Biochemistry Problem

Fluhardy Pharmaceuticals has just sent you a sample of mythomcyin, a newly discovered antibiotic that inactivates **one** enzyme involved in carbohydrate metabolism. Although they omitted to inform you which enzyme was inhibited by mythomycin, they did include the following information.

When yeast are grown anerobically, i.e., no O_2 is present, in the absence of the antibiotic and glucose is the sole carbon source the ATP/glucose ratio is 2.00. In contrast, when the yeast are grown anerobically in the presence of the antibiotic, the ATP/glucose is 1.67.

When the yeast are grown with ^{14}C-glucose labeled in the following positions, and the position of the label in pyruvate that is formed is analyzed, the following data are obtained.

	Position of ^{14}C-label in pyruvate	
Radiolabeled glucose	Antibiotic Absent	Antibiotic Present
Glucose-1-^{14}C	C3	NONE
Glucose-2-^{14}C	C2	C1 and C3
Glucose-3-^{14}C	C1	C1 and C2
Glucose-4-^{14}C	C1	C1
Glucose-5-^{14}C	C2	C2
Glucose-6-^{14}C	C3	C3

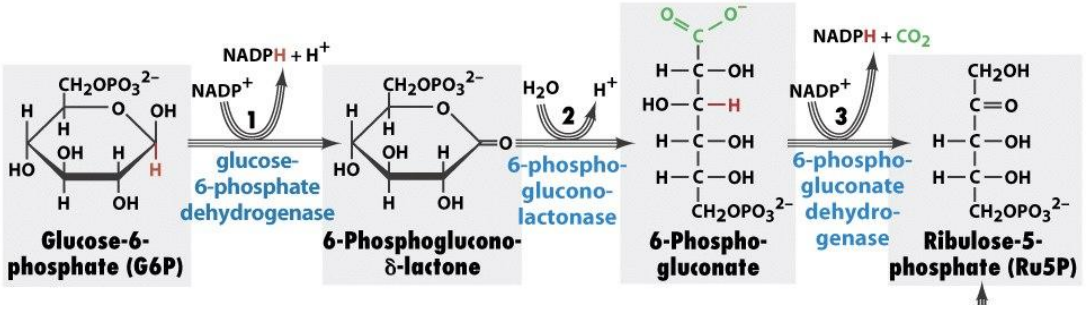
[Note: yeast are capable of synthesizing and degrading glycogen, they perform glycolysis, gluconeogenesis, and the hexose monophosphate shunt (phosphogluconate pathway.]

Based on the data provided above, indicate which enzyme is inhibited. Show your work and discuss your reasoning. (10 pts)

Solution:

Key Observations:

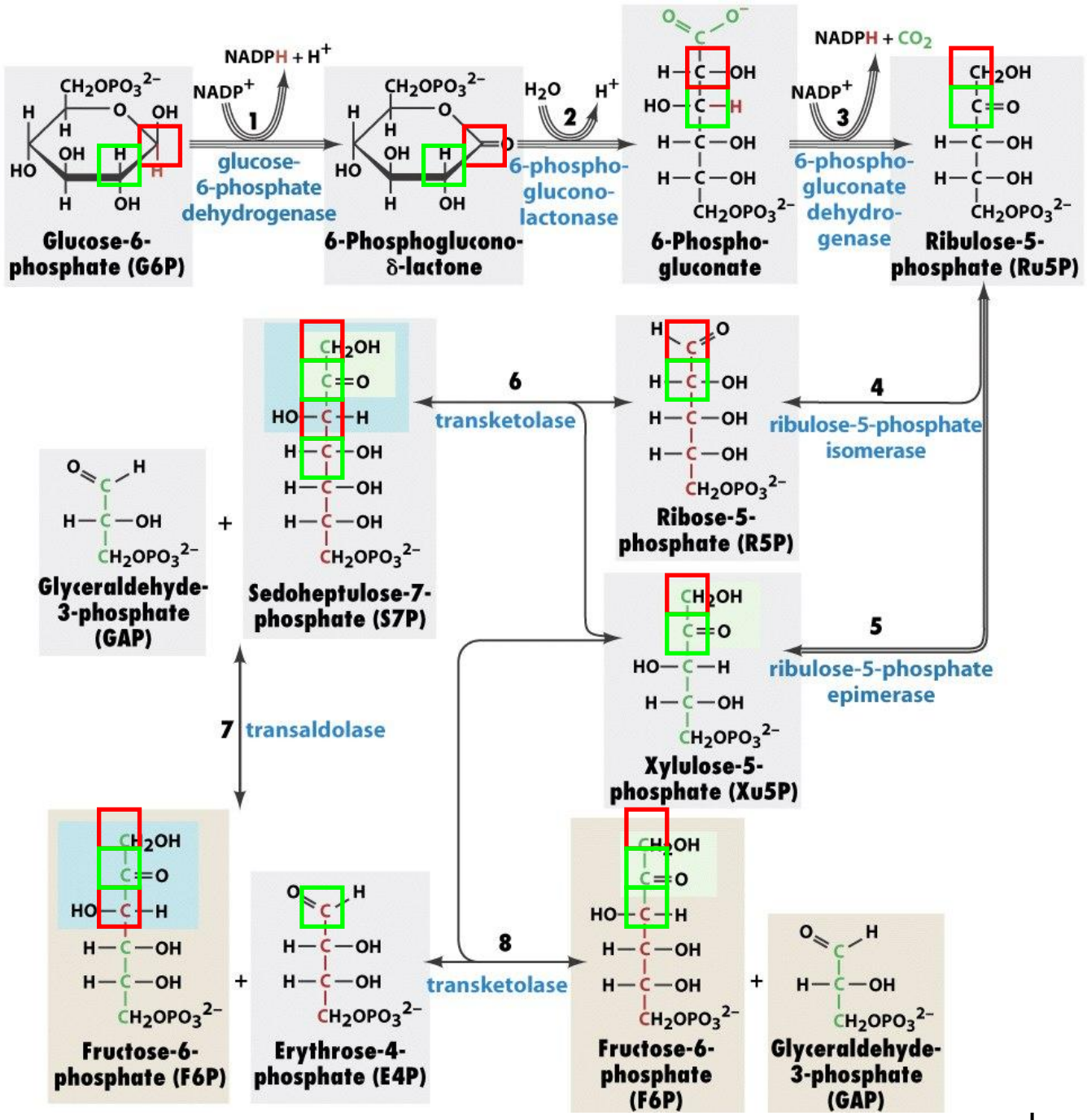
- In absence of antibiotic, ATP/glucose ratio is 2.00 or 6 ATP for every 3 glucose, which is the normal ATP yield for glycolysis.
- In the presence of antibiotic, ATP/glucose ratio is 1.67 or 5 ATP for every 3 glucose, which is the normal ATP yield if glucose metabolism completely channeled through hexose monophosphate shunt.
  - Net reaction of hexose monophosphate shunt:
    $3G6P + 6NADP^+ + 3H_2O \rightarrow 6NADPH + 6H^+ + 3CO_2 + 2F6P + GAP$
    - 2 F6P produce 3 ATP when funneled through glycolytic pathways
    - 1 GAP produce 2 ATP when funneled through glycolytic pathways
    - 5 ATP produced for every 3 glucose
- Glucose-1-^{14}C does not appear in pyruvate in the presence of antibiotic. This oxidative decarboxylation of glucose at the 1 position is characteristic of the transition from 6-phosphogluconate to ribulose-5-phosphate (catalyzed by 6-phosphogluconate dehydrogenase).



Hypothesis: Inhibition of **phosphoglucose isomerase (PGI)**

The antibiotic inhibits an enzyme that allows all glucose to be funneled into the hexose monophosphate shunt in place of proceeding through the sequences of steps in glycolysis. Because the hexose monophosphate shunt converts G6P to F6P, then glycolysis must not be isomerizing G6P directly into F6P. This would indicate an inhibition in the phosphoglucose isomerase (PGI).

I will confirm this hypothesis by reproducing experimental results using proposed carbohydrate metabolic pathway in presence of antibiotic. Because (1) it is apparent that glucose labeled at the 4, 5 and 6 positions do not change positions in pyruvate in presence or absence of antibiotic, and (2) I discussed a mechanism by which all carbons in the 1 position of glucose are excised, I will verify the experimental results for positions 2 and 3. [next page]



Outcomes:

- The 1 position on F6P becomes the 3 position on pyruvate, which is occupied by a carbon label originally at the 2 position of glucose. This agrees with the experiment.
- The 2 position of F6P becomes the 2 position on pyruvate, which is occupied by a carbon label originally at the 3 position of glucose. This agrees with the experiment.
- The 3 position of F6P becomes the 1 position on pyruvate, which is occupied by a carbon label originally at the 2 or 3 position of glucose. This agees with the experiment.