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Structural Insights into the Specific Binding of Huntingtin Proline-Rich Region with the SH3 and WW Domains

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Summary

The interactions of huntingtin (Htt) with the SH3 domain- or WW domain-containing proteins have been implicated in the pathogenesis of Huntington's disease (HD). We report the specific interactions of Htt proline-rich region (PRR) with the SH3GL3-SH3 domain and HYPA-WW1-2 domain pair by NMR. The results show that Htt PRR binds with the SH3 domain through nearly its entire chain, and that the binding region on the domain includes the canonical PxxP-binding site and the specificity pocket. The C terminus of PRR orients to the specificity pocket, whereas the N terminus orients to the PxxP-binding site. Htt PRR can also specifically bind to WW1-2; the N-terminal portion preferentially binds to WW1, while the C-terminal portion binds to WW2. This study provides structural insights into the specific interactions between Htt PRR and its binding partners as well as the alteration of these interactions that involve PRR, which may have implications for the understanding of HD.

Introduction

Huntington's disease (HD) is a dominant neurodegenerative disorder characterized by movement abnormalities, cognitive impairment, and psychiatric disturbances due to neuronal cell loss, especially in the basal ganglia and the cerebral cortex (Martin and Gusella, 1986; Vonsattel et al., 1985). Accumulating evidence supports the finding that a polyglutamine (polyQ) expansion tract in huntingtin (Htt), a ubiquitously expressed protein of yet unknown function, is the cause of this disease (MacDonald et al., 1993; DiFiglia et al., 1995).

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Human Htt is a large multidomain protein of 3144 amino acid residues with a polyQ domain at the N terminus (MacDonald et al., 1993). The polyQ domain ranges from 11 to 34 glutamine residues in unaffected individuals, whereas that of HD patients extends to 37 or more glutamine residues (Bates et al., 2002). A proline-rich region (PRR) containing 40 residues (normally residues 41–80) directly follows the polyQ domain in sequence (Figure 1A).

Recently, many investigations revealed that mutant Htt with expanded polyQ impairs the normal interactions of several proteins involved in gene transcription, trafficking, endocytosis, and cell signaling (Goehler et al., 2004; Harjes and Wanker, 2003; Li and Li, 2004). Thus, it is speculative that HD neuropathology is related to the interference of the normal function of cellular proteins by the aberrant Htt protein (Landles and Bates, 2004; Li et al., 2003; Li and Li, 2004).

There are many Htt-interacting partners identified; some contain Src homology 3 (SH3) or tryptophan (WW) domains, such as SH3GL3/endophilin3 (Sittler et al., 1998), protein kinase C and casein kinase substrate in neurons 1 (PACSIN1/syndapin) (Modregger et al., 2002), HYPA/FBP11 (Faber et al., 1998), PSD-95 (Sun et al., 2001), RasGAP (Liu et al., 1997), and CA150 (Holbert et al., 2001). Among them, SH3GL3 is reported to be preferentially expressed in human brain and testis, and its C-terminal SH3 domain is essential for the interaction with Htt PRR (Sittler et al., 1998). The characteristics of the interaction between SH3GL3 and Htt and the colocalization of these two proteins suggest that SH3GL3 could be involved in the selective neuronal cell death in HD (Sittler et al., 1998). SH3GL3 was also found to bind to the shell of the Htt body, suggesting that the SH3GL3-associated HD pathology may be caused by sequestering the Htt inclusion bodies (Qin et al., 2004). PACSIN1 has been implicated in clathrin-mediated endocytosis (DiProspero et al., 2004), and its abnormal binding behavior and altered intracellular distribution in pathological tissues suggest that it plays a role during the early stages of the selective neuropathology of HD (DiProspero et al., 2004; Modregger et al., 2002). Human HYPA interacts with the N-terminal region of Htt protein through its tandem WW domains, as identified by yeast two-hybrid assay (Faber et al., 1998; Passani et al., 2000). FBP11, the murine ortholog of human HYPA, is one of the several proteins that bind with formins involved in murine limb and kidney development (Bedford, et al., 1997). FBP11 also participates in pre-mRNA splicing (Lin et al., 2004) and regulation of N-WASP localization (Mizutani et al., 2004).

The involvement of PRR in the pathological process of HD can also be observed from the fact that MW7 scFv, a monoclonal antibody recognizing Htt PRR, significantly inhibits aggregation as well as the cell death induced by mutant Htt protein (Khoshnan et al., 2002). Another observation indicates that removal of a series of prolines adjacent to the polyQ tract in Htt blocks formation of the shell of the Htt body and redistributes sequestration of many vesicle-associated proteins, a process

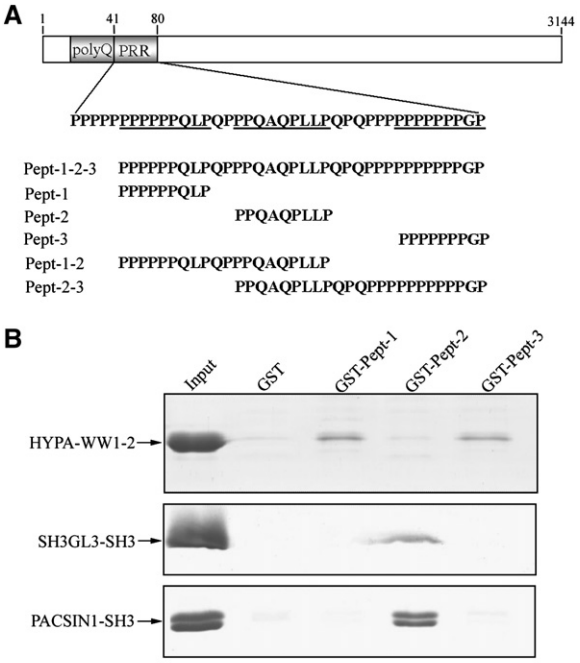

Figure 1. The N-Terminal, Central, and C-Terminal Portions of Htt PRR Have Different Binding Preferences for the SH3 and WW Domains

(A) The domain architecture of the N-terminal Htt protein and the amino acid sequence of the PRR region. Six peptides corresponding to different portions of Htt PRR were generated by peptide synthesis. Pept-1, Pept-2, and Pept-3 were also generated and purified as recombinant GST fusion proteins for pull-down assay.

(B) GST pull-down assay showing that the tandem WW domains of HYPA-WW1-2 interact with GST-Pept-1 and GST-Pept-3, while SH3GL3-SH3 and PACSIN1-SH3 only interact with GST-Pept-2 in vitro. The "Input" lane represents the band from 10% of the amount of protein in each sample.

that may be related to neuronal dysfunction (Qin et al., 2004).

The typical PRR segment recognized by SH3 or WW domains contains less than 10 amino acid residues (Kay et al., 2000), and the molecular mechanism underlying the specific recognition has been elucidated and reviewed (Ilsley et al., 2002; Musacchio, 2002; Sudol, 1996; Zarrinpar et al., 2003). However, many proteins, such as Htt, formin (Bedford et al., 1997), and N-WASP (Bompard and Caron, 2004), contain PRR far longer than 10 residues. How these long PRRs recognize various SH3 and WW domains remains largely unknown. Obtaining the structural knowledge of the interaction between Htt PRR and the SH3 or WW domain will be the first step toward understanding how the abnormally expanded polyQ tract interferes with the normal function of cellular proteins that contain the SH3 or WW domain.

We have determined the solution structure of the SH3 domain of SH3GL3 and have assigned the backbone resonances of the WW domain pair of HYPA by heteronuclear NMR. Based on the structure, the binding specificities between Htt PRR and these domains have been elucidated in detail. This study reveals that Htt PRR recognizes the SH3 domain and the WW domain pair with high specificities, but with different mechanisms.

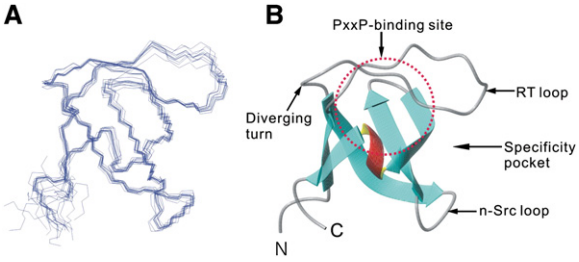

Figure 2. 3D Solution Structure of the SH3 Domain from SH3GL3

(A) Backbone atom superposition of the final ten structures. The structures are superimposed adopting residues 3–58.

(B) Ribbon diagram representation of the SH3 domain of SH3GL3. The canonical PxxP-binding pocket is indicated in a circle, while the specificity pocket between the RT loop and the n-Src loop is also highlighted.

Results

The Three Portions of Htt PRR Have Different Preferences for the SH3 and WW Domains

To identify which portion of the Htt PRR region (residues 41–80) is responsible for the interaction with the SH3 or WW domain, we subdivided the region into three peptide parts, corresponding to the N-terminal (Pept-1), central (Pept-2), and C-terminal (Pept-3) portions of Htt PRR, respectively (Figure 1A). The three segmental sequences were cloned and expressed as GST fusion proteins for a pull-down assay. Figure 1B suggests that SH3GL3-SH3 and PACSIN1-SH3 only bind to Pept-2, not to Pept-1 or Pept-3, while HYPA-WW1-2 (the tandem domains of HYPA) binds to Pept-1 and Pept-3, but not to Pept-2. This is consistent with the previous study that an expanded Pept-2 specifically binds with PACSIN1-SH3 (Modregger et al., 2002). The results imply that the three portions of Htt PRR have different binding affinities for the SH3 domain of SH3GL3 or PACSIN1 and the tandem WW domains of HYPA.

Solution Structure of the SH3GL3-SH3 Domain

To study the interaction between SH3GL3-SH3 and Htt PRR in detail, we solved the structure of the SH3 domain in solution by heteronuclear multidimensional NMR techniques. A summary of the NMR experimental restraints for structure calculation and statistics is presented in Table S1 (see the Supplemental Data available with this article online). All ten of the lowest-energy final structures converge with an NOE or dihedral angle violation no greater than 0.3 Å or 5°, respectively. The average root-mean-square deviations (rmsds) for the ten structures for backbone and all heavy atoms are 0.64 and 1.76 Å, respectively. Figure 2A depicts a superimposition of the ten lowest-energy structures and a ribbon representation of one of the ten NMR structures. The solution structure of SH3GL3-SH3 maintains a typical SH3 fold containing five β strands organized in an antiparallel β barrel structure with a short 3_{10} helix between the last two β strands (Figure 2B). Similar to most SH3 fold domains (Dalgarno et al., 1997), this structure also contains two characteristic loops, namely, the RT loop between β_1 and β_2 and the n-Src loop between β_2 and β_3. There is also a broad pocket called the specificity pocket