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Thermodynamic Aspects of Coupled Binding and Folding of an Intrinsically Disordered Protein: A Computational Alanine Scanning Study[†]

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ABSTRACT: We have performed computational alanine scanning to gain insight into thermodynamic aspects of coupled folding and binding of the phosphorylated kinase-inducible activation domain (pKID) of CREB, and intrinsically disorder protein (IDP), to KIX. Residues of pKID directly involved in nativelike interactions at the interface were found to steeply lower their contribution to the free energy of binding. The results suggest that, with a steep lowering of the free energy of binding, it is ensured that pKID has full control of binding specificity, is not trapped in a non-native conformational state, has full plasticity for folding, and forms a thermodynamically stable complex with KIX without compromising binding kinetics.

Protein−protein interactions (PPIs) are of vital importance in the cellular machinery, as they participate in a broad range of functional roles. Given their biological relevance, PPIs between proteins possessing a well-defined structure have been studied from the structural, biochemical, and thermodynamic standpoints. However, recent experimental evidence revealed the existence of intrinsically disordered proteins (IDPs), which do not possess a well-defined three-dimensional structure under physiological conditions (*1*−*4*). IDPs are functional in their native state and often participate in PPIs, thus fulfilling a number of key functional roles such as signaling and regulation. Many IDPs are capable of undergoing disorder-to-order transitions upon binding, ranging from the restriction of the dynamics in the primary structure to the formation of a folded structure (*5*). Among these scenarios, particular attention has been paid to the latter case, commonly known as coupled folding and binding (*6*, *7*). Here, an IDP becomes structured only upon binding, a mechanism that provides binding specificity to multiple targets and balanced free energy of binding. One of the best studied cases of this mechanism is the association between the transcription factor cAMP response-element binding protein (CREB) and its coactivator CREB binding protein (CBP). The phosphorylated kinase-inducible activation domain (pKID) of CREB interacts with the kinase-induced domain interacting domain (KIX) of CBP via a coupled folding and binding mechanism (*8*). NMR studies have shown that residues 119−146 of pKID fold upon binding, forming two helices (α_A and α_B) connected by a short loop (Figure S1 of the Supporting Information) (*8*). Recent experimental (*9*) and computational (*10*) studies have suggested that pKID is structured only upon binding, forming intermediates that evolve to a thermodynamically stable complex. In this study, computational alanine scanning with a simple physical model was used to gain insight into the thermodynamic aspects of coupled folding and binding of pKID to KIX. The approach proved to be robust for a modest computational cost, capturing features of the coupled folding and binding that are in agreement with state-of-art experimental and computational data.

The approach, based on the thermodynamic analysis of the native interactions at the interface, is justified because (a) the mechanism of binding of pKID to KIX is largely determined by the formation of specific nativelike interactions (*10*) and (b) it has been experimentally shown that an increase in the helical content of helix α_B does not have an appreciable effect on binding affinity (*11*), indicating that mutations to alanine will not significantly affect the binding equilibrium. NMR structures of the pKID−KIX complex were retrieved from the Protein Data Bank (entry 1KDX). To account for backbone and side chain flexibility, all 17 structures that satisfied the NMR-derived constraints were used. Each structure was subjected to energy minimization for 500 steps, and polar hydrogens were added. Computational alanine scanning was performed using a program developed by Kortemme and Baker, which identifies all residues in a protein−protein interface and computes the changes in binding free energy for alanine mutations ($\Delta\Delta G$) (*12*, *13*). The method uses a free energy function consisting of Lennard-Jones and hydrogen bond potentials as well as Coulomb electrostatics and an implicit solvation model. A detailed description of the method is given in refs *12* and *13*. As this approach does not take phosphorylation into account, the effect of the alanine mutations of pS133 from pKID as well as K662 and Y658 from KIX was studied using the MM/PBSA method (*14*). Moreover, as computer simulations recently showed that A654 of KIX has critical hydrophobic contacts with pKID (*10*), glycine scanning was performed for this residue. Likewise, the A145G mutant was also studied. A complete description of the MM/PBSA approach is provided as Supporting Information.

To assess the robustness of the method, we compared calculated $\Delta\Delta G$ values with available experimental data (*11*, *15*) (Table 1). Experimentally, most mutations to alanine have been studied on KIX; nevertheless, a good agreement was observed between the calculated and experimental $\Delta\Delta G$ values. Except for Y650A, whose destabilizing effect is overestimated, the method is very accurate in capturing both qualitative and quantitative trends observed in the experiments.

Furthermore, the method was able to correctly identify the key residues that have been suggested to play a major role in the mechanism of coupled folding and binding of pKID. NMR titration studies showed that pS133, Y134, I137, and L138 are essential for high-affinity binding (*9*), in agreement with

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Table 1: Comparison between Calculated and Experimental $\Delta\Delta G$ Values

mutation[a]	$\Delta\Delta G_{calc}$ (kcal/mol)	$\Delta\Delta G_{exp}$ (kcal/mol)
L599A	1.1 ± 0.2	0.7 [Parker et al. (*15*)]
K606A	1.3 ± 0.5	0.8 [Parker et al. (*15*)]
Y650A	2.6 ± 0.5	1.6 [Zor et al. (*11*)]
K662A	1.4 ± 0.2	1.6 [Zor et al. (*11*)]
pS133A	2.5 ± 0.3	2.3 [Zor et al. (*11*)]

[a]Except for pS133, all alanine mutations are reported for KIX.

computational alanine scanning (Figure 1A). Here, these residues appear to contribute to binding with more than 1 kcal/mol, in conformity with the notion of "hot spot". Likewise, coarse-grained molecular dynamics simulations suggested that residue L141 possesses a fundamental role as an anchor residue for the formation of the encounter complex (*10*). This observation is in accord with a large $\Delta\Delta G$ value (2.5 kcal/mol), reduced solvent accessibility, and low mobility (*16*).

Analysis of calculated $\Delta\Delta G$ values revealed that the α_B segment of pKID and several interface residues of KIX are susceptible to alanine mutations. If we consider the contribution of residues with a $\Delta\Delta G$ of ≥1 kcal/mol to the contact area, the overall contribution to binding is equally distributed between pKID and KIX. However, alanine scanning showed that residues from pKID and KIX have a differential role in the binding mechanism: while residues from pKID with a $\Delta\Delta G$ of ≥1 kcal/mol are distributed along the α_B segment, similar residues from KIX appeared to be localized around residues of pKID with a $\Delta\Delta G$ of ≥1 (Figure 1B). This observation suggests that KIX side chains actively act as anchoring sites that pKID uses to efficiently fold and bind. This observation is in agreement with previous computational studies on protein−protein complexes involving IDPs, which have suggested that IDPs use most of their binding energy to fold (*17*).

While it is not entirely unexpected that the α_B region of pKID plays the most important role in folding and binding, how each residue contributes to folding and binding is very surprising. Upon binding the formation of the encounter complex via anchoring of L141, one would expect that, as coupled folding and binding proceed, each residue directly involved in nativelike contacts will evenly contribute to the free energy. In principle, this should ensure that the contribution of each residue to the rates of association and dissociation is similar, as suggested by experiments (*9*). However, alanine scanning revealed a very different pattern: when the anchor residue (L141) binds, spatially neighboring residues at the protein−protein interface steeply increase their contribution to the free energy of binding, with a maximum $\Delta\Delta G$ value of ~2.4 kcal/mol for pSer133.

In analogy to protein folding, coarse-grained molecular dynamics simulations were recently used to calculate the Φ values for residues of pKID involved in native contacts with KIX (*10*). The analysis suggested that residues in helix α_B and particularly L141 govern the association mechanism. Moreover, large Φ values of L141 and its neighboring residues, D140 and S142, indicate that, in the transition state, this region already possesses a nativelike structure (*10*). Data extracted from computational alanine scanning are in remarkable agreement with Φ value analysis, as $\Delta\Delta G$ values of helix α_B are significantly larger compared to those obtained for helix α_A. Together, Φ value analysis and computational alanine scanning on pKID also indicate that pKID binds to KIX following a "synergistic" mechanism, which suggests that both conformational selection and coupled folding and binding account for the formation of the bound state (*5*).

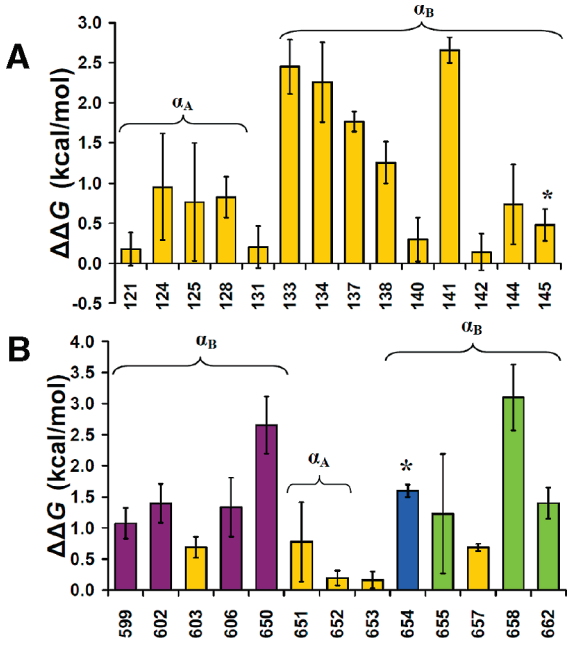

FIGURE 1: Calculated changes in binding free energy for alanine mutations of (A) pKID and (B) KIX. (B) Residues of KIX that interact with pS133/Y134 and L141 of pKID are colored green and purple, respectively. The asterisk indicates the use of glycine instead of alanine scanning.

Why would pKID choose such a distribution of free energy for coupled folding and binding? Upon the formation of the encounter complex, L141 provides a stable anchoring point for folding and full binding, allowing pKID to search through its free energy landscape toward the formation of the folded, high-affinity complex. As intermolecular interactions provide the driving force to lower the free energy toward the formation of the high-affinity complex, a steep decrease in the free energy will provide an extremely efficient enthalpy−entropy balance at all times during coupled binding and folding. In a scenario where each residue contributes evenly to binding, a very large gain in enthalpy will surpass the loss of entropy, thus significantly affecting binding reversibility. More importantly, such a free energy pattern should allow pKID to fully control the specificity of binding to KIX.

Stronger interactions near the anchoring site might also have significant implications for the formation of native contacts and binding kinetics. Recently, it has been suggested that the formation of specific nativelike interactions determine the mechanism of binding of pKID to KIX (*10*). The results from computational alanine scanning support this observation, as stronger interactions involving the α_B region of pKID will dramatically reduce the probability of formation of non-native interactions, thus facilitating the formation of the native helical pattern of pKID in the bound state. More importantly, a steep lowering of the free energy of binding will have a dramatic effect on binding kinetics. Contrary to the notion that nonspecific interactions [via a "fly casting" mechanism (*18*)] accelerate the binding kinetics of IDPs, our results from computational alanine scanning indicate that strong nativelike interactions may actually accelerate the binding process. This scenario was recently demonstrated for the association of pKID with KIX, showing that the origins of faster binding for IDPs are the fewer encounter times before the formation of the complex (*19*).

In conclusion, the results indicate that the mechanism of coupled folding and binding of pKID heavily relies on thermodynamic control. Although residues from KIX and pKID were found to provide the energy gradient required for coupled folding and binding, they possessed a differential role in the binding mechanism. With a steep lowering of the free energy of binding, it is ensured that pKID (a) has full control of binding specificity, (b) is not trapped in a non-native conformational state, (c) has full plasticity for folding, and (d) forms a thermodynamically stable complex with KIX without compromising binding kinetics.

This study demonstrates that despite its simplicity and inherent limitations, computational alanine scanning is a complementary technique that is sufficiently robust to extract relevant information regarding the thermodynamic aspects of coupled folding and binding of IDPs. Computational alanine scanning could be further used for the design of small molecules that bind to sites necessary for anchoring or folding, leading to the inhibition of protein−protein interactions involving IDPs via specific perturbation of the coupled binding and folding mechanism.

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SUPPORTING INFORMATION AVAILABLE

Figure S1 and a complete description of the MM/PBSA approach. This material is available free of charge via the Internet at http://pubs.acs.org.

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