Phase Separation and Single-Chain Compactness of Charged Disordered Proteins are Strongly Correlated

Yi-Hsuan Lin1,2 and Hue Sun Chan1,3
1Department of Biochemistry, University of Toronto, 1 King’s College Circle, Toronto, Ontario M5S 1A8, Canada; 2Molecular Medicine, Hospital for Sick Children, 686 Bay Street, Toronto, Ontario M5G 0A4, Canada; 3Department of Molecular Genetics, University of Toronto, 1 King’s College Circle, Toronto, Ontario M5S 1A8, Canada

ABSTRACT Liquid-liquid phase separation of intrinsically disordered proteins (IDPs) is a major undergirding factor in the regulated formation of membraneless organelles in the cell. The phase behavior of an IDP is sensitive to its amino acid sequence. Here we apply a recent random-phase-approximation polymer theory to investigate how the tendency for multiple chains of a protein to phase separate, as characterized by the critical temperature $T^{*}_{cr}$, is related to the protein’s single-chain average radius of gyration $⟨R_g⟩$. For a set of sequences containing different permutations of an equal number of positively and negatively charged residues, we found a striking correlation $T^{*}_{cr} \sim ⟨R_g⟩^{-γ}$ with $γ$ as large as $\sim 6.0$, indicating that electrostatic effects have similarly significant impact on promoting single-chain conformational compactness and phase separation. Moreover, $T^{*}_{cr} \propto −SCD$, where SCD is a recently proposed “sequence charge decoration” parameter determined solely by sequence information. Ramifications of our findings for deciphering the sequence dependence of IDP phase separation are discussed.

Received for publication 1 Jan 0000 and in final form 1 Jan 0000.

Address reprint requests and inquiries to H. S. Chan, E-mail: chan@arrhenius.med.toronto.edu

The biological function and disease-causing malfunction of proteins are underpinned by their structures, dynamics, and myriad intra- and inter-molecular interactions. Many critical cellular functions are carried out by intrinsically disordered proteins or protein regions (collectively abbreviated as IDPs here) with sequences that are less hydrophobic than those of globular proteins but are enriched in charged, polar, and aromatic residues (1–6). At least 75% of IDPs are polyampholytes (7, 8) in that they contain both positively and negatively charged residues (9, 10). Accordingly, electrostatic effects are important in determining individual IDPs’ conformational dimensions (8, 11, 12) and binding (13, 14). Charge-charge interactions are often significant in the recently discovered phenomenon of functional IDP liquid-liquid phase separation as well (15–22). IDP phase separation appears to be the physical basis of membraneless organelles, performing many vital tasks. Recent examples include subcompartmentalization within the nucleolus (22) and synaptic plasticity (21). Malfunction of phase separation processes can lead to disease-causing amyloidogenesis (18) and neurological disorders (21). Speculatively, membraneless liquid-liquid phase separation of biomolecules might even have played a role in the origins of life (23).

Electrostatic effects encoded by a sequence of charges depend not only on the total positive and negative charges or net charge (24, 25) but also the charge pattern (8). For IDPs, this was demonstrated by Das and Pappu who conducted explicit-chain, implicit-solvent conformational sampling of thirty different sequences each composing of 25 lysine (K) and 25 glutamic acid (E) residues (termed KE sequences hereafter). They found that the average radius of gyration, $⟨R_g⟩$, is strongly sequence-dependent, and is correlated with a charge pattern parameter $κ$ that quantifies local deviations from global charge asymmetry (8). A subsequent analytical treatment of the KE sequences by Sawle and Ghosh rationalized the trend through another charge pattern parameter “sequence charge decoration” (SCD) that also correlates well with $⟨R_g⟩$ (26). For IDP phase separation, a recent sequence-dependent random-phase-approximation (RPA) approach we put forth (27, 28) accounted for the experimental difference in phase-separation tendency between the wildtype and a charge-scrambled mutant of the 236-residue N-terminal fragment of DEAD-box RNA helicase Ddx4 (16).

These advances suggest that a deeper understanding of the fundamental relationship between single- and multiple-chain IDP properties is in order. It would be helpful, for instance, if experiments on single-chain properties can infer the conditions under which a protein sequence would undergo multiple-chain phase separation. We embark on this endeavor by first focusing on electrostatics, while leaving aromatic and other $π$-interactions—which can figure prominently in IDP behavior (16, 27, 29)—to future effort. To reach this initial goal, we apply RPA to the thirty KE sequences of length $N = 50$ to ascertain their phase-separation properties under salt-free conditions. Adopting our previous notation and making the same simplifying assumption that amino acid residues and water molecules are of equal size in the theory (27, 28), the free energy $F_{RPA}$ of the multiple-chain system of a given polyampholytic sequence with charge pattern $\{σ_1, σ_2, ..., σ_N\}$,

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where \( \sigma_i = \pm 1 \) is the sign of electronic charge of the \( i \)-th residue, is given by [see Eqs. (13) and (40) of Ref. (28)]:

\[
\frac{F_{\text{RPA}} a^3}{k_B T} = \phi_m N \ln \phi_m + (1 - \phi_m) \ln (1 - \phi_m)
+ \int_0^\infty dk k^2 \frac{4}{4\pi^2} \{ \ln [1 + G(k)] - G(k) \},
\]

(1)

where \( a = 3.8\AA \) is the C-C distance, \( V \) is system volume, \( k_B \) is Boltzmann constant, \( T \) is absolute temperature, \( \phi_m = \rho_m a^3 \) is the volume ratio of amino residues wherein \( \rho_m / N \) is protein density, and

\[
G(k) = \frac{4\pi\phi_m}{k^2(1 + k^2)T^* N} \sum_{i,j=1}^N \sigma_i \sigma_j \exp \left( -\frac{k^2}{6} |i - j| \right).
\]

(2)

Here \( T^* \equiv a/l_B \) is reduced temperature; the Bjerrum length \( l_B = e^2/(4\pi\epsilon_0\epsilon_\infty k_B T) \) where \( e \) is elementary charge, \( \epsilon_0 \) is vacuum permittivity, and \( \epsilon_\infty \) is relative permittivity (27, 28); \( \epsilon_r \approx 80 \) for water but can be significantly lower for water-IDP solutions (28). Here \( \epsilon_r \) is treated largely as an unspecified constant because our main concern is the relative \( T^*_c \)s of different sequences.

We determined the phase diagrams of the 30 KE sequences from the free energy expression Eq. (1) using standard procedures (28). For each sequence, the highest temperature on the coexistence curve is the critical temperature \( T^*_c \), which is the highest \( T^* \) at which phase separation can occur (Fig. 1(a)). The critical temperatures of the KE sequences are highly diverse, ranging from \( T^*_c = 0.089 \) (sv1) to 8.570 (sv30). The variation of critical volume fraction \( \phi_{c,r} \equiv \phi_m(T^*_c) \) from 0.0123 (sv30, sv24) to 0.0398 (sv1) is narrower. The KE sequences were originally labeled as sv1, sv2, . . . , sv30 in ascending values for Das and Pappu’s charge pattern parameter \( \kappa \), from the strictly alternating sequence sv1 with \( \kappa = 0.0009 \) (minimum segregation of opposite charges) to the diblock sequence sv30 with \( \kappa = 1.0 \) (maximum charge segregation) (8). Our RPA-predicted \( T^*_c \)s follow largely, though not exactly, the same order: sv1 and sv30 have the lowest and highest \( T^*_c \)s, respectively; but, e.g., sv24 rather than sv27 has the fourth largest \( T^*_c \) and sv5, not sv2, has the second lowest \( T^*_c \). If \( \epsilon_r = 80 \) is assumed, RPA predicts that 21 KE sequences can, but 9 KE sequences cannot phase separate at \( T \gtrsim 300 \) K (Fig. 1(b,c)).

Because \( \langle R_g \rangle \) correlates positively with \( \kappa \) (8), the present \( T^*_c \) trend suggests that multiple-chain \( T^*_c \)s should correlate with single-chain \( \langle R_g \rangle \). Indeed, a striking correlation (Fig. 1(b)) satisfying the approximate power-law

\[
T^*_c \approx 9.8 \times 10^7 \left( \frac{\langle R_g \rangle}{\AA} \right)^{-5.83},
\]

(3)

with \( R_g \) in units of \( \AA \), is observed for the KE sequences. The variation of \( T^*_c \) with \( \langle R_g \rangle \) is very sharp: \( T^*_c \) increases \( \sim 100 \) times while \( \langle R_g \rangle \) decreases by \( \lesssim 50\% \). Qualitatively, the positive \( \langle T^*_c \rangle \sim \langle R_g \rangle \) correlation may be understood by considering two extreme cases: The diblock and the strictly alternating sequences (Fig. 2). For the diblock, attractive Coulomb interactions are absent—cannot be satisfied—within most stretches of several (e.g. < 6) residues. However, once a pair of opposite charges are in spatial proximity, chain connectivity brings two oppositely charged blocks together, leading to a strong Coulomb attraction, thus a small \( \langle R_g \rangle \) and a higher tendency to phase separate (higher \( T^*_c \)). In contrast, for the strictly alternating sequence, attractive Coulomb interactions that are already weakened relative to that of the diblock sequence require more conformational restriction, resulting in more open, large-\( \langle R_g \rangle \) single-chain conformations and less tendency to phase separate (lower \( T^*_c \)).

It is instructive to compare the predictive power of \( \kappa \) and another charge pattern parameter SCD ≡ \( \sum_{i<j} \sigma_i \sigma_j \sqrt{j-i} / N \) that has emerged from the analysis of Sawle and Ghosh (26). The two parameters are well correlated \( (r^2 = 0.95, \text{see Fig. 7 of Ref. (26)}) \), yet the variation of both \( T^*_c \) and \( \langle R_g \rangle \) of the KE sequences is significantly smoother with respect to SCD than \( \kappa \) (Fig. 3). For example,
despite the large variation in $\kappa$ for sv24, sv26, and sv28 (0.45, 0.61, and 0.77, respectively), their $\langle R_g \rangle = 17.6, 17.5$, and 17.9 Å (8), and their $T_{cr}^\ast = 5.16, 5.08$, and 5.18 are almost identical (Fig. 3(b)). This similarity, however, is well reflected by their similar $\text{SCD} = -17.0, -16.2$, and -16.0. Indeed, a near-linear relationship ($r^2 = 0.997$),

$$T_{cr}^\ast \approx -0.314(\text{SCD}),$$  

is observed (Fig. 3(b)). A likely origin of $\text{SCD}$’s better performance is that it accounts for potential interactions between charges far apart along the sequence whereas $\kappa$ relies on averaging over 5 or 6 consecutive charges. Accordingly, $\text{SCD}$ is less sensitive than $\kappa$ to isolated charge reversals. The rather smooth $\langle R_g \rangle$ dependence is remarkable because the simulated $\langle R_g \rangle$ (8) bears no formal relationship with the variational theory from which $\text{SCD}$ emerges (26). Future effort should be directed toward further assessment of these and other possible charge pattern parameters (31) as predictors for IDP conformational properties.

In summary, we have quantified a close relationship between single-chain conformational compactness of polyampholytes and their phase separation tendency. The above RPA results were derived with a short-range cutoff for Coulomb interactions to account for residue sizes (28, 30). If we had adopted an unphysical interaction scheme without such a cutoff, similar trends would still hold although the scaling relations Eqs. (3) and (4) would be modified, respectively, to $T_{cr}^\ast \sim (R_g)_{cr}^{-3.57}$ and $T_{cr}^\ast \approx -0.490(\text{SCD})$. Thus, in any event, basic physics dictates a rather sharp positive correlation between $T_{cr}^\ast$ and $\langle R_g \rangle$. This connection should be further explored by both theory and simulation (31, 32) to help decipher the sequence determinants of IDP phase separation.

ACKNOWLEDGMENTS

We thank Julie Forman-Kay and Robert Vernon for helpful discussions. This work was supported by Canadian Cancer Society Research Institute grant no. 703477, Canadian Institutes of Health Research grant MOP-84281, and computational resources provided by SciNet of Compute Canada.

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