

**NONCOVALENT COMPLEXES OF PROTEINS: COMPARISONS OF SOLUTION AND GAS
PHASE BINDING ENERGIES**

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The observation of ions of noncovalent complexes of proteins with small molecules and other proteins raises the question of whether the same specific interactions that bind the partners in solution remain in the gas phase ions. If so, it may be possible to measure at least relative binding energies of biomolecules using mass spectrometry. We have previously reported¹ that the hydrogen bonds that bind heme in holomyoglobin in solution persist in the gas phase ions, at least for charge states +11-+14. These bonds were detected by measuring relative dissociation energies for a series of mutant myoglobins in which the bonds were systematically removed. We have now extended this work to holomyoglobin in higher charge states (up to +21). Collision cross section measurements show that the protein has unfolded appreciably in these higher charge states. However measurements of the relative energies needed to cause heme dissociation show the heme binding energy is decreased only slightly in the more highly charged ions. Therefore much of the heme pocket appears to remain in this protein small molecule complex as it unfolds in the gas phase. We have also examined noncovalent complexes of bovine pancreatic trypsin inhibitor (BPTI, m.w. 6512) with trypsin (m.w. 23,322), chymotrypsin (25,224) and trypsinogen (m.w.23,981) which have dissociation constants for solution binding of 10^{-14} , 10^{-9} and 10^{-6} M respectively. Thus these three proteins form a system of natural mutants with different solution binding energies. For these protein-protein complexes we find no correlation between solution binding energy and the gas phase binding energies.

1. Hunter, C. A., Mauk, A. G., Douglas, D. J. *Biochemistry*, 1997, 36, 1018.