

## Case 18

# Purification of Phosphofructokinase 1-C

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*Last modified 19 March 2004, K. Cornely, Providence College*

### Focus concept

The purification of the C isozyme of PFK-1 is presented and the kinetic properties of the purified enzyme are examined.

### Prerequisites

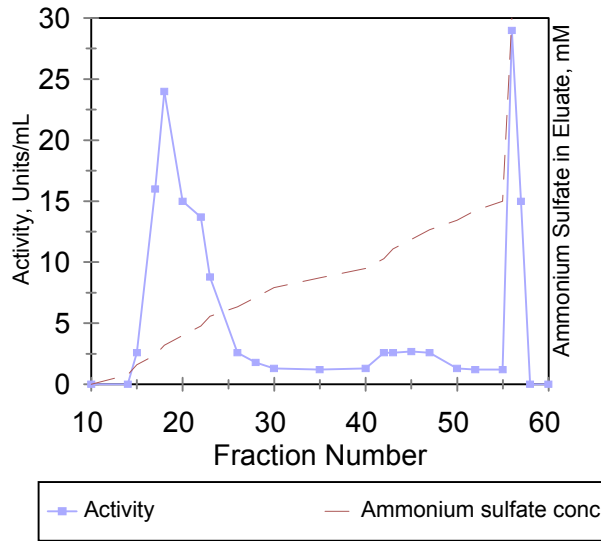
- C Protein purification techniques.
- C Enzyme kinetics and inhibition.
- C The glycolytic pathway.

### Background

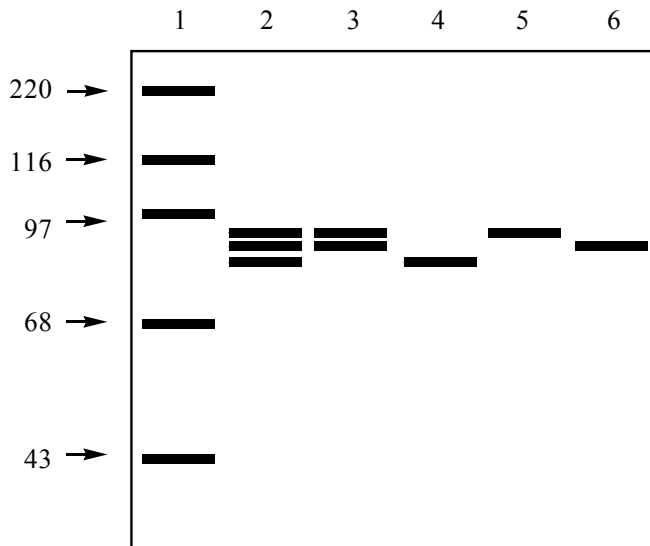
In this case, Foe and Kemp purified the isozyme phosphofructokinase-1 C (PFK-1 C) from brain tissue. There are three isozymes of PFK-1 and they are designated A, B, and C. The A isozyme ( $M_r = 84,000$  dal) is found in the muscle and the brain; the B isozyme ( $M_r = 80,000$  dal) is found in the liver and the brain; and the C isozyme ( $M_r = 86,000$ ) is found in the brain. Because the brain contains all three isozymes and there isn't a location where the C isozyme is found exclusively, the enzyme has been difficult to purify. In this case, the investigators purified the desired enzyme to homogeneity, and also presented ample evidence that the C isozyme is distinct from the A and B isozymes. The availability of a pure C preparation means that antibodies can be generated which can be used to detect the isozyme. Since the levels of PFK-1 isozymes have been shown to change during malignant transformation of cells, the availability of a C antibody might be a valuable diagnostic tool.

### Questions

1. To accomplish the purification, rabbit brain tissue was homogenized and centrifuged to remove insoluble material. Next, the soluble preparation was loaded on top of an ATP-Sepharose column. This is an affinity column in which ATP is covalently linked to a polysaccharide bead. The sample is loaded on top of the column, washed with a low-salt buffer, followed by a wash with a high salt buffer. What is the rationale for using this procedure? Draw a diagram of the expected elution profile.
2. Next, the fractions containing PFK-1 activity were applied to a DEAE-Sephadex (anion exchange) column. The column was equilibrated with a pH = 8.2 buffer. The column was eluted with a salt (ammonium sulfate) gradient and the results are shown in Figure 18.1. Using the elution profile as well as the results from SDS-PAGE analysis shown in Figure 18.2, identify which isozyme is found in each of the two peaks. How might the amino acid composition of PFK-1 B differ from that of PFK-1 A and C based on the manner of elution from the DEAE column?

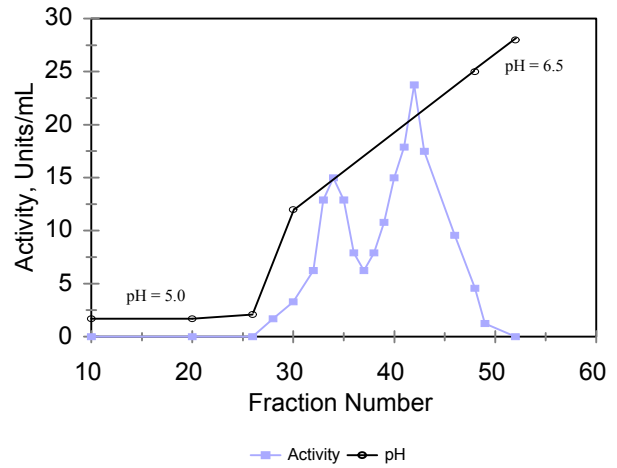


**Figure 18.1:** Purification of PFK-1C by DEAE Sephadex (anion exchange) chromatography. (Based on Foe and Kemp, 1985.)



**Figure 18.2:** SDS-PAGE analysis of the PFK-1C purification. Lane 1: Molecular weight standards. Lane 2: Eluant from the ATP-Sepharose affinity column. Lane 3: Pooled fractions 16-26 from the DEAE-Sephadex column. Lane 4: Pooled fractions 55-60 from the DEAE-Sephadex column. Lane 5: Fractions 30-35 from the CM-52 column (elution profile shown in Figure 18.3). Lane 6: Fractions 41-50 from the CM-52 column. (Based on Foe and Kemp, 1985.)

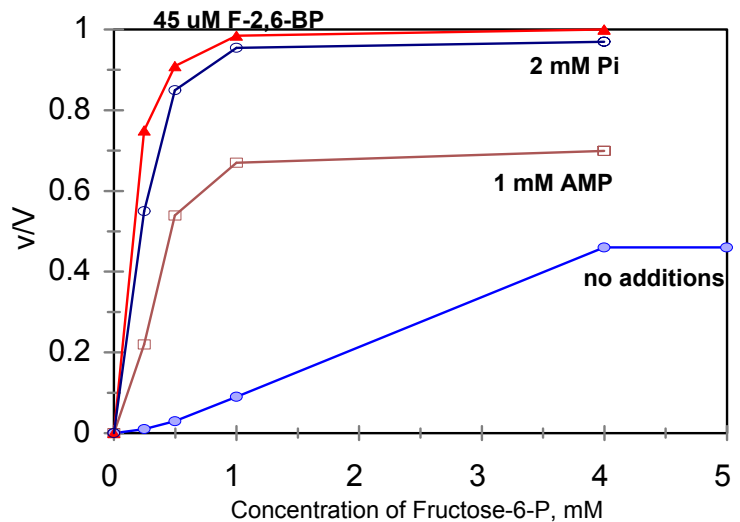
3. Next, the investigators took Fractions 16-26 from the DEAE-Sephadex column, pooled them, and adjusted the pH to 5.0. This preparation was then loaded onto a CM-52 (cation exchange) chromatography column and eluted with a pH gradient. Fractions 30-35 were collected and pooled, as were Fractions 41-50. Identify the peaks in the chromatogram in Figure 18.3, using information in the SDS-PAGE gel. Based on their elution from the cation exchange column, how might the amino acid compositions of these two proteins differ?



**Figure 18.3:** Cation exchange chromatography of PFK-1. (Based on Foe and Kemp, 1985.)

4. Write the reaction catalyzed by PFK-1.  
 5. There are several allosteric effectors that influence the activity of PFK-1 in the cell. What are they? List both activators and inhibitors of the enzyme.

6. The investigators next carried out kinetic studies using their newly purified PFK-1C isozyme. They studied the catalytic behavior of the enzyme in the presence of the metabolites AMP, inorganic phosphate ( $P_i$ ) and fructose-2,6-bisphosphate (F-2,6-BP). The results are shown in Figure 18.4. Additional information concerning the three isozymes' response to allosteric effectors is presented in Tables 18.1 and 18.2.



**Figure 18.4:** Activation of PFK-1C.  $v$  is  $V_{max}$  and is defined as the activity at 0.5 mM ATP, 10 mM fructose-6-phosphate and 2 mM  $P_i$ . (Based on Foe and Kemp, 1985.)

- a. Compare the ability of PFK-1C to catalyze the phosphorylation of fructose-6-phosphate in the absence of, and in the presence of AMP, F-2,6-BP or  $P_i$ . How do these allosteric effectors influence the velocity of the reaction?
- b. Evaluate whether the investigators have shown that PFK-1 C is different from the PFK-1 A and PFK-1 B isozymes. Speculate why there might be functional differences among the isozymes.

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**Table 18.1:** Relative potency of the allosteric effector citrate on PFK isozymes. The concentration given is the micromolar concentration of citrate required to inhibit 50% of the enzyme activity.

<i>Isozyme</i>	<i>Citrate, : M</i>
A	100
B	> 2000
C	750

**Table 18.2:** Relative potency of allosteric effectors on PFK isozymes. Numbers given are the micromolar concentrations of each effector required to achieve 50% of the maximal velocity.

<i>Isozyme</i>	<i>Phosphate</i>	<i>AMP</i>	<i>Fructose-2,6-BP</i>
A	80 : M	10 : M	0.05 : M
B	200 : M	10 : M	0.05 : M
C	350 : M	75 : M	4.5 : M

## Reference

Foe, L. G., and Kemp, R. G. (1985) *J. Biol. Chem.* **260** pp. 726-730.