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Damien Hall

Protein self-association in the cell: a mechanism for fine tuning the level of macromolecular crowding?

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Abstract A new role for protein self-association in the cell is discussed. An argument is advanced that when cellular protein is in its associated state the excluded volume component of the solution is minimized. Conversely, when cellular protein is in its dissociated state the excluded volume component of the solution is maximized. For proteins that make up a substantial fraction of the intracellular protein concentration, control of the self-association event thus presents itself as a means of regulating cellular processes that are influenced by different levels of volume exclusion. In this communication we examine how the control of protein association/dissociation might influence one such important process, namely the folding of a protein to a compact state.

Keywords Macromolecular crowding · Cytoskeleton · Protein association

Introduction

That the intracellular environment is a crowded and complex solution has been known since the development of high resolution cellular imaging technologies. However, appreciation of the physico-chemical effects that such a solution media exerts upon biological interactions taking place within it is only now beginning to receive widespread recognition within the broader biochemical community (Ellis 2001; Hall and Minton 2003). Under conditions of physiological ionic strength, such physico-chemical effects can frequently be quantitatively accounted for by using simple models based on the volume excluding properties of the solution components (Ellis 2001; Hall and Minton 2005; Zimmerman and Minton 1993; Lindner and Ralston 1995). This situation has led to the description of highly volume occupied biological solution environments as 'crowded' (Zimmerman and Minton 1993). Use of such 'crowding' models for the interpretation of experimental data has yielded important insights into many intracellular processes such as DNA compaction (Miyoshi et al. 2004), cell volume regulation (Minton et al. 1992; Hall 2002), aggregate formation (Hatters et al. 2002; Hall and Minton 2004; Van den Berg et al. 1999) and protein folding (Minton 2005; Sasahara et al. 2003; Hall 2005).

In general, experimental manifestations of the crowding phenomena have relied upon constructing in vitro mimics of a biological solution environment resembling that of simple cell types such as the bacterial cell *E. coli* (Zimmerman and Trach 1991). However, the complexity of the intracellular environment varies starkly between prokaryotic and eukaryotic cells due to the existence of a number of additional features, notably the presence of a highly developed and *abundant* cytoskeletal network (Graumann 2004; Alberts et al. 1989). Although prokaryotes have been shown recently to possess some of the rudimentary facets of a cytoskeletal lattice (Graumann 2004) the evolutionary accumulation of increasingly larger amounts of self-assembling protein structures has conferred upon eukaryotes selective advantages with regard to structural support and intracellular transport (Graumann 2004; Alberts et al. 1989) helping them to become larger and more complex than their prokaryotic ancestors. Here I explore the hypothesis that the presence, to a significant extent, of such self-assembling proteins might provide eukaryotic cell types with an additional advantage, namely the ability to regulate intracellular processes by 'fine tuning' the excluded volume component of the solution. In particular I have examined the effect of this proposed fine tuning mechanism upon the transition of a protein between an unfolded expanded state and a folded compact state (Fig. 1)—a process already known to be influenced by molecular crowding (Minton 2005; Sasahara et al. 2003).

D. Hall
Chemistry Department, University of Cambridge,
Lensfield Road, Cambridge, CB21EW, UK
E-mail: drh32@cam.ac.uk
Fax: +44-0-1223-763418

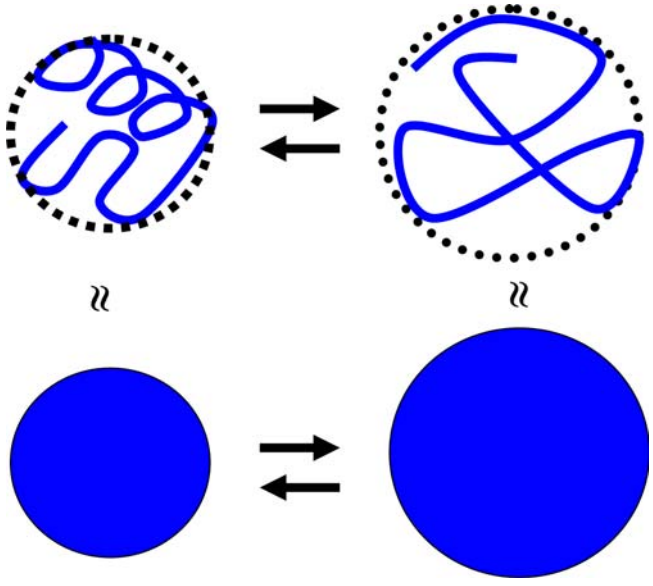

Fig. 1 Approximation of a two state protein folding/unfolding transition as a transition between hard spheres of two different sizes

Materials and methods

For the simulation we used a direct Monte Carlo procedure—the basics of which can be found (Leach 1997). Briefly the procedure involved consideration of a solution of red spheres (radius = 2 nm) and blue spheres (compact state radius = 2 nm; expanded state radius 2.5 nm) in a region of solution volume defined by a cubic box of side length 25 nm. The red spheres were allowed to self-associate to form larger spheres. As we were studying the effect of differential extents of volume occupation by the red spheres on the equilibrium behaviour of a blue sphere able to transition between a compact and expanded state (and not the self-association reaction of the red sphere) we used an empirical mass action relationship $C_i = K_R^{(i-1)} C_1^i$ (Hall and Minton 2003) to define the association state of the red sphere component. This empirical formulation utilizes an equilibrium constant K_R (units M^{-1}) to describe the relationship between the concentration of species composed of i red sphere monomers, C_i, and the equilibrium concentration of red sphere monomers, C_1. The equilibrium red sphere size distribution was determined by iterative application of the above empirical relationship under the constraint of a constant concentration of monomers present within the distribution (C_{TOT} = 10.63 mM; equivalent to 100 monomers in the box). The red spheres of different sizes making up the distribution were then selected in random order and then randomly inserted into the box. Successful insertion was judged by not violating a hard sphere overlap criterion (Leach 1997) with the other balls present in solution. The walls of the box were subject to periodic boundary conditions with their opposing face. Once all red balls were placed in the box a blue ball in a compact state (radius = 2 nm) was repeatedly inserted into, and withdrawn from, the box by the random selection of x, y and z coordinates. Each time the blue ball did not physically intersect with a red ball in solution the insertion was judged to be successful. The procedure was repeated until a total of 500 successful insertions were achieved. The ratio of successful to non successful insertions was recorded and plotted in Fig. 1e. This insertion/withdrawal procedure was repeated again for a blue ball in an expanded state (radius = 2.5 nm) (Fig. 1e). The ratio of the two insertion factors (Ψ - red line Fig. 1e) describes the effect of the crowded solution environment on the unfolding equilibrium of the blue ball. This quantity, $\Psi = F_{BIG}/F_{SMALL}$, provides the relationship between the measured apparent unfolding equilibrium constant, K_B, {where $K_B = [Blue]_{BIG}/[Blue]_{SMALL}$ under crowded conditions} and the unfolding equilibrium constant under ideal conditions, K_B^o, {where $K_B^o = [Blue]_{BIG}/[Blue]_{SMALL}$ under ideal conditions} such that $K_B = K_B^o \Psi$ (see[1]). For each size distribution of red spheres the total procedure of blue ball insertion was repeated for 8 different random placements of the red sphere distribution. Although in this instance we have used a Monte Carlo procedure to estimate the non-ideal effect closed form analytical approximations are available in the form of either virial expansions (for spheres of the same size) or scaled particle expansions (for spheres of different sizes and particles of different shapes). Descriptions of these alternative approaches can be found (Zimmerman and Minton 1993)

Results

The fine tuning hypothesis has been investigated via the use of simulation. The simulation method utilized a Monte Carlo procedure to calculate the probability of successfully placing either of two differently sized blue spheres (representing the protein undergoing the folding/unfolding transition in either a compact or expanded state) into a highly volume occupied solution of a red sphere that is able to change its size composition (the red sphere representing a protein which is able to reversibly self-assemble into higher order structures) (Fig. 2a–c). From the simulations we note the following points

(a) The effect of changing the equilibrium association constant governing self assembly of the red sphere, K_R, is to alter the average size and number concentration of the red type sphere (Fig. 2d).

[1]The physical justification of this formulation lies in the definition of the equilibrium constant and the separable nature of the chemical potential of species i into ideal and non-ideal components ($\mu_i = \mu_{i\text{IDEAL}} + \mu_{i\text{NON-IDEAL}}$). For example we note that $K_B = \exp\{-(\mu_{BIG}-\mu_{SMALL})/(RT)\} = \exp\{-\Delta\mu_{IDEAL}/(RT)\}\times \exp\{-\Delta\mu_{NON\text{-}IDEAL}/(RT)\}$. Thus we may identify K_B^o with the ideal component $\exp\{-\Delta\mu_{IDEAL}/(RT)\}$ and Ψ with the non-ideal component $\exp\{-\Delta\mu_{NON\text{-}IDEAL}/(RT)\}$. We calculate $\psi = \exp\{-\Delta\mu_{NON\text{-}IDEAL}/(RT)\} = f_{BIG}/f_{SMALL}$ by using the Monte-Carlo procedure to define the ratio f_{BIG}/f_{SMALL}. Further information on equilibrium constants and non-ideality can be found (Section 2 of Hall and Minton 2003; Dill and Bromberg 2003).

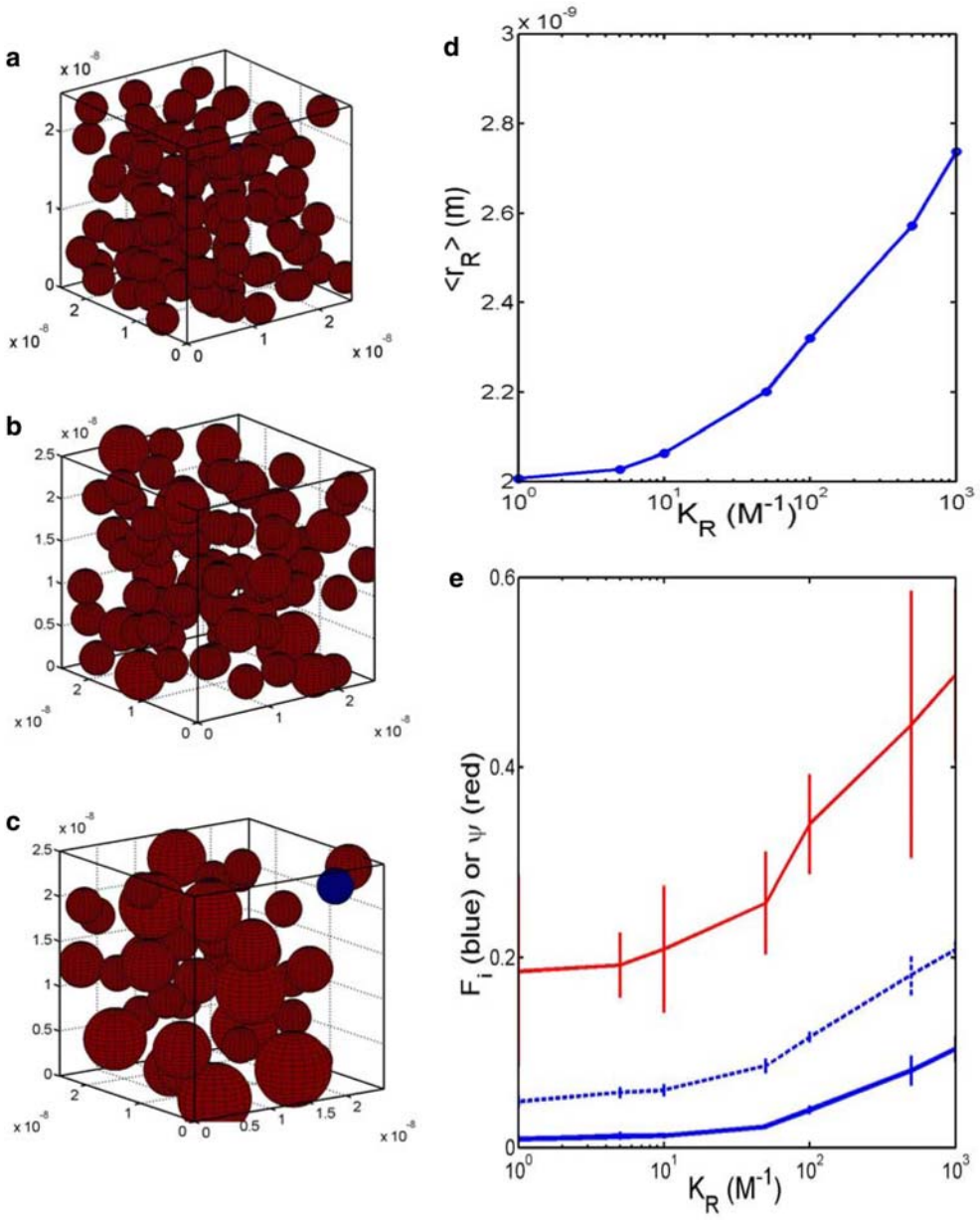

Fig. 2 Effect of varying the red sphere association equilibrium constant (K_R) on the size distribution of the red sphere solution **a** K_R = 1 M^{-1} **b** K_R = 50 M^{-1} **c** K_R = 1000 M^{-1} (total concentration 10.63 mM of red sphere monomers, monomer radius = 2 nm). (d) Effect of varying K_R on number average radius of red spheres. **e** Fractional success of random insertion of blue sphere when in compact form (F_{SMALL}, *blue dotted line*) and when in expanded form (F_{BIG} *blue solid line*). *Red line* describes the value of Ψ which represents the ratio of the fraction of successful insertions of big versus *small blue spheres* ($\Psi = F_{BIG}/F_{SMALL}$) and therefore the degree to which the ideal blue sphere unfolding equilibrium constant K_B^o would be modified by the crowded solution conditions to yield an apparent unfolding equilibrium constant K_B ($K_B = K_B^o \Psi$). *Error bars* represent ± 1SD

(b) The modulation of the assembly state of the red type protein, in turn, alters the effective excluded volume component of the solution as evidenced by the change in fractional success of placing either of the differently sized blue spheres into the different solution environments (Fig. 2e blue lines)

(c) The effect of modulating the solution excluded volume component is to displace the effective equilibrium of the expansion/contraction transition of the blue sphere (Fig. 2e red line).

Discussion

The effect of the 'fine tuning' mechanism explored in this paper will be greater for larger proteins undergoing a folding/unfolding transition and for proteins with a greater degree of expansion upon unfolding. Although general in nature[2] the above findings raise a number of intriguing questions about the nature of protein

[2]Reviewer 1 and 2 have posed the following questions worthy of further study. (1) How would the proposed fine tuning effect discussed above be itself affected by different combinations of time scales for protein folding (blue protein) and protein self-association (red protein) events?. (2) How local might the proposed effects be within the cell?. (3) How would increasing the complexity of the starting mixture (eg. various sizes and shapes) and the geometry of the aggregate affect the proposed fine tuning mechanism? Answers to questions 1 and 2 might be provided by more detailed kinetic based simulations of the type conducted (Eleock 2003). An answer to question 3 would require reworking the simulations with size and shape parameters chosen from a more detailed consideration of the relevant composition of macromolecules inside a particular area of the cell.

regulation in vivo. It is an established fact that protein function can be modulated by changes in tertiary or quaternary structure (Cantor and Schimmel 1980; Van Holde et al. 1998). Such changes can be brought about by altering temperature, general solvent conditions, absence or presence of substrate/product, allosteric effectors or via simple mass action effects (Cantor and Schimmel 1980; Van Holde et al. 1998). An increasing number of experimental (Eggers and Valentine 2001; Zhou et al. 2004; Tokuriki et al. 2004) and theoretical (Minton 2005; Elcock 2003; Kinjo and Takada 2003; Zhou 2004) studies show that crowding and confinement effects are additionally capable of modulating protein tertiary and quaternary structure. With regard to this point it is important to realise that the degree of crowding effect exerted on a particular macromolecule is determined by the amount of volume excluded to it by the other macromolecules in solution rather than just the actual physical volume occupied by these macromolecules (Hall and Minton 2003; Zimmerman and Minton 1993). As such there are finite size effects on the magnitude of the crowding 'pseudo-force' determined by the size of the crowding agent in relation to the size of the macromolecule of interest (Zhou et al. 2004). The ability to dynamically change the size composition of a crowded solution would hence posit an extra dimension for regulating the magnitude of the crowding effect and concomitantly for regulation of protein structure and enzyme function.

There is significantly more protein capable of self-association in the more highly evolved eukaryotic cell types as compared to the more primitive prokaryotic cell type (Graumann 2004; Alberts et al. 1989). Examples of such self-associating proteins present at very significant volume fractions in eukaryotic, but not prokaryotic cell types, include the cytoskeletal proteins actin and tubulin. Although speculative it is interesting to consider that the presence of such a large pool of protein capable of transitioning between associated and dissociated states could confer upon eukaryotes a selective advantage, in evolutionary terms, distinct from their traditionally accepted roles in facilitating intracellular transport and providing structural integrity ((Graumann 2004; Alberts et al. 1989)) – that additional advantage being an extra mechanism for regulating protein/enzyme behaviour via modulation of cellular excluded volume. As a final point it has not escaped the author's notice that the different levels of the crowding effect exerted by a self-associating protein in its different stages of complexation also exhibits a protein chaperone like behaviour of a type not conforming to the current 'holdase' or 'foldase' type paradigms (Hall 2005; Young et al. 2004).

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