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The *cis* conformation of proline leads to weaker binding of a p53 peptide to MDM2 compared to *trans*

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ABSTRACT

The *cis* and *trans* conformations of the Xaa–Pro (Xaa: any amino acid) peptide bond are thermodynamically stable while other peptide bonds strongly prefer *trans*. The effect of proline *cis–trans* isomerization on protein binding has not been thoroughly investigated. In this study, computer simulations were used to calculate the absolute binding affinity for a p53 peptide (residues 17–29) to MDM2 for both *cis* and *trans* isomers of the p53 proline in position 27. Results show that the *cis* isomer of p53(17–29) binds more weakly to MDM2 than the *trans* isomer, and that this is primarily due to the difference in the free energy cost associated with the loss of conformational entropy of p53(17–29) when it binds to MDM2. The population of *cis* p53(17–29) was estimated to be 0.8% of the total population in the bound state. The stronger binding of *trans* p53(17–29) to MDM2 compared to *cis* may leave a minimal level of p53 available to respond to cellular stress. This study demonstrates that it is feasible to estimate the absolute binding affinity for an intrinsically disordered protein fragment binding to an ordered protein that are in good agreement with experimental results.

Introduction

Although the *cis* conformation of proline residues represents a very small fraction of peptide bonds, it is still biologically important. The vast majority of peptide bonds in proteins are observed in *trans* conformation ($\omega \sim 180°$) due to favored interactions between the amide hydrogen and the preceding alpha carbon [1]. However, peptide bonds in *cis* conformation ($\omega \sim 0°$) are also found in some cases [2]. In a nonredundant set of 571 proteins, a very small fraction (0.03%) of *cis* conformation are observed in Xaa–nonPro and this increases to 5.2% for Xaa–Pro (Xaa: any amino acid, nonPro: any amino acid but proline) [3–6]. Many studies have stressed the importance of the *cis–trans* isomerization of peptide bonds for protein folding processes [7–10]. It has been shown that the isomerization processes is likely to play roles in cell signaling, ion channel gating, and gene expression [11–14]. The unique structure of proline allows for a smaller entropic loss than other amino acids when undergoing isomerization from *trans* to *cis* [10,15]. The slow inter-conversion between *cis* and *trans* isomers of Xaa–Pro peptide bonds can be catalyzed by peptidyl-prolyl *cis–trans* isomerase to regulate biological processes [16–19]. Dysfunction of the isomerization process may result in diseases such as cancer and Alzheimer's [20–22,19,23].

Prolines play important roles in the structure and dynamics of intrinsically disordered proteins (IDPs)[1]. The frequency of prolines in IDP sequences is twice that of ordered proteins [24]. The ring structure of proline that links to the peptide backbone tends to disrupt the alpha helical structure of proteins if it is not at a capping position [25–27]. Prolines in N-terminal flanking regions of pre-structured motifs have been predicted to promote helical structure whereas prolines in C-terminal flanking regions tend to terminate helix formation in IDPs [28]. Mutations on prolines that cause increased helicity may enhance the binding between an IDP and its partner, and affect signaling in cells [29,30].

The accurate estimation of binding affinities for IDPs could be valuable for designing therapeutic drugs [31], or in protein engineering since IDPs play important roles in cell signaling and transcription [32–35]. The networks of protein–protein interactions regulate a wide range of biological activities from cellular metabolism to signal transduction [36]. The functions of IDPs are carefully tuned by the structures, dynamics and binding affinities [37,38,30].

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[1] *Abbreviations used:* IDPs, intrinsically disordered proteins; MD, molecular dynamics; PMF, potential of mean force; MDM2, murine double minute clone 2; P27, position 27.

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The estimation of absolute binding affinities for protein–protein systems is a key challenge in computational biology [39]. Various methods with differing levels of complexity and accuracy have been used to calculate protein–protein binding affinities. Empirical energy functions and scoring schemes are used to screen large protein databases in the search of a good binding partner [40–43]. This class of approaches is designed to handle a large amount of molecules with high throughput, but tend to be inaccurate due to the simplicity of the scoring functions. Other methods such as linear interaction energy method [44] and the molecular mechanical and continuum solvent approach [45], combine the use of conformations from molecular dynamics (MD) simulations in explicit solvent with binding affinity functions. This class of methods is widely used but suffers from inaccuracy in some cases due to insufficient sampling of MD simulations and/or functions that are not general enough. Another group of methods, for example free energy perturbation [46] and thermodynamic integration [47], are based on statistical mechanics principles and depend entirely on simulations, typically with explicit solvent. These methods provide the most accurate binding affinity estimates, in principle, but can be hampered by insufficient sampling and/or very long simulation times. Another class of methods for calculating absolute binding affinities that also provides information about the binding/unbinding pathway is to estimate the potential of mean force (PMF) using restraining potentials to enhance convergence [48–51]. The slope of the PMF provides information about the average force over all conformations along a defined reaction coordinate [47]. The PMF can be integrated to estimate the free energy difference between two states. Restraints on the degrees of freedom of the system reduce the conformational space available enhancing the convergence of simulations. The free energies associated with the restraints are rigorously accounted for in order to generate an unbiased estimate of the binding affinity [49,52]. Some specific examples of this approach have been reported for AcpYEEI peptide binding with the human $p56^{lck}$ SH2 domain [49], KID protein in association with KIX protein [53], and peptide APSYSPPPPP interacting with the SH3 domain of the Abl kinase [52].

The model system used in the current study is a disordered fragment of p53 (residues 17–29) binding to the E3 ubiquitin ligase murine double minute clone 2 (MDM2). Protein p53 activates the expression of MDM2 [54–56]. In turn, MDM2 binds p53 for ubiquitination causing p53 to be transported out of the nucleus for degradation by the proteasome [57–59]. This elegant feedback loop maintains low levels of p53 in non-stressed cells. Under stressed conditions, the binding between p53 and MDM2 is abrogated by post-translational modifications, resulting in increased levels of p53 [60,61]. The activated p53 then leads to cell cycle arrest and the subsequent transcription of target genes to revive the cell [62]. The binding site for MDM2 contains residues 18–26 that are transiently helical in the unbound state [63]. The proline at position 27 (P27) in p53 is in the C-terminal flanking region and is adjacent to L26, one of three critical residues for binding MDM2 (F19, W23, and L26) [45,64]. The residue P27 was established as a disrupter to the MDM2-binding motif of p53 as confirmed in recent studies [28,65,30].

In this study, we used computer simulations to calculate PMFs and corresponding binding affinities to understand how the *cis* and *trans* conformations of P27 in a p53 fragment (residue 17–29) affect the binding with MDM2. Nuclear magnetic resonance (NMR) spectroscopy revealed that around 5.5% of the L26–P27 peptide bonds are in the *cis* conformation for the unbound p53(1–63), but a *cis* signal could not be resolved for the same peptide bond in the p53–MDM2 complex (unpublished NMR data from Dr. François-Xavier Theillet). A PMF-based approach was used to compute the absolute binding affinity for both *trans* and *cis* isomers binding with MDM2 and found to be −11.8 (1.0) kcal/mol and −8.9 (0.8) kcal/mol, respectively. Based on these affinity calculations the *cis* isomer was estimated to be 0.8% of the total bound state population with the rest in *trans*. It was found that N29 of the *trans* isomer contributes to the binding by having stronger electrostatic attraction to MDM2 than the *cis* isomer. In addition, the *cis* isomer has more flexibility in the unbound state compared to *trans* that decreases the binding affinity for *cis*. The stronger binding of *trans* p53(17–29) to MDM2 compared to *cis* may suggest a mechanism to help maintain minimal levels of p53 in unstressed cells and allow for rapid response to cellular stress. Our results suggest that around 5% of the p53 proteins in the cell may not be targeted for degradation because they are in *cis* and thus essentially unavailable for binding to MDM2.

Methods

Thermodynamic cycle

The binding free energy, or binding affinity, is the free energy difference between bound and unbound states of a system. When restraints are added to the system the unbiased free energy can be calculated by accounting for the free energies of releasing these restraints. This process can be illustrated by a thermodynamic cycle shown in Fig. 1 [50]. In this study, restraining potentials were applied to the p53 to limit the freedom of the system and allow the simulations to converge more quickly. Conformational, axial, and orientational restraints were applied to p53 as shown in Fig. 2. Since free energy is a state function, the change from unbound to bound state is independent of the path taken [66] and so the unbiased binding affinity ΔG_{bind} was calculated as:

$$\Delta G_{bind} = \Delta G^{u}_{conf} + \Delta G^{u}_{axial} + \Delta G^{u}_{orient} + \Delta G^{res}_{bind} + \Delta G^{b}_{conf} + \Delta G^{b}_{axial} + \Delta G^{b}_{orient} \quad (1)$$

Binding affinity with restraints

The binding affinity with restraints, ΔG^{res}_{bind}, was calculated using the PMF $w(r)$, where r the center of mass distance between p53 and MDM2. The formula used in this study was reported previously [53,67] and given by:

$$e^{-\beta \Delta G^{res}_{bind}} = 4\pi r^{*2} C^0 \int_0^{r^*} e^{-\beta[w(r)-w(r^*)]} dr \quad (2)$$

where $\beta = 1/k_bT$ (k_b is the Boltzmann constant.); C^0 is the standard state concentration 1.0 mol/l (i.e. 1/1661 Å^3); r^* is an arbitrary reference distance where the interaction between the two molecules is negligible.

Contributions from conformational restraints

The free energy cost to impose the conformational restraint for either the bound or unbound state was computed from the PMF $w(\xi)$, where ξ is the root mean square deviation (RMSD) of p53 relative to an equilibrated conformation. The conformation of p53 is restrained by a harmonic potential $u_c(\xi)$.

$$e^{\beta \Delta G^{b}_{conf}} = \frac{\int d\xi e^{-\beta(w^b(\xi)+u_c(\xi))}}{\int d\xi e^{-\beta w^b(\xi)}}, e^{-\beta \Delta G^{u}_{conf}} = \frac{\int d\xi e^{-\beta(w^u(\xi)+u_c(\xi))}}{\int d\xi e^{-\beta w^u(\xi)}} \quad (3)$$

Contributions from axial and orientational restraints

For the axial and orientational restraints in the bound state the free energy change was calculated using the Bennett acceptance ratio approach [68].