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Purifications and Characterizations of UCH-1 and UCH-10 from Chick Skeletal Muscle

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Ubiquitin (Ub), a highly conserved 76-amino acid polypeptide, is generated from its fusion gene products and re-generated from Ub-protein conjugates. Ubiquitin C-terminal hydrolases (UCHs) are responsible for the specific cleavage of the α -peptide and/or ϵ -isopeptide bond after C-terminus of Ub. Recently, we have shown that chick muscle extract contained at least 10 distinct UCH's activities and purified one of them, UCH-6, to apparent homogeneity. Here, we report purification and characterization of two UCHs that are distinct from UCH-6 using ^{125}I -labeled Ub-PEST as a substrate. Purified UCH-1 and -10 had molecular masses of about 37 and 210 kDa, respectively. Both the activities of UCH-1 and UCH-10 were inhibited by sulfhydryl-blocking agents, such as iodoacetamide and N-ethylmaleimide. UCH-1 was maximally active at near pH 8.5, while UCH-10 was at near pH 7.5. Although both the enzymes could generate monomeric Ub from Ub-PEST, Ub-CEP80 and Ub-DHFR fusion proteins, only UCH-1 was capable of cleaving the peptide bond between two repeated ubiquitins and the isopeptide bond of Ub-lysozyme conjugate. These results suggest that UCH-1 and UCH-10 are different in their biochemical properties and may be involved in different steps in the metabolic pathway of ubiquitin.

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Primary Structures of Two Homologous Subunits of PA28, a γ -interferon-inducible Protein Activator of the 20S Proteasome

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The primary structures of two proteins that comprise PA28, an activator of the 20S proteasome, have been determined by cDNA cloning and sequencing. These protein subunits, termed PA28 α and PA28 β , are about 50% identical to one another and are highly conserved between rat and human. PA28 α and PA28 β are homologous to a previously described protein, Ki antigen, whose function is unknown. PA28 α , but neither PA28 β nor Ki antigen, contains a "KEKE motif", which has been postulated to promote the binding of proteins having this structural feature. PA28 α and PA28 β were coordinately regulated by γ -interferon, which greatly induced mRNA levels of both proteins in cultured cells. The mRNA level of the Ki antigen also increased in response to γ -interferon treatment, but the magnitude of the increase was less than that for the PA28s, and the effect was transient. These results demonstrate the existence of a new protein family, at least two of whose members are involved in proteasome activation. They also provide the basis for future structure/function studies of PA28 subunits and the determination of their relative physiological roles in the regulation of proteasome activity.