Compression of random coils due to macromolecular crowding

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The conformation of a linear polymer chain is studied as a function of the concentration of a macromolecular crowding agent by neutron scattering. Excluded volume to random coil due to macromolecular crowding in cells is predicted to exert a compressive force that will tend to reduce its size. It is shown that when reducing free volume due to macromolecular crowding, we observe a compression of the polymer chain with a reduction in its radius of gyration of up to $\simeq 30\%$ and that the effective chain-chain interactions are strongly modified.

I. INTRODUCTION

The interior of the cells is filled with a very high quantity of space-filling macromolecules with very different sizes and shapes. Generally the concentration of each species is rather low, but the overall occupied volume fraction can range up to 0.3; the cellular environment is highly packed. The excluded volume due to this macromolecular crowding affects a number of phenomena of biological importance. Although the first quantitative theory was developed in the early 1980s [1], real effort to quantify the effect of macromolecular crowding of the cytoplasm on various processes was developed only during the last 10 years [2–4].

Theoretical works [5,6] predicted that excluded-volume interactions in cells [4,7] could significantly influence protein stability. Model calculations suggest that macromolecular crowding stabilizes the native state of proteins relative to unfolded states. The excluded volume to a polypeptide chain by high concentrations of rigid macromolecule would be expected to exert a compressive force reducing its average dimension. The more extended conformations of the unfolded chain ensemble are predicted to be preferentially destabilized relative to more compact conformations, including the native state. The equilibrium ($N \rightleftharpoons S_i$) between the native (N) and the unfolded states ensemble (S_i) of proteins is thus shifted toward the native state. The calculations were performed on a model system of hard spheres (to account for crowding effects of the cytoplasm) and the unfolded states of the protein were assumed to adopt random coil conformations [8]. The validity of this assumption is supported by diffraction studies on unfolded protein conformation. At sufficiently high chemical denaturant concentration, it was shown that the unfolded state of a protein adopts a Gaussian-type conformation [9].

Experimental studies to verify whether proteins are stabilized by excluded-volume effects were performed on test macromolecules in presence of polysaccharides such as Ficoll 70 of Dextran to mimic the crowding of the cytoplasm. Protein activities [10] or circular dichroism [11,12] was measured in denaturing solvent conditions as a function of the volume fraction of cosolute molecules to check change in the $N \rightleftharpoons S_i$ equilibrium. These results tend to confirm, at least qualitatively, the prediction of Minton [5,6]. It was also demonstrated that temperature has a little impact on the stabilization effect due to crowding; heat and cold denaturation are suppressed in the presence of crowded environment [13]. Numerical simulations also confirm the theoretical predictions [14]. A recent experimental study on the effect of macromolecular crowding on native protein seems to show that excluded volume increases the structural content of folded proteins [15]. To our knowledge there was no direct evidence of the changes in Gaussian chain conformation due to the presence of macromolecular crowder, by scattering techniques, which could help to understand the physical mechanism inducing stabilization.

The conformations of polymer chains are studied by scattering methods. Small-angle neutron scattering (SANS) is especially suited to study polymer conformations in polymer blends or mixtures because of the strong ability of neutron to discriminate between hydrogenated and deuterated isotopes [16]. Using isotope contrast one can look at the conformation of a single marked chain in a polymer bulk or mixture. Scattering methods tell us the size of polymer coils. Polymer conformation is refined by simple Gaussian chain models whose approximate form factor was computed by Debye [17] in the absence of any excluded-volume effects. The experimental studies range from chemically identical polymers to multicomponent polymer mixtures. Generally, chemically different polymers are not miscible, although some homogeneous mixtures were reported. To overcome this problem and more generally the effects of interactions, measurements of marked chains at very low concentrations are performed and the single-chain parameters are determined by extrapolation to zero concentration. Using Debye-type form factors one can obtain the radius of gyration R_g of the polymer, its average mass weight M, and the second virial coefficient which provides information on intermolecular interactions.

In this paper, we report a study by SANS of the conformation of a Gaussian chain as a function of the weight fraction of a macromolecular crowding agent, with the attempt to figure out the conformational changes induced by increasing concentration of the latter. In particular, we want to verify whether significant diminution of the radius of gyration of the Gaussian chain is observed as was theoretically predicted [6]. As a first step, we choose polymer and crowding agent with similar radii of gyration.

II. MATERIAL AND METHODS

A. Sample preparation

Our model system is constituted of deuterated poly(ethylene glycol) [D-PEG (for deuterated), the Gaussian chain which will be referred to as polymer in the text] and an almost spherical molecule: Ficoll 70® (F70), which will be referred to as the crowding agent. The osmotic pressure of a Ficoll solution with $\Phi_f \simeq 0.3$ is around 3×10^5 Pa. The weight-averaged molecular weight of the PEG was $M_w = 18\,000$ Da with $M_w/M_n = 1.05$ provided by Polymer Source Inc. The F70 is a highly ramified polysaccharide with molecular weight of 70 kDa and was purchased from Sigma [18]. F70 is highly soluble in water, where it can be dissolved up to concentrations of more than 450 mg ml^{-1}. In our measurements, the F70 fraction weights range from $\Phi_f = 0$ up to more than $\Phi_f \simeq 0.4$ to simulate the macromolecular crowding of the cytoplasm.

B. Neutron-scattering experiments

The neutron-scattering experiments were carried out on the SANS instrument PACE at the Laboratoire Léon Brillouin (France) and D22 at the Institut Laue-Langevin (France) at room temperature. Both instruments were used to cover the full wave-vector range necessary for the experiments. The very high neutron flux available on D22 is a strong advantage for getting high statistical accuracy due to the reduced contrast between the solvent and the PEG (due to F70 matching) and the necessity to extrapolate to zero PEG concentration.

Due to the coherent-scattering length differences between hydrogen and deuterium, the neutron-scattering length density differences between fully hydrogenated F70 and the deuterated polymers are very significant. It is thus possible to match the solvent [xD$_2$O+$(1-x)$H$_2$O] and F70 scattering length densities (for $x \simeq 0.41$) and keep a significant contrast between the solvent and the D-PEG. Under such conditions only the PEG contribution appears as a wave-vector-dependent intensity in the spectra whatever the F70 concentration is. For each F70 weight fraction different D-PEG concentrations were studied in order to trace Zimm-type plots (from a few mg ml^{-1} to few tens of mg ml^{-1} of D-PEG). It is then possible to follow the conformational changes in the D-PEG induced by changes in F70 concentration. The polymer concentrations are chosen to remain far from the overlap concentration.

III. EXPERIMENTAL RESULTS

The spectra were refined using

$$I(q) = \frac{c_p(\Delta\rho)^2}{N_A v_s^2} M_a(c_p, \Phi_f) P(q)(1 + 2M_a c_p A_2). \quad (1)$$

$\Delta\rho$ is the neutron-scattering length density contrast between the deuterated PEG and the solvent, N_A is the Avogadro number, and $P(q) = \frac{2}{x^2}(x - 1 + e^{-x})$ is the molecular form factor of a Gaussian chain, with $x = (qR_g)^2$. A_2 is the second virial coefficient and $M_a(c_p, \Phi_f)$ is the concentration-dependent molar
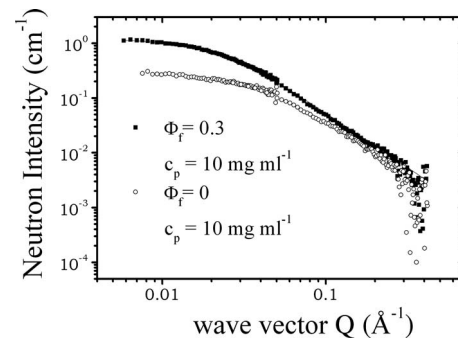

FIG. 1. D-PEG spectra measured on D22 at concentration $c_p = 10$ mg ml^{-1} for two different F70 mass fractions (0 and 0.3). The solvent scattering length density is matched to the F70: only the PEG appears as a wave-vector-dependent intensity.

mass. We obtain the mass of the polymer, M_a, by extrapolation to zero concentration $M_a(c_p \to 0, \Phi)$. v_s is the specific volume of the macromolecule. Figure 1 shows the measured spectra for the two different F70 mass fractions $\Phi_f = 0$ and $\Phi_f = 0.3$ on D22 after standard corrections and background subtraction. The lines are the results of refinement of the spectra by a Gaussian chain form factor.

Figure 2 is a plot of the PEG radius of gyration measured for a concentration of $c_p \simeq 10$ mg ml^{-1} as a function of the Ficoll mass fraction Φ_f (open circle). One can clearly observe an increase in the radius of gyration of the scattering objects with increasing Φ_f, which reflects the tendency to aggregation. Above a given F70 fraction, which depends on the PEG concentration, the solution even becomes turbid. The F70 is a polysaccharide of sucrose. In order to verify if specific interaction occurs between the PEG and the monomer, we measured spectra with equivalent mass fraction of sucrose (open diamond). The radius of gyration of the polymer in the presence of sucrose at almost the same mass fraction is also shown in Fig. 2. The conformation of the polymer is not modified by the presence of the sucrose, and R_g is almost equal to the one measured at $\Phi_f = 0$ whatever Φ_f is. This observation supports the fact that the quality of the solvent is not significantly modified by the presence of the F70 monomer. Thus no preferential interactions occur between the monomer of the PEG and the sucrose. We interpret this feature as an indication that the PEG-F70 interactions are mainly steric (crowding effects).
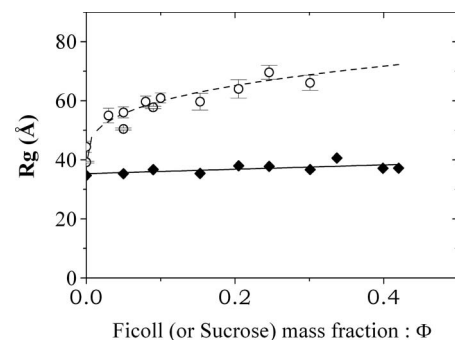

FIG. 2. R_g of the D-PEG at concentration $c_p = 10$ mg ml^{-1} as a function of Φ, the mass fraction of macromolecular crowding agent added. ◇: sucrose, $\Phi = \Phi_s$; ○: F70, $\Phi = \Phi_f$.
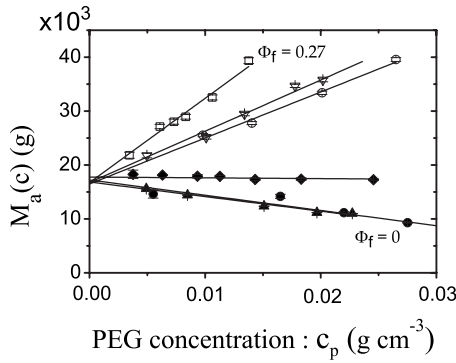
FIG. 3. $M_a(c_p)$ for different Φ_f ranging from $\Phi_f=0$ to $\Phi_f=0.27$.

To determine the effect of the presence of F70 at high mass fraction, we have to overcome the tendency to clustering or aggregation. This implies the necessity to extrapolate the effect of increasing Φ_f to zero PEG concentration. Figure 3 presents the PEG concentration-dependent apparent molecular mass deduced from zero wave-vector intensity:

$$M_a(c_p) = \frac{N_A v_s^2}{c_p(\Delta\rho)^2} I(q \to 0, c_p). \qquad (2)$$

These results are presented as a function of the PEG concentration c_p for different mass fractions Φ_f of F70, ranging from $\Phi_f=0$ (full circle) to $\Phi_f=0.27$. The six concentrations converge to the same intercept at zero PEG concentration which corresponds to the mass of the polymer given by the provider. This feature is particularly interesting for our purpose because it means that by extrapolation to zero PEG concentration, the molecular form factor of a single chain can be obtained whatever the F70 concentration is. The PEG mass concentration dependence $[M(c_p)]$ exhibits a linear behavior as a function of c_p whatever the F70 concentration is, and the slopes are very strongly dependent on Φ_f. These slopes are proportional to the second virial coefficient A_2. When $\Phi_f \to 0$, A_2 is positive, which states the small repulsive interaction between PEG chains. The transition, when Φ_f increases, to a negative value of the pseudo-second-virial term A_2 reflects the tendency of PEG chains to associate, which is fully consistent with the plot in Fig. 2. When increasing Φ_f, the PEG concentration at which the spectra significantly differ from Gaussian-chain-like form factors diminishes. This reflects the fact that when increasing Φ_f the aggregation occurs at smaller c_p.

Figure 4 shows the dependence of the R_g deduced from the Debye formula as a function of the PEG concentration c_p for different F70 mass fractions Φ_f. As with Fig. 3, the clear tendency to clustering is observed when increasing F70 concentration, but this time with the strong increase in the radius of gyration of the scattering objects. The slope changes from negative value for $\Phi_f < 0.1$ up to strongly positive ones for $\Phi_f \simeq 0.3$. This slope is proportional to B_2, a quantity which is similar in nature to the second virial coefficient of the osmotic pressure and reflects molecular interactions. This observation can be related to the previous experimental observation [10] and theoretical predictions [19] that macromo-

FIG. 4. Concentration dependence of the radius of gyration of the D-PEG for different F70 mass fractions.

lecular crowding increases the unfolded protein aggregation. No specific interactions occur between the F70 and the PEG chains. Thus the changes in the second virial coefficient from small positive to large negative value reflect this tendency of clustering simply due to macromolecular crowding.

The particularly interesting point here concerns the extrapolation of the radius of gyration to $c_p=0$ for different Φ_f (Fig. 5). It clearly diminishes when F70 concentration Φ_f increases. For $\Phi_f=0$ (only the PEG is in solution) $R_g \simeq 57$ Å, whereas it reduces to $R_g \simeq 35$ Å for $\Phi_f \simeq 0.27$. This result, together with the conclusion for Fig. 3 that only one polymer chain contributes to the signal at zero PEG concentration, is a clear experimental observation of the compression of the chain due to the presence of macromolecular crowding. The R_g reduction of the polymer chain from a solvent without any macromolecular crowding to a solution of 270 mg ml^{-1} is nearly 30%, although experimental uncertainties become significant when the reduction reaches 50% for 330 mg ml^{-1} of F70.

IV. DISCUSSION

Direct comparison to the theoretical predictions [5,6] is not straightforward for various reasons. First the F70 does certainly not behave as a hard sphere. Although it is much more compact than a Gaussian chain, its radius of gyration is the same as the one of the PEG with an average weight of 70 kDa, whereas the latter is only 18 kDa. The molecular form factor of F70 $[F(q)]$ measured in D$_2$O at almost the same
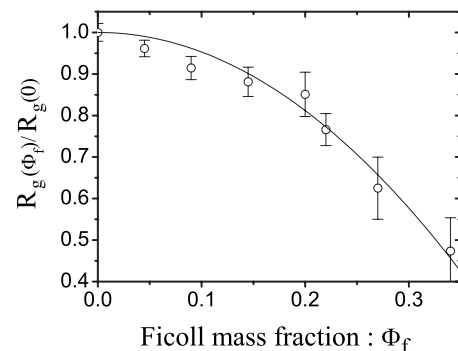

FIG. 5. $R_g(\Phi)/R_g(0)$ of the D-PEG as a function of the mass fraction Φ_f of the macromolecular crowding agent (F70).

concentration as the PEG does not fit to the hard-sphere one. At high q the slope of $F(q)$ is closer to q^{-2} (Gaussian chain) than q^{-4} predicted for hard sphere. However such decay at high q of the form factor can be quite easily reproduced with a hard-sphere core decorated with some polymers on its surface or with random-walk polymer confined in a sphere [20]. Thus the true F70 potential is certainly not a hard-sphere one but softer than that. Nevertheless the results of the computation of Minton [5,6] for different unfolded proteins predict a reduction in the R_g of nearly 30% due to macromolecular crowding, which is comparable to our results obtained for homopolymers.

Homopolymer collapse, as a result of a delicate balance between enthalpic and entropic effects, was a subject of intense research activity over the past, both theoretically [21,22] and experimentally [23–26]. The first observations of the collapse were driven by the solvent quality, by change in temperature, or by mixture of solvent of different qualities at rather low polymer concentration. "The better the solvent the greater the 'swelling' of the molecule; conversely the poorer the solvent the smaller the molecule" [21]. Later the compression of a polymer chain was observed in a matrix of cross-linked polymers [26] by neutron scattering. In our experiments the situation is different because the solvent quality is not modified; therefore we speak of compression of the chain rather than collapse, the mechanism of which is basically different [27]. Because of the large loss of configurational entropy associated with total collapse and monomer-excluded interactions, volume exclusion is not predicted to induce collapse of simple polymers such as PEG. However it may induce collapse in more complex polymers such as polypeptides, where short-ranged intramolecular hydrogen bonding between segments of the backbone and hydrophobic interactions between nonpolar side chains can stabilize a collapsed (or molten globulelike) state and compensate for the loss of configurational entropy.

V. CONCLUSION

We have measured the compression of a polymer chain due to macromolecular crowding by neutron scattering using contrast method. When increasing cosolute mass fraction (Ficoll 70) up to $\Phi \simeq 0.3$, the radius of gyration of the chain is decreased by a factor of 2. Models for excluded-volume interactions [6] due to hard spheres are consistent with our experimental results. If such a compression behavior could be observed for polypeptide chains, it could have significant influence on our understanding of protein stability in cytoplasmic environment.

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