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A targeted molecular dynamics study of WPD loop movement in PTP1B

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Abstract

Targeted molecular dynamics was used to examine the mechanism of WPD loop closure in PTP1B, which is essential for the activity of the enzyme. Two important regions are identified: the R-loop (residues 113–118), which assists in substrate binding, and the S-loop (residues 198–209), which undergoes a conformational change that appears to be vital for the movement of the WPD loop. The S-loop is adjacent to the α3-helix, and its conformational change is coupled with a change of interactions between the α3- and α7-helices. This latter observation is of particular interest in connection with a novel class of allosteric inhibitors of PTP1B [Wiesmann et al., Nat. Struc. Mol. Biol. 11 (2004) 730–737]. These compounds prevent the closure of the WPD loop, forcing the enzyme to remain in a catalytically inactive conformation, by blocking the rearrangement of the α3-helix relative to the α7-helix.
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Initially considered to be mere household-enzymes with low substrate specificity, protein tyrosine phosphatases (PTPs) have been shown to play an important role in any process governed by protein tyrosine (pTyr) phosphorylation [1]. Protein tyrosine phosphatase 1B (PTP1B) [2] was the first PTP to be isolated in homogeneous form and has since become somewhat of a model system for studying the properties of PTPs. The demonstration that the knock-out of PTP1B on receptor PTP in mice results in insulin hypersensitivity [3] has made this protein an important drug target for the treatment of diabetes (in particular disease states with insulin resistance such as non-insulin dependent diabetes mellitus [4]), as current therapies are limited and often ineffective. Because of the multiple roles of pTyr in signalling, the availability of potent and selective inhibitors, i.e. pTyr mimetics, is essential—not only as potential drugs, but also as tools for further elucidation of signal pathways.

However, the development of selective pTyr mimetics has proven to be difficult, as even only distantly related members of the PTP superfamily share the PTP signature motif in the catalytic domain [5,6]. Recently, Wiesmann et al. described a group of non-pTyr-like allosteric inhibitors which bind to a novel site located ~20 Å away from the catalytic site [7]. Crystallography indicates that these inhibitors prevent the closure of the WPD loop, a conformational change which is essential for the catalytic mechanism of PTP1B since it positions Asp^{181} so that it can act as a general acid, facilitating leaving group departure [8]. This novel class of inhibitors may help to overcome the issue of selectivity and thus may allow for the development of novel therapies for type II diabetes and obesity. Also, in contrast to singly or doubly charged pTyr mimetics, these compounds are neutral, which is likely to be beneficial for bioavailability.

The unique role of the WPD loop in PTP1B and, in particular, its conformational change upon pTyr binding has been commented on several times [6,7,9]. As illustrated by the work of Wiesmann et al., understanding the mechanism of closure of the catalytic flap is of great importance as it gives insight into how this conformational change can potentially be manipulated. The changes in structure and dynamics in PTP1B upon ligand binding have been the subject of a detailed computational study [10]. Peters

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et al. carried out molecular dynamics (MD) simulations of both uncomplexed PTP1B (open form) and of PTP1B complexed with a pTyr containing hexapeptide (closed form) in aqueous solution, which were analysed by the essential dynamics method. Loop opening/closing could not be observed directly. However, from the combined essential dynamics analysis of the trajectories Peters et al. could identify regions of the protein which are involved in the WPD loop conformational change.

This study uses targeted molecular dynamics (tMD) and standard molecular dynamics to perform a more detailed examination of WPD loop movement. The tMD method makes it possible to observe full loop opening and closure. The significant structural changes in the immediate vicinity of this loop are analysed. It is hoped that an improved understanding of the mechanism of the closure of the catalytic flap will allow for the design of better inhibitors for PTP1B.

Materials and methods

The atomic coordinates for the open [6] and closed [9] conformations of PTP1B were obtained from the protein data bank [11] (PDB codes: 2HNP and 1PTV, respectively). Using 1PTV as the template, we employed WHAT IF [12] to add the residues missing at the N-terminus in 2HNP (compared with 1PTV). WHAT IF was also used to replace Ser^{215} by a cysteine residue in the 1PTV based system (cf. [10]). All subsequent computational work was carried out using the CHARMM software package [13]. In preparation for MD and tMD the initial structures were minimised. The resulting structures were then placed in a pre-equilibrated truncated octahedron of 21179 TIP3P [14,15] water molecules with a side-length of 85 Å. Deletion of waters overlapping with the protein resulted in systems containing 14146 and 13888 TIP3P waters [14,15] for 2HNP and 1PTV, respectively. Electrostatic interactions were computed by the particle-mesh-Ewald method [16]. The two systems were equilibrated for 600 ps MD. From these two starting structures we carried constant pressure, constant temperature MD simulations (Berendsen thermostat and barostat) of both the open (uncomplexed) and closed (complexed to pTyr) conformations. The length of each of the two simulations was 2 ns; the time step was 2 ps and SHAKE [17] was used to keep all bonds involving hydrogen atoms fixed.

Targeted molecular dynamics (tMD) is a tool to study the pathways of conformational changes [18,19]; the method has been available in CHARMM [13] since version c28. In tMD, one uses standard MD in conjunction with a constraining force, which is adapted at each time step and which gradually forces the starting conformation towards that of the target structure. In this work, the constraining force was changed in 0.00001 Å increments at each MD step, and the calculation was terminated once the root mean square deviation between moving and target structure reached 0.05 Å. The actual tMD simulations for the PTP1B system were quite short (400 ps on average). Both the movements of the WPD loop in the forward (open to closed) and reverse (closed to open) directions were simulated; each calculation was repeated three times by assigning different random, initial velocities. Thus, in total six simulations were carried out, over which observations were averaged.

Results and discussion

Fig. 1 shows the closed form of PTP1B with several regions of potential relevance for WPD loop movement highlighted. While there is no standard definition of the WPD loop, an accepted definition is that the full WPD

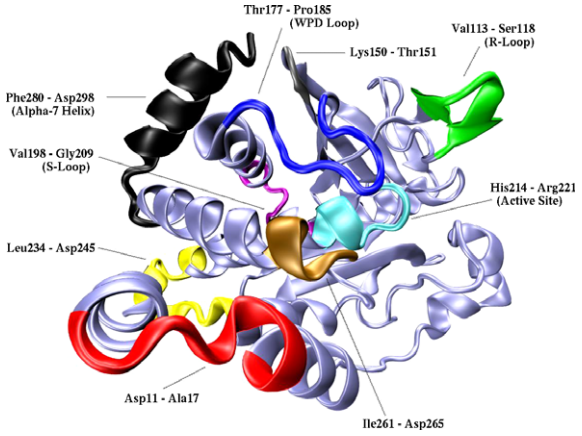

Fig. 1. A schematic diagram of PTP1B in the closed conformation, with regions of interest highlighted (cf. [11,13] and main text). The WPD loop (residues 177–185) is shown in blue, the active site (residues 214–221) in cyan, the R-loop (residues 113–118) in green, the S-loop (residues 198–209) in purple, the α7-helix (residues 280–298) in black, residues 11–17 in red, residues 150–151 in gray, residues 234–245 in yellow, and residues 261–265 in brown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

loop (shown in blue in Fig. 1) comprises residues Thr^{177} to Pro^{185} [10]. However, visually, most of the loop activity is confined to residues Trp^{179}-Val^{184}. These residues appear to act as hinges during the movement of the WPD loop, and will therefore be referred to as hinge residues throughout. By calculating the differences between equivalent C_α-trace dihedral angles using DSSP [20] (data not shown), one can identify several additional structural differences. Aside from the WPD loop and adjacent residues, residues 105–130, 150–151, 234–245, and 261–265 have deviations of more than 20°. We also calculated the mean RMSD fluctuation per residue over the course of standard MD simulations of the open and closed conformations. Here, the largest differences between the apo-enzyme and PTP1B with pTyr bound were observed in the residues 113–142, 198–209, 280–298, and, of course, the WPD loop. Interestingly, barely any deviation was observed for the active site residues (residues 214–221, shown in cyan in Fig. 1). Residues 280–298 form the α7-helix (shown in black in Fig. 1), which according to Wiesmann et al. is important for allosteric inhibition of PTP1B [7]. Peters et al. suggested that residues Val^{113}-Ser^{118} and Val^{198}-Gly^{209} are important for WPD loop opening/closing [10]. Both regions, to which we refer henceforth as the R-loop (shown in green in Fig. 1) and as the S-loop (shown in purple in Fig. 1), are part of longer residue ranges that can be detected either by the differences in C_α-trace dihedral angles of the crystal structures or by differences in mobility during the MD simulations. The distance of the WPD loop to the active site was also monitored. Its value fluctuates, but there is no net change in the average distances as compared to the respective starting value. This suggests that no significant movement of the WPD loop occurs over the course of 2 ns of MD, which is in good agreement with both computational and experimental studies [10,21].

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