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Purification and characterization of recombinant pyruvate dehydrogenase kinases from pea and soybean plants

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The pyruvate dehydrogenase complex (PDC) is a large multienzyme complex catalyzing the oxidative decarboxylation of pyruvate and concomitant reduction of NAD to yield acetyl-CoA and NADH. The plant PDCs have vital roles in catabolic and anabolic metabolism. The plant complexes contain three primary components: pyruvate dehydrogenase (E_1), dihydrolipoyl acetyltransferase (E_2) and dihydrolipoyl dehydrogenase (E_3). Additionally, mitochondrial PDC (mtPDC) contains two associated regulatory enzymes: pyruvate dehydrogenase kinase (PDK) and phospho-pyruvate dehydrogenase phosphatase. PDK catalyzes phosphorylation on the α subunit of E_1 , resulting in inactivation of the complex. We have cloned two PDKs from soybean and recently we have cloned three PDKs from pea. cDNAs encoding soybean PDK 1 and 2 and pea PDK 1, 2 and 3 were subcloned into pET expression vector and *E. coli* BL21 (DE3) cells were transformed with each pET-28-H₆-PDK construct. Recombinant proteins were expressed and purified by Ni-NTA agarose column chromatography to approximately 95% homogeneity. Biochemical characterization of these proteins is underway.