Novel Isolation and Filamentation of Drosophila melanogaster Phosphofructokinase-1

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This study aimed to determine if Drosophila melanogaster PFK (DmPFK) forms filaments, and if so, under what conditions. Enzyme filamentation, or the reversible formation of oligomers, has been seen in metabolic enzymes including the liver isoform of human PFK. Filamentation may be important to the regulation of metabolic enzymes and the pathogenesis of human diseases. Drosophila melanogaster, the fruit fly, is commonly used as a model system for studying human development and disease. The rate-limiting step of glycolysis, the process by which glucose is broken down to produce energy that sustains cells, is catalyzed by the allosteric enzyme phosphofructokinase-1 (PFK). I hypothesized that filamentation occurs in the presence of factors that inhibit PFK, such as ATP. To determine the conditions under which PFK forms filaments, the protein was purified from Escherichia coli cells by capturing it with nickel resin and using anion exchange fast protein liquid chromatography. Successful purification was detected via western blot and Coomassie stained SDS-PAGE gel with bands at 89 kDa, the molecular weight of DmPFK. Buffer screening determined that DmPFK was concentrated the most efficiently and had the highest enzymatic activity rate in a low salt and sodium phosphate buffer adjusted to pH 7.5 at room temperature. DmPFK required ATP and MgCl2, in the presence of F6P and (NH4)2SO4, to form filaments. The structures matched the physical description of those from human liver PFK, supporting the hypothesis. Future research may investigate how DmPFK filamentation can affect cancer cell proliferation, autoimmune diseases, and neurodegenerative diseases.

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