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Review

Principles and equations for measuring and interpreting protein stability: From monomer to tetramer

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ABSTRACT

The ability to measure the thermodynamic stability of proteins with precision is important for both academic and applied research. Such measurements rely on mathematical models of the protein denaturation profile, i.e. the relation between a global protein signal, corresponding to the folding states in equilibrium, and the variable value of a denaturing agent, either heat or a chemical molecule, e.g. urea or guanidinium hydrochloride. In turn, such models rely on a handful of physical laws: the laws of mass action and conservation, the law that relates the protein signal and concentration, and the one that relates stability and denaturant value. So far, equations have been derived mainly for the denaturation profiles of homomeric proteins. Here, we review the underlying basic physical laws and show in detail how to derive model equations for the unfolding equilibria of homomeric or heteromeric proteins up to trimers and potentially tetramers, with or without folding intermediates, and give full demonstrations. We show that such equations cannot be derived for pentamers or higher oligomers except in special degenerate cases. We expand the method to signals that do not correspond to extensive protein properties. We review and expand methods for uncovering hidden intermediates of unfolding. Finally, we review methods for comparing and interpreting the thermodynamic parameters that derive from stability measurements for cognate wild-type and mutant proteins. This work should provide a robust theoretical basis for measuring the stability of complex proteins.

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1. Introduction

The possibility of measuring the stability of proteins with precision finds many applications in fundamental and applied research. It has allowed one to understand and quantify the forces that contribute to the conformational stability of proteins in their aqueous environment, and the effects of sequence changes on this stability [1,2]. The data on the stability of proteins and their mutants are important to develop reliable energy functions for proteins [3,4]. These force fields are used in algorithms to predict the structure or docking of proteins, and to design new proteins and stabilizing changes. The precise measurement of stability is also important to understand and describe the unfolding and folding of proteins at atomic resolution, by a combination of experimental and theoretical approaches, i.e. the analysis of the Φ values and molecular dynamics [5].

By definition, the conformational stability ΔG of a protein is equal to the variation of free energy between its native and unfolded states. It can be deduced from the constant of equilibrium between these two conformational states, and thus from the measurement of concentrations. The stability depends on the physico-chemical conditions and must therefore be given in standard conditions, e.g. $\Delta G(H_2O)$ in aqueous buffer at 20 °C. The concentration of the unfolded state is usually very low in physiological conditions; therefore the values of the stability are measured in variable physico-chemical conditions and extrapolated to the standard conditions. A physical quantity that is sensitive to the conformational state of the protein, is used for the measurement of concentrations. Likewise, the difference of stability between two folding states of a protein is equal to the variation of free energy between these two states.

Excellent reviews on theoretical and practical aspects of measuring protein stability have been published recently [6–8] and the aim of the present paper is not to duplicate them. Rather, it focuses on the derivation of the equations that are necessary to quantify stability from experimental data, from a handful of basic physical laws and principles. It shows that a rigorous process for deriving such equations enables one to expand the scope of the proteins that can be studied quantitatively, and allows one to define new meaningful protein parameters. More specifically:

1) I review the improvements that have been made over the years to the equations that are used to deduce thermodynamic parameters of protein stability from unfolding equilibria, induced with denaturants.
2) Some of these improvements have been made to replace empirical parameters with intrinsic protein parameters, i.e. parameters that are specific to the protein and independent of the measuring device.
3) Up to now, equations have been published mainly for monomeric, homodimeric and homotrimeric proteins, although there is at least one report of equations for a heterodimeric protein [9]. I show that one can deduce the equations necessary to measure the stability of a large variety of proteins, from monomers to heterotrimers and tetramers, using a small set of principles.
4) I mention the intrinsic mathematical limitations of these methods.
5) The equations that have been derived to measure the stability of proteins, are only valid when the unfolding equilibria are monitored with a signal that correspond to an extensive property of the protein under study, e.g. the intensity of fluorescence emission at a given wavelength. I show how these equations can also be used when the unfolding equilibria are monitored with such intensive properties as the wavelength λ_{max} of maximal fluorescence emission or the mean partition coefficient in size exclusion chromatography.
6) I review a method that enables one to determine whether a monomeric protein unfolds according to a two state mechanism or with an unfolding intermediate, and extend it to other classes of proteins.
7) Finally I review the equations that allow one to determine the respective contributions of the resistance to denaturation and cooperativity of unfolding to the stability of proteins, and the questions of additivity, synergy or antagonism of multiple mutations on protein stability.

Only the general principles are presented in the main part of this paper. Their application to the derivation of fitting equations for different species of proteins is developed in the Supplementary Informations. Table 1 lists the different cases that are considered.

2. Chemicals and heat as protein denaturants

Many chemicals can be used as protein denaturants. The two chemical denaturants that are most often used, are urea and guanidinium hydrochloride (GdmCl). Urea is considered to give more reliable stability measurements than GdmCl [10]. However, some proteins are not fully denatured in 8 M urea and therefore GdmCl is used in such cases, as it is a stronger denaturant than urea. It is often assumed that the stabilities of proteins, measured with either urea or GdmCl as denaturant, should be the same [11]. However, this assumption is erroneous. GdmCl is charged whereas urea is uncharged. The ionic nature of GdmCl can mask electrostatic interactions in proteins, a phenomenon that is absent when the uncharged urea is used. Thus, GdmCl and urea denaturations may give vastly different estimates of protein stability, depending on how important electrostatic interactions are to the protein stability [12]. Heat is another widely used denaturant.

3. Equation of equilibrium, laws of mass action and conservation

The first equations that one should write, are the equation of the equilibria under study, which defines the stoechiometries of the