ラット肝培養細胞におけるリソゾーム カテプシンB及びHの生合成

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Biosynthesis of Lysosomal Cathepsins B and H in Cultured Rat Hepatocytes

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ABSTRACT The biosynthesis of lysosmal cysteine proteases, cathepsins B and H, was investigated by using pulse–chase experiments *in vivo* in primary cultures of rat hepatocytes. Cathepsins B and H were isolated from either total cell extracts or culture medium labeled with [^{35}S] methionine by immunoprecipitation and analyzed for their molecular forms. Within 60 min of chase, cellular proforms of cathepsins B of 39 kDa and H of 41 kDa were converted to single–chain form cathepsins B of 29 kDa and H of 28 kDa, respectively, and persisted as these forms even after 12–h chase periods. The proforms of cathepsins B and H derived from pulse–labeling experiments showed complete susceptibility to endoglycosidase H treatment, indicating that these proenzymes bear high–mannose–type oligosaccharides at the stage of initial events of biosynthesis. In the presence of tunicamycin, unglycosylated proenzymes of cathepsins B of 35 kDa and H of 34 kDa were found to be secreted into the extracellular medium without undergoing proteolytic processing. Furthermore, in the presence of swainsonine, a potent inhibitor of Golgi mannosidase II, considerable amounts of the proenzymes were secreted and accumulated in the medium during chasing periods. These results suggest that the oligosaccharide moiety of these enzymes would be necessary for the intracellular sorting mechanism. In monensin–treated cells, the conversion of intracellular proenzymes to mature enzymes was significantly inhibited and the proenzymes were secreted into the medium. In the presence of chloroquine or ammonium chloride, proteolytic processing of the proenzymes was completely prevented and the enhanced secretion

of proenzymes was observed. These results suggest that in the presence of lysosomotropic amines the intracellular sorting of proenzymes might not occur properly during biosynthesis.

抄録 ラット肝培養細胞におけるリソゾームカテプシンB及びHの生合成について検討した。カテプシンB及びHは共に分子量の大きいN-グルコシド糖鎖を持ったプロ型として生合成され，その後これらプロ酵素はプロテオリシスを受け成熟型酵素になることが判った。さらに，これら生合成過程におけるトニカマイシン，スワンソニン，モネンシン，クロロキシン，塩化アンモニウムの影響を調べ，プロ型より成熟型へのプロセシング機構について検討した。

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