E133 Ubiquitin C-terminal Hydrolase-1 and -8 (UCH-1 and UCH-8), Two Novel Isopeptidases from Chick Skeletal Muscle

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Ubiquitin (Ub) is a highly conserved 76-amino acid polypeptide and synthesized as a fusion protein with ribosomal polypeptides and as a polyubiquitin. Ubiquitination of proteins is involved in a variety of cellular processes, such as cell cycle regulation and MHC class I antigen presentation. Therefore, generation of free Ub from Ub precursors and multi-ubiqutinated proteins should be essential for the cellular function of Ub molecules. Ub C-terminal hydrolases (UCHs) are responsible for specific cleavage of the α-peptide and/or ε-isopeptide bond. Here we report the purification and characterization of two novel UCHs that can generate free Ubs from multi-ubiquitinated proteins in which Ubs are linked through isopeptide bonds as well as from Ub-αNH-polypeptide conjugates. These enzymes were purified to apparent homogeneity from chick skeletal muscle. The purified UCH-1 and UCH-8 behave as 37- and 210-kDa proteins, respectively. Remarkably UCH-8 was activated dramatically by poly-L-Lys, while the activity of UCH-1 was not. UCH-1 and UCH-8 had different inhibition patterns by salt and Ub-aldehyde. In addition, UCH-1 was active at broad pH range from 6 to 10, but UCH-8 had narrow pH optimum near pH 7.8. These results suggest that two UCH activities are distinct from each other, and must play a role in maintenance of cellular Ub pool.

E134 Development-specific Phosphorylation of the 63-kDa Protein by the θ-type Protein Kinase C in Chick Embryonic Skeletal Muscle

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The pattern of protein phosphorylation was found to change during development of chick embryonic muscle. In particular, phosphorylation of the 63-kDa protein increased dramatically during the early period of development(i.e., from day-9 to -12). It reached to a maximal level in 15-day embryonic muscle but could not be detected from day 21 or in adult muscle. The changes in the extent of the protein phosphorylation were primarily due to the alteration in the level of the 63-kDa protein but not that of the activity or protein level of the protein kinase. In addition, the 63-kDa protein phosphorylation could not be seen in other embryonic or adult tissues(including heart, kidney, liver and brain), except that in embryonic lung the protein phosphorylation occurred to about 10% of that in embryonic muscle. Therefore, it appears that the 63-kDa protein phosphorylation is embryonic muscle-specific and is under developmental control. Treatment of Ca^{2+} and/or calmodulin or EGTA showed little or no effect on the protein phosphorylation. However, the specific inhibitors of protein kinase C(PKC), such as H7 and sphingosine, blocked the 63-kDa protein phosphorylation, while 12-*O*-tetradecanoyl phorbol 13-acetate, an activator of PKC, increased it. Furthermore, the antibody against the θ-type PKC specifically interacted with the 82-kDa protein in the partially purified kinase preparation, and inhibited the 63-kDa protein phosphorylation. In addition, phosphoamino acid analysis revealed that the 63-kDa protein was exclusively phosphorylated on its Ser residue(s). These results indicate that the Ca^{2+} -independent, θ-type isoform of PKC is responsible for the development-specific phosphorylation of the 63-kDa protein in chick embryonic muscle.