

INFLUENCE OF MACROMOLECULAR CROWDING ON PROTEIN STABILITY

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Following the synthesis by the ribosome, to carry out its biological function, a protein much fold into a single, well defined conformational state: the native state. Protein folding is thus the physico-chemical process by which a polypeptidic chain undergoes a structural change from an ensemble of coil like structure up to the unique structure encoded in its amino-acid sequence. This process is fascinating and remains one of the most challenging problems of structural biology. Protein misfolding is involved in number of pathologies such as BSE an Alzheimer diseases.

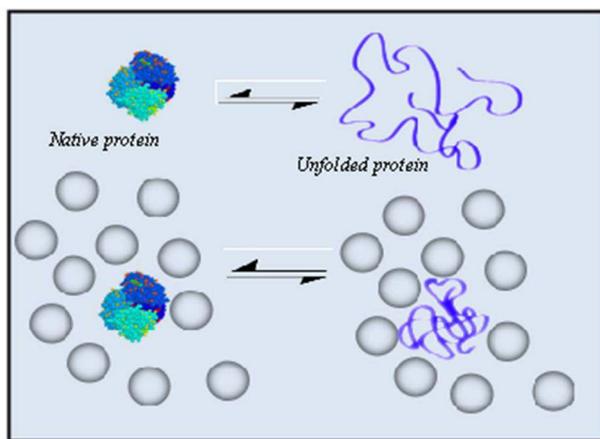


Fig. 1: Equilibrium reaction between the Unfolded ensemble and the Native state of a protein with or without crowding agent.

Experimental studies of protein folding are often (if not always) performed in diluted solutions. Under such conditions small globular proteins can fold and unfold relatively rapidly depending mostly on the nature of the solvent (presence of unfolding agents, change of temperature or pressure ...). In-vivo, under physiological conditions, the proteins are surrounded by a huge number of macromolecules different in nature, size and shape (proteins, nucleic acids, ribosome, carbohydrates ...). Each macromolecule is generally present at very low concentration, but the overall mass concentrations of macromolecules in the cytoplasm can reach values as high as 300 to 400 mg.ml⁻¹, which correspond to volume fractions of the order of 0.2 to 0.3 [1,2]. This environment is qualified of crowded. The chemistry of life, as opposed to the biochemistry of the laboratory, will occur in a very densely packed media. For such volume fraction the protein-protein interactions play a fundamental role. These interactions can be either specific (protein associations ...) or non-specific (sterical or

electrostatic interactions). Non specific interactions are generally much weaker than specific ones.

Theoretical works [3,4] predicted that macromolecular crowding in cells could significantly influence protein stability. The predictions suggest that the equilibrium ($N \leftrightarrow S_i$) between the native (N) and the unfolded states ensemble (S_i) of proteins is shifted towards the native state due to the excluded volume effect induced by macromolecular crowding. The volume excluded to a polypeptide chain by high concentrations of rigid macromolecule would be expected to exert a compressive force reducing its average dimension. More extended conformations of the unfolded chain ensemble are predicted to be preferentially destabilized relative to more compact conformations, including the native state.

Experimental studies intending to verify whether the proteins are stabilized by excluded volume effects were performed on test macromolecules in presence of polysaccharides such as Ficoll 70 or Dextran to mimic the crowding of the cytoplasm. Fluorescence measurements [6] or enzymatic tests [7] that serve to measure protein activities showed protein stabilization (the concentration of unfolded agent needs to be higher to unfold the protein or the denaturation temperature is higher...). Such stabilization effects were also observed by using circular dichroism [7] that quantifies the amount of secondary structures of the proteins. All these experiments have unambiguously stressed protein stabilization due to macromolecular crowding, but the physical mechanism remains unknown because they cannot capture directly the change in conformation of the unfolded state and observe the decrease of its radius of gyration that is associated with the compression of the chain.

Small-angle neutron scattering (SANS) is the ideal tool to evidence such mechanism because contrast matching methods allow to suppress the macromolecular crowding agent signal and to observe the scattering of very dilute species in a huge fraction of agents mimicking the crowded cytoplasm

To achieve this, either the protein or the crowding agent must be deuterated to get enough contrast. As the protein in the unfolded state has a Gaussian chain like conformation [8], and protein deuteration is tricky (especially in rather high quantity as is useful for neutron scattering) we decided to first study the evolution of a deuterated polymer in rather good solvent in water: the polyethylene-glycol (PEG) in order to observe the compression. To obtain information on one single chain we need, for each volume fraction in macromolecular crowder, to extrapolate the information to zero polymer concentration (Zimm plot).

We have shown that macromolecular crowding strongly reduces the radius of gyration of the D-PEG (fig. 2). For macromolecular crowder and polymer chain of similar radii of gyration, at a mass fraction of F70 of $\Phi_m \sim 0.27$, the reduction of R_g of the chain is 30% compared to that measured in water [9].

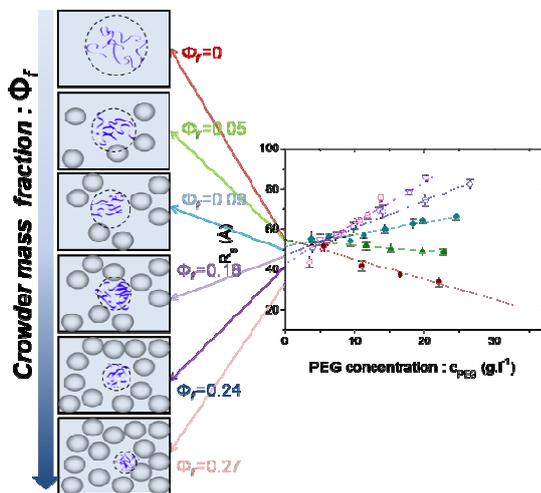


Fig. 2: Zimm plot of the evolution of the radius of gyration of a Gaussian chain when increasing the mass fraction of the crowding agent (Ficoll) up to $\Phi_c = 0.27$.

As can be shown on fig. 2, the crowding not only affects the radius of gyration of the chain but also the protein-protein interactions (slope of the curves).

For a homopolymer like the PEG, we observe a strong compression of the chain but not its total collapse, which would have a too large entropic cost.

We started a second step in order to mimic the situation in biological systems, by performing similar experiments on deuterated proteins. We aim to correlate the destabilization of the unfolded state with the stabilization of the protein observed with biological techniques. One can speculate that, for a heteropolymer like an amino-acid chain a total collapse is possible. For such chains the short range interactions (hydrogen bonds, hydrophobic interactions ...) would bring a sufficient enthalpic gain to compensate for the entropic lost caused by the collapse, and hence significantly stabilize the native state of the protein.

References:

- [1] R. J. Ellis, Trends in Biochem. Sci. 26, 597 (2001).
- [2] A. P. Minton, The J. of Biol. Chem. 276, 10577 (2001).
- [3] Zhou Y. and C. K. Hall, Biopolymers 38, 273 (1996).
- [4] A. P. Minton, Biophysical J. 88, 971 (2005).
- [5] Y. Que and D.W. Bolen, Biophys. Chem., 101, 155 (2002).
- [6] B. Van den Berg, *et al*, The EMBO J., 19, 3870 (2000).
- [7] L. Stagg *et al.*, PNAS 104, 18976 (2007).
- [8] P. Calmettes *et al*, Biophys. Chem. 53, 105 (1994).
- [9] C. Le Coeur *et al*, Phys. Rev. E, 79, 031910 (2009).

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