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Rapid Report

Enzymatic control of 3-phosphoglycerate reduction in chloroplasts

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The kinetic equation of 3-phosphoglycerate reduction in chloroplasts is presented. It is shown that, under sufficient lighting, the rate of 3-phosphoglycerate reduction may be estimated just on the basis of the measured activities of phosphoglycerate kinase and NADP-linked glyceraldehyde-3-phosphate dehydrogenase and [ATP], [ADP] and 3-phosphoglycerate concentrations.

While reducing PGA to triose phosphate, ATP and NADPH are utilized and so ADP, $NADP^+$ and P_i are regenerated in the chloroplast. The catalyzed reactions are as follows:

$$PGA + ATP \rightarrow DPGA + ADP$$

$$DPGA + NADPH + H^+ \rightarrow GAP + NADP^+ + P_i$$

$$GAP \rightarrow DHAP$$

The first reaction is catalyzed by phosphoglycerate kinase, the second by NADP-linked glyceraldehyde-3-phosphate dehydrogenase and the third by triosephosphate isomerase.

The concentrations of intermediate metabolites (DPGA and GAP) are very low in this system [1,2]. Using this feature of the enzyme system I have attempted to find the general rate equation for these three reactions.

The mechanism of phosphoglycerate kinase catalysis is not yet determined [3]. It is possible that there is a rapid equilibrium random mechanism [4]. From the general rate equation for this mechanism, a formula can be derived in accordance with the forward reaction equation from Ref. 4 and the assumption that the terms of the general rate equation numerator that include [DPGA] are equal to zero

$$\nu_1 = V_{1max}([ATP][PGA] - [DPGA][ADP]/K_{1e})/D_1 \quad (1)$$

where

$$D_1 = K_A(1 + [ATP]/K_m^{ATP} + [PGA]/K_m^{PGA} + [ADP]/K_m^{ADP} + [ATP][PGA]/K_A + [PGA][ADP]/K_B + [ATP][ADP]/K_C)$$

The actual rates are denoted by ν; maximum rates for each enzyme by V_{max}; equilibrium constants by K_e; apparent Michaelis constants by K_m; dissociation constants by K_S; K_A, K_B and K_C are complex constants. The reaction components are indicated by the corresponding superscript letters and the reaction number by subscript numerals. The constants: $K_m^{ATP} = 0.24$ mM, $K_m^{PGA} = 0.63$ mM, $K_m^{ADP} = 0.1$ mM, $K_A = 0.151$ mM^2, $K_B = 0.189$ mM^2 and $K_C = 0.048$ mM^2 were calculated from the data [4]. $K_{1e} = 7.59 \cdot 10^{-4}$ [5], $V_{1max} = 5$ mM/mg Chl per h [6].

The kinetic equation of the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase is described by Cerff [7]. I have reduced this equation in accordance with the assumption that the terms of the numerator that include [DPGA] and [GAP] are equal to zero:

$$\nu_2 = V_{2max}([DPGA][NADPH] - [GAP][P_i][NADP^+]/K_{2e}[H^+])/D_2 \quad (2)$$

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Abbreviations: DHAP, dihydroxyacetone-phosphate; DPGA, 1,3-diphosphoglycerate; GAP, glyceraldehyde 3-phosphate; PGA, 3-phosphoglycerate; P_i, inorganic phosphate.

where

$$D_2 = K_m^{dPGA}\left([NADPH] + \frac{K_m^{NADP^+}[P^+][NADPH]}{K_S^{NADP^+}K_m^{Pi}} + \frac{K_S^{NADPH}K_m^{GAP}[P_i][NADP^+]}{K_m^{Pi}K_S^{NADP^+}K_S^{GAP}}\right)$$

$K_S^{GAP} = 0.29$ mM and $K_S^{NADP^+} = 0.048$ mM [7]: $K_m^{DPGA} = 0.0075$ mM (mean value from [7]): $K_m^{Pi} - 5.5$ mM (taken from Ref 7 as a product inhibition constant). The constants $K_m^{GAP} = 0.29$ mM, $K_S^{NADPH} = 0.023$ mM and $K_m^{NADP^+} = 0.041$ mM are taken from Ref. 8 (1st form). $K_{2e}[H^+] = 9.764$ mM at pH = 7 [5]. $V_{2max} = 1.25$ mM/mg Chl per h [6].

The equation for the isomerization of GAP to DHAP has been characterized by Anderson [9].

$$v_3 = V_{3max}([GAP] - [DHAP]/K_{3e})/D_3 \quad (3)$$

where $D_3 = K_m^{GAP}(1 + [DHAP]/K_m^{DHAP})$, $K_m^{GAP} = 0.42$ mM and $K_m^{DHAP} = 1.1$ mM [9], $K_{3e} = 20.9$ [5], $V_{3max} = 6$ mM/mg Chl per h [6].

According to Ref. 10, from Eqns. 1–3 the general steady-state rate of PGA reduction (v) has been found.

$$v = \frac{K_{1e}K_{2e}[H^+]K_{3e}F_A[PGA]/[DHAP] - 1}{\frac{K_{3e}}{[DHAP]}\left(\frac{D_1K_{1e}K_{2e}[H^+]F_A}{V_{1max}[ATP]} + \frac{D_2K_{2e}[H^+]}{V_{2max}[P_i][NADP^+]} + \frac{D_3}{V_{3max}}\right)} \quad (4)$$

where

$$F_A = [ATP][NADPH]/[ADP][P_i][NADP^+]$$

F_A has been termed the assimilatory force [11].

It is necessary to have all metabolite concentrations and enzyme activities in order to compare the results of calculations with experimental data. We have not found such complete set of data. However, there is a good collection of data in the articles of Dietz and Heber [2,12], that may be used if supplementary suppositions are made. For example, the values of ([ATP] + [ADP]) and ([NADP$^+$] + [NADPH]) were taken from [6] and pH = 8 [5]. The temperature dependence of enzyme activities was expressed by formula $V_{max} = Q_{10} \cdot V_{max}$, where V_{max} is maximum rate at 25°C, Q_{10} is a thermal coefficient that is supposed to be equal to 2.

While using the concentrations of metabolites from Refs. 2 and 12 one can find out that the rate of PGA reduction depends linearly on [NADPH]/[NADP$^+$] only in the first region (Fig. 1). This peculiarity may be used to estimate the PGA reduction rate at low light [13]. Furthermore, this rate depends strongly on [P_i] (Fig. 1). While using the selected coefficients and concentrations of metabolites, the evaluated rate of PGA reduction becomes very low or negative as increasing P_i concentration to 1 mM and higher (not shown), if the ratios of NADPH to NADP$^+$ are between 2 and 9, as usually observed under strong light [11,14,15].

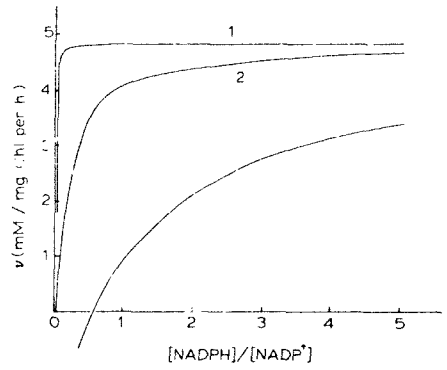

Fig. 1. Calculation of PGA reduction rate (Eqn. 4) as dependent on [NADPH]/[NADP$^+$] ratio at different values of [P_i]: (1) 10^{-4} mM; (2) 0.01 mM and (3) 0.1 mM. [PGA] = 2.4 mM, [DHAP] = 0.48 mM and [ATP]/[ADP] = 2.3 are from Refs. 2,12 at [CO_2] = 350 cm^3/m^3, 25°C and light equalling 200 W/m^2. The mean values of ([ATP] + [ADP]) = 2 mM and ([NADP$^+$] + [NADPH]) = 0.3 mM are taken from Ref. 6.

Though it is difficult to measure the P_i concentration directly in the chloroplasts, it is thought that P_i stromal concentration may be about 1 mM and above [16,17]. Therefore, the evaluated rate of PGA reduction must be small or even lower than the rate of the back reaction.

However, it should be noted that, when [P_i] was induced over the optimal level (several mM) in the intact chloroplasts, the rate of PGA reduction was decreased only slightly [18,19]. Therefore, one has to consider that change in [P_i] has insignificant influence upon the general reaction rate, i.e., the value [P_i] should be considered as very small.

At this moment we can explain this contradiction only through a supposition about low P_i concentration in the site of its binding with NADP-linked glyceraldehyde-3-phosphate dehydrogenase. The existence of a multienzyme complex which restricts the free access of P_i into it is the simplest explanation. The existence of a multienzyme complex at this stage is known [20]. However, the question of influence of P_i concentration upon the rate of PGA reduction requires further research.

The rate of PGA reduction changes insignificantly when [P_i] is very low, [NADPH]/[NADP$^+$] is over 0.5 and activities of enzymes and concentrations of other metabolites are within usually observed values (Fig. 1). Therefore, terms of Eqn. 4 containing [NADPH]/[NADP$^+$] and some others can be neglected in this

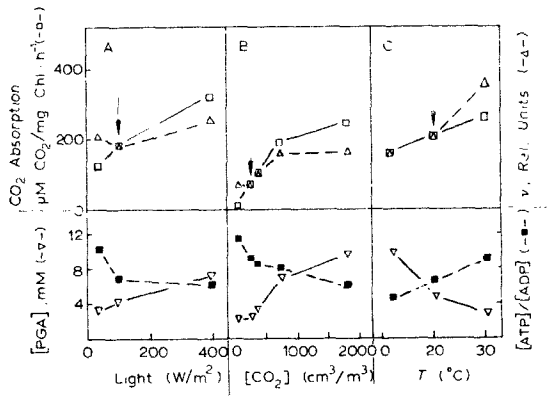

Fig. 2. Comparison of model calculations (Eqn. 4) in respect to relative rate of PGA reduction with the CO_2 absorption rate and concentration of corresponding metabolites from [2,12]. Arrows show the experimental points used for estimation of relative rate of PGA reduction equal to 1. The values [DHAP] and [NADPH]/[NADP$^+$] from Refs. 2, 12 are not shown, $[P_i] = 10^{-4}$ mM. (A) Light dependence. (B) CO_2 dependence. (C) Temperature dependence.

case. Then the steady-state rate can be approximated as:

$$v = \frac{k_{1e}k_{2e}[H^+][ATP][PGA]}{[ADP](D_1K_{1e}K_{2e}[H^+]/[ADP]V_{1max} + k_m^{DPGA}/V_{2max})} \quad (5)$$

The value of D_1 (Eqn. 1) depends only on [PGA], [ATF] and [ADP]. Therefore, the solution of Eqn. 5 depends only on the concentrations of these metabolites.

Only relative rates can be compared because the actual enzyme activities are not known in articles [2,12] (Fig. 2). The model solutions are in agreement with experimental data, with the exception of low light and low $[CO_2]$. If we ignore the P_i inhibition of PGA reduction, the differences between the solutions of Eqns. 4 and 5 are always lower than 2% (not shown).

At low light, the measured value of CO_2 assimilation is considerably lower in comparison with its estimated value (Fig. 2A). The analogous dependence could be obtained from data of Siebke et al. [17] (not shown). This discrepancy may be explained by deactivation of phosphoglycerate kinase and NADP-linked glyceraldehyde 3-phosphate dehydrogenase at low light, because the estimated value of D (Eqns. 4 and 5) has to be decreased while diminishing maximum enzyme activities. The decrease in the activities of these enzymes at low light is well known [6].

The reason for the discrepancy at low CO_2 concentration may be associated with the contribution of photorespiration to the rate of PGA reduction. It is known that a part of the metabolites formed at oxygenation of ribulose 1,5-bisphosphate returns into chloroplasts as PGA [6]. Therefore, the estimated rate of PGA reduction must exceed the rate of CO_2 assimilation because it includes regeneration rate of photorespiration metabolites. The participation of photorespiration is increased when the CO_2 concentration decreases. Therefore, the apparent CO_2 assimilation has to be decreased more than the rate of PGA reduction as the CO_2 concentration decreases.

It should be noted that Eqn. 5 agrees well with the dependence of O_2 evolution on [PGA] and the ratio [ATP]/[ADP] obtained by Robinson and Walker [21] for the reconstructed chloroplast system (not shown).

If the value V_{1max} changes proportionally to V_{2max}, then, instead of Eqn. 5, a more simple equation may be used to study of the relative rate changes:

$$v = SV_{2max}[PGA][ATP]/[ADP] \quad (6)$$

where S is a proportionality coefficient.

Calculated according to Eqn. 5 and Eqn. 6, the relative rates of PGA reduction change in one direction, but the quantitative solutions may be different.

However, a number of papers are known in which the PGA reduction rate is determined using the assumption that the reaction chain involved in reduction of PGA is close to the thermodynamic equilibrium and the value of the assimilatory force (F_A) is used as a motive force of reaction [2,11,12,17,22].

The attempt to prove this suggestion was based on the measurement of metabolite concentrations [2,14, 22]. However, the concentration of P_i and NADPH cannot be determined exactly [11,17,23]. Therefore, the conclusion of the thermodynamic equilibrium existence may be incorrect. The concept of assimilatory force cannot be used either, at least because redox ratios of NADPH to NADP$^+$ were usually between 2 and 9 at strong light [11,14,15] and as a result the PGA reduction rate should not depend linearly on this ratio (Fig. 1).

There was also a failure to observe any direct relationship between light-dependent changes of assimilatory force and photosynthetic flux. It was observed that F_A was often decreased when photosynthetic flux was increased due to higher light intensity [11,12]. The F_A value was also decreased while the CO_2 concentration was increased [17]. It is difficult to explain these data if we proceed from the assumption that F_A determines the photosynthetic flux. In my opinion, the attempt to use the changes in enzyme activities in order to explain those data [17] is incorrect because the assumption about existence of thermodynamic equilibrium excludes the influence of enzyme activities on general rate of the process.

It should be noted that a considerable deviation from equilibrium metabolite concentration was found recently in chloroplast stroma in the light [24].

It is possible that simple thermodynamic correlations can not be applied to the reductive pentose phosphate cycle [1].

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