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Redox exchange of the disulfides of human two-domain CD4 regulates the conformational dynamics of each domain, providing insight into its mechanisms of control

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ABSTRACT:

CD4, a membrane glycoprotein expressed by specific leukocytes, plays a vital role in the human immune response and acts as a primary receptor for HIV entry. Of its four ecto-domains (D1−D4), D1, D2, and D4 each contain a distinctive disulfide bond. Whereas the disulfides of D1 and D4 are more traditional in nature, providing structural functions, that of D2 is referred to as an “allosteric” disulfide due to its high dihedral strain energy and relative ease of reduction that is thought to regulate CD4 structure and function by shuffling its redox state. While we have shown previously that elimination of the pre-stressed D2 disulfide results in a favorable structural collapse that increases the stability of a CD4 variant comprising only D1 and D2 (2dCD4), we sought to further localize and determine the nature of the biophysical modifications that take place upon redox exchange of the D1 and D2 disulfides by using amide hydrogen-deuterium exchange mass spectrometry (HDX-MS) to measure induced changes in conformational dynamics. By analyzing various redox isomers of 2dCD4, we demonstrate that ablation of the D1 disulfide enhances the dynamics of the domain considerably, with little effect on that of D2. Reduction of the D2 disulfide however decreases the conformational dynamics of many of the β-strands of the domain that enclose the bond, suggesting a model in which inward collapse of secondary structure occurs around the allosteric disulfide upon its eradication, resulting in a marked decrease in hydrodynamic volume and increase in stability as previously described. Increases in the dynamics of regions important for HIV gp120 and MHCII binding

in D1 also result allosterically after reducing the D2 disulfide, which are likely a consequence of the structural changes that take place in D2, findings that advance our understanding of the mechanisms by which redox exchange of the CD4 disulfides regulates its function.