influence of protein-protein interactions in this complex on reaction mechanism, substrate binding, and kinetic parameters and whether information is still transferred from reduced glyceraldehyde-3-phosphate dehydrogenase to reduced phosphoribulokinase. The answers to these important questions should provide a better understanding of carbon metabolism, as it is well documented that the enzymes are predominantly in a reduced state in illuminated chloroplasts.

EXPERIMENTAL PROCEDURES

Materials—Ru-5-P, xylulose 5-phosphate, and RuBP were obtained from Sigma. Ribose 5-phosphate, ATP, and DTT were supplied by Roche Molecular Biochemicals.

Strains and Culture Conditions—The wild type WM3 “strain of C. reinhardtii” was mixotrophically grown at 25 °C on Tris-acetate-phosphate medium at a light intensity of about 2500 lux (34).

** Purification of Isolated and Embedded PRK—The bi-enzyme complex made up of PRK and GAPDH and the isolated PRK were purified as described previously (13).

Enzyme Assays and Protein Measurements—PRK activity was continuously monitored in a spectrophotometric assay in which generation

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed. Tel.: 33 1 44 27 40 21; Fax: 33 1 44 27 59 94; E-mail: meunier@ijm.jussieu.fr.

‡ The abbreviations used are: PRK, phosphoribulokinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; DTT, dithiothreitol; Ru-5-P, ribulose 5-phosphate; RuBP, ribulose 1,5-bisphosphate.
of ADP was coupled to NADH oxidation via phosphoenolpyruvate, pyruvate kinase, and lactate dehydrogenase (35). The protein concentration was determined with the Bradford assay using bovine serum albumin as a standard (36).

Data Analysis—The experimental data were fitted to the equations developed in the text, with Simplex (37) or Marquardt (38) algorithms using a VAX computer.

RESULTS

The bi-enzyme complex was not dissociated by incubation with 5 mM DTT for 1 h, and the integrity of the complex was checked by electrophoresis under native conditions (data not shown). The PRK activity of this mixture was then monitored using fixed concentrations of both substrates (ATP and Ru-5-P) without reducing agents (Fig. 1). The lag was similar to the curve obtained with the bi-enzyme complex under the oxidized

![Image](image_url)

**FIG. 1. Time curves of the reaction catalyzed by reduced PRK.** The product (q) of the reaction catalyzed by PRK in the complex was continuously monitored. The complex had been incubated with 5 mM DTT for 1 h. The data are fitted using Equation 1 in the main text. Solid lines are theoretical curves. The PRK in the complex was assumed to be inactive (A) or active (B). The insets show the distribution of residuals (d) in both cases. The total enzyme concentration in the assay mixture was 1.5 nM; the ATP and Ru-5-P concentrations were 1 and 0.2 mM, respectively.

**FIG. 2. The apparent dissociation rate constant of reduced complex as a function of substrate concentrations.** The apparent dissociation rate constant (k*) was measured as a function of Ru-5-P concentration with [ATP] held constant (1 mM) (A). The apparent dissociation rate constant was measured as a function of ATP concentration, whereas [Ru-5-P] was held constant (1 mM) (B). All apparent dissociation rate constants (k*) were derived by fitting experimental progress curves to Equation 1 in the main text. The values of k* were then obtained using Equation 18 in Lebreton et al. (31).

**TABLE I**

Kinetic parameters for the different forms of PRK in oxidized and reduced states

| Kinetic parameters          | Oxido-reduction states | Enzyme forms             | Ru-5-P | ATP  |
|----------------------------|------------------------|--------------------------|--------|------|
| **K_m** (μM)               |                        | Stable PRK               | 115    | 89   |
|                            | Oxidized               | PRK inserted in the complex | 30     | 46   |
|                            | Metastable PRK         | 59                       | 48     |
|                            | Reduced                | Stable PRK               | 55     | 55   |
|                            | PRK inserted in the complex | 61               | 60     |
|                            | Metastable PRK         | 94                       | 51     |
| **k_cat** (s⁻¹/site)       |                        | Stable PRK               | 0.062  | 0.065|
|                            | Oxidized               | PRK inserted in the complex | 3.25   | 3.25 |
|                            | Metastable PRK         | 56.3                     | 56.5   |
|                            | Reduced                | Stable PRK               | 23.3   | 23   |
|                            | PRK inserted in the complex | 32.4            | 32.7   |
|                            | Metastable PRK         | 300                      | 303.8  |
| **k_cat/K_m** (mM⁻¹s⁻¹/site)| Oxidized              | Stable PRK               | 0.54   | 0.73 |
|                            | PRK inserted in the complex | 108             | 70     |
|                            | Metastable PRK         | 954                      | 1177   |
|                            | Reduced                | Stable PRK               | 424    | 418  |
|                            | PRK inserted in the complex | 531              | 545    |
|                            | Metastable PRK         | 3191                     | 5957   |
state (31). It was probably due to dissociation of the bi-enzyme complex upon dilution in the assay cuvette. It corresponds to the conversion of the PRK inserted in the complex in a dissociated form, the so-called metastable PRK. This curve may be fitted to

\[ q = P_T \left( \frac{p(1)}{r} + \frac{(p(1) - p(2))}{k^* - (e^{k^* t} - 1)} \right) \quad \text{(Eq. 1)} \]

where \( q \) is the product of the reaction catalyzed by PRK, and \( k^* \) is the apparent dissociation rate constant of the bi-enzyme complex. \( p(1) \) is the steady-state rate, and \( p(2) \) is the initial velocity. The parameter \( p(1) \) is the rate of the metastable form of PRK, and \( p(2) \) is the rate of the PRK in the complex. \( P_T \) is the total protein concentration. This equation is similar to an expression given by Neet and Ainslie (39) in a different context (31). The distribution of the residuals was much better when the bi-enzyme complex was assumed to be active, which implies \( p(2) \neq 0 \). The metastable form was more active than the PRK in the complex (Fig. 1).

The effects of the substrates on the apparent dissociation

---

**Fig. 3.** Double-reciprocal plots of rates against substrate concentrations with the three forms of reduced PRK in the presence of the product RuBP. The reaction catalyzed by reduced stable PRK was measured with 1 mM ATP, 0 mM (○), 1 mM (△), or 2 mM RuBP (□), and different concentrations of Ru-5-P (A) or with 1 mM Ru-5-P, 0 mM (○), 1.7 mM (△), or 3.4 mM RuBP (□), and different concentrations of ATP (B). The stable reduced enzyme concentration was 2.8 nM. The reaction catalyzed by reduced PRK in the complex was measured with 0 mM (○), 3 mM (△), or 6 mM RuBP (□) with 1 mM ATP and different concentrations of Ru-5-P (C) or with 1 mM Ru-5-P and different concentrations of ATP (D). The concentration of the reduced complexed PRK was 1.7 nM. The reaction catalyzed by reduced metastable PRK was measured with 0 mM (○), 3 mM (△), or 6 mM RuBP (□) with 1 mM ATP and different concentrations of Ru-5-P (E) or with 1 mM Ru-5-P and different concentrations of ATP (F). The concentration of the reduced metastable PRK was 0.5 nM.
rate constant \( k^* \) of the complex to the metastable form of PRK (Fig. 2) were examined. Ru-5-P tended to stabilize the complex, whereas ATP only slightly destabilized it. We then measured the kinetic parameters of the PRK in the complex and the reduced metastable form. Another form of PRK, the isolated stable form, can be obtained during purification (13). This form may also be reduced by DTT, and its kinetic parameters can be measured. All the enzyme forms followed Michaelis-Menten law with both substrates (Ru-5-P and ATP), (Table I). These data also allow comparison of the oxidized and reduced states of these various forms of PRK. The reduced metastable PRK seemed to be more stable than the oxidized form. Oxidizing the reduced metastable form with glutathione and reducing it again with DTT gave the same values for the kinetic parameters as those of the stable reduced enzyme (data not shown).

We determined the mechanism of substrate binding to PRK using inhibition by one of the products (RuBP) of the reduced enzymes. The method of alternative substrates, which can discriminate between homeomorphic kinetic models (40), was used instead of inhibition by ADP.

Various concentrations of RuBP were placed in the assay cuvette with one substrate concentration held constant and the other varied (Fig. 3). The reaction was started by adding reduced complex. This product is competitive relative to Ru-5-P and noncompetitive relative to ATP for the stable reduced enzyme but was noncompetitive for both substrates for reduced PRK. When it was in the bi-enzyme complex and in the metastable state (it is impossible to discriminate between random and ordered sequential kinetic mechanism), an alternative substrate \( S'_2 \) to Ru-5-P \( S'_2 \) such as xylulose-5-phosphate or ribose-5-phosphate was used. The rate expressions do not take into account the order in which products (ADP or RuBP) are released in the method of alternative substrates. Kinetic experiments were performed with the ATP concentration constant and variable concentrations of Ru-5-P or alternative substrates. The data were fitted using the Dalziel formalism (41),

\[
[\text{[E]}_0 / V = \Phi_0 + \Phi_1 + \Phi_2 + \Phi_{12}
\]  
(Eq. 2)

where \([\text{[E]}_0\) is the total enzyme concentration, \(V\) the maximal velocity, and the parameters \(\Phi_j\) correspond to the Dalziel coefficients. \(S_1\) is Ru-5-P, and \(S_2\) is ATP. With an alternative substrate, \(S'_2\) becomes \(S'_1\), and the \(\Phi_j\) coefficients become \(\Phi'_j\). These Dalziel coefficients, \textit{a priori}, are quite different. Nonetheless, constraints between these parameters may emerge using the models shown in Fig. 4 (40).

In the case of an ordered model with \(S_1\) binding first to the enzyme (Fig. 4A), there is no constraint between \(\Phi_0\) and \(\Phi'_1\). For an ordered model with \(S'_1\) binding after \(S_2\) (Fig. 4B), there are two constraints, \(\Phi_2 = \Phi'_2\) and \(\Phi_{12}/\Phi_1 = \Phi'_{12}/\Phi'_{11}\). For a random mechanism (Fig. 4C), the mathematical development only leads to \(\Phi_{12}/\Phi_1 = \Phi'_{12}/\Phi'_{11}\). All \(\Phi_j\) and \(\Phi'_j\) parameters were obtained by fitting the experimental data to Dalziel equations (Table II).

None of the constraints mentioned above was found with the stable reduced PRK. This, plus the product inhibition data, point to Ru-5-P binding first and RuBP being the last product to be released. The situations were quite different for reduced PRK in the complex and the metastable form. The parameters \(\Phi_2\) and \(\Phi'_2\) were not required in Dalziel equation for reduced, complexed PRK. Thus, \(\Phi_{12}/\Phi_1 = \Phi'_{12}/\Phi'_{11}\) and, therefore, the models B and C could account for the results obtained with the complex. The double-reciprocal plots of the ATP intersect on the rate axis indicated that ATP was bound first (data not shown). The inhibition study, and particularly the noncompetitive inhibition of RuBP relative to Ru-5-P, also supported this conclusion. We therefore adopted the ordered model (Fig. 4B) with ATP binding first.

The constraints obtained with the reduced metastable PRK and the results of RuBP inhibition indicated that the mechanism is ordered, with ATP binding first and ADP being the last product to be released. Therefore, the PRK in the complex and the PRK resulting from the dissociation of the complex have the same kinetic mechanism.

The complex and metastable PRKs also behave quite similarly with regard to RuBP inhibition. These results indicate that PRK bore the imprinting from GAPDH and retained for a while the conformation it had in the complex. But the kinetic parameters \(K_m\) and \(k_{cat}\) were different. The kinetic parameters and the kinetic mechanism of the metastable form were different from those of the stable one. The differences between the kinetic parameters were used to quantify the imprinting effect of GAPDH on PRK, which altered the kinetic parameters. Thermodynamic analysis (42) can be used to express the \(K_m\) and \(k_{cat}\) with stabilization-destabilization energy terms which take into account the protein-protein interactions; thus,

\[
K_m = K_m^{exp} (\Psi_{0,0}, \Psi_{1,1}) / RT
\]  
(Eq. 3)

and

\[
k_{cat} = k_{cat}^{exp} (\Psi_{0,0}, \Psi_{1,1}) / RT
\]  
(Eq. 4)

where \(K_m\), \(K_m^{exp} \), \(k_{cat}\), and \(k_{cat}^{exp}\) are the apparent association constants (equivalent to \(1/K_m\)) of the substrates (either ATP or Ru-5-P) and the catalytic constants \(k_{cat}\) of the metastable (\(\mu\)) and stable (\(\sigma\)) forms of PRK. Similarly, the catalytic efficiency of the metastable form \(K_m^{exp} \) may be defined as the following.

\[
K_m^{exp} = K_m^{exp} (\Psi_{0,0}, \Psi_{1,1}) / RT
\]  
(Eq. 5)

The differences \(\Psi_{0,0} - \Psi_{1,1}\), \(\Psi_{0,0} - \Psi_{1,1}\), and \(\Psi_{0,0} - \Psi_{1,1}\) are stabilization-destabilization energies. They therefore reflect the apparent free energies of substrate binding \(\Delta G^0\) and activation \(\Delta G^0\) associated with the reaction catalyzed by the stable and the metastable forms of PRK (Table III). The imprinting effect of GAPDH on PRK may be assessed through thermodynamic boxes, which associate the free energies of the processes catalyzed by the two forms of PRK (Fig. 5).

**Discussion**

We have shown that PRK may exist in a reduced stable or metastable form and as part of a bi-enzyme complex. The reduced bi-enzyme complex can depolymerize in vitro to a metastable form. This conversion is substrate-dependent and tends to be prevented by Ru-5-P rather than ATP. Dissociation is due to dilution, as it also occurs with the oxidized bi-enzyme complex (31). The rate of conversion \(k^*\) is faster than that of the oxidized enzyme; reduction weakens the bi-enzyme complex.
**Imprinting Effect of GAPDH on Reduced PRK**

**TABLE II**  
Dalziel coefficients with alternative substrates

All these parameters were obtained by fitting the experimental data to Dalziel equations. Ri5P, ribose 5-phosphate; Xu5P, xylulose 5-phosphate.

| Substrates | Stable reduced PRK | Reduced PRK inserted in the complex | Metastable reduced PRK |
|------------|--------------------|----------------------------------|------------------------|
|            | $\Phi_2$           | $(\Phi_{12}/\Phi_1)$             | $\Phi_2$               | $(\Phi_{12}/\Phi_1)$             |
| Ru-5-P     | $7.76 \times 10^{-4} \pm 1.2 \times 10^{-4}$ | $2.5 \times 10^{-2} \pm 5.2 \times 10^{-2} | 0.11 \pm 0.06 | $4.3 \times 10^{-2} \pm 2.3 \times 10^{-6}$ | $0.028 \pm 7 \times 10^{-3}$ |
| Ri5P       | $9 \times 10^{-4} \pm 1.5 \times 10^{-4}$ | $1.2 \times 10^{-2} \pm 4.2 \times 10^{-2}$ | $0.12 \pm 0.02 | $3.7 \times 10^{-2} \pm 4.7 \times 10^{-6}$ | $0.023 \pm 4 \times 10^{-3}$ |
| Xu5P       | $1.3 \times 10^{-3} \pm 2.1 \times 10^{-4}$ | $1.7 \times 10^{-2} \pm 5.3 \times 10^{-3}$ | $0.13 \pm 0.01 | $4.8 \times 10^{-2} \pm 1.3 \times 10^{-5}$ | $0.028 \pm 6.7 \times 10^{-3}$ |

**TABLE III**  
Thermodynamic parameters for stable and metastable reduced PRK

| Substrates | Stable reduced PRK | Reduced PRK inserted in the complex | Metastable reduced PRK |
|------------|--------------------|----------------------------------|------------------------|
|            | $\Psi_{0,1} - \Psi_{1,1}$ (kJ/mol) | $-1.24 \pm 0.18$ | $0.03 \pm 0.2$ | $-23.53 \pm 0.4$ | $-23.26 \pm 0.02$ | $23.7 \pm 0.02$ | $63.81 \pm 0.07$ | $63.84 \pm 0.12$ | $57.73 \pm 0.03$ | $57.74 \pm 0.02$ | $6.35$ kJ/mol |

**Fig. 5. Thermodynamic boxes associated with apparent substrate binding and catalysis.** Apparent free energies of Ru-5-P (A) or ATP (B) binding to stable reduced PRK ($\Delta G^*_o$) and metastable reduced PRK ($\Delta G^*_m$). Free activation energies of the reaction (C) catalyzed by stable reduced PRK ($\Delta G^*_o$) and metastable reduced PRK ($\Delta G^*_m$). The constants ($\psi_{0,1} - \psi_{1,1}$) and ($\psi_{0,1} - \psi_{1,1}$) are the stabilization-destabilization energies responsible for imprinting.

(13). For instance, at 1 mM ATP and 1 mM Ru-5-P, the value of $k^*$ is about 0.1 min$^{-1}$ for the oxidized bi-enzyme complex and up to 1 min$^{-1}$ for the reduced one. Thus, the conversion of the complex to the metastable form of PRK is 10-fold faster under reducing conditions than under oxidizing conditions.

Although oxidized PRK is active when bound to glyceraldehyde-3-phosphate dehydrogenase, reduced PRK is much more active (Table I). All the reduced forms of PRK are more active than their oxidized counterparts. But the $K_m$ values of the oxidized and reduced PRK are very similar. The increase in $k_{cat}$, with no major change in $K_m$, results in a greater catalytic efficiency. This is without doubt of physiological relevance, since PRK is in a reduced state upon illumination (18, 26–27, 30). The published kinetic parameters of the isolated reduced PRK are quite similar to those we obtained with the metastable reduced form. The $K_m$ and the $k_{cat}$ for the metastable enzyme are quite similar to those of Roesler and Ogren (43). Extraction and purification are usually done in 10 mM DTT, which destroys the bi-enzyme complex. Hence, the authors probably studied the metastable reduced form. This form of the enzyme persists for some time in the presence of DTT, and conversion to the stable form is slowed down. The kinetic parameters of the oxidized reduced PRK are similar to those of the oxidized stable PRK. If this form is reduced again with DTT, the kinetic parameters are those of the reduced stable enzyme, which is less active. Thus, PRK is influenced by being a partner of GAPDH in a supramolecular unit.

The stable oxidized PRK and PRK in the complex have different kinetic properties because information is transferred between PRK and GAPDH via conformation changes (5, 42). We have not detected any difference in the kinetic parameters ($k_m$ and $k_{cat}$) of the stable reduced form of PRK and the PRK in the complex, but this does not rule out the possibility that information is transferred, since the kinetic mechanism is dramatically changed. Ru-5-P binds first to the stable reduced form, but ATP is the first substrate when this enzyme is bound to GAPDH. The simplest explanation for this is that GAPDH is involved.

The metastable form even seems to retain the “memory” or the “imprint” (5) of GAPDH for a while after its release from the bi-enzyme complex and behaves according to the ordered kinetic model, with ATP binding first. Therefore, not only is information transferred between GAPDH and PRK, there is also a imprinting by GAPDH on PRK.

The catalytic efficiency of the metastable form of PRK (Table I) is 10-fold greater than that of the isolated reduced PRK. This is probably due to the memory of a conformation change that results from the interaction between PRK and GAPDH. The difference between the catalytic constants of the metastable PRK and of PRK in the bi-enzyme complex results from the greater flexibility of the metastable enzyme in solution. This flexibility is compatible with the great freedom required for catalysis.

The information transfer corresponds to stabilization-destabilization energies, which come from a conformation change in PRK within the complex. These energies are stored during association and are used after disruption via the imprinting effect. The difference in free activation energies (Table III) of the metastable and stable reduced forms of PRK corresponds to energy transferred from GAPDH to PRK by imprinting. This stabilization energy ($-6$ kJ/mol) helps lower the energy barrier during catalysis.

Thermodynamic analysis also shows that imprinting has no effect on the apparent pseudo-affinity constant for ATP and only a slight effect on that for Ru-5-P. This effect relies on a destabilization energy (1.24 kJ/mol). Despite this destabilization energy, the catalytic efficiency ($k_{cat}/K_m$) expressed by $k_{cat}/K_m$ is higher for metastable PRK than for the stable form. The difference ($\psi_{0,1} - \psi_{1,1}$) responsible for a best catalytic effect corresponds to a fraction ($\approx -5.5$ kJ/mol) of the energy stored by PRK when associated with GAPDH.

PRK and GAPDH are associated and use ATP and NADPH produced by the primary phase of photosynthesis. Thus, the bi-enzyme complex may allow concerted regulation of these two enzymes and, therefore, may be defined as a “unit control.” As a consequence, the study of isolated enzymes, although necessary, must be biased. The “unit control” that is the bi-enzyme...
complex is the starting point of regulation by light. Even if the complex dissociates, a conformation change resulting from a depolymerization in vitro probably governs regulation. Therefore, the effects of pH, metabolites, and thioredoxin activation must all be examined using this supramolecular entity. One possible function of this enzyme complex in eukaryotic cells may be to maintain, permanently or temporarily, some enzymes in a state of "super reactivity." The protein-protein interactions between PRK and GAPDH may well provide for the fine regulation of the Benson-Calvin cycle.

Acknowledgment—This manuscript was edited by Dr. Owen Parkes.

REFERENCES
1. Goodsell, D. S. (1991) Trends Biochem. Sci. 16, 203–206
2. Minton, A. P. (1981) Biopolymers 20, 2093–2120
3. Fulton, A. B. (1982) Cell 30, 345–347
4. Srere, P. A. (1982) Trends Biochem. Sci. 7, 373–378
5. Ricard, J., Gontero, B., Avilan, L., and Lebreton, S. (1998) Cell. Mol. Life Sci. 54, 1231–1248
6. Srere, P. A. (1987) Annu. Rev. Biochem. 56, 89–124
7. Droux, M., Ruffet, M. L., Douce, R., and Job, D. (1998) Eur. J. Biochem. 255, 235–245
8. Suss K. H., Prokhorenko, I., and Adler, K. (1995) Plant Physiol. 107, 1387–1397
9. Anderson, L. E., Goldhaber-Gordon, I. M., Li D., Tang, X., Xiang, M., and Prakash, N. (1995) Planta 196, 245–255
10. Nicholson, S., Easterby, J. S., and Powis, R. (1987) Eur. J. Biochem. 162, 423–431
11. Wedel, N., Soll, J., and Paap, B. K. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 10479–10484
12. Wedel, N., and Soll, J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 9699–9704
13. Avilan, L., Gontero, B., Lebreton, S., and Ricard, J. (1997) Eur. J. Biochem. 246, 78–84
14. McDonald, F. D., and Buchanan, B. B. (1987) in Photosynthesis (Amez, J., ed) pp. 175–197, New Elsevier Science Publishing Co., Inc., New York
15. Bowyer, J. R., and Leegood, R. C. (1997) in Plant Biochemistry (Dey, P. M., and Harbone, J. B., eds) pp. 49–110, Academic Press, Inc., San Diego, CA
16. Heldt, H. W., Werdan, K., Milevancev, M., and Geller, G. (1973) Biochim. Biophys. Acts 314, 224–241
17. Gardemann, A., Stitt, M., and Heldt, H. W. (1983) Biochim. Biophys. Acts 722, 51–60
18. Faske, M., Holtgrefe, S., Ochertina, O., Meister, M., Backhausen, J. E., and Scheibe, R. (1985) Biochim. Biophys. Acts 1247, 135–142
19. Jacquot, J. P., Lancelin, J. M., and Meyer, Y. (1997) New Phytol. 136, 543–570
20. Buchanan, B. B. (1980) Annu. Rev. Plant Physiol. 31, 341–374
21. Pradel, J., Soulé, J. M., Buc, J., Meunier, J. C., and Ricard, J. (1981) Eur. J. Biochem. 113, 507–511
22. Cadet, F., and Meunier, J. C. (1988) Biochem. J. 253, 249–254
23. Woosuk, A., Balleria, M. A., and Hagelin, K. (1993) PASEB J. 7, 622–637
24. Runquist, J. A., Harrison, D. H., and Miziaiko, H. M. (1998) Biochemistry 37, 1221–1226
25. Tabita, R. F. (1988) Microbiol. Rev. 52, 155–189
26. Porter, M. A., Stringer, C., and Hartman, F. C. (1988) J. Biol. Chem. 263, 123–129
27. Brandes, H. K., Larimer, F. W., and Hartman, F. C. (1986) J. Biol. Chem. 271, 3333–3335
28. Holmgren, A., and Bjornstedt, M. (1995) Methods Enzymol. 252, 199–208
29. Schurmann, P. (1996) Methods Enzymol. 252, 274–283
30. Scheibe, R. (1991) Plant Physiol. 96, 1–3
31. Lebreton, S., Gontero, B., Avilan, L., and Ricard, J. (1997) Eur. J. Biochem. 246, 85–91
32. Lebreton, S., Mulliert, G., Rault, M., Giulivi-Orticoni, M. T., and Ricard, J. (1993) Eur. J. Biochem. 217, 1075–1082
33. Rault, M., Lebreton, S., and Ricard, J. (1991) Eur. J. Biochem. 197, 791–797
34. Harris, E. H. (1989) The Chlamydomonas Source Book. A Comprehensive Guide to Biology and Laboratory Use, pp. 25–31, 1st Ed., Academic Press, Inc., San Diego, CA
35. Gontero, B. Cardenas, M. L. and Ricard, J. (1988) Eur. J. Biochem. 173, 437–443
36. Bradford, M. M. (1976) Anal. Biochem. 72, 248–252
37. Nelder, J. A., and Mead, R. (1965) Computer J. 7, 308–313
38. Marquardt, D. W. (1963) J. Soc. Ind. Appl. Math. 11, 431–441
39. Neet, K. E., and Ainslie, G. R. (1980) Methods Enzymol. 64, 192–226
40. Ricard, J., Notat, G., Got, C., and Borel, M. (1972) Eur. J. Biochem. 31, 14–24
41. Dalziel, K. (1957) Acta Chem. Scand. 11, 1706–1723
42. Lebreton, S., Gontero, B., Avilan, L., and Ricard, J. (1997) Eur. J. Biochem. 250, 396–392
43. Roestel, R. K., and Ogren, W. L. (1990) Plant Physiol. 93, 188–190