Published in final edited form as:

Arch Biochem Biophys. 2015 June 01; 575: 22–29. doi:10.1016/j.abb.2015.03.021.

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.

Keywords

Intrinsically disordered protein; *cis-trans* isomerization; Binding affinity; Molecular dynamics; Potential of mean force

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 ($\omega \sim 180^\circ$) due to favored interactions between the amide hydrogen and the preceding alpha carbon [1]. However, peptide bonds in *cis* conformation ($\omega \sim 0^\circ$) 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

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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 prestructured 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].

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

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

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 G_{bind} was calculated as:

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

Binding affinity with restraints

The binding affinity with restraints, ΔG_{bind}^{res} , 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:

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

where $\beta = 1/k_b T$ (k_b is the Boltzmann constant.); C^0 is the standard state concentration 1.0 mol/l (i.e. $1/1661 \text{ \AA}^3$); 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 $u_c(\xi)$.

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

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].

For the unbound state, the free energy cost associated with imposing axial and orientational restraints were calculated from numerical integration over the spherical angles, θ and φ , and Euler angles, Ψ , Θ , and Φ . The formulas for the axial and orientational restraints are:

$$e^{-\beta\Delta G_{axial}^u} = \frac{\int d\tau e^{-\beta u_a}}{\int d\tau} = \frac{\int_0^\pi d\theta \sin\theta \int_0^{2\pi} d\varphi e^{-\beta u_a}}{4\pi} \quad (4)$$

$$e^{-\beta\Delta G_{orient}^u} = \frac{\int d\Omega e^{-\beta u_o}}{\int d\Omega} = \frac{\int_0^\pi d\Phi \sin\Phi \int_0^{2\pi} d\Theta \int_0^{2\pi} d\Psi e^{-\beta u_o}}{8\pi^2} \quad (5)$$

where u_a and u_o are respectively the axial and orientational restraint potentials.

Population of p53^{cis}-MDM2

The ratio of the population of p53^{cis}-MDM2 and p53^{trans}-MDM2 were derived from their binding affinities. According to Boltzmann statistics, the ratio of probabilities of two states in a system is equal to a ratio of their Boltzmann factors [69]. The probability of p53^{cis}-MDM2 is the population of this complex divided by the total population of p53-MDM2, similarly for p53^{trans}-MDM2. The ratio of population of p53^{cis}-MDM2 and p53^{trans}-MDM2 was calculated using:

$$R = \frac{\text{Population of p53}^{cis}\text{-MDM2}}{\text{Population of p53}^{trans}\text{-MDM2}} = \frac{e^{-\beta\Delta G_{bind}^{cis}}}{e^{-\beta\Delta G_{bind}^{trans}}} = e^{-\beta(\Delta G_{bind}^{cis} - \Delta G_{bind}^{trans})} \quad (6)$$

Assuming no other proline conformation besides *cis* and *trans* are present, the population of p53^{cis}-MDM2 is given by $\frac{R}{R+1}$.

Computational details

The initial structure of the p53^{trans}-MDM2 complex was obtained from the protein databank (PDB ID: 1YCR) [70] and includes residues 17–29 of p53 and residues 25–109 of MDM2. The coordinates for the missing hydrogen atoms in the crystal structure were guessed by VMD psfgen package. [71] The simulations of unbound p53^{trans} were also initiated from PDB ID: 1YCR in the absence of MDM2.

The initial structure of the p53^{cis}-MDM2 complex was obtained by rotating the dihedral angle omega of L26–P27 peptide bond in p53 (PDB ID: 1YCR) from 180° to 0° using VMD [71]. This did not produce any steric clashes. Similarly, the unbound p53^{cis} was transformed from unbound p53^{trans}.

Each system was solvated in a TIP3P [72] water box that extended 17 Å in each direction from the solute and was given a neutral charge by adding 150 mM sodium chloride (NaCl). [71] All simulations were carried out at 300 K using the CHARMM22 force field [73] as

implemented in NAMD 2.9. [74] The long range electrostatic interactions were calculated using particle mesh Ewald [75] with a real-space cutoff of 12 Å. Nonbonded van der Waals interactions were smoothly switched to zero between 10 and 12 Å. The vibration of the bonds involving hydrogen atoms were constrained using SHAKE [76] to allow for a time step of 2 fs. Trajectory snapshots were saved every 2 ps. Each system was initially minimized for 1000 steps with the backbone atoms fixed and then minimized again for additional 1000 steps with all constraints removed. The minimized structures were gradually heated up to 300 K in 300 ps followed by equilibration at constant pressure 1 atm (Langevin piston) and temperature 300 K (Langevin dynamics) for 100 ps. [77] Independent 100 ns simulations were performed three times for p53^{trans}-MDM2 and p53^{trans}, three times for p53^{cis}, and six times for p53^{cis}-MDM2.

For p53^{trans}-MDM2 and p53^{cis}-MDM2 systems PMF calculations were performed using umbrella sampling [78] with WHAM (weighted histogram analysis method) [79]. Umbrella sampling introduces a biasing potential energy that keeps the simulation near a particular value of the reaction coordinate [78]. The WHAM package version 2.0 from Alan Grossfield was used for processing the umbrella sampling simulation results with error estimated by bootstrapping [80].

We calculated the PMF $w(r)$ for p53 binding to MDM2 using various restraints to enhance the convergence of the simulations. A harmonic biasing potential $u_r = k_r(r - r_0)^2/2$ was applied to each window centered at different distances r_0 using a force constant $k_r = 10$ kcal/mol Å². We used 37 windows with inter-window spacing 0.5 Å for $r_0 \leq 26$ Å and spacing of 1 Å for $r_0 > 26$ Å. For each window, 2 ns simulations were performed and the last 1 ns was used for analysis. The reference distance (see Eq. (8)), r^* , was set to be 35 Å, where p53 and MDM2 were not interacting.

The PMFs for the conformational restraints $w^u(\xi)$ and $w^b(\xi)$ were calculated from 21 umbrella sampling simulations separated by 0.5 Å for the unbound system and 0.4 Å for the bound system. For each window, 2 ns simulations were performed and the last 1 ns was used for analysis. A value of 1 kcal/mol Å² was set to the force constant in the conformational restraining potential $u_c(\xi) = k_c \xi^2/2$. Harmonic biasing potentials $k_\xi(\xi - \xi_0)^2/2$ were used with force constants of 15 kcal/mol Å².

The free energy differences, ΔG_{axial}^b and ΔG_{orient}^b , associated with the axial and orientational restraints were calculated by the Bennett acceptance ratio method [4]. The axial

restraining potential was $u_a = \frac{1}{2}k_a \left[(\theta - \theta^{ref})^2 + (\varphi - \varphi^{ref})^2 \right]$ and the orientational potential

was $u_o = \frac{1}{2}k_o \left[(\Phi - \Phi^{ref})^2 + (\Theta - \Theta^{ref})^2 + (\Psi - \Psi^{ref})^2 \right]$ with $k_a = k_o = 0.03$ kcal/mol degree². For both axial and orientational restraint calculations, simulations of the complex were 2 ns for each value of k_a or $k_o = 0.03, 0.021, 0.015, 0.01, 0.006, 0.003, 0.001, 0$. The first 1 ns was discarded for equilibration and the remaining 1 ns was used to compute the corresponding free energy difference.

Results

In this study, a PMF-based approach with restraints was used to calculate the absolute binding affinity of the *trans* and *cis* isomers of p53(17–29) (p53^{*trans/cis*}) to MDM2. The binding affinities were calculated via Eq. (1) as depicted in the thermodynamic cycle (Fig. 1). All the contributions to the affinity are discussed below and summarized in Table 1.

Binding affinity with restraints

Fig. 3 shows PMFs for the transition from the p53^{*trans/cis*}-MDM2 complex to the unbound state as a function of the center of mass separation between MDM2 and p53^{*trans/cis*} with all restraints present. All simulations were initiated from long independent MD simulations of the protein complexes leading to differences in the most favorable center of mass separations (PMF minima). All PMF curves are very flat for $r > 30$ Å where the interaction between the proteins is negligible. The corresponding free energy differences ΔG_{bind}^{res} are calculated from the PMFs using Eq. (2) using a reference distance of $r^* = 35$ Å. Table 1 shows that the average ΔG_{bind}^{res} values are very similar for the two isomers: -23.89 (0.84) kcal/mol for *trans* and -23.92 (1.42) kcal/mol for *cis*.

Contributions from conformational restraints

Fig. 4 shows the PMFs as a function of RMSD calculated for p53^{*trans/cis*} in the bound state and unbound states. The conformational restraints are based on the RMSD of p53^{*trans/cis*} relative to an equilibrated structure of the complex for *trans* and *cis* isomers respectively. The width of the PMF is an indication of the range of conformational states. As one would expect, the PMFs for the unbound p53 (Fig. 4(b) and (d)) are wider than the bound p53 (Fig. 4(a) and (c)) for both *trans* and *cis*, indicating the larger conformational freedom of the unbound p53. The RMSD PMFs for the bound state p53^{*trans*} in Fig. 4(a) exhibit packed profiles overall, peaking sharply around 1–1.5 Å. On the other hand, the RMSD PMFs for the bound state p53^{*cis*} in Fig. 4(c) have more diverse profiles. For the unbound state the RMSD PMFs for p53^{*cis*} in Fig. 4(d) have broader profiles than p53^{*trans*} in Fig. 4(b) in general. These observations suggest a wider range of accessible conformational states is allowed for p53^{*cis*} compared to p53^{*trans*}. It is possible that broader PMFs would be seen if more than three trials of p53^{*trans*} were performed, however, we believe that this is not likely given the consistency of the p53^{*trans*} trials compared to p53^{*cis*}.

The free energy changes due to the restraints on the RMSD were calculated using Eq. (3).

Taken together, ΔG_{conf}^u and ΔG_{conf}^b determine the free energy cost associated with the loss of conformational freedom of p53 when adopting a specific conformation in the bound state.

As shown in Table 1, this free energy cost, $\Delta G_{conf}^u + \Delta G_{conf}^b$, is calculated as 4.25 (1.29) kcal/mol for p53^{*trans*} and 6.56 (1.00) kcal/mol for p53^{*cis*}. As a result, the difference of the free energy cost due to conformational restraints between p53^{*trans*} and p53^{*cis*} is 2.31 (1.63) kcal/mol. This accounts for most of the 2.90 (1.28) kcal/mol difference between *cis* and *trans* G_{bind} suggesting that the conformational flexibility is a major contributor to how p53^{*cis*} binds differently to MDM2 than p53^{*trans*}.

Contributions from axial and orientational restraints

Table 1 shows the free energy costs for restraining the axial and orientational degrees of freedom of p53(17–29) for both *trans* and *cis* conformations (see Supplemental Information for more detailed results). The axial restraint cost $\Delta G_{axial}^u + \Delta G_{axial}^b$ is 2.58 (0.25) kcal/mol for p53^{trans} and 3.05 (0.15) kcal/mol for p53^{cis} and suggests that axial degrees of freedom are not contributing much to the difference between *cis* and *trans* p53(17–29) binding to MDM2. Similarly, the orientational restraint cost $\Delta G_{orient}^u + \Delta G_{orient}^b$ is 5.24 (0.22) kcal/mol for p53^{trans} and 5.38 (0.09) kcal/mol for p53^{cis} suggesting that orientational degrees of freedom are also not contributing much to the difference between *cis* and *trans* p53(17–29) binding to MDM2.

Calculation of binding affinity

Table 1 shows the binding affinities for p53^{trans/cis}–MDM2, G_{bind} calculated using Eq. (1). The affinities are –11.83 (1.02) kcal/mol for p53^{trans}–MDM2 and –8.93 (0.78) kcal/mol for p53^{cis}–MDM2. The p53^{trans}–MDM2 affinity estimate compares favorably (within several kcal/mol) to the reported experimental result of –9.3 kcal/mol for peptide p53(16–29) binding with MDM2(17–125) [81]. For comparison, we also provide the value calculated using the MM-PBSA/GBSA approach of –16.3 kcal/mol [29].

Equilibrium simulations

To gain insight into the structures and interactions for *trans* and *cis* equilibrium simulations were performed of the unbound p53(17–29) and p53(17–29)–MDM2 complexes (see “Methods”). The structures of *trans* and *cis* at L26–P27 in p53(17–29) stayed in *trans* and *cis* respectively during the course of all simulations for both bound and unbound p53(17–29). RMSD-based clustering analysis was performed on all of the bound state p53(17–29) structures using the VMD clustering plugin [71,82] with cutoff 1.0 Å. Fig. 5 shows representative structures from the largest clusters for *trans* (1040 out of 15,000 structures) and *cis* (1165 out of 30,000 structures) p53(17–29) while bound to MDM2. These structures are representative of the most common structures seen in the simulations. Fig. 5 shows that the tail region (residues 27–29) of p53^{cis} in the complex tended to point away from the MDM2 binding pocket while the tail of p53^{trans} remained close to the binding pocket. This trend was observed for all clusters (data not shown). Unfortunately, there is no experimental evidence to support this conclusion, however, we believe that the 100 ns simulations are sufficiently long for the C-terminus to have found energetically favorable structures.

Discussion

Our results show that the binding affinity for p53^{cis}–MDM2 is around 2.90 (1.28) kcal/mol weaker than p53^{trans}–MDM2. Based on this binding affinity difference, for the bound state, the *cis* conformation of L26–P27 in p53(17–29) is estimated to be 0.8% of the total population. NMR spectroscopy has shown that, for the unbound state, around 5.5% of the L26–P27 peptide bonds in p53(1–63) are in the *cis* conformation (unpublished NMR data from Dr. François-Xavier Theillet).

The most significant contributor to the binding affinity difference between *cis* and *trans* is the free energy change due to the conformational restraints for unbound p53(17–29), ΔG_{conf}^{u} , that was found to be 2.61 (1.30) kcal/mol larger for *cis* than for *trans*. This term is the free energy cost associated with adding the conformational restraint on p53 in the unbound state. The larger cost for *cis* indicates that p53^{*cis*} in the unbound state has more accessible conformations than unbound p53^{*trans*}. Fig. 6 shows the backbone root mean square fluctuations (RMSF) for p53^{*trans*} and p53^{*cis*} in the unbound state and suggests that *cis* is more flexible than *trans* for the helical region (residues 19 to 24) and the region around the L26–P27 peptide bond (residues 26 and 27). Taken together, Fig. 6 and the ΔG_{conf}^{u} values in Table 1 suggest that the primary reason that p53^{*cis*} binds more weakly to MDM2 than p53^{*trans*} is because the *cis* conformation of p53(17–29) is more flexible in the unbound state leading to a greater conformational entropy loss upon binding.

To gain insight into how the interaction energies differ between p53^{*trans*}–MDM2 and p53^{*cis*}–MDM2, we studied intra- and intermolecular interactions of p53^{*trans/cis*}–MDM2 from 300/600 ns molecular dynamic simulations. The intra-molecular interactions of bound and unbound p53 and MDM2 are indistinguishable between the *trans* and *cis* isomers. The intermolecular interaction energy of p53^{*trans*}–MDM2 is stronger than that of p53^{*cis*}–MDM2 primarily due to the electrostatic interaction (shown in Fig. 7). A detailed analysis of individual contributions to the electrostatic energy for every p53 residue (Fig. 8) shows that the electrostatic interaction between N29 of p53^{*trans*} and MDM2 is ~33.2 kcal/mol stronger than for p53^{*cis*} and MDM2. Although the error bars are partially overlapping, the difference is appreciable. We think this difference is consistent with the observation that N29 of p53^{*cis*} tends to point away from MDM2 (Fig. 5). Note that both the protein fragments used in this study and the fragments used in the experimental comparison study in Table 1 were not capped, that is, the C-termini were negatively charged. The N-termini of the protein fragments used in this study were positively charged and the N-terminus of the fragments used in the experiments were negatively charged due to conjugating fluorescein isothiocyanate at the N-terminus [81].

The stronger binding of p53^{*trans*} to MDM2 compared to p53^{*cis*} suggests a possible mechanism to help maintain a minimal level of p53 in unstressed cells. While the isomerization and binding rates of p53 in vitro have not been determined to our knowledge, it is known that the timescale for the conversion of *cis* to *trans* is minutes without any catalyzer at room temperature [19,83], and that typical timescales for binding are in the range of nanoseconds to milliseconds [84,85]. This separation of timescales, together with the NMR data (unpublished data from Dr. François-Xavier Theillet) and our binding affinity results suggest that up to 5.5% of the p53 proteins in the cell may be essentially unavailable for binding MDM2 because they are in the *cis* conformation at that moment. Since MDM2 binding signals the ubiquitination process that leads to nuclear export and degradation of p53 [57–59], the preferential binding of the *trans* conformation may leave a small population of unbound p53 molecules that remain available to respond to cellular stress. Given the complex network of interactions in vivo, experimental evidence will be required to justify the above claim.

This study demonstrates that it is feasible to estimate absolute binding affinities that are in good agreement with experimental data for a system involving an IDP binding to an ordered protein. We found that it was necessary to restrain the axial, orientational, and conformational degrees of freedom to enhance the convergence of the simulations, as has been discussed previously [48–53]. The final (unbiased) binding affinities were calculated by rigorously accounting for all the restraints used in the simulations. The affinities are estimated -11.83 (1.02) kcal/mol for p53^{trans}-MDM2 and -8.93 (0.78) kcal/mol for p53^{cis}-MDM2. The p53^{trans}-MDM2 affinity estimate compares favorably (within several kcal/mol) with the experimental binding affinity of -9.3 kcal/mol on a fragment of p53 (residues 16–29) binding to MDM2 (residues 17–125) [81]. Our estimate is also similar to experimental affinities measured on the transactivation domain of p53, -8.9 kcal/mol, [86] and full-length p53, -8.8 kcal/mol [87].

Conclusions

In this study we used a PMF approach to compute the absolute binding affinity for both *trans* and *cis* isomers of the L26–P27 peptide bond of p53(17–29) binding to MDM2. We find that the *trans* conformation of p53(17–29) binds more strongly by around 2 kcal/mol. Based on the binding affinity difference between p53^{trans}-MDM2 and p53^{cis}-MDM2, the *cis* isomer was estimated to be $\sim 0.8\%$ of the total population. A more detailed analysis revealed that N29 at C-terminal of the *trans* isomer contributes to the binding by having stronger electrostatic attraction to MDM2 than the *cis* isomer. In addition, the *cis* isomer exhibits higher flexibility at unbound state and lowers the binding affinity. NMR spectroscopy showed that when p53(1–63) is unbound around 5.5% of the L26–P27 peptide bonds are in the *cis* conformation (unpublished NMR data from Dr. François-Xavier Theillet). NMR could not determine the *cis* population for p53(1–63) when bound to MDM2 due to possible peak overlapping. The stronger binding of p53^{trans} to MDM2 compared to p53^{cis} may suggest a mechanism to help maintain minimal levels of p53 in unstressed cells, which could help for rapid response to cellular stress. Our results suggest that around 5.5% of the p53 proteins in the cell will not be targeted for degradation because they are in *cis* and thus essentially unavailable for binding to MDM2. Finally, this study demonstrates that it is feasible to estimate absolute binding affinities that are in good agreement with experimental data for a system involving an IDP binding to an ordered protein, provided that restraints are used to enhance convergence of the simulations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank Dr. François-Xavier Theillet for providing NMR data and analysis (In-cell NMR Laboratory, Department of NMR-supported Structural Biology, Leibniz Institute of Molecular Pharmacology, Berlin, Germany). This project was supported by the National Institutes of Health (NIH R21 GM83827 to F.M.Y.), the INBRE Program (NIH Grant P20 GM103408), and the IBEST Computing Core (NIH Grant P30 GM103324). The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views of NIH.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.abb.2015.03.021>.

Fig. 1.

Thermodynamic cycle used to compute absolute binding affinities G_{bind} . Conformational (conf), axial, and orientational (orient) restraints were applied to the unbound state (u) and bound state (b) of p53. The absolute binding free energy G_{bind} was calculated by adding the restrained binding free energy ΔG_{bind}^{res} to the free energy differences associated with all the restraints via Eq. (1).

Fig. 2. Schematic representation of the internal coordinates used to define the position and orientation of p53 relative to MDM2 and construct the restraining potentials.

Fig. 3. Potential of mean force (PMF) as a function of center of mass separation for the restrained (a) *trans* and (b) *cis* conformations of p53 binding to MDM2. Three independent trials were performed for the *trans* isomer and six trials were performed for the *cis*. Independent trials were initiated from the last frames of 100 ns simulations of the p53–MDM2 complex that resulted in the differences in the location of the PMF minima.

Fig. 4.

Conformational potential of mean force (PMF) results as a function of the root mean square distance (RMSD) in the bound and unbound states. Three independent trials were performed for the (a) bound state *trans* isomer and (b) for the unbound state *trans* isomer. Six independent trials were performed for the (c) bound state *cis* isomer and (d) unbound state *cis* isomer. Independent trials were initiated from the last frames of 100 ns simulations of the p53-MDM2 complex.

Fig. 5. Most common *trans* (yellow) and *cis* (green) conformations of p53 while bound to MDM2 (white surface). These structures are representative of the largest cluster obtained from clustering analyses using all equilibrium simulations of the *trans* and *cis* complexes of lengths 300 ns and 600 ns respectively. Residues 26–29 of p53 are shown in licorice and the other p53 residues are shown in cartoon.

Fig. 6. Backbone root mean square fluctuations (RMSF) for the unbound p53 fragment. Each curve is an average obtained from three independent 100 ns molecular dynamics simulations (last 50 ns used to compute averages) for *cis* (green line) and *trans* (cyan line) isomers. The shading shows the standard errors for the independent simulations.

Fig. 7. Van der Waals (VDW) and electrostatic (ELECT) interaction energies for the p53–MDM2 complex. Each bar is an average obtained from three/six independent 100 ns molecular dynamics simulations (last 50 ns used to compute averages) for the *trans/cis* isomers (white/black bars). The error bars are the standard errors for the independent simulations.

Fig. 8.

Electrostatic interaction energy between p53 and MDM2. Each data point is an average obtained from three/six independent 100 ns molecular dynamics simulations (last 50 ns used to compute averages) for the *trans/cis* isomers (solid/dashed lines). The error bars are the standard error for the independent simulations.

Table 1

Computation of the free-energy contributions to MDM2 and p53 binding. The value of G_{bind} was calculated by summing all the free energy components and is shown in bold; see Eq. (1). Uncertainties are given by the standard errors and were estimated from six independent trials for *cis* and three trials for *trans*. Experimental and MM-PBSA values are obtained from references [81,29], respectively.

Component	<i>trans</i> (kcal/mol)	<i>cis</i> (kcal/mol)
ΔG_{bind}^{res}	-23.89 ± 0.84	-23.92 ± 1.42
ΔG_{conf}^u	11.28 ± 1.01	13.89 ± 0.82
ΔG_{axial}^u	3.48 ± 0.00	3.50 ± 0.00
ΔG_{orient}^u	5.97 ± 0.08	5.82 ± 0.06
ΔG_{conf}^b	-7.03 ± 0.80	-7.33 ± 0.57
ΔG_{axial}^b	-0.90 ± 0.25	-0.45 ± 0.15
ΔG_{orient}^b	-0.73 ± 0.20	-0.44 ± 0.07
G_{bind} (this study, p53(17–29))	-11.83 ± 1.02	-8.93 ± 0.78
G_{bind} (experiment, p53(16–29))	-9.3	
G_{bind} (MM-PBSA, p53(17–29))	-16.3	