Fluorescence Studies on Denaturation and Stability of Recombinant Human Interferon-Gamma

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Unfolding/folding transitions of recombinant human interferon-gamma (hIFNγ) in urea and guanidine chloride (Gn.HCl) solutions were studied by fluorescence spectroscopy. At pH 7.4 Gn.HCl was a much more efficient denaturant (midpoint of unfolding C* = 1.1 M and ΔG^0 = 13.4 kJ/mol) than urea (C* = 2.8 M and ΔG^0 = 11.7 kJ/mol). The close ΔG^0 values indicate that the contribution of electrostatic interactions to the stability of hIFNγ is insignificant. Both the pH dependence of the fluorescence intensity and the unfolding experiments in urea at variable pH showed that hIFNγ remains native in the pH range of 4.8–9.5. Using two quenchers, iodide and acrylamide, and applying the Stern-Volmer equation, a cluster of acidic groups situated in close proximity to the single tryptophan residue was identified. Based on the denaturation experiments at different pH values and on our earlier calculations of the electrostatic interactions in hIFNγ, we assume that the protonation of Asp63 causes conformational changes having a substantial impact on the stability of hIFNγ.

Key words: Human Interferon-Gamma (hIFNγ), Fluorescence Spectroscopy, Denaturation