Effects of salts on the conformation and stability of proteins

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Summary

Conformation and stability of proteins, in particular in the denatured state, critically depend on the salt conditions. Whereas several proteins including apomyoglobin and cytochrome *c* are unfolded significantly at pH 2 in the absence of salt, the addition of salts stabilizes the molten globule state. Although the molten globule (MG) state has been proposed as a major intermediate of protein folding, it has proven difficult to obtain thermodynamic data characterizing this state. To explore novel approach for characterizing the MG state, salt-induced formation of the MG states of horse cytochrome *c* and horse apomyoglobin at pH 2 was studied by isothermal titration calorimetry.

By titrating the acid-unfolded cytochrome *c* or apomyoglobin with sodium perchlorate, an exothermic reaction was observed. The titration curve obtained from the heat was cooperative and agreed well with the conformational transition curved measured by circular dichroism (CD) at 222 nm. These results indicated that the salt-induced conformational change of these proteins is well approximated by a two-state transition between the acid-unfolded and MG states and that isothermal titration calorimetry will become a useful approach for investigating the effects of salts on the conformation of proteins. In the case of cytochrome *c*, the heat for formation of the MG state estimated by isothermal titration calorimetry was consistent with the enthalpy change for unfolding of the sodium perchlorate-stabilized MG state at pH 2, which was measured by differential scanning calorimetry and CD. These indicate that the heat of titration largely reflects the enthalpy change of the conformational transition and confirm that the unfolding transition of the MG state is a two-state transition. On the other hand, in the case of apomyoglobin, the heat for formation of the MG state estimated by titration calorimetry was slightly larger in magnitude than the enthalpy change for unfolding of the salt-stabilized MG state at pH 2, suggesting a relatively small contribution of heat other than the conformational change. The conformational transition of the MG state of apomyoglobin may be explained on the basis of a mechanism involving both the cooperative and gradual characteristics.