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NON-CYCLIC PHOTOREDUCTIVE CARBON FIXATION IN PHOTOSYNTHESIS. LIGHT AND DARK TRANSIENTS OF THE GLYCERATE-3-P SPECIAL PAIR

Francis K. Fong and Karen A. Butcher

Department of Chemistry
Purdue University
West Lafayette, Indiana 47907

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SUMMARY: It is demonstrated that carbon fixation in photosynthesis is regulated in two kinetically coupled pathways involving the specialized pair of non-equivalent, enzyme-bound glycerate-3-P (3-PGA) molecules obtained from ribulose 1,5-bisphosphate (RuBP) carboxylation in the light. A non-cyclic pathway is suggested (reaction 2) for the direct biosynthesis of sucrose from the 3-PGA obtained from C-3, C-4 and C-5 of the six-carbon carboxylation adduct. Concomitant to the appearance of sucrose as the principal product, the Mg^{2+}-bound 3-PGA molecule formed from C-1, C-2 and C-2′ of the C_6 intermediate is released and subsequently reduced in regenerating the RuBP. It is proposed that the nocturnal inhibitor, 2-carboxyarabinitol-1-phosphate (1-PCA) is obtained from a condensation of 3-PGA and glyceraldehyde.

For more than thirty years, biochemists and plant physiologists have been brought up with the universal belief that the path of carbon fixation in plant photosynthesis occurs in the dark, according to the Calvin cycle detailed in the late fifties by J.A. Bassham and M. Calvin. In this communication we wish to demonstrate that the "dark" cycle, as described in all the current textbooks, is in fact unsupported by the experimental observations by Calvin and his co-workers, which instead corroborate a non-cyclic, light reaction for carbon fixation in plant photosynthesis.

An important clue to the path of carbon in photosynthesis is provided by the stereochemistry of reaction products in the carboxylation of RuBP (ribulose 1,5-bisphosphate). Two molecules of

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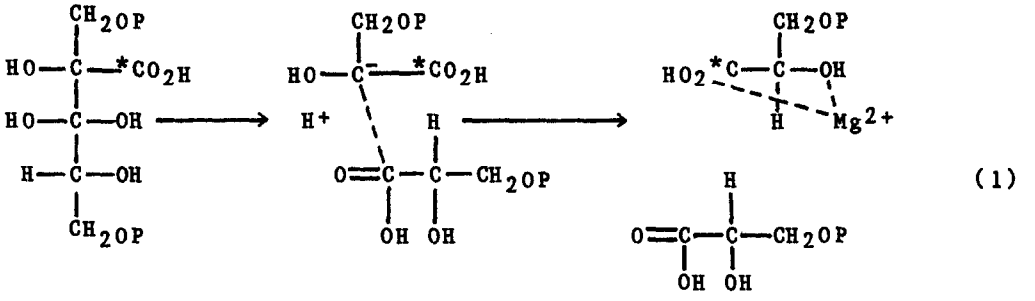
D-glycerate-3-P (3-PGA) are produced in the enzymatic hydrolysis of the six-carbon intermediate, 2-carboxy-3-keto-arabinitol 1,5-bisphosphate (CKABP), obtained from the carboxylation of RuBP (ribulose 1,5-bisphosphate).[1,2] Based on this fact, we proposed[3] a reaction pathway in which CKABP undergoes an alkoxide cleavage to form a specialized pair of non-equivalent 3-PGA molecules bound to the active site of the RuBP carboxylase (RuBPCase):



(1)

Hydrated 2-Carboxy-3-keto-arabinitol 1,5-bisphosphate

Carbanion Intermediate

Glycerate-3-P Special Pair

in which the newly assimilated carbon is labeled *C. A stereochemical inversion at C-2 results from the conversion of CKABP to the 3-PGA special pair.[3] The proposed binding interactions of the upper 3-PGA molecule to a divalent metal cation in the unsymmetrical pair are consistent with experimental observations on an enzyme-Cu^{2+}-3-PGA complex containing two molecules of 3-PGA.[4]

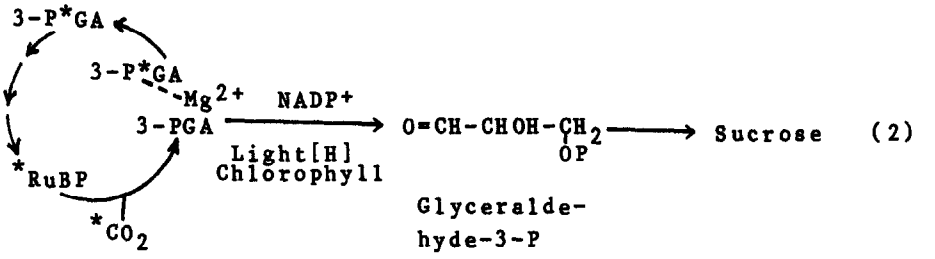
In examining the biochemical consequences of the asymmetric configuration of the 3-PGA special pair, we consider the recent identification of 2-carboxyarabinitol 1-P (1-PCA), an inhibitor [5,6] that binds to the active site of the RuBP carboxylase. In addition, we re-analyze the following important conclusions drawn

from two sets of experiments published by Calvin et al:

(a) **Experiments in which all carbon atoms of the intermediate compounds are saturated with ^{14}C** (Fig. 5, Ref. 7). [1] Wilson and Calvin concluded[7] from these experiments that the observed total carbon disappearing from RuBP quantitatively accounts for the sum of new carbon incorporated into sucrose and 3-PGA. Clearly, as correctly noted by Wilson and Calvin,[7] *in vivo* RuBP carboxylation in the light does not result in the release of two free 3-PGA molecules, as was demonstrated *in vitro*.[1,2] [2] We confirm that the reciprocal changes in reservoir sizes of RuBP and 3-PGA relate the production of 3-PGA from RuBP carboxylation to the regeneration of RuBP on 3-PGA reduction. [3] We further conclude that, in addition to this cyclic pathway, a monotonic increase in the new carbon content in sucrose is observed. This steady increase contrasts the fluctuating reservoir sizes of RuBP and 3-PGA, and supports a *non-cyclic pathway as the principal mechanism for energy storage in the photosynthetic conversion of sunlight into non-volatile fuel molecules*.

(b) **Carbon labeling experiments carried out with short exposures to $^{14}CO_2$ to obtain selective labeling of compounds and carbon atoms** (Figs. 3 and 4, Ref. 8). [1] From these experiments we note that the newly incorporated radiocarbon occurs principally in the 3-PGA appearing in the earliest product distribution. [2] The rapid appearance of sucrose as the principal product, as above described in paragraph a, is not observed in these experiments. Products from the unlabeled, lower 3-PGA in reaction 1 would not be detectable in experiments using short exposures of $^{14}CO_2$. We accordingly conclude that the upper (labeled) and lower molecules of the 3-PGA special pair respectively give rise to the simultaneous appearance of 3-PGA and sucrose.

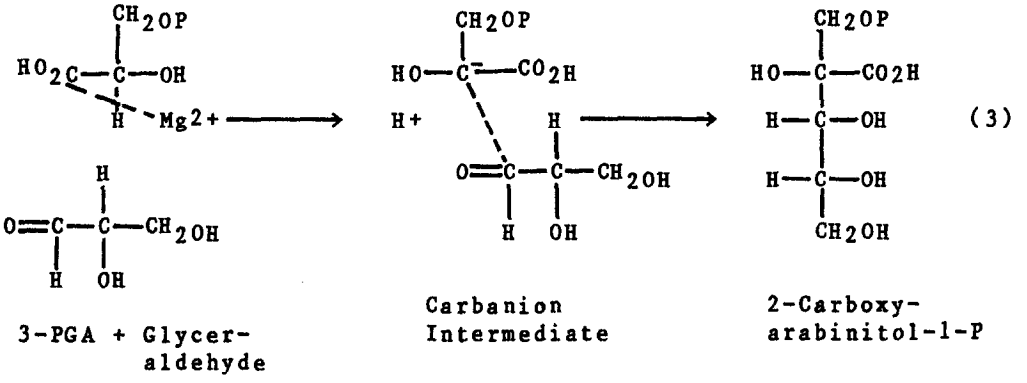
The collective conclusions in a and b make possible a molecular description for the path of carbon:



in which the cyclic conversion of 3-P*GA to *RuBP would require the reduction of 3-PGA to glyceraldehyde-3-P, followed by an appropriate sequence of transaldolase,[9] epimerase and kinase reactions. In reaction 2 the asymmetric arrangement of the special pair enables a branching of two different reductive 3-PGA pathways. From this point the lower (unlabeled) 3-PGA molecule exits the cyclic path of 3-P*GA, to yield sucrose. Meanwhile the upper, labeled 3-PGA is returned to the metabolic pool of the reductive pentosephosphate cycle. Thus the Mg^{2+} chelation of the upper 3-PGA in the special pair[3] gives rise to two separate 3-PGA pools, and the resultant non-equivalence in the labeling of the 3-PGA in the cyclic and non-cyclic paths conceivably accounts for the asymmetrical labeling reported by Kandler and Gibbs.[10,11]

The concentration of the nocturnal inhibitor, 1-PCA, varies according to a diurnal pattern, such that the activity of CO_2 fixation is low at nightfall and maximal at midday.[5,6] The biosynthesis of 1-PCA from the reaction transients of RuBP carboxylation is therefore relevant. The similarity between 1-PCA and CKABP is obvious. However, the reduction of the keto group at C-3 of CKABP would result in 2-carboxyarabinitol 1,5-bisphosphate (CABP), which would irreversibly bind to RuBPCase, permanently inactivating it.[6] The removal of the C-5 phosphate group prior to binding of the 1-PCA to RuBPCase is thus essential in order for

1-PCA to function as a regulator of the enzyme activity. Reaction 2 suggests that 1-PCA could conceivably result from a back addition of 3-PGA to the carboxylic group of glyceraldehyde



(3)

in which an inversion at C-2 of 1-PCA is obtained by means of a carbanion adduct, consistent with a microscopic reversal of a mechanism similar to that shown in reaction 1.

The Calvin cycle has become recognized[3,9,12-15], occasionally but widely through the decades, to be "irreconcilable"[12] with biochemical data accumulated ever since the fifties, when Bassham and Calvin dismissed[16] the evidence[7] for the photoreductive carboxylation of RuBP. We have presented above a pathway for reaction transients that is corroborated by experiment, in which the path of carbon fixation is presumably mechanistically, as well as stoichiometrically, coupled to the photolytic splitting of water by the chlorophyll light reaction. We are cognizant of the requirement of a supermolecular mechanism involving the lower 3-PGA in reaction 2 relative to triose phosphate transport and cytosolic localization of sucrose synthesizing enzymes outside of the chloroplast.

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