

Structural and Functional relationship of the recombinant catalytic subunit of pyruvate dehydrogenase phosphatase.

Young Mi Kim, Ki Hwa Jung

Department of Pharmacy, Duksung Womens University,
419 Ssangmoon-dong, Tobong-gu, Seoul 132-714, Korea

Catalytic subunit of pyruvate dehydrogenase phosphatase (PDPc) has been suggested to have three major functional domains such as dihydrolipoamide acetyltransferase(E2)-binding domain, regulatory subunit of PDP(PDP_r)-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 (L2) of E2 of pyruvate dehydrogenase complex (PDC) in the presence of Ca²⁺, 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.