

분류번호

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<p>제 목</p>	<p>Partial Purification of Protein X from the Pyruvate Dehydrogenase Complex of Bovine Kidney</p>
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<p>내 용</p>	<p>Mammalian pyruvate dehydrogenase complex(PDC) enzyme consists of multiple copies of three major oligomeric enzymes-E1, E2, E3. And protein X is one of the enzymatic constituents which is tightly bound to E2 subunit. This complex enzyme is responsible for the oxidative decarboxylation of pyruvate producing of acetyl CoA, which is a key intermediate for the entry of carbohydrates into the TCA cycle for its complete metabolic conversion to CO<sub>2</sub>. And the overall activity of the complex enzyme is regulated <i>via</i> covalent modification of E1 subunit by a E1 specific phosphatase and kinase. Protein X has lipoyl moiety that undergoes reduction and acetylation during enzymatic reaction, and has been known to be involved in the binding of E3 subunit to E2 core and in the regulatory activity of kinase. The purification of protein X has not been achieved majorly because of its tight binding to E2 subunit. The E2-protein X subcomplex was obtained by the established methods and the detachment of protein X from E2 was accomplished in the 0.1M borate buffer containing 150mM NaCl. During the storage of the subcomplex in frozen state at -70°C, the E2 subunit was precipitated and the dissociated protein X was obtained by centrifugation into the supernatant. The verification of protein X was accomplished by (1)the migration on SDS-PAGE, (2)acetylation by [2-<sup>14</sup>C] pyruvate, and (3)internal amino acid sequence analysis of tryptic digested enzyme.</p>