Cooperative regulation of human exonuclease1 (hExo1) by 14-3-3 proteins

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ABSTRACT

Human exonuclease1 (hExo1) is a member of the eukaryotic nuclease family that includes Rad2/Xeroderma pigmentosum complementation group G (XPG), flap endonuclease1 (FEN1) and gap endonuclease1 (GEN1). Human exonuclease1 is involved in multiple DNA metabolism processes, including DNA repair and replication. Most of the fundamental roles of Exo1 have been described in yeast. The DNA end resection process is a guiding principle to cellular response during DNA double strand break lesion and is pivotal for genome maintenance. Even though insufficient DNA resection restrains homology-directed repair mechanisms and the activation of ATR (ataxia telangiectasia and Rad3 related)-dependent checkpoint, over-resection results in production of an excessive single-stranded DNA that could lead to genomic instability. Nonetheless, the control mechanisms for DNA end resection are not yet fully understood especially with regards to hExo1 structure/function point of view.

In this study, hExo1 protein was over-expressed from both insect cells and bacteria and over-expressed protein was purified to near homogeneity. There is no experimental evidence relating hExo1 structure to its function and/or regulation. To address this, we investigated the biophysical characteristics as well as the *in vitro* regulation of the hExo1-mediated DNA end resection.

This study show that hExo1 resection activity is both positively and negatively controlled by protein-protein interactions to enable a proper DNA end resection mechanism. Both 14-3-3ζ and 14-3-3ε proteins cooperatively interact with the C-terminus region of hExo1 to negatively control DNA end-resection activity of hExo1 while PCNA sliding clamp increases the DNA end resection activity by hExo1. Our results show that the C-terminal region on hExo1 is intrinsically disordered and there is an allosteric inhibition of the hExo1/PCNA complex by 14-3-3: the strength of the binding reaction varies with 14-3-3 isotype, and involves at least three 14-3-3 dimers.

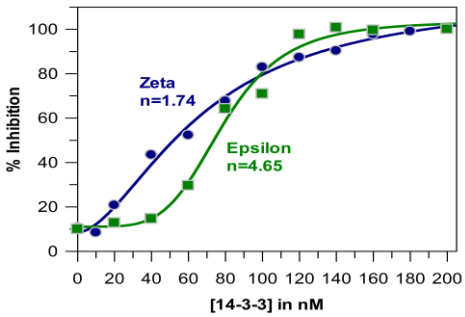


Figure 1. Binding curve. The cooperative binding of 14-3-3 zeta and 14-3-3 epsilon to hExo1 protein.

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