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Disordered Protein Diffusion under Crowded Conditions

Yaqiang Wang[†], **Laura A. Benton**[†], **Vishavpreet Singh**[†], and **Gary J. Pielak**[†,‡,⊥,*]

[†]Chemistry, University of North Carolina, Chapel Hill, NC 27599

[‡]Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC 27599

[⊥]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599

Abstract

Intrinsically disordered proteins are important in signaling, regulation, and translocation. Understanding their diffusion under physiologically relevant conditions will yield insight into their functions. We used NMR to quantify the translational diffusion of a globular and a disordered protein in dilute solution and under crowded conditions. In dilute solution, the globular protein chymotrypsin inhibitor 2 (CI2, 7.4 kDa) diffuses faster than the disordered protein α-synuclein (14 kDa). Surprisingly, the opposite occurs under crowded conditions; α-synuclein diffuses faster than CI2, even though α-synuclein is larger than CI2. These data show that shape is a key parameter determining protein diffusion under crowded conditions, adding to the properties known to be affected by macromolecular crowding. The results also offer a clue about why many signaling proteins are disordered.

Keywords

^{19}F NMR; Crowding; Diffusion; Disordered proteins

Intrinsically disordered proteins lack a stable tertiary structure.[1,2] In bacteria, between 6% and 33% of proteins are disordered and 35 – 51% are disordered in eukaryotes.[3] Despite the lack of well-defined structure, disordered proteins are involved in numerous cellular functions, including regulation, signaling, and translocation.[1–7] Bioinformatics studies show that more than 70% of signaling proteins are unstructured.[4] Diffusion is the major mode of macromolecular transport in cells, and therefore is expected to play a vital role in signaling.

The cellular interior, however, is an exceptionally complex environment where macromolecules can reach concentrations of 300 g/L and occupy 30% of the volume.[8,9] This crowded environment is vastly different from the dilute, idealized conditions used in most biophysical studies. Fluorescence results show that cytoplasm of *Escherichia coli* slows the translational diffusion of a globular protein, green florescent protein, 10-fold compared to dilute solution.[10,11] Results from *in vitro* experiments using synthetic polymers also show that crowding can dramatically decrease the translational diffusion of test globular proteins.[12–14] However, there is no quantitative information about disordered protein diffusion under the crowded conditions.

The measurement of translational diffusion in cells has been dominated by fluorescence methods.[15,16] Despite progress in site-specific protein labeling in living cells, most such studies are still limited to green fluorescent protein.[17] Nuclear magnetic resonance spectroscopy (NMR) is a noninvasive approach that can assess protein biophysics in cells

[*]Corresponding Author: gary_pielak@unc.edu.

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and under crowded in vitro conditions.[18] Here, we use NMR to quantify the translational diffusion of the disordered 14-kDa Parkinson's disease related protein α-synuclein in dilute solution and in 300 g/L solutions of glycerol, the synthetic polymers polyvinylpyrrolidone 40 (PVP, 40 kDa), and Ficoll 70 (a 70 kDa cross-linked sucrose polymer), and the globular proteins lysozyme (15 kDa), and bovine serum albumin (BSA, 67 kDa), and then compared the diffusion coefficients to those from parallel studies on the 7.4-kDa globular protein chymotrypsin inhibitor 2 (CI2).[19,20]

We used ^{19}F NMR because of its high sensitivity, spectral simplicity, and large chemical shift range[21–23] to assess the diffusion of α-synuclein. The protein was labeled with 3-fluorotyrosine.[22] Translational diffusion was measured with the Dbppste sequence.[24] Spectra of the protein in 300 g/L BSA solution as a function of gradient strength are shown in Figure 1. The slope of plots like the one shown in Figure 2 gives the translational diffusion coefficient (The uncertainties from repeating the experiment are less than 0.4×10^{-11} m2/s.). The linearity of the plot indicates that proteolysis of α-synuclein is not a problem, consistent with other investigations of α-synuclein under crowded conditions.[25] The linearity of all the plots analyzed for this report is also consistent with simple- rather than sub-diffusion.[26]

To assess the veracity of ^{19}F NMR as a probe, we measured the diffusion coefficient ^{19}F-labeled CI2[22] and α-synuclein in dilute solution (pH 7.4, 25 °C). The value for CI2, 16.0×10^{-11} m^2/s, is consistent with that obtained from ^{15}N enrichment (15.51×10^{-11} m^2/s).[19,20] Our value for 19F-labeled α-synuclein, 7.8×10^{-11} m^2/s, is reasonable agreement with the value of 5.7×10^{-11} m^2/s obtained at 10 °C,[28] given that the properties of disordered proteins depend more heavily on conditions than those of globular proteins. For instance, the hydrated radius of α-synuclein increases by 8 Å between 10 °C and 35 °C.[25] In summary our comparisons suggest that ^{19}F is a reliable tool for measuring diffusion.

Next, we compared the diffusion of CI2 to that of α-synuclein in dilute solution and in a 300 g/L solution of the small-molecule co-solute, glycerol (Figure 3). CI2 diffuses faster for three reasons. First, CI2 has a smaller molecule weight. Second, CI2 is folded and compact, making its hydrodynamic radius smaller than that of α-synuclein.[25] Third, when the radius of a protein is comparable to or greater than the radius of the co-solute (e.g., glycerol), a larger protein will diffuse more slowly.[19,20,29] In addition, the slowing of CI2 diffusion by glycerol is consistent with the Stokes-Einstein equation.[20]

We then studied macromolecular crowding. Compared to dilute solution, CI2 and α-synuclein diffusion is slowed 5- to 10-fold in solutions crowded by PVP, Ficoll, BSA and lysozyme (Figure 3). The slowing by protein crowders is consistent with studies of green fluorescent protein in *E. coli*,[10,11] suggesting that our data have some biological relevance. Circular dichroism and NMR data collected under crowded conditions rule out large crowding-induced conformational changes of CI2 and α-synuclein as the cause of the slowing.[30–36]

Synthetic polymers impede the diffusion of both CI2 and α-synuclein more than the protein crowders (Figure 3). The explanation is that the synthetic polymers overlap to form a mesh that impedes translation,[37] but proteins, being quasi-spherical, cannot overlap and offer less of an impediment. In summary, CI2 and α-synuclein diffuse faster in crowded protein solutions than they do in crowded synthetic polymer solutions.

The key observation of this study is that macromolecular crowding affects the diffusion of the disordered and the globular protein differently (Figure 3). While CI2 diffusion is slowed 5- to 10-fold, α-synuclein diffusion is slowed only 3- to 6-fold, despite the fact that α-synuclein has a larger molecular weight. The consequence is that α-synuclein diffusion is

now faster than CI2 diffusion; the opposite of what is observed in dilute solution and in glycerol. These results do not arise from a structural change because CI2 remains compact[20] and α-synuclein remains collapsed[30,33–36] under crowded conditions. We suggest that α-synuclein adopts a different diffusion strategy under crowded conditions. For instance, we know that the cellular environment dampens that the rotational diffusion of disordered proteins less than the rotational diffusion of globular proteins.[33] This relative increase in rotation motion for disordered proteins arises because they possess more internal (segmental) motion. Perhaps the inherent internal motion of disordered proteins is converted to translational motion under crowded conditions.

Our results may help explain an observation about FlgM, an intrinsically disordered protein that regulates flagella and chemotaxis genes in *E. coli* and *Salmonella typhimurium*. Specifically, the disorder would facilitate its export from the cell via the narrow central channel of the flagella filament.[38,39] More generally, this facilitation of diffusion would decrease the apparent size of disordered signaling proteins, making it easier for them to shuttle through the small cavities between macromolecules inside the crowded cell.

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ABBREVIATIONS

CI2 chymotrypsin inhibitor 2

PVP polyvinylpyrrolidone

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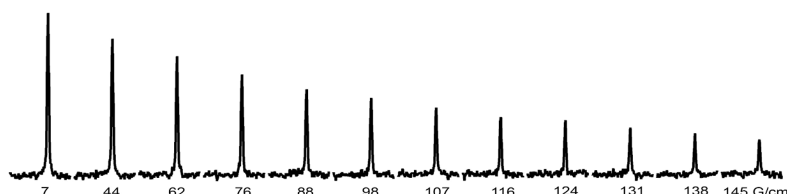

Figure 1.
^{19}F spectra of 1 mM ^{15}N-enriched, 3-fluorotyrosine labeled α-synuclein in 300-g/L bovine serum albumin [pH 7.4, 200 mM potassium phosphate buffer, 1 mM EDTA, 0.5 mM, 25 °C] with increasing gradient strength. Materials and Methods: Chicken lysozyme, BSA, glycerol, Ficoll and PVP were purchased from Sigma-Aldrich and used without further purification. For α-synuclein experiments, glycerol, PVP and Ficoll were dissolved in 50 mM potassium phosphate buffer, 1 mM EDTA, 0.5 mM phenylmethanesulfonyl fluoride (PMSF), pH 7.4, whereas lysozyme and BSA were dissolved in 200 mM potassium phosphate buffer, 1 mM EDTA, and 0.5 mM PMSF, pH 7.4. The final concentration of CI2 and α-synuclein was ~1 mM. ^{19}F data were acquired without decoupling on a Varian Inova 600 spectrometer equipped with a ^{19}F-{^{1}H} z-gradient probe. Conventional 5-mm NMR tubes were used. Gradient strengths ranged from 5.4 G/cm to 129.1 G/cm and the diffusion delay was 0.15 s in solutions containing Ficoll and PVP. Gradient strengths ranged from 5.4 G/cm to 118.4 G/cm and the diffusion delay was 0.08 s in solutions containing lysozyme and BSA.

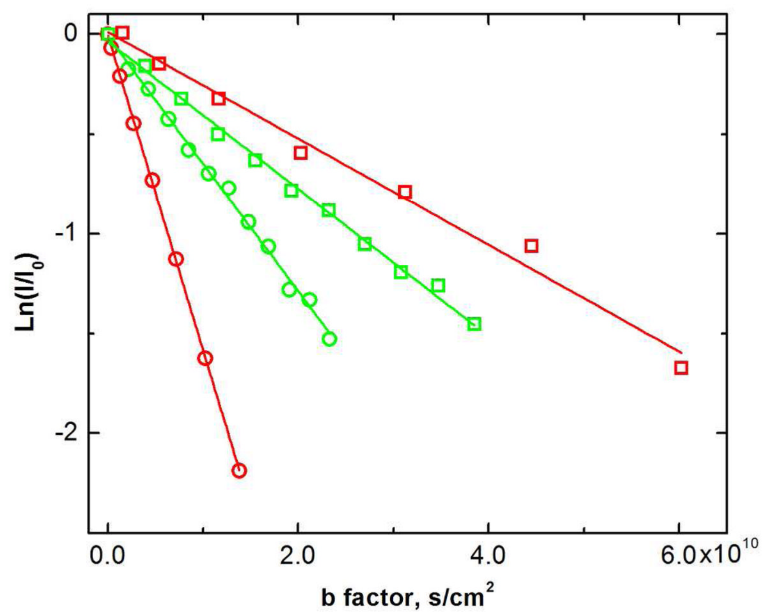

Figure 2.
Translational diffusion of CI2 (red)[19,20] and α-synuclein (green) in dilute solution (circles) and in a 300 g/L solution of bovine serum albumin (squares). b factor = $(\gamma G d)^2\Delta$ where γ is the gyromagnetic ratio, G is the gradient strength, d is the duration of the gradient pulse, and Δ is the delay between gradient pulses.[27]

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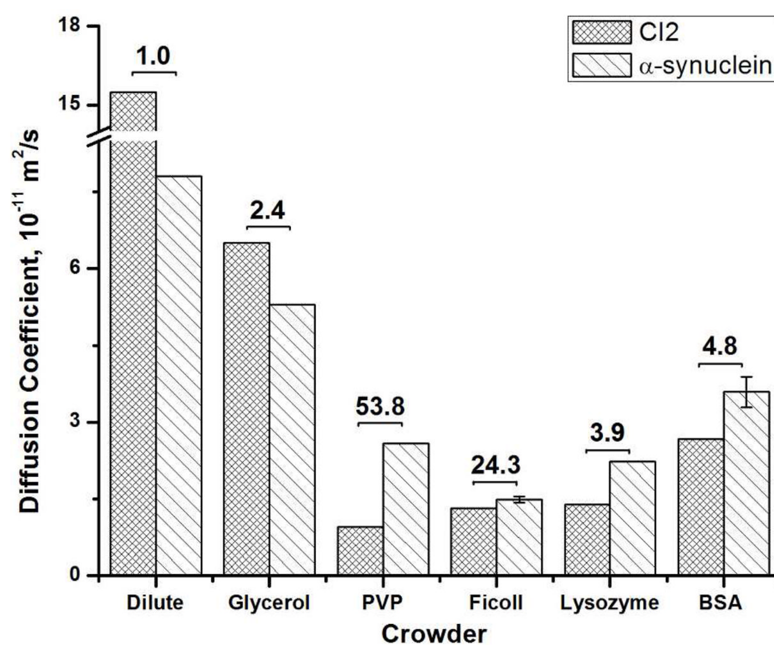

Figure 3.

Histogram showing the translational diffusion coefficients of CI2 and α-synuclein in dilute solution and 300 g/L solutions of glycerol, PVP, Ficoll 70, lysozyme and bovine serum albumin at 25 °C. Error bars represent the standard deviation of the mean from three trials. The corresponding macroviscosity (in cP) is indicated at the top of each entry. The CI2 data have been published.[19,20]

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