THE EFFECT OF pH ON THERMAL STABILITY OF GLOBULAR PROTEINS
A critical insight

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Abstract

In this study we try to re-analyze the pH dependence of thermal stability of small globular proteins. From the thermodynamic point of view a long series of calorimetric and spectroscopic investigations has shown that the decreased stability in very acidic conditions can be ascribed to entropic effects. The same conclusion is reached, from a microscopic point of view, by assuming that a binding of protons on equal and noninteracting sites takes place as a consequence of unfolding process. By linking the conformational unfolding equilibrium to the proton binding equilibrium, a model is developed that is able to describe the dependence on the pH of the thermal denaturation processes of small globular proteins. The application of the model to hen lysozyme and T4 lysozyme correctly accounts for the experimental results.

Keywords: calorimetry, globular proteins, pH dependence of thermal stability, spectroscopic investigations, thermodynamics

Introduction

It is well known that all the physico-chemical properties of globular proteins, and in particular the catalytic efficiency of enzymes, are strongly pH dependent. Performing spectroscopic or calorimetric measurements it has been established that the unfolding temperature considerably varies at changing the pH of solution for all the proteins [1-5]. Usually, at decreasing the pH from neutrality, the thermal stability diminishes and in very acidic conditions the native structure is just denatured at room temperature. The traditional explanation of this behaviour relies on the classical electrostatics and follows the lines of the
At decreasing the $pH$ of the solution, the net charge on the folded form increases, due to the protonation of some acidic groups and this causes an enhancement of the electrostatic repulsion which tends to destabilize the native structure, because the charge density is greater for the native state than for the denatured one. Thus, in very acidic conditions, the unfolding process results thermodynamically favoured, because it gives rise to a state with a lower electrostatic free energy. Similar arguments account for the stability decrease observed in highly basic solutions due to repulsion of negative charges. The theory predicts maximum stability near the isoelectric point of the protein, where the net charge is zero. However a number of proteins, with acidic or basic isoelectric points, show their maximum thermodynamic stability still near to neutrality. This observation suggests that, in addition to overall charge, other considerations are important in determining the contribution of ionizable groups to the overall folding energy of globular proteins. For example Dill and Stigter show, in a theoretical study, that when there are buried, nontitratable ionic groups, the electrostatic contribution becomes asymmetric and the maximum stability of the protein is not at the isoelectric point [7]. Moreover, in a recent paper, Dahlquist and coworkers [8], have pointed out that the presence, in the native structure, of a salt bridge between the side chains of histidine 31 and aspartic acid 70 with $pK_a$ values very different from those in the unfolded state, can account for the maximum of thermodynamic stability near $pH$ 5.5 of bacteriophage T4 lysozyme, whose isoelectric point is above $pH$ 10.

Most thermodynamic studies on the influence of $pH$ on stability of native conformation have been devoted to the investigation of temperature-induced denaturation at different $pH$ values. In this manner it is possible to determine how protein stability depends on solution $pH$. The election technique for these purposes is the differential scanning calorimetry, DSC, because it provides a direct thermodynamic method for evaluating the stability of native conformation [9]. Indeed in a DSC measurement the heat capacity of protein solution is recorded as function of temperature. Being the heat capacity the second temperature derivative of the Gibbs energy, every of variations reflects on stability of native conformation. It is worth noting that throughout this work we will always refer to proteins whose denaturation process is well represented by a two-state transition. Because in a two-state transition the number of moles does not change and the activity coefficients in very dilute solutions can be assumed as constant in the whole investigated temperature range, we believe right to consider as standard the changes of thermodynamic functions associated to the denaturation process of globular proteins [10].

The aim of this paper is to combine the well established general thermodynamic features with a reasonable average microscopic model of the effect of $pH$. 

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