カテプシンL前駆体のプロセシングにおける アスパルティックプロテアーゼの関与について

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Evidence that Aspartic Proteinase is involved in the Proteolytic Processing Event of Procathepsin L in Lysosomes[1]

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ABSTRACT Our recent studies have shown that cathepsin L is first synthesized as an enzymatically inactive proform in endoplasmic reticulum and is successively converted into an active form during intracellular transport and we postulated that as partic proteinases would be responsible for the intracellular propeptide-processing step of procathepsin L accompanied by the activation of enzyme (Y. NishimurA, T. Kawabata, and K. Kato(1988) *Arch, Biochem, Biophys. 261,* 64-71). To better understand this proposed mechanism, we investigated the effect of pepstatin, a potent inhibitor of aspartic proteinases, on the intracellular processing kinetics of cathepsin L analyzed by pulse-chase experiments *in vivo* with [^{35}S] methiohine in the primary cultures of rat hepatocytes. In the pepstatin-treated cells, the proteolytic conversion of cellular procathepsin L of 39 kDa to the mature enzyme was significantly inhibited and considerable amounts of proenzyme we re found in the cell after 5-h chase periods. Further, the subcellular fraction ation experiments demonstrated that the intracellular processing of procathepsin L in the high desity lysosomal fraction was significantly inhibited and that co nsiderable amounts of the procathepsin L form were still observed in the light density microsomal fraction after 2 h of chase. These results suggest that pep statin treatment caused a significant inhibitory effect on the intracellular proce ssing and also on the intracellular movement of procathepsin L from the endo plasmic reticulum to the lysosomes. These findings provide the first evidence showing hat aspartic proteinase may play an important role in the intracellular

proteolytic processing and activation of lysosomal cathepsin L *in vivo*. Therefore, we suggest that cathepsin D, a major lysosomal aspartic proteinase, is more likely to be involved in this proposed model in the lysosomes.

抄録 カテプシンL前駆体の成熟型への変換にアスパラティックプロテアーゼが関与していることを証明するため，酵素阻害剤であるペプスタチンを培養肝細胞に働かせ，[^{35}S]メチオニン標識酵素のプロセシングを経時的に解析した。

ペプスタチン処理細胞では，カテプシンLは39 kDaの前駆体として長時間細胞内に依存し，成熟酵素への変換が強く阻害されていた。この細胞を細胞分画してみると，本酵素はライソゾーム中においても前駆体のままで存在し，又かなりの量が，小胞体中に留まっていた。従って，カテプシンDがカテプシンLの前駆体の成熟型への変換に係わっており，前駆体はライソゾーム中で成熟酵素として活性化されることが判明した。

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