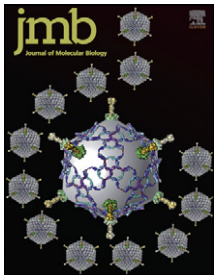

Sequence Specificity in the Entropy-Driven Binding of a Small Molecule and a Disordered Peptide

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Abstract

Approximately one-third of the human proteome is made up of proteins that are entirely disordered or that contain extended disordered regions. Although these disordered proteins are closely linked with many major diseases, their binding mechanisms with small molecules remain poorly understood, and a major concern is whether their specificity can be sufficient for drug development. Here, by studying the interaction of a small molecule and a disordered peptide from the oncogene protein c-Myc, we describe a "specific-diffuse" binding mechanism that exhibits sequence specificity despite being of entropic nature. By combining NMR spectroscopy, biophysical measurements, statistical inference, and molecular simulations, we provide a quantitative measure of such sequence specificity and compare it to the case of the interaction of urea, which is diffuse but not specific. To investigate whether this type of binding can generally modify intermolecular interactions, we show that it leads to an inhibition of the aggregation of the peptide. These results suggest that the binding mechanism that we report may create novel opportunities to discover drugs that target disordered proteins in their monomeric states in a specific manner.

Disordered proteins [1–8] represent a major untapped potential for drug discovery [9–12]. The identification of small molecules that interact with the monomeric forms of these proteins, however, is hindered by a limited understanding of the corresponding mechanisms of binding and by the uncertainty about whether specificity is possible for these interactions. Traditional drug discovery methods, which have been developed mainly to target globular proteins, focus on the optimization of interactions between small molecules and binding pockets with well-defined structures [13,14]. Disordered proteins do not readily lend themselves to this type of binding [1–7,9–12,15,16] as they lack stable conformations and are better represented as ensembles of many structures with relatively low populations. Consequently, disordered proteins often do not adopt suitable binding pockets amenable to traditional drug discovery programs. Therefore, developing a greater understanding of the mechanisms by which small molecules can interact with disordered proteins, and hence potentially alter their disease-promoting behavior, could have profound implications for the search of new drugs. However, the identification of small molecules that interact with disordered proteins is particularly challenging because their highly dynamic nature makes it extremely difficult to study them experimentally [17], and notably, few monomeric disordered proteins have been successfully targeted by small molecules [9–12,16–20].

In order to investigate how small molecules bind disordered proteins, here we characterized the interaction between a small molecule and the disordered protein, c-Myc. High-throughput screening approaches have already yielded several compounds able to prevent the oncogenic dimerization of c-Myc to its partner Max, identifying in particular a small molecule, 10058-F4, that binds the 11-residue region comprising residues 402–412 (c-Myc$_{402–412}$)

[10,21–23]. In this study, we combined experimental thermodynamic techniques with all-atom NMR-restrained metadynamic metainference simulations to determine how c-Myc$_{402–412}$ binds 10058-F4, revealing a "diffuse-specific" mechanism, which is sequence-specific while being of predominantly entropic nature.

Thermodynamic characterization

As c-Myc$_{402–412}$ is disordered, we asked whether the binding mechanism of this monomeric peptide to 10058-F4 could be characterized in terms of equilibrium thermodynamics. To ensure that we were indeed working with the monomeric peptide, dynamic light scattering measurements were taken of the sample prior to any other measurements (Fig. S1).

Given the absence of well-defined binding pockets within this short, disordered peptide, the identification of a thermodynamic signature of binding is fundamental for understanding the mechanism of this interaction. Therefore, we performed isothermal titration calorimetry (ITC) on this system (Fig. 1a), which is one of the most conventional and well-established methods for direct, label-free measurements of enthalpic changes [13,17]. Using this method, we detected heats of dilution indicative of low enthalpic contributions to the binding at 25 °C (Fig. 1b). As similar results were obtained by repeating the experiments at 15 °C, we were prompted to more closely consider entropic contributions.

To unambiguously elucidate the contributions of enthalpy and entropy in this interaction, we performed a van't Hoff analysis (Fig. 1d) using fluorescence titration experiments (Fig. S2) at different temperatures (Fig. 1c), taking advantage of the intrinsic fluorescence of a tyrosine residue within the peptide sequence, and found consistent results with the ITC experiments. At room temperature, we observed an affinity of 14 μM with a 95% confidence interval (95% CI 12.2, 15.9 μM), consistent with the affinity previously reported of 13 ± 1 μM [10]. This analysis showed that the binding free energy (−27.6 ± −8.5 kJ/mol at 25 °C) predominantly comprised entropic contributions (−20.7 ± −4.2 kJ/mol), while enthalpic contributions were also observed (−7.0 ± −4.3 kJ/mol). We thus concluded that the binding of 10058-F4 to the disordered c-Myc peptide is associated with an increase of entropy of the system.

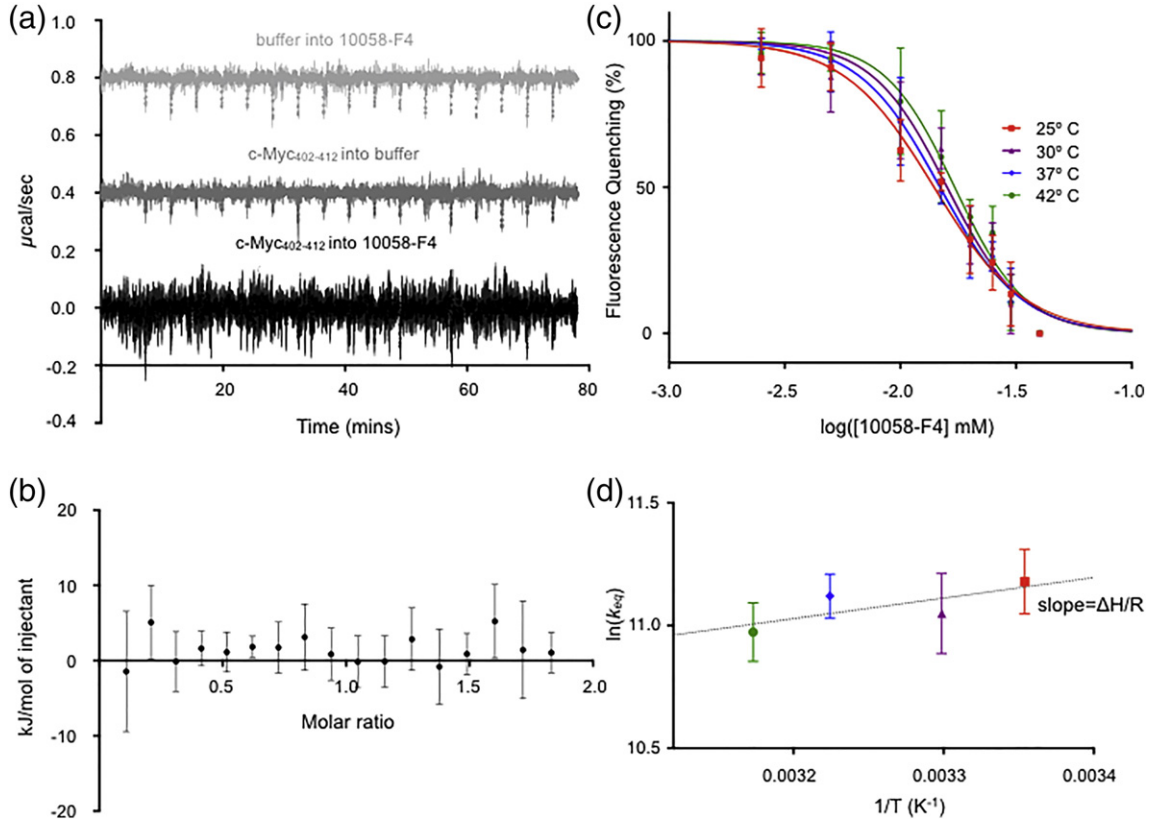

Fig. 1. Thermodynamic characterization of the interaction between the disordered 11-residue c-Myc peptide, Ac-YILSVQAEEK-NH$_2$ (c-Myc$_{402–412}$) and 10058-F4. (a) ITC binding isotherms in which c-Myc$_{402–412}$ (590 μM) was titrated into the compound solution 10058-F4 (30 μM) with corresponding heats of dilution. (b) Integrated peaks from ITC, accounting for heats of dilution. (c) Intrinsic fluorescence titrations performed at various temperatures. Error bars represent the SD from three independent experiments. (d) Van't Hoff analysis of the data shown in panel c.