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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 PA28α and PA28β, are about 50% identical to one another and are highly conserved between rat and human. PA28α and PA28β are homologous to a previously described protein, Ki antigen, whose function is unknown. PA28α, but neither PA28β nor Ki antigen, contains a "KEKE motif", which has been postulated to promote the binding of proteins having this structural feature. PA28α and PA28β 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.