Photosynthesis : Dark reaction

Carbon dioxide fixation Pathway

Dark reaction is light independent enzyme mediated series of reactions in photosynthesis. In this step, NADPH and ATP produced in the light reaction are utilized along with carbon dioxide taken from atmosphere.. It is also known as Blackman reaction or carbon dioxide fixation pathway. In fact, Blackman was the first person who indicated that photosynthesis dark reaction besides light reaction. Later on it was established that dark reaction takes place in the stroma region of the chloroplast.

Mechanism of carbon dioxide (CO_2) fixation was first established by the landmark discovery made by Melvin Calvin, Andrews Benson, James Bassham and others at the University of California from 1946 to 1953, who identified the intermediate compounds in the dark reaction. They did their experiments using culture of a unicellular green alga, *Chlorella* and proposed a cyclic chain of reactions, which is called as Calvin cycle or Calvin Benson cycle. For his great work, Calvin was awarded Nobel Prize in the year 1961.

Calvin and his team workers used a unicellular alga *Chlorella pyrenoidosa*, which contains a well distinguished chloroplast and performs photosynthesis like higher plants. The advantage of using *Chlorella* was that the alga grows well in water medium and could be easily handled. It multiples fast and grows luxuriantly in diverse conditions.

Calvin *et al.*, used CO_2 labelled with Isotopic carbon (C^{14}) in their studies. They fed Chlorella with $C^{14}O_2$ providing all required factors in photosynthesis. Chlorella cells were grown in a flask and were removed at 1/16 to 5 seconds of intervals in the boiling alcohol. The collected samples were homogenized and chromatographic studies were done to detect the compounds formed at regular interval of time. It was found that 3 phosphoglyceric acid (3PGA) was the first stable compound formed in the dark reaction. They identified that, a five carbon sugar phosphate Ribulose1, 5- bisphosphate (RuBP) was the initial acceptor of CO_2 in the cycle. Their work explained the synthesis of sugars and regeneration of RuBP.

Since, the first stable intermediate compound in the dark reaction is 3PGA, a three carbon compound, this cycle was named as C_3 cycle.

Further researches were carried on by different workers on different higher plants, it was found that some other pathways of CO_2 fixation do also exist, Viz., Cane type photosynthesis / C_4 cycle or Hatch and Slack pathway found in sugar cane plants, Crassulacean acid Metabolism (CAM) Cycle in succulent plants of *Crassulaceae* family.

Therefore, there are following three types of CO_2 fixation pathway in photosynthesis:-

1. Calvin cycle (C_3 plants)

2. Hatch and Slack cycle (C_4 plants)

3. CAM cycle (CAM plants)

Calvin cycle (C_3 cycle)

Fixation of atmospheric carbon dioxide in photosynthesis by C_3 plants is known as Calvin cycle. This is the most prevalent pathway of CO_2 fixation on the earth. This is also named as Bassham-Calvin cycle, Calvin-Benson cycle, C_3 cycle or Reductive pentose phosphate cycle.

This cycle is divided into three steps of reactions:-

1. Carboxylation

2. Reduction / Glycolytic reversal

3. Regeneration of Ribulose1, 5-bisphosphate

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Carboxylation-

Addition of carbon dioxide to a compound is called as carboxylation. This step includes carboxylation of 6molecules of RuBP by 6 molecules of CO_2 in presence of an enzyme ***Carboxy dismutase*** or ***Ribulose phosphate carboxylase (RubisCo***). As a result of carboxylation twelve molecules of Phosphoglyceric acid. The enzyme *RubisCo* is the most abundant enzyme on the earth.

RuBP (6 mol) $+6CO_2+6H_2O$---***RubisCo***----→ 3-Phosphoglyceric acid (12 mol.)

Reduction (Glycolytic reversal)

This step includes some of the reactions in photosynthesis, which are just reversal of glycolysis of respiration.

Twelve molecules of 3PGA are phosphorylated by 12 molecules of ATP in presence of enzyme ***Phospho- glycerate kinase (PGK).***In this step 12 molecules of ADP are produced.

3PGA + ATP---------- ***PGK***----------→ 1, 3- Bisphosphoglycerate (BPG) +ADP

In the next step 12 molecules of 1, 3-bisphosphoglyceric acid are reduced to 12 mol. of 3-phosphoglyceraldehyde by utilizing 12 mol. of $NADPH+H^+$ obtained from light reaction. In this step 12 mol. of inorganic phosphate (Pi) are released

1, 3-BPG is reduced by NADPH+H^+ by the enzyme ***Glyceraldehyde-3-phosphate dehydrogenase (Gly3PD).***.

1, 3-BPG+ NADPH+H^+---------***Gly.3PD***---→ Glyceraldehyde-3-phosphate (GAP) + Pi

3PGAldehyde/GAP is isomerised to Dihydroxy acetone phosphate in presence of enzyme ***Triose phosphate isomerase (TPI).***

3Phosphoglyceraldehyde (PGAld) ←-------***TPI***--------→ Dihydroxy acetone phosphate (DHAP)

3 molecules of 3-PGAld and 3 mol. of DHAP combine to form 3 mol. of fructose 1, 6-bisphosphate (Hexose sugar), catalyzed by the enzyme aldolase.

3PGAld + DHAP---------***Aldolase***-----→ Fructose1, 6-bisphosphate

Each fructose 1, 6-bisphosphate loses one phosphate with the help of enzyme phosphatase.

Fructose1, 6-bisphosphate + H_2O--------***Phosphatase***--→ Fructose- 6-phosphate+Pi

Out of 3 molecules of Fructose-6-phosphate one mol. is converted to other sugars and further to starch.

Regeneration of Ribulose bisphosphate-

(i) 2 mol. of Fructose- 6-phosphate combine with 2 mol. of 3PGAld to produce 2 mol. of Xylulose-5-phosphate and 2 mol. of Erythrose-4-phosphate. This reaction is catalyzed by the enzyme ***Transketolase.***

Fructose-6-p +3 PGAld**----------*Transketolase***----------→ Eryhrose-4-p + Xylulose-5-p

(ii) 2 molecules of erythrose-4-phosphate combine with 2 molecules of dihydroxy acetone phosphate to produce 2 molecules of Sedoheptulose- 1, 7-bisphosphate.This reaction is catalyzed by the enzyme ***Transaldolase.***

Dihydroxy acetone phosphate + Erythrose-4-phosphate-------***Transaldolase***---→Sedoheptulose-1, 7- bisphosphate

(iii) Each molecule of Sedoheptulose -1, 7-bisphosphate phosphate is dephosphorylated by the enzyme ***Sedoheptulose bisphosphatase*** to produce Sedoheptulose-7-phosphate.

Sedoheptulose-1, 7-bisphosphate---***Sedoheptulose bisphosphatase***---=→ Sedoheptulose-7-phosphate+ Pi

(iv) In the next step, 2 molecules of Sedoheptulose-7-phosphate combine with two molecules of 3-PGAldehyde to produce two molecules each of ribose -5-Phosphate and xylulose-5-phosphate.This reaction is catalysed by the enzyme Transketolase.

Sedoheptulose-7-p + 3 PGAld-------**Transketolase**----→ Ribose-5-p + Xylulose-5-p

(v) **2 molecules** of ribose-5-p are isomerised to **2 molecules ribulose-5-p** by the enzyme ***Ribose phosphate isomerase.***(**RPI**)

Ribose-5-p←====== (**RPI**) ==========➔**Ribulose-5p** (**2 molecules**)

(vi) **4 molecules** of xylulose-5-p are converted to **4 molecules of ribulose-5-p** by the enzyme ***Ribulose phosphate epimerase*** (**RPE**). In this reaction, the HO-C-H group of third carbon of xylulose-5-p is rotated in its epimeric form as H-C-OH.

Xylulose-5-p←==== (**RPE**) ==========➔**Ribulose-5-p** (**4 molecules**)

(vii) Thus, generated **6 molecules of ribulose-5-p** are phosphorylated by **6 molecules of ATP**, which were produced during the light reaction.

Ribulose-5-p is converted to Ribulose-1, 5-biphosphate.

Ribulose -5-p + ATP----------→ Ribulose1, 5-biphosphate

Now, Ribulose1, 5-bisphosphate is regenerated and ready to accept CO_2 in the next cycle.

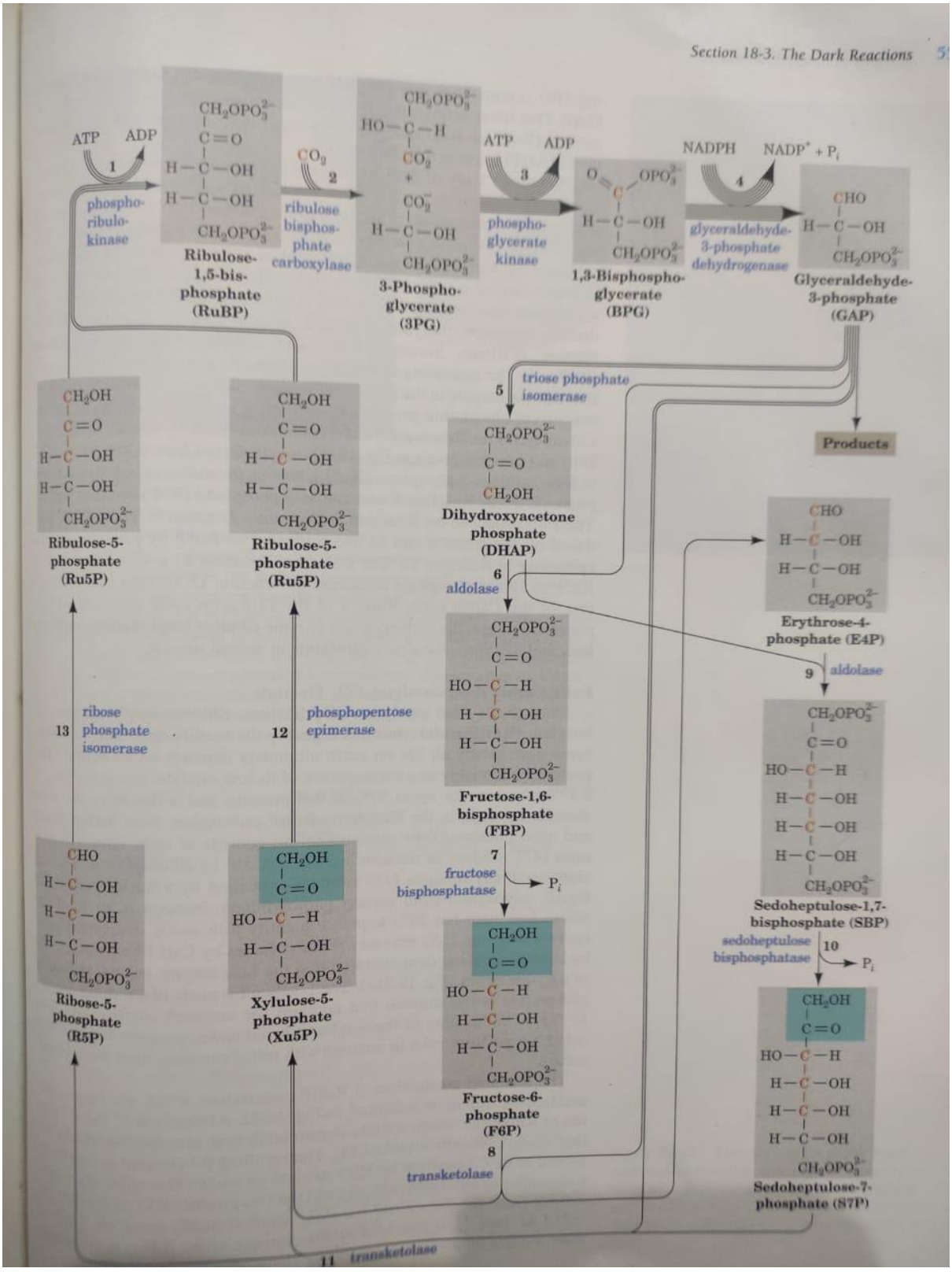

Fig. Calvin cycle (courtesy; Fundamentals of Biochemistry: Voet, Voet & Pratt)