BI 32

CATHEPSIN E IS A PLASMA MEMBRANE-BOUND ASPARTIC PROTEINASE IN MAMMALS.

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Cathepsin E is a non-secretory acid proteinase in mammals. In an attempt to define its destination site in the cell, we examined the subcellular localization pattern of the enzyme in rat livers: Perfused rat livers were homogenized in 0.25M sucrose and differentially centrifuged to obtain nuclear, mitochondrial, lysosomal, microsomal and cytosol fractions. Electrophoretic analysis clearly showed a predominant association of cathepsin E with microsomal fraction, in contrast with an well-known lysosomal association of cathepsin D. When microsomal fraction was resuspended in 57% sucrose, placed under 1M and 0.25M sucrose layers and centrifuged at 75,500xg for 16h to segregate ER-rich (P4) and plasma membrane-rich (P2) fractions, cathepsin E was found to be cencentrated in P2 fraction. Although this fraction is known to be heterogeneous and contain Golgi bodies, further sucrose density centrifugation experiments of P2 revealed a distinct difference in distribution pattern between cathepsin E and UDP-GlcNAc:lysosomal enzyme N-acetylglucosaminylphosphotransferase (a marker of cis Golgi). The results thus strongly suggested that cathepsin E is transported to the plasma membrane in rat liver.

BI 33

α,α-TREHALASE FROM ARTEMIA NAUPLIUS

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A modified procedure of purifying trehalase from Artemia nauplius and the newly investigated nature of the enzyme were presented in this report.

The soluble enzyme was purified more than 1000 fold in the presence of soybean trypsin inhibitor, leupeptin and EDTA by the following 4-step procedures; acetone treatment, DEAE-Sepharose CL-6B, Sephadex G-75 and Con A-Sepharose. The yield of the activity was 30 % and the specific activity was 45 units/mg protein. The enzyme was further purified by chromatofocusing and Sephadex G-75. Although pI of the enzyme was pH 4.8-4.5, most of the enzyme was eluted at pH 4.15-4.00 in the presence of 0.1 % Triton X-100 in the chromatofocusing. Residual activity still retained in the Polybuffer exchanger-94 was eluted by 1.0 M NaCl. Trehalase activity and its molecular weight were not affected by 2-mercaptoethanol. Temperature dependency of the trehalase was investigated; its activity was strongly inactivated over 60° C, and the activation energy was calculated to be 11.0 kcal. The trehalase activity was completely inhibited by 0.1 mM $HgCl_2$, and monovalent inorganic anions such as I^-, Br^- and Cl^- were effective to counteract the inhibition by $HgCl_2$.

BI 34

CELLOBIASE FROM ARTEMIA NAUPLIUS -PURIFICATION AND CHARACTERIZATION-

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In the course of purifying trehalase from Artemia nauplius, cellobiase activity was found to be coexistent in the beginning steps, so that we tried to isolate the enzyme.

Soluble cellobiase was isolated from Artemia nauplius in the following 5-step procedures; acetone treatment, DEAE-Sepharose CL-6B, Con A-Sepharose, Chromatofocusing and Sephadex G-75. The purified enzyme was shown to be a single band of protein with its activity on SDS-PAGE, its molecular weight being determined to be 86K. The enzyme retained its activity even after the treatment with 2-mercaptoethanol, showing its molecular weight to be 57K. The cellobiase was highly specific for cellobiose and pNP-β-D-glucoside. The Km value for cellobiose was estimated to be 0.28 mM at the optimum pH of 6.0. Isoelectric point of the enzyme was shown to be pH 6.3-6.6. Effect of temperature on the activity was investigated; the activity was strongly inactivated over 70 °C, and the activation energy was calculated to be 11.0 kcal. The activity of the cellobiase was strongly inhibited by 0.1 mM D-glucono δ-lactone and 0.1 mM $HgCl_2$.

BI 35

MITOCHONDRIAL CYLINDRIN IS A SPECIFIC SUBSTRATE OF ATP-DEPENDENT PROTEASE

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Mitochondrial cylindrin forming hollow cylinder tube structure was isolated from mitochondrial matrix of pig liver in our laboratory. The molecular weight of this protein was estimated as 25,000 by SDS gel electrophoresis. On the other hand, it was found that a 25K protein (SP-25) in mitochondrial matrix of adrenal cortex is specifically digested by ATP-dependent protease in the mitochondrial matrix. Then comparison of molecular features between both proteins was conducted in this study. Molecular structure of SP-25 observed with electron microscope indicated that SP-25 shows a hollow cylinder tube structure which is completely identical as mitochondrial cylindrin. Both tubes show 13nm and 7nm in outer and inner diameter respectively and consist of six subunits. The tube structure was dissociated in the addition of 1M KI or sonication and reconstructed in the presence of magnesium ions. Furthermore mitochondrial cylindrin from pig liver was crossreacted by polyclonal antibody of SP-25. These common features between both proteins indicate that mitochondrial cylindrin is a specific substrate of ATP-dependent protease.