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Determination of amino acids involved in specificity and activity of ChlaDUB2

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ABSTRACT

Chlamydia trachomatis is a pathogen which infects humans as a sexually transmitted disease or through ocular infection, causing ocular trachoma. Ocular trachoma is the leading cause of non-congenital blindness in developing countries. The bacteria employs the deubiquitinating enzyme ChlaDUB2 to remove ubiquitin from its inclusion membrane in order to avoid lysosomal degradation. Key amino acids involved in ubiquitin recognition and cleavage were mutated in order to probe substrate specificity and catalytic activity of ChlaDUB2. Mutants were used in fluorometry assays in order to determine how the mutations affect the ability of ChlaDUB2 to release the amino methyl coumarin (AMC) group from ubiquitin-AMC. It was found that point mutants C282A, M190A, Q275A, L333A, and a deletion mutant in which a helix (VR3) was removed all reduced deubiquitinating activity. These results indicate that the mutated residues contribute to ubiquitin binding and hence catalysis. These results lead to a better understanding of the deubiquitinating activity of ChlaDUB2.

KEYWORDS

Ubiquitin, *Chlamydia trachomatis*, deubiquitinating enzymes, mutagenesis, ubiquitin-AMC