In Silico Biology 2 (2002) 305–311
IOS Press

Communication

Molecular Dynamics Simulations on the Free and Complexed N-Terminal SH2 Domain of SHP-2

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Edited by E. Wingender; received 18 December, 2001; revised and accepted 27 March, 2002; published 28 March, 2002

ABSTRACT: Signal transduction events are often mediated by small protein domains such as SH2 (Src homology 2) domains that recognize phosphotyrosines (pY) and flanking sequences. In case of the SHP-2 receptor tyrosine phosphatase an N-terminal SH2 domain binds and inactivates the phosphatase (PTP) domain. The pY-peptide-binding site on the N-terminal SH2 domain does not overlap with the PTP binding region. Nevertheless, pY-peptide binding causes domain dissociation and phosphatase activation. Comparative multi-nanosecond molecular dynamics simulations on the N-SH2 domain in ligand-bound and free states have been performed to study the allosteric mechanism that leads to domain dissociation upon pY-peptide binding.
Significant ligand-dependent differences in the conformational flexibility of regions that are involved in SH2-PTP domain association have been observed. The results support a mechanism of signal transduction where SH2-peptide binding modulates the domain flexibility and reduces its capacity to fit into the entrance of the PTP catalytic domain of SHP-2.

KEYWORDS: allosteric conformational change, signal transducution, ligand-receptor binding, molecular dynamics, SH2 domains, SHP-2 phosphatase, conformational flexibility

INTRODUCTION

The Src family of tyrosine phosphatases are highly conserved signaling proteins in which a catalytic phosphatase domain (PTP) is preceded by two Src homology 2 domains (SH2). The crystal structure of the SHP-2 phosphatase shows that the N-terminal SH2 domain (N-SH2) inactivates the phosphatase by binding to the entrance of the catalytic PTP domain [5]. The N-terminal SH2 domain of SHP-2 specifically binds phosphotyrosine (pY)-containing peptides (or proteins) with the consensus sequence

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Electronic publication can be found in *In Silico Biol.* **2**, 020328<http://www.bioinfo.de/isb/2002/02/0028/>, 28 March 2002

(L/V)XpY(T/A)X(L/V) [6]. Phosphotyrosylpeptide binding induces a change in conformation or flexibility that propagates to the PTP binding site of the N-SH2 domain and causes dissociation and thereby activation of the PTP domain [10]. Phosphatase activation in turn results in further downstream signal transduction events.

Since the pY-peptide binds to a region of the N-SH2 domain several Ångstrøms apart from the PTP binding surface, SHP-2 is a good model system to investigate the crosstalk between distant protein regions in atomic detail. Such ligand-induced allosteric conformational changes play a role in many signal transduction processes.

RESULTS AND DISCUSSION

The crystal structures of the isolated N-terminal SH2 domain (N-SH2) of SHP-2 in free- and ligand-bound forms as well as the crystal structure of the complete three-domain SHP-2 molecule in ligand-free form are available [5,9,11]. The conformational difference between the isolated peptide-bound and free N-SH2 crystal structures in terms of an atomic root mean square deviation (RMSD) is only 1.6 Å (Figure 1A). This is smaller than the RMSD of 2.1 Å between the isolated peptide-free N-SH2 and the corresponding peptide-free structure in the intact SHP-2 molecule (Figure 1B). Hence, the static crystal struc-

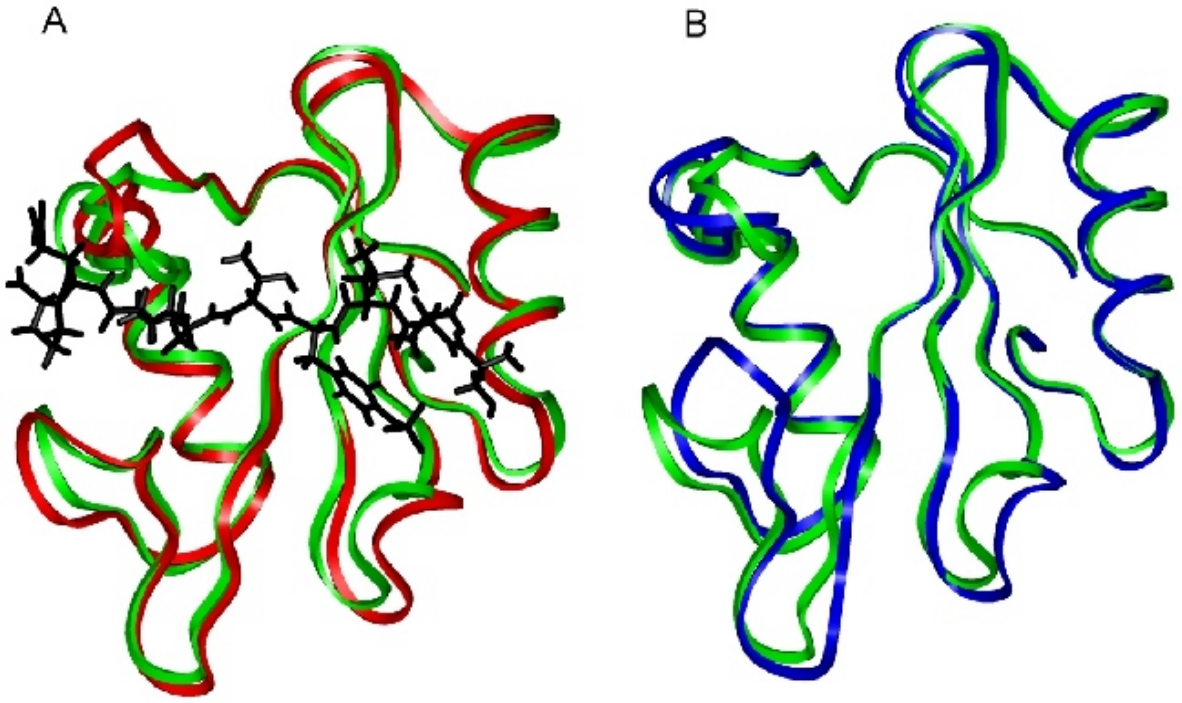

Fig. 1. Influence of pY-peptide binding and PTP domain association on the 3D conformation of the N-terminal SH2 domain of the SHP-2 phosphatase. (A) Influence of pY-peptide binding: Superposition of the crystal structures (as ribbons) of the isolated N-SH2 domain in pY-peptide-bound (red, PDB ID: 1AYD) and pY-peptide-free form (green, PDB ID:1AYA [9]). The pY-peptide (PDGFR-1009) is shown at atomic resolution (stick representation) and comprises Ser (−3), Val (−2), Leu (−1), P-Tyr (0), Thr (+1), Ala (+2), Val (+3), Gln (+4) and Pro (+5). (B) Influence of PTP-domain association: Superposition of the crystal structures (as ribbons) of the peptide-free N-SH2 domain, in isolated (green, PDB ID:1AYA [9]) and PTP-domain-associated form. The PTP-domain-associated form is extracted from the crystal structure of the complete SHP-2 phosphatase (blue, PDB ID 2SHP [5]).

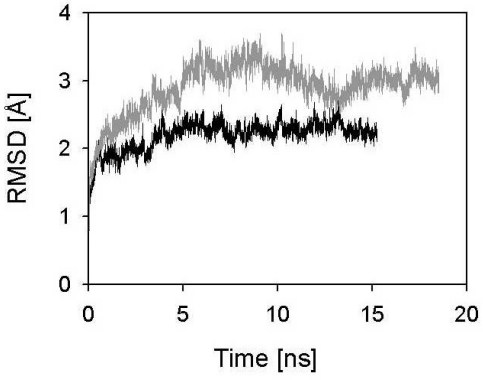

Fig. 2. The root mean square deviation time course of the 15 ns and 18 ns simulations of the ligand-bound (black) and free (grey) N-SH2 domains, respectively. The drift is shown with respect to the starting structures (PDB Ids: 1AYA and 1AYD, respectively) over the course of the simulation.

tures themselves are not sufficient to explain differences in ligand-dependent binding behaviour to the PTP domain. Especially, the large conformational difference between the ligand-free N-SH2 domain in isolated and PTP-associated state (Figure 1B) may indicate that ligand-induced changes in N-SH2 conformational flexibility may play a significant role for the domain association behaviour.

In the present study, comparative multi-nanosecond molecular dynamics (MD) simulations on the isolated N-terminal SH2 domain (in absence of the rest of the SHP-2 molecule) were performed for the peptide-bound and free states. The aim was to investigate how pY-peptide binding at one site affects the structure and dynamic at a distant region that interacts with the PTP domain in the complete SHP-2 molecule. The room temperature MD simulations resulted in stable trajectories as indicated by well conserved overall SH2-domain folds. Convergence to a stable RMSD with respect to the starting structure was obtained after 2–3 ns for the ligand-bound form. However, in case of the free N-SH2 form an equilibration time of 6 ns was necessary, since larger time scale motions could be observed (Figure 2).

During the MD simulation no significant drift of the ligand-free N-SH2-domain towards the PTP-bound N-SH2 conformation, as found in the crystal structure of the complete SHP-2 molecule, was observed (Figure 3). This result was obtained by comparing the complete N-SH2 structures (Figure 3A) as well as by comparing only the regions that form the binding interface to the PTP domain (Figure 3B).

In addition, the analysis of the backbone C-C distances between individual residues of the ligand-free N-SH2 domain during the simulations gave no conclusive hint for a stable conformational drift towards the PTP-bound conformation (Figures 4 and 5). Some C-C distances monitored during the simulation show good agreement with the corresponding distances found experimentally in the PTP-bound SHP-2 crystal structure (Figure 5A). In other cases, however, the residue distances of the pY-peptide-bound form are more resembling to those of the PTP-bound conformation (crystal structure of SHP-2) than to those in the ligand-free form (Figure 5B, E and F). However, the broader peaks of the distance-distribution in case of the ligand-free form (Figure 5C–F) indicate a greater conformational flexibility.

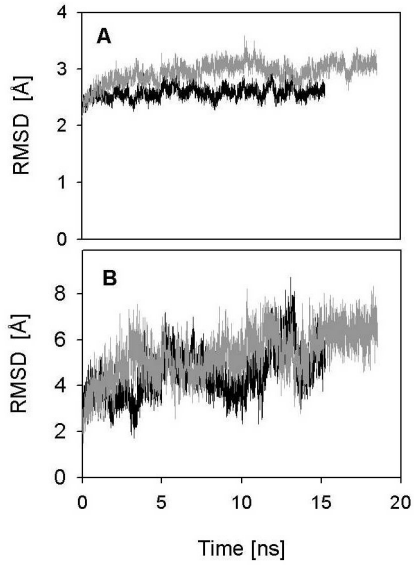

Fig. 3. The root mean square deviation time course of the 15 ns and 18 ns simulations of the ligand-bound (black) and free (grey) N-SH2 domains, respectively, (PDB Ids: 1AYD and 1AYA) relative to the crystal structure of the ligand-free N-terminal SH2 domain associated to the PTP domain in the complete SHP-2 phosphatase (PDB ID 2SHP). (A) Superposition on the whole domain. (B) Superposition on the PTP-binding region, only (residues 57–80).

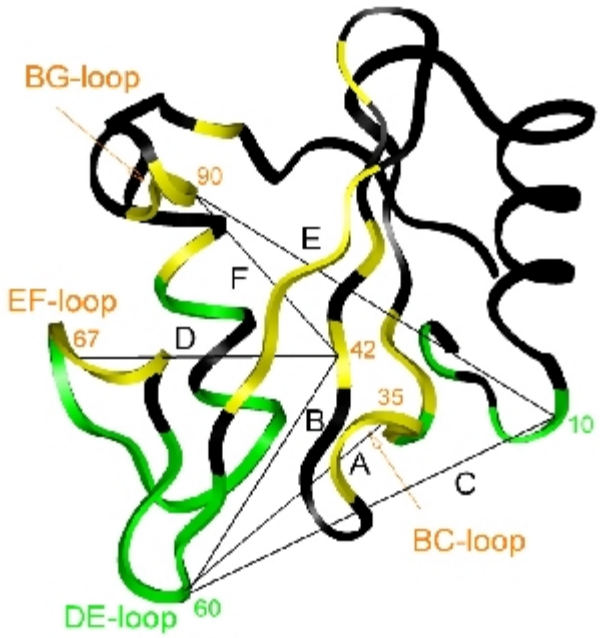

Fig. 4. Ribbon diagram of the N-SH2 domain, illustrating the distances (A, B, C, D, E and F), that have been recorded during the MD trajectory and are presented in Figure 5 A–F. The residues that are contacting the PTP domain (according to Hof *et al*. [5]) are coloured in dark grey and the residues that are involved in pY-peptide-binding (according to Lee *et al*. [9]) are coloured in light grey.

Significantly larger atomic fluctuations and a larger conformational drift from the start structure were found for the free SH2-domain compared to the pY-peptide-bound form (Figures 2 and 6). As it would be expected, absence of the ligand leads to an increased flexibility of regions that are directly involved in ligand binding. This is the case for the EF-loop (66–69) and the BG-loop (90–94) that directly contact the pY- peptide ligand. In addition, the DE-loop (58–62) shows significant larger conformational flexibility in the absence of the ligand, although it does not contact the ligand in the pY-peptide complexed SH2 domain but goes into a deep cleft of the associated PTP domain [5]. The increased flexibility induced by ligand dissociation of the above mentioned regions compared to the rest of the molecule becomes even more apparent when superimposed on only the rigid -helical and -sheet core regions (Figure 6 B). Comparison of Figure 6 A and B indicates not only a higher intrinsic EF-, BG- and DE-loop flexibility in the free state but also larger loop motions relative to the protein core made up of two -helices and one central -sheet.

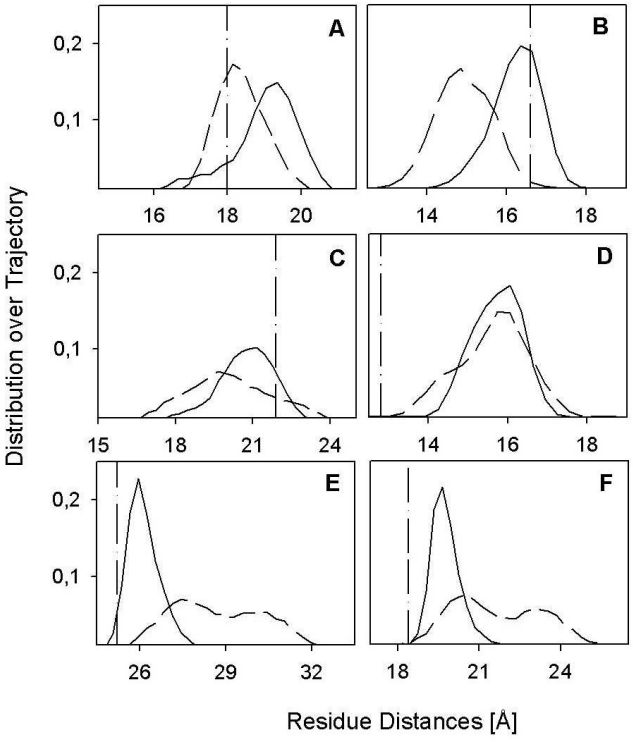

Fig. 5. Distribution of particular distances during the simulation gathering time (starting after 6 ns equilibration time) of the ligand-bound (straight line)and ligand-free state (dashed line). The corresponding distance in the crystal structure of N-SH2 in complex with the intact SHP-2 molecule is marked by the vertical dashed line. C-C distance-distributions between residues (A) Gly60 and Lys35, (B) Gly60 and Thr42, (C) Gly60 and Asn10, (D) Gly67 and Thr42, (E) Glu90 and Asn10, (F) Glu90 and Arg42 are shown.

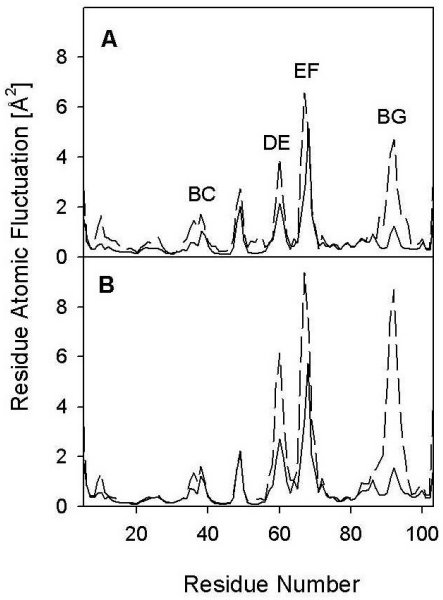

Fig. 6. Identification of flexible regions: (A) Atomic fluctuations of backbone (C) atoms averaged over the trajectory. (B) same as (A) but superposition on the two -helices and the central -sheet.

CONCLUSIONS

The comparative MD simulations indicate that the conformational flexibility of the C-terminal half (57–95) of the N-SH2 domain that forms the interface to the PTP domain is significantly larger in the absence of a pY-peptide. Greater conformational freedom implies a greater capacity for conformational adaptation. Such enhanced capacity for conformational adaptation of the N-SH2 domain might be necessary to form a binding interface that exactly fits to the entrance of the PTP domain. The present results support a mechanism of SHP-2 activation where N-SH2-peptide binding reduces the flexibility of the N-SH2 domain and in turn decrease the capacity to associate with the PTP catalytic domain.

METHODS

The atomic coordinates for the crystal structures of recombinant mouse N-terminal SH2 domain of SHP-2 in peptide-free form (PDB ID 1AYD) and complexed with the phosphotyrosylpeptide PDGFR-1009, with the sequence: S V L pY T A V Q P (PDB ID 1AYA) [9] were used as starting structures for molecular dynamics calculations. The AMBER (Assisted Model Building with Energy Restraints) suite of programs, version 5.0 [1], with the parm94 force field [2] was used for all simulations. The crystal structures were prepared for the dynamics using the Leap module of AMBER. The net charge of the protein was neutralized by adding 6 Na^+ / 4 Cl^- ions and roughly 3400 TIP3 water molecules [7]. For the nonbonded short-range interactions a 9.0 Å cutoff was used. The particle mesh Ewald summation technique with a grid size of 1.0 Å was employed to calculate long-range electrostatic interactions for distances greater than 9.0 Å [3]. The conformation of the solvated protein was first relaxed via energy minimization. Following the minimization the system was gradually heated from 20 to 300 K, thereby

slowly increasing the atomic velocities over a period of 200 ps. The solvent was separately heated before heating of the complete system including the solute. The equilibration time at 300 K was 6 ns, followed by a gathering time of 9 ns (1AYA) and 12 ns (1AYD), respectively. During both MD simulations a constant pressure of 1 bar and a relaxation time of 0.2 ps was used. Coordinates were stored each 1 ps simulation time. Root mean square Cartesian coordinate fluctuations for all heavy atoms were calculated after deduction of overall translation and rotation.

REFERENCES

[1] Case, D.A., Pearlman, D.A., Caldwell, J.W., Cheathman III, T.E., Ross, W.S., Simmerling, C., Darden, T., Merz, K.M., Stanton, R.V., Cheng, A., Vincent, J.J., Crowley, M., Ferguson, D.M., Radmer, R., Seibel, G.L., Singh, U.C., Weiner, P. and Kollman, P.A., (1997). AMBER 5.0 (San Francisco, CA, UCSF).

[2] Cornell, W.D., Cieplak, P., Bayly, C.I., Gould, I.R., Merz jr., K.M., Ferguson, D.M., Spellmeyer, D.C., Fox, T., Caldwell, J.W. and Kollman, P.A., (1995). A second generation force field for the simulation of proteins, nucleic acids, and organic molecules. *J. Am. Chem. Soc.* **117**, 5179–5197.

[3] Darden, T., York, D. and Pedersen, L., (1993). Particle mesh Ewald: An N·log(N) method for Ewald sums in large systems. *J. Chem. Phys.* **98**, 10089–10092.

[4] Eck, M.J., Pluskey, S., Trüb, T., Harrison, S.C. and Shoelson, S.E., (1996). Spatial constraints on the recognition of phosphoproteins by tandem SH2 domains of the phosphatase SH-PTP2. *Nature* **379**, 277–280.

[5] Hof, P., Pluskey, S., Dhe-Paganon, S., Eck, M.J. and Shoelson, S.E., (1998). Crystal Structure of the Tyrosine Phosphatase SHP-2. *Cell* **92**, 441–450.

[6] Huyer, G. and Ramachandran, C., (1998). The specifity of the N-terminal SH2 domain of SHP-2 is modified by a single point mutation. *Biochemistry* **37**, 2741–2747.

[7] Jorgensen, W.L., Chandrasekhar, J., Madura, J.D., Impey, R.W. and Klein M.L., (1983). Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* **79**, 926–935.

[8] Kuriyan, J. and Cowburn, D., (1993). Structures of SH2 and SH3 domains. *Curr. Opin. Struct. Biol.* **3**, 828–837.

[9] Lee, C.H., Kominos, D., Jacques, S., Margolis, B., Schlessinger, J., Shoelson, S.E. and Kuriyan, J., (1994). Crystal structures of peptide complexes of the amino-terminal SH2 domain of the Syp tyrosine phosphatase. *Structure* **2**, 423–438.

[10] Shoelson, S.E., (1997). SH2 and PTB domain interactions in tyrosine kinase signal transduction. *Curr. Opin. Chem. Biol.* **1**, 227–234.

[11] Waksman, G., Shoelson, S.E., Pant, N., Cowburn, D. and Kuriyan, J. (1993). Binding of a High Affinity Phosphotyrosyl Peptide to the Src SH2 Domain: Crystal Structures of the Complexed and Peptide-free Forms. *Cell* **72**, 779–790.