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High-pressure NMR techniques for the study of protein dynamics, folding and aggregation

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ABSTRACT

High-pressure is a well-known perturbation method used to destabilize globular proteins and dissociate protein complexes or aggregates. The heterogeneity of the response to pressure offers a unique opportunity to dissect the thermodynamic contributions to protein stability. In addition, pressure perturbation is generally reversible, which is essential for a proper thermodynamic characterization of a protein equilibrium. When combined with NMR spectroscopy, hydrostatic pressure offers the possibility of monitoring at an atomic resolution the structural transitions occurring upon unfolding and determining the kinetic properties of the process. The recent development of commercially available high-pressure sample cells greatly increased the potential applications for high-pressure NMR experiments that can now be routinely performed. This review summarizes the recent applications and future directions of high-pressure NMR techniques for the characterization of protein conformational fluctuations, protein folding and the stability of protein complexes and aggregates.

1. Introduction

Hydrostatic pressure has been widely used over the past decades to characterize the stability of globular proteins and protein complexes, mostly by fluorescence spectroscopy but also small angle X-ray scattering and infrared spectroscopy. The combination of high-pressure perturbation with NMR spectroscopy emerged in the mid-50s with the development of "autoclave" high pressure probes for which the entire radiofrequency transmitters and detection coils are placed in a high-pressure vessel [1]. This method was later developed by Jonas and coworkers for the study of biomolecules under pressure [2] but the incompatibility of this probe design with modern NMR probe electronics and thermal shielding has limited the further application of this approach. These limitations were largely circumvented by the development of pressure-resistant capillary cells that could be used with standard NMR probes and allowed the measurement of any multidimensional experiments [3], an approach that was then popularized by Akasaka and coworkers who used high-pressure perturbation to characterize the folding mechanism of numerous globular proteins [4]. Nevertheless, the capillary cell method also suffers from certain limitations, including the hand-made manufacturing of the cells and the small sample volume (about 40 μL). A large volume NMR tube capable of kilobars of pressure was first introduced by Wand and coworkers in 1996 using a novel method for joining a sapphire tube to a pressure manifold [5,6]. A subsequent shift in materials (to aluminum-toughened zirconia) and manufacturing process led to the development of the high-pressure NMR tubes currently commercially available (Daedalus Innovation™), rated to pressures up to 3 kbar [7]. These ceramic tubes with an inner diameter of 2.75 mm (3.0 mm for the tubes rated at 2.5 kbar) can be used with any commercial NMR probe and maintain a sensitivity of ~50% of a standard Shigemi™ tube with a similar sample volume [7].

When combined with NMR spectroscopy, high-pressure has been shown to be a very sensitive and perfectly reversible method of perturbation, allowing a detailed characterization of the factors governing the stability of globular proteins and protein complexes. Pressure can also be combined with other perturbation methods such as pH, temperature, or chemical denaturants to provide an in-depth description of protein free-energy landscapes. An overview of the high-pressure NMR techniques will be presented here, from the thermodynamic aspects of pressure perturbation to the detection and structural characterization of high-pressure conformers and the effects of pressure on protein folding equilibrium and kinetics. We will finally present a brief overview of the possible applications of pressure to study the stability of protein complexes and aggregates.

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2. Thermodynamic aspects

An increase in pressure shifts the thermodynamic equilibrium toward the states with the lower molar volume. When applied to proteins, pressure in a range of a few thousand bar tends to destabilize or completely unfold proteins by increasing the relative population of the lower volume unfolded states compared to the higher volume folded state [8]. Assuming a two-state folding reaction, the difference in free-energy can be expressed through a 2nd order Taylor series expansion around the reference pressure p_0:

$$\Delta G_u(p) = \Delta G_u^0 + \Delta V_u(p - p_0) - \frac{\Delta\beta_u}{2}(p - p_0)^2$$

where ΔG_u^0 stands for the free energy difference between the unfolded and folded states at atmospheric pressure, ΔV_u the volume change upon unfolding and $\Delta\beta_u$, the difference in compressibility between the unfolded and folded states.

Because the difference in compressibility upon unfolding is small for globular proteins [9], it is often assumed that the relative stability of the folded state with respect to the unfolded states changes linearly with pressure in the typical pressure range used in NMR experiments (1–3 kbar) (Fig. 1). Nevertheless, it has been observed in several cases that a non-null difference in compressibility is necessary to fully explain the experimental data [10,11].

The magnitude of ΔV_u values measured for globular proteins typically lies around 50–100 ml/mol, which represent only 0.5–2% of the protein's molar volume [8]. The intriguingly small magnitude of ΔV_u has generated a large number of different interpretations over the last 40 years. Brandts et al. pointed out that the small ΔV_u values measured for proteins likely originate from an almost perfect compensation of large magnitude negative and positive contributions [12]. They also noticed that due to the numerous negative contributions, as (i) the transfer of apolar groups (−23 ml/mol from methane model), (ii) the transfer of polar groups (−4.5 ml/mol from propanol), (iii) the exchange of a intrapeptide to a peptide-water hydrogen bond (−2 ml/mol), (iv) the ionization of amino and carboxyl groups (∼−10 ml/mol) and (v) the elimination of cavities and void volume in the folded states, the resulting ΔV_u values should be at least one order of magnitude larger than the actual values. The large difference between the expected ΔV_u and the experimental values, suggesting the existence of a missing positive contribution, has been termed the "protein volume paradox" by Chalikian and Breslauer [13].

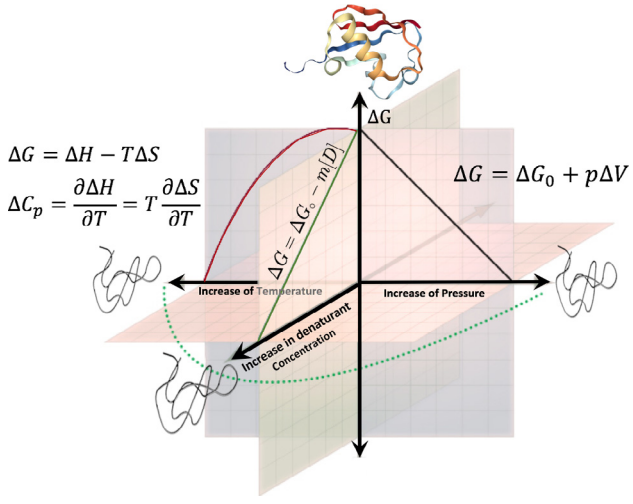

Fig. 1. Effects of pressure, temperature, and chemical denaturant on the unfolding free-energy of a globular protein. Because the difference in compressibility between the folded and unfolded states is very small, the unfolding free-energy, ΔG, decreases quasi linearly with pressure. The volume change upon unfolding, ΔV, corresponds to the steepness of the decrease of ΔG with pressure. The degree of pressure sensitivity of a globular protein (i.e. the magnitude of the ΔV value) is largely dependent on the amount of packing defects and internal cavities present in the core of the structure. Similarly, the unfolding free-energy decreases also linearly with an increase of chemical denaturant concentration. The so-called *m*-value is correlated with the difference in solvent-accessible surface area exposed upon unfolding. On the other hand, the temperature dependence of ΔG is more complex due to the fact the second-order parameter ΔC_p, the difference in heat capacity between the unfolded and folded states, is rather large for globular proteins.

Recent high-pressure NMR and computational studies have provided evidence that the elimination of the solvent-excluded internal voids due to imperfect protein packing, rather than the differential hydration of individual atoms, likely represents the largest contribution to the magnitude of ΔV_u [8,14,15]. Under the influence of high pressure, water molecules are believed to penetrate into internal cavities of the protein core and to induce the destabilization of hydrophobic interactions [14–16]. De Oliveira and Silva recently proposed a push-and-pull hypothesis to describe the opposite mechanisms of chemical and pressure denaturation of proteins [17]. In this model, urea molecules preferentially bind to the protein backbone creating a pulling effect, whereas pressure favored the hydration of the solvent-excluded cavities creating a pushing effect [17].

3. Pressure-induced chemical shift perturbation for the detection of low lying conformational states

The pressure dependence of chemical shifts has been recognized since the late 80's as exquisitely sensitive parameters to monitor subtle structural changes occurring within the folded state ensemble [18]. Akasaka and Li compiled the pressure dependence of ^{1}H chemical shifts for a set of 8 globular proteins and observed that the mean value of the chemical shift linear pressure dependence was similar for all the proteins under study [19]. This observation suggests a general, non-specific, downfield shift of the ^{1}H chemical shifts resulting from the compression of the hydrogen bonds at high-pressure. On the other hand, the non-linear pressure response of the ^{1}H chemical shifts showed much more variations among the 8 proteins and was attributed to the presence of low lying conformational substates within the folded states basin. Interestingly, the authors found a slight correlation between the mean value of the non-linear pressure response and the cavity density calculated from the protein structures [19].

Many efforts have since been directed at confirming the presence of these high-energy conformational substates which are commonly referred to as "low-lying" excited states because they lie within about 10 kJ/mol just above the lowest energy conformation at the bottom of the folding funnel [19–24]. As mentioned above, high-energy conformers are usually detected indirectly, by observing non-linear changes in the ^{1}H chemical shifts as a function of pressure. A general framework for the interpretation of these experimental data was proposed by Akasaka and coworkers through the "protein volume theorem" stipulating that *the partial molar volume of a protein decreases in parallel with the decrease of the conformational order* [25,26] (Fig. 2).

Further analysis on the effect of pressure on ^{15}N chemical shifts of different proteins by Kitahara et al. revealed that residues around water-excluded cavities exhibit large deviations from the average values, indicating again that cavities can be an important source of conformational fluctuation in globular proteins [27]. An example of such structural fluctuations around internal cavities has been recently reported in a study of the human prion protein, showing a correlation between the xenon binding sites and the regions exhibiting non-linear chemical shift perturbation as a function of pressure [28]. It has also been demonstrated for several globular proteins that high-pressure was able to stabilize partially