

Phosphofructokinase (PFK) Exercise

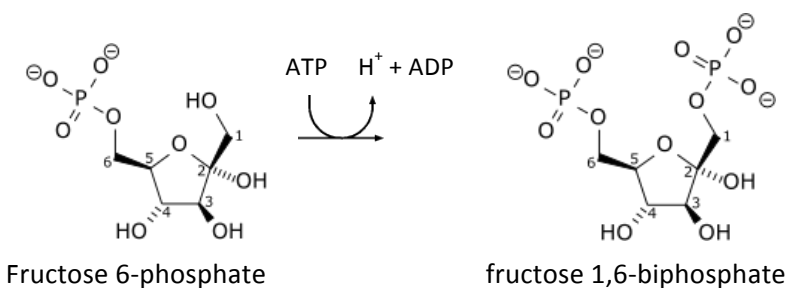
Learning Objectives

In this exercise, you will use StarBiochem, a protein 3D viewer, to explore the structure and function of the enzyme phosphofructokinase (PFK).

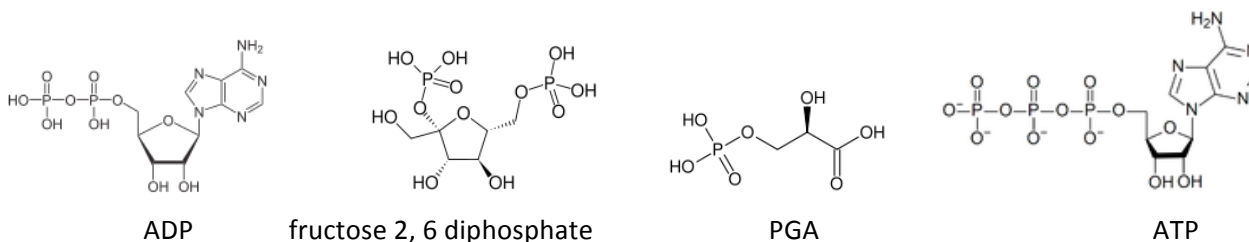
Background

Living organisms, both unicellular and multicellular, require energy to live and function. Energy is stored and provided in the form of adenosine triphosphate (ATP). ATP is produced by multiple cellular processes including glycolysis, as well as aerobic and anaerobic respiration.

PFK is the most important regulatory enzyme of glycolysis. It irreversibly catalyzes the rate-limiting step of glycolysis: the conversion of fructose 6-phosphate and ATP to fructose-1, 6-diphosphate and ADP.



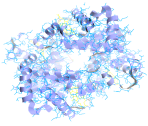
PFK exists as a homotetramer in bacteria and mammals. Each of the four monomers of PFK is composed of two similar domains. One domain has an ATP binding pocket. The other domain contains the substrate-binding site and the allosteric site. An allosteric site is a regulatory binding site that affects enzyme activity and is distinct from the substrate-binding site of an enzyme. Various activators and inhibitors regulate PFK. Examples of PFK activators are ADP, AMP and fructose 2, 6 diphosphate. Examples of PFK inhibitors are ATP, phosphoglycerate (PGA) and citrate.



Getting started with StarBiochem

- To begin using StarBiochem, please navigate to: <http://web.mit.edu/star/biochem>.
- Click on the **Start** button to launch the application.
- Click **Trust** when a prompt appears asking if you trust the certificate.
- Under **Samples**, click on **Select from Sample** and select "Phosphofructokinase – E. coli (2PFK)" and click **Open**.

You are now viewing the structure of PFK (2PFK) with each bond in the protein drawn as a line ("bonds only" view). You will see two independent PFK structures within the 2PFK file. This is an artifact of how the structure was determined. Focus on one of the two structures while answering the questions in this exercise.



Protein Structure Questions

1 How many amino acids comprise PFK (2PFK)?

Answer

2 How many monomers/chains do you see in the current view of PFK (2PFK)?

Answer

3 Does the current view of PFK (2PFK) represent the active form of this enzyme? Answer Yes /No and explain your answer.

Answer

4 Let us highlight amino acids #195, #196 and #224 in the polypeptide chain.

a) What is the most likely type of interaction between amino acids #196 and #224? Your choices are 'hydrogen bond', 'ionic bond', 'hydrophobic interaction', 'covalent bond', 'peptide bonds' or 'van der Waals forces'.

Answer

b) What is the most likely type of interaction between amino acids #195 and #196? Your choices are 'hydrogen bond', 'ionic bond', 'hydrophobic interaction', 'covalent bond', 'peptide bonds' or 'van der Waals forces'.

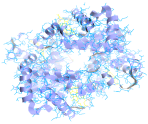
Answer

c) Draw amino acids #195 and #196 and circle the reactive group(s) or atoms that account for their interaction. Explain why you selected these groups (atoms).

Answer

d) Are these three amino acids part of the same or different polypeptide chain?

Answer



e) Highlight amino acids #241 and #261. **What is the most likely type of interaction between these two amino acids?** Your choices are 'hydrogen bond', 'ionic bond', 'hydrophobic interaction', 'covalent bond', 'peptide bonds' or 'van der Waals forces'.

Answer

5 Individual amino acids fold into different local secondary structures.

a) **Name the different secondary structures observed in PFK (2PFK)?**

Answer

b) **Please list the number of individual structures you observe for each of the secondary structures in PFK (2PFK). Focus on just one of the PFK monomers.**

Answer

c) A cleft is found between the two domains that form the active site within each monomer. This cleft is formed by two sheets which are angled towards each other. **Identify the sheets within the structure that contribute to the formation of the active site.** Choose only one of the two independent PFK structures when answering this question.

Answer

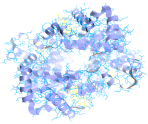
d) We will now examine one of the sheets found within PFK (2PFK): S1C. **Is S1C an example of a parallel or an anti-parallel sheet?**

Answer

6 Explore the tertiary structure of PFK (2PFK) and its relationship with the environment.

Why is the observed distribution of non-polar amino acids within PFK (2PFK) critical for the localization and function of this enzyme in the cytosol?

Answer



Protein Structure -> Function Questions

7 We will now explore PFK's catalytic activity. To do this we will compare the structure of PFK in different states.

a) *Which of these three structures represents the resting (ligand-unbound) state of PFK?* Explain your answer.

Answer

b) Now compare the structure of the two remaining molecules that you did not select while answering the question above (7a). *Which of the two structures represents the active state and which one represents the inactive state of PFK?* Explain your choices.

Answer

Keywords:

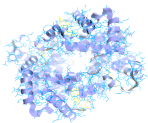
Enzymes, regulatory/rate-limiting reactions, glycolysis, aerobic respiration, anaerobic respiration, catalytic reactions, reversible and irreversible reactions, allosteric modulators, allosteric activators, and allosteric inhibitors.

Thought Questions

1 Deficiency in PFK leads to Tarui's disease. This disorder is characterized by severe nausea, vomiting and muscle cramps. Patients with this genetic disorder are advised not to exercise vigorously. Explain why reduced physical activity can help these patients.

2 Activity of PFK influences the production of ATP both by aerobic and anaerobic respiration. Explain why this is so.

3 ATP is required for the reaction catalyzed by PFK. However, ATP is also an inhibitor of PFK. How could ATP perform two different regulatory effects on the same enzyme?



PROTEIN STRUCTURE BASICS

Each protein has the following three levels of protein structure:

Primary structure

Lists the amino acids that make up a protein's sequence, but does not describe its shape.

Secondary structure

Describes regions of local folding that form a specific shape, like a helix, a sheet, or a coil.

Tertiary structure

Describes the entire folded shape of a whole protein chain.

In addition, some proteins interact with themselves or with other proteins to form larger protein structures. How these proteins interact and fold to form a larger protein complex is termed **Quaternary structure**.

CHEMICAL STRUCTURES OF THE AMINO ACIDS

The 20 amino acids share a common backbone and are distinguished by different 'R' groups, highlighted in various colors below.

