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Expression, Purification and Functional and structural relationship of pyruvate dehydrogenase phosphatase

Young Mi Kim, Ki Hwa Jung
Department of Pharmacy, Duksung Women's University, Seoul, Korea

ABSTRACT

Pyruvate dehydrogenase phosphatase (PDP) is a mitochondrial protein serine/threonine phosphatase that catalyzes the dephosphorylation and concomitant reactivation of the pyruvate dehydrogenase componant of the pyruvate dehydrogenase complex (PDC). PDP consists of a Mg^{+2}-dependent and Ca^{+2}-stimulated catalytic subunit (PDPc) of *Mr* 52,600 and a FAD-containing regulatory subunit (PDPr) of *Mr* 95,600. Catalytic subunit of pyruvate dehydrogenase phosphatase (PDPc) has been suggested to have three major functional domains such as dihydrolipoamide acetyltransferase(E_2)-binding domain, regulatory subunit of PDP(PDPr)-binding domain, and calcium-binding domain. In order to identify functional domains, recombinant catalytic subunit of pyruvate dehydrogenase phosphatase (rPDPc) was expressed in E. coli JM101 and purified to near homogeneity using the unique property of PDPc: PDPc binds to the inner lipoyl domain (L_2) of E_2 of pyruvate dehydrogenase complex (PDC) in the presence of Ca^{+2}, not under EGTA. PDPc was limited-proteolysed by trypsin, chymotrypsin, Arg-C, and elastase at pH 7.0 and 30°C and N-terminal analysis of the fragments was done. Chymotrypsin, trypsin, and elastase made two major fragments: N-terminal large fragment, approx. 50 kD and C-terminal small fragment, approx. 10 kDa. Arg-C made three major fragments: N-terminal fragment, approx. 35 kD, and central fragment, approx. 15 kD, and C-terminal fragment, approx. 10 kD. This study strongly suggest that PDPc consists of three major functional domains. However, further study should be necessary to identify the functional role.