Solution NMR Studies on the Mechanisms underlying Activation of Deubiquitinase A by Phosphorylation

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Deubiquitinase A (DUBA) was recently identified as a negative regulator of interferon-I (INF-I) production by cleaving the Lys63-linked poly-ubiquitin chains on TRAF3, which is an E3 ubiquitin ligase critical for INF-I production. Overproduction of INF-I has been linked to autoimmune diseases, such as systemic lupus erythematous. DUBA is activated by phosphorylation of Ser177 located at the N-terminal disordered region of the catalytic domain. The structural mechanisms of DUBA activation are not well understood, because previous NMR studies indicated no significant structural changes due to phosphorylation. We used solution NMR, in combination with biochemical assays, to define the intramolecular interactions mediated by the phosphorylated serine, essential for DUBA activity. The weak interactions between the N-terminal disordered fragment and the well-folded domain of DUBA were characterized by paramagnetic relaxation enhancement. In addition, we characterized conformational dynamics of DUBA on the microsecond-to-millisecond time scale by amide ^{1}H Carr-Purcell-Meiboom-Gill experiments. The effects of phosphorylation on the dynamical properties of DUBA were quantified by comparison of phosphorylated and unphosphorylated forms of DUBA. Overall, the NMR data suggest that the effects of phosphorylation are not limited to the modulation of substrate affinity to the enzyme and the dynamical properties of DUBA essential for activity may also be modulated by phosphorylation.

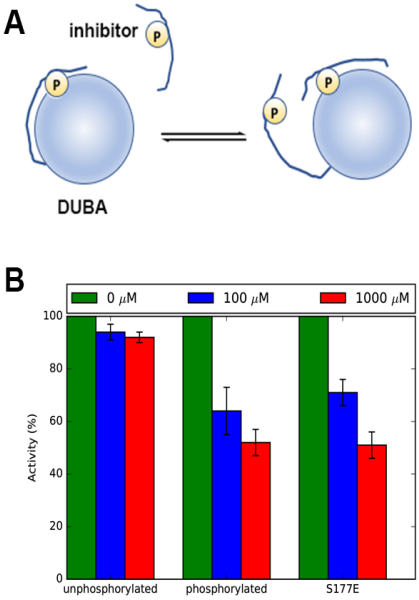

Figure 1 DUBA inhibition assay. (A) Illustration of the mode of inhibition of DUBA activity by the phosphorylated peptide derived from DUBA. (B) Activity of DUBA in the presence of inhibitors at two concentrations (100 μM and 1000 μM). The enzyme concentration is 0.2 μM. The peptide sequence is GAGYNSEDEYEA. In the phospho-mimetic mutant peptide, S is replaced by E.

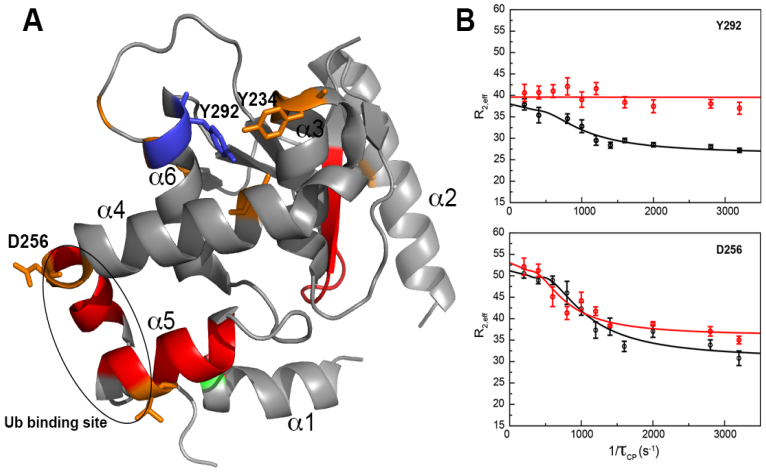

Figure 2 (A) Structure of DUBA (in bound conformation) with residues undergoing millisecond time scale dynamics highlighted in color. Residues in orange show similar relaxation dispersion profiles in both phosphorylated and unphosphorylated forms of DUBA, whereas residues in blue show different dispersion profiles. The residues in red are not detected due to extreme line broadening (B) Typical ^{1}H relaxation dispersion profiles of residues in phosphorylated (red) and unphosphorylated (black) DUBA.