Solution structure of the isolated Pelle death domain

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Received 12 January 2005; revised 24 May 2005; accepted 3 June 2005

Available online 23 June 2005

Edited by Christian Griesinger

Abstract The interaction between the death domains (DDs) of Tube and the protein kinase Pelle is an important component of the Toll pathway. Published crystallographic data suggests that the Pelle–Tube DD interface is plastic and implies that in addition to the two predominant Pelle–Tube interfaces, a third interaction is possible. We present the NMR solution structure of the isolated death domain of Pelle and a study of the interaction between the DDs of Pelle and Tube. Our data suggests the solution structure of the isolated Pelle DD is similar to that of Pelle DD in complex with Tube. Additionally, they suggest that the plasticity observed in the crystal structure may not be relevant in the functioning death domain complex.

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Keywords: Death domains; Tube; Pelle; NMR

1. Introduction

The Toll pathway in Drosophila functions in establishing dorsoventral polarity in embryos and additionally, in the innate immune response to Gram-positive bacteria [1]. In dorso- ventral patterning, activation of the transmembrane Toll receptor results in a signal transduction event that induces the proteolysis of Cactus which relieves the inhibition of Dorsal, a transcription factor of the Rel/NF-κB family. In the initiation of the innate immune response, the Toll pathway again involves Dorsal and another protein of the Rel/NF-κB family, Dif [2,3]. In mammals, an innate immune response is mounted as a result of bacterial challenge with the activation of an homologous pathway consisting of the mammalian Toll-like receptors TLR2 and TLR4 [4].

Central to the Toll pathway in Drosophila are two proteins Tube (T) and Pelle (P) that are required to transmit the signal from Toll to the Dorsal/Cactus complex [5,6]. Tube lacks recognizable catalytic activity but possesses an interaction motif belonging to the death domain (DD) superfamily as well as C-terminal repeats that interact with Dorsal [7]. Pelle possesses a kinase domain and an N-terminal DD. Recently, an adaptor protein, dMyD88 [8,9] that possesses a DD has been identified as a component of the Toll pathway and the evidence suggests that Tube, Pelle and dMyD88 form a heterotrimmer [10]. Additionally, it has been shown that alleles of dMyD88 (Kra/ dMyd88) which affect dorsoventral patterning interact with Tube but not Pelle [11]. Homologues of Pelle have been found in mammals in the form of the interleukin-1 receptor associated kinases (IRAKs) [12–14] but no Tube homologues have been identified to date. In mammalian toll signaling, the adaptor MyD88 associates with a conserved cytoplasmic signaling motif (TIR domain) of Toll-like receptors and the IL-1 receptor and also the DD of IRAKs [15,16].

The importance of DD interactions in the innate immune response as well as their role as the prime mediator of the interactions required for apoptosis have made them important candidates for both structural and functional studies. The death domain superfamily consists of the DD, death effector domain (DED) and caspase recruitment domain (CARD) families. They are characterized by a conserved six-helical bundle fold [17,18] and typically associate via homotypic interactions in which DD–DD, DED–DED and CARD–CARD contacts are formed exclusively. Despite similarities in the overall fold of the superfamily members, a common interaction surface has not been identified. However, the finding that the ERK-binding epitope on the DED of PEA-15 [19] is similar to that of Tube DD [20] suggests that there may be conserved features, at least among some family members.

The complementary interactions formed by members of the DD superfamily are strong implying high-affinity protein–protein interactions. For example, the dissociation constant for the interaction between the death domains of Tube and Pelle is approximately 0.5 μM [21]. The three-dimensional structures of two heterodimers, Tube–Pelle [20] (DD–DD) and Apaf-1–procaspase-9 [22] (CARD–CARD) have been solved crystallographically. The residues comprising the interface in these two heterodimers are different; the CARD–CARD interface predominantly involves charge–charge interactions between the second and third helices of Apaf-1 and helices 1 and 4 of procaspase-9 which has been termed a type 1 interaction [23]. In the DD–DD interface, helix-4 of Pelle interacts with a groove formed by helices 1, 2 and 6 of Tube and additionally, the C-terminal tail of Tube interacts with a groove on Pelle created by the helices 2 and 3 and 4 and 5 [20] and this is termed a type 2 interaction [23]. Another difference between the CARD–CARD and DD–DD interfaces arises because the crystal structure of the Pelle–Tube DD complex [20] contains two different Pelle–Tube dimers in the crystallographic asymmetric unit. Consequently, the DD–DD interface has been described as being “plastic” and this plasticity is inferred to be absent in the CARD–CARD interaction.

The differences in the interfaces observed in the Pelle–Tube complex [20] presumably arise because of the insertion of a Tube molecule from a second asymmetric unit between one Pelle–Tube interface (see Fig. 5, Supplementary Materials). It is therefore important to ascertain if the plasticity is merely a...
forced adaptation by the interface to accommodate the insertion or whether it is an inherent feature of the interface. Additionally, the crystal structure suggests that the Pelle–Tube DD complex can exist as a tetramer comprised of Pelle and Tube heterodimers arranged in a linear sequence P1:T1:P2:T2 and whether this persists in solution needs to be determined as it may have implications for the function of the DD complex. To address these issues, we have solved the structure of the isolated Pelle-DD in solution and have studied the interaction between the DDs of Pelle and Tube using nuclear magnetic resonance (NMR) spectroscopy. Our results suggest that: in the α-helical regions, the structure of Pelle-DD is similar to that of Pelle-DD in complex with the DD of Tube; of the two types of Pelle–Tube dimer interfaces observed in the crystal structure (P1:T1 and P2:T2) only one is likely to persist in solution.

2. Materials and methods

2.1. Sample preparation

Pelle-DD (26–131) and Tube-DD (1–184) were amplified by polymerase chain reaction using Tube or Pelle cDNA as the template. The amplified fragment was confirmed by DNA sequencing and inserted into the NdeI/EcoR1 sites of the pET28a expression vector (Novagen) and transformed into BL21 (DE3) cells. Transformations with the Pelle-DD were grown in MOPS media [24] (in H2O or D2O) containing 0.4 g 15N-ammonium chloride (Martek Biosciences) as the sole nitrogen source, 2 g glucose, uniformly labelled 13C- or 2H-glucose (Cambridge Isotopes) and 60 µg/ml kanamycin (Sigma) at 310 K to an optical density of 600 nm of 0.9–1.0 and induced with 0.8 mM isopropyl-1-thiogalactopyranoside. Induction was continued at 293 K for an additional 12 h and the cells harvested by centrifugation at 6500 × g for 15 min. Transformed cells with Tube-DD were grown in MOPS media (D2O) supplemented with 2H-glucose and 15N-ammonium chloride. Subsequent treatment was the same as for cells containing Pelle-DD.

Cells containing Pelle-DD or Tube-DD were resuspended in a solution containing 20 mM sodium phosphate, 100 mM NaCl, pH 7.6 (buffer A) and lysed. The lysate was centrifuged at 10 500 × g for 1 h and the supernatant loaded on to a Ni-NTA resin pre-equilibrated with buffer A. The column was washed with 10-column volumes of buffer A followed by buffer A containing 500 mM imidazole. To the column eluate, 0.5 mM EDTA and 60 units thrombin (Boehringer Mannheim) were added (for Pelle-DD only) and digestion allowed to proceed overnight at room temperature or until cleavage of the hexa-HIS tag was complete as judged by SDS-PAGE. Following digestion, the protein solution was concentrated using a Vivaspin-20 concentrator (Vivasence) and loaded onto a Superdex-75 size-exclusion column and eluted with buffer A containing 0.5 mM EDTA. Pelle-DD and Tube-DD elute as apparent monomers and pooled fractions were concentrated as above.

To confirm that the expressed Pelle DD was folded in solution, a far-uv CD spectrum was recorded. The spectrum is characterized by double minima at 209 and 223 nm (Fig. 6, Supplementary Materials) which is indicative of an α-helical secondary structure. Analysis of the CD spectrum revealed a secondary structure content of 45% α-helix; 8.3% β-sheet; 18.9% β-turn and 29.8% unordered. Additionally, the number of predicted helices was 6 and the average length of the helical segments was 8 residues. The thermostability of Pelle DD was determined by measuring the temperature dependence of the Δσ value at 222 nm and the midpoint of the melting transition was 64 °C. This suggests that the isolated Pelle DD is stable in solution and is consistent with previous findings [43]. Given the relatively low resolution of the CD technique, the calculated secondary structure content of Pelle DD as well as the number of α-helices is consistent with that expected of a death domain [44] and additionally, with the Pelle DD observed in the crystal structure of the Pelle–Tube DD complex [20].

Table 1 lists the NMR restraints and the conformational energies obtained for the isolated Pelle-DD. The twenty lowest energy NMR solution structures of the Pelle-DD are shown in Fig. 1. The isolated Pelle-DD forms a well defined anti-parallel six-helix bundle. The residues forming the six helices (α1–α6) are: L39–L52; W55–V62; P66–N77; A82–W90; V98–K106 and H111–I117. Resonance assignments were possible for only one (G22) of the first six residues of the N-terminal domain and H111–I117. Resonance assignments were possible for only one (G22) of the first six residues of the N-terminal domain and this suggests that this region is locally unstructured in solution. Four of the first six residues result from the cloning vector used, and consequently, this finding is not surprising.
The six-helix bundle conformation of the isolated Pelle-DD observed by NMR is similar to that of the Pelle-DD in complex with the DD of Tube[20]. Fig. 1 (b) shows the lowest energy NMR structure superimposed on that determined crystallographically. The \( \alpha \)-helices (\( \alpha_1–\alpha_6 \)) superimpose with RMSDs of: 1.812 ± 0.840; 0.896 ± 0.276; 0.730 ± 0.238; 0.751 ± 0.226; 1.542 ± 0.541 and 1.359 ± 0.290, respectively. The largest differences between the isolated DD of Pelle and that of Pelle in the Pelle–Tube complex in the well ordered region (residues 35–119) occur at P40 in \( \alpha \)-helix-1 and L108 which is in the loop between \( \alpha \)-helix-5 and \( \alpha \)-helix-6.

### 3.2. Interface analysis

The X-ray crystal structure of the Pelle–Tube DD complex contains two Pelle-DD (P1, P2) and two Tube-DD (T1, T2) molecules in the crystallographic asymmetric unit. These are arranged in an approximately linear sequence, P1:T1:P2:T2 [20], yielding three Pelle–Tube DD interfaces (see Fig. 5, Supplementary Materials). Additionally, the third helix of a Tube molecule from another asymmetric unit (T2A) is inserted between the P1:T1 interface [20] and there are also interactions between the P1 molecule from this second asymmetric unit and T2. The absence of tetrameric species in previous solution studies of the Pelle–Tube death domain complex, suggests that the predominant species is dimeric [20,21]. Given the many interactions observed in the crystal structure [20], it is important to ascertain whether the Pelle–Tube death domain interface, as defined crystallographically, is the same in solution.

The residues comprising the P1:T1, P2:T2 and T1:P2 Pelle–Tube DD interfaces seen in the crystal structure [20] obtained using the program probe [45] with a probe radius of 1.4 \( \text{Å} \) are listed in Table 2. The interaction between the death domains is extensive with thirty-three and thirty-two Pelle-DD residues, respectively, contributing to the P1:T1 and P2:T2 interface; twenty-seven residues are common to both the P1:T1 and P2:T2 interface. Residues in the P1:T1 interface that are not in the P2:T2 interface are L28, A61, Q99, M114, V121 and L125 while G22, S23, H24, M25 and K127 are found in the P2:T2 interface but not in the P1:T1 interface. For this latter group, the first four residues (G22, S23, H24 and M25) are derived from the expression plasmid and thus do not strictly belong to the DD. Analysis of the T1:P2 interface reveals that it is comprised of 16 residues and that two of these, K106 and Y128 are found in both the P1:T1 and P2:T2 interface; twenty-seven residues are common to both the P1:T1 and P2:T2 interface. Residues in the P1:T1 interface that are not in the P2:T2 interface are L28, A61, Q99, M114, V121 and L125 while G22, S23, H24, M25 and K127 are found in the P2:T2 interface but not in the P1:T1 interface. For this latter group, the first four residues (G22, S23, H24 and M25) are derived from the expression plasmid and thus do not strictly belong to the DD. Analysis of the T1:P2 interface reveals that it is comprised of 16 residues and that two of these, K106 and Y128 are found in both the P1:T1 and P2:T2 interface.

### Table 1

<table>
<thead>
<tr>
<th>Number of experimental restraints</th>
<th>Unambiguous</th>
<th>1502</th>
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<tbody>
<tr>
<td>Ambiguous</td>
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<td></td>
</tr>
<tr>
<td>Dihedral restraints</td>
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<td></td>
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<tr>
<td>Talos derived</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>(^{3}\text{HONHA})</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Coordinate precision</td>
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<td></td>
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<tr>
<td>RMSD of backbone atoms (35–119)</td>
<td>0.56 ± 0.11b</td>
<td>0.40b</td>
</tr>
<tr>
<td>RMSD of all heavy atoms (35–119)</td>
<td>0.88 ± 0.21</td>
<td>0.81</td>
</tr>
<tr>
<td>RMS deviations from experimental restraints</td>
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</tr>
<tr>
<td>NOE distances (Å)</td>
<td>0.007 ± 0.003</td>
<td>0.009</td>
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<tr>
<td>Talos dihedrals (°)</td>
<td>0.434 ± 0.279</td>
<td>0.178</td>
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<tr>
<td>RMS deviations from idealized geometry</td>
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<tr>
<td>Bonds (Å)</td>
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<td>0.001</td>
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<tr>
<td>Angles (°)</td>
<td>0.295 ± 0.011</td>
<td>0.293</td>
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<tr>
<td>Improper s (°)</td>
<td>0.140 ± 0.017</td>
<td>0.143</td>
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<tr>
<td>Final energy ( E_{LJ} ) (kcal/mol)</td>
<td>−997.12 ± 7.80</td>
<td>−981.36</td>
</tr>
<tr>
<td>Ramachandran analysis(^3)</td>
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<td></td>
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<tr>
<td>Residues in the most favored regions (%)</td>
<td>87.7 (79.9)</td>
<td>88.6 (82.3)</td>
</tr>
<tr>
<td>Residues in additionally allowed regions (%)</td>
<td>10.6 (16.6)</td>
<td>10.1 (14.6)</td>
</tr>
<tr>
<td>Residues in generously allowed regions (%)</td>
<td>1.6 (2.7)</td>
<td>1.3 (1.1)</td>
</tr>
<tr>
<td>Residues in disallowed regions (%)</td>
<td>0.2 (0.8)</td>
<td>0.0 (1.0)</td>
</tr>
</tbody>
</table>

\(^{a}\) This column represents the average RMS deviations for the ensemble.  
\(^{b}\) This column represents values for structures closest to the mean.  
\(^{c}\) The Lennard-Jones potential was not used during refinement.  
\(^{d}\) Residues 35–119; 28–131 in parentheses.
the complex with the DD of Tube is formed. Such shifts are clearly seen, for example, H96 and R35. The latter residue, R35, forms an ion pair with E50 of Tube-DD and charge reversal mutations of this residue have been found to inhibit the formation of dorsoventral patterning elements [20]. A closer inspection of Figs. 2 and 3 reveals that the $^1$H-$^1$5N chemical shifts of most residues common to the P1:T1 and P2:T2 interfaces (column $P_1:T_1\cap P_2:T_2$ in Table 2) are different between the free and complexed states. Quantitatively, the largest chemical shift differences are observed for R35, V62, K63, L64, D67, Q68, G91, G92, Y94, N95, H96, A103, and L104 with shifts for the other residues being somewhat less. That shifts are observed for these common residues suggests that the interactions obtained in solution are similar to that seen in the crystal structure. The $^1$H-$^1$5N chemical shift of the six residues that are in the P1:T1 but not the P2:T2 interface also show differences between the free and complexed Pelle-DD while of the residues that are only in P2:T2, only K127, which also belongs to the T1:P2 interface is shifted. Note that G22, which is the only residue of the first six to be assigned only shows a very small shift when the Pelle–Tube DD complex is formed.

As mentioned, the T1:P2 interface is comprised of sixteen residues. Some of these, for example, A47, show very small differences in chemical shift between the free and complexed state while others (H48, H127) have more substantial chemical shift differences between the free and complexed forms. If these shifts are not the result of conformational differences, the data implies that there may be an interaction similar to that seen in the crystal (T1:P2) albeit with an apparently reduced interaction surface.

In the crystal structure of the Pelle–Tube DD complex, the differences between the P1:T1 and P2:T2 interfaces were observed to arise because of the insertion of a second symmetry related Tube molecule (T2A) into the P1:T1 interface; a condition that is not expected to occur in solution. There are nine

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**Table 2**

Analysis of the Pelle–Tube death domain interfaces seen in the X-ray crystal structure

<table>
<thead>
<tr>
<th>$P_1:T_1\cap P_2:T_2$</th>
<th>$P_1:T_1^0$</th>
<th>$P_2:T_2^0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>D29, R35, V62, K63, L64</td>
<td>L28, A61</td>
<td>G22, S23</td>
</tr>
<tr>
<td>Y65, D67, Q68, Q71, E85</td>
<td>Q99, M114</td>
<td>H24, M25</td>
</tr>
<tr>
<td>N88, I89, W90, G91, G92</td>
<td>V121, L125</td>
<td>K127</td>
</tr>
<tr>
<td>Q93, Y94, N95, H96, T100</td>
<td></td>
<td>K109, H111</td>
</tr>
<tr>
<td>L101, F102, A103, L104</td>
<td></td>
<td>N112, R115</td>
</tr>
<tr>
<td>K106, K107, Y128</td>
<td></td>
<td>K118, H126</td>
</tr>
</tbody>
</table>

$P_1:T_1\cap P_2:T_2$ are residues common to the P1:T1 and P2:T2 interfaces; $P_2:T_2^0$ are residues in the P1:T1 interface that are not in the P2:T2 interface and vice-versa. The other headings are defined analogously.

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![Fig. 2](image1.png)

**Fig. 2.** $^1$H-$^1$5N HSQC spectrum of the isolated Pelle DD (black) and $^1$H-$^1$5N-HSQC-TROSY spectrum of Pelle-DD in complex with the DD of Tube (red). Arrows indicate peaks that are aliased in the $^1$5N dimension.

![Fig. 3](image2.png)

**Fig. 3.** $^1$H-$^1$5N chemical shift deviations ($\Delta$) between the isolated Pelle-DD and Pelle-DD in complex with the DD of Tube. $\Delta$ was calculated using $((N_{PT}-N_{P})/4.1)^2 - (H_{PT}-H_{P})^2)/2$. $N_{PT}$ and $N_{P}$ are the chemical shifts of the amide nitrogen in the Pelle–Tube complex and the isolated Pelle-DD and $H_{PT}$ and $H_{P}$ the corresponding chemical shift for the amide protons, respectively. Arrows denote residues common to both P1:T1 and P2:T2 for which shifts are observed but whose magnitude is uncertain. The correction factor for the amide nitrogen chemical shift difference is the ratio of the spectral width of $^1$5N to $^1$H [46].
residues (Q56, Y65, P66, D67, Q93 and Y94) on P1 that interact with T2A. If the residues that are in the P1:T1 interface are removed from this list, only Q56, P66, V69 and Q70 remain. Of these, small shifts in the HSQC spectra are observed for Q56 and Q70 and a slightly large shift for V69. Thus, it is unlikely that in solution, the P1:T1 interface would be disrupted as seen in the crystal implying that this specific interface is absent.

3.3. Sedimentation velocity

As mentioned, the NMR shifts suggest that in solution there may be an interaction analogous to the T1:P2 interaction and this would imply the existence of a tetramer. Previous data [20,21] had found no evidence of a tetrameric species and the issue of whether the tetramer observed in the crystal structure is an artifact is unresolved. To address this, we have performed sedimentation velocity experiments on a solution containing Pelle- and Tube death domains. Fig. 4 shows the experimental sedimentation profiles, the sedimentation coefficient distribution, $c(s)$, the molecular weight distribution, $c(M)$, calculated using SEDFIT [42] and the residuals obtained from fitting the data. The r.m.s.d of the fit was 0.014 and the recovered frictional coefficient was 1.39. The $c(s)$ distribution shows peaks at 1.91 S, 2.54 S and a smaller peak at 3.7 S. From the $c(M)$ distribution these correspond to species with the following molecular weights: 21558.4 Da (T), 33191.4 Da (PT) and 58811.8 (PTPT) and these are within 5%, 0.1% and 12%, respectively, of the expected molecular weights based on sequence. From the distributions, it is evident that the predominant complex of Pelle- and Tube-DD is indeed dimeric and the area under the $c(s)$ distribution suggests that the concentration of the tetrameric Pelle–Tube DD complex is approximately 0.4% that of the Pelle–Tube dimer.

4. Discussion

An understanding of the interactions that occur between death domains is an important step towards dissecting the structural basis of signal transmission between these motifs and the processes that they regulate. The X-ray crystal structures of two heterodimers from the death domain superfamily – Pelle–Tube (DD) and Apaf-1-procaspase-9 (CARD) – have been solved. The residues involved in the interface and the mode of binding was different in both complexes. Additionally, two similar but distinct interfaces were observed in the Pelle–Tube DD complex which has led to the suggestion that the Pelle–Tube dimer interface is plastic. Because the differences in the Pelle–Tube DD interface observed crystallographically were due to the insertion of Tube from a second asymmetric unit (T2A) between the P1:T1 interface, a primary objective was to determine if the plasticity inferred from the crystal structure also occurred in solution. To ensure that the changes seen in the HSQC spectrum of Pelle-DD when in complex with Tube-DD could be interpreted as arising from complex formation and not necessarily because of differences between the structures of Pelle in both states a high resolution structure of the isolated Pelle-DD was required. Previous attempts at obtaining such data by X-ray crystallography were unsuccessful [43] and consequently, NMR spectroscopy was used.

The solution structure of Pelle-DD in solution is similar to that of Pelle in the Pelle–Tube complex [20]. The protein adopts a six-helix bundle fold which is characteristic of death domains and the helical regions superimpose reasonably well on the corresponding residues from the crystal structure. The largest structural differences are predominantly observed in the regions N-terminal of helix-1 and C-terminal of helix-6. This is not unexpected as the residues in these regions have

Fig. 4. Sedimentation velocity profiles of the Pelle-DD, Tube-DD complex obtained at 293 K and a rotor speed of 55 000 rpm (a) and the residuals obtained after fitting (b). The peaks at 1.39 S, 2.54 S and 3.7 S in the $c(s)$ distribution (d) correspond to free Tube, the Pelle–Tube dimer and the Pelle–Tube tetramer, respectively. The molecular weights of these species are shown in the corresponding $c(M)$ distribution (c). Data were analyzed using the program SEDFIT [42].
no regular secondary structure content and, additionally, their B-factors are among the highest of either P1 or P2 in the crystal structure of the Pelle–Tube complex [20]. Interestingly, the first six residues of Pelle-DD appear to be quite mobile as only one (G22) is observed in the NMR data and they are not observed in P1 of the crystal structure. Additionally, from the NMR data, the presence of Tube-DD does not appear to have an effect on the mobility of these residues as there are no additional peaks which could be assigned to them and the chemical shift of G1 is unaltered which would not be expected if these residues adopted the conformation seen in the P2 molecule [20]. This implies that packing constraints are the likely reason why they are observed in P2 and not necessarily the presence of the C-terminal tail of Tube-DD.

The X-ray structure of the Pelle–Tube complex led to the suggestion that the Pelle–Tube interface is plastic. The T2A insertion observed in the crystal should not occur in solution and this is supported by the observation that most residues on the Pelle-DD that belong to this interface exhibit no shift or only marginal shifts in their $^1$H-$^15$N frequencies. Consequently, if the plasticity observed crystallographically is a result of the T2A insertion, it does not appear to exist in solution, at least at the concentrations used in our experiments.

May be, however, that the plasticity is an inherent feature of the Pelle–Tube DD interface. Our results do not explicitly refute this assertion. For example, while chemical shifts are observed for the amide-proton and amide nitrogen of A61 and Q99 (P1:T1), these residues are surrounded by common interaction residues and it is difficult to determine if such shifts are due to direct interaction with Tube. For others, for example (M114) there appear to be only small shifts and this residue is sandwiched between N112 and R115 which belong to the T1:P2 interface. In addition, some residues, for example Y65, observed in both the T1:P1 and T2:P2 interfaces bind differently. If there were two distinctly different binding modes in solution, two sets of peaks reflecting the different environments of the residues involved should be observed. In this regard, the indole ring of W90 appears to sample two distinct chemical environments in the P:T complex as there are two peaks for the H$_n$ proton of this residue (Fig. 2). Interestingly, if one superimposes P1 and P2, from the X-ray crystal structure, the indole rings of W90 are in the same orientation and appear to be in similar environments. Our results therefore suggest that there is a predominant interface in solution and that the plasticity is likely to exist on a small scale.

The NMR data presented confirm the extensive nature of the Pelle–Tube death domain interface as all twenty seven residues that constitute the core interaction surface on Pelle-DD show significant chemical shift differences between the $^1$H-$^15$N HSQC spectrum of Pelle-DD and the $^1$H-$^15$N HSQC-NMROSY spectrum of the Pelle–Tube DD complex. The NMR data also suggest that the T1:P2 interaction observed in the crystals, may persist in solution. This implies that the death domains of Pelle and Tube are capable of forming a tetramer and this is corroborated by the sedimentation velocity data which reveals that the concentration of the tetrameric complex is very small (0.4%) relative to that of the dimer. The absence of larger oligomeric species in the sedimentation velocity data suggests that the tetramer may be arranged thus: P1T1·P2T2 as seen in the asymmetric unit or, alternatively, P1T1·T2A·P2A as would be obtained if the tetramer arises from stacking of PT heterodimers. While the NMR data does not unambiguously distinguish between these possibilities, the fact that more substantial chemical shifts are observed for residues in the T1:P2 interface may suggest that the more likely arrangement of the tetramer is P1T1·P2T2. Thus, the dominant species formed by the interaction of Pelle-DD and Tube-DD is a dimer and whether the very small amount of the tetrameric species plays a role in signaling is unknown; for example, a mutant of Pelle bearing a mutation in the T1:P2 interface (D50K) [20] failed to express protein. It is conceivable that the residues in Pelle that are part of the T1:P2 interface are sites of interaction for other members of the signaling complex. A recent report [10] argues against that partner being dMyD88 as this component is thought to bind predominantly to Tube. A more likely scenario however, given that dMyD88 appears to bind predominantly to Tube and not Pelle [10,11] is that in the functioning complex, P2 is occupied by dMyD88. Thus, the residues in Tube-DD that contribute to the T1:P2 interface are likely to be the scaffold that recruits dMyD88.

In summary, the structure of the isolated Pelle DD is similar to that of the Pelle DD in complex with the DD of Tube. While the death domains of Pelle and Tube may form a tetramer, consistent with the crystallographic observation [20] the concentration of this species is extremely low. Consequently, inferences regarding the “plasticity” of the Pelle-Tube DD complex in vivo should be reevaluated.

Acknowledgement: We thank Dr. Helen Mott for help with setting up the NMR calculations using Aria 1.2 and Dr. Wayne Boucher for help with the Azara suite of programs. This work was supported by a grant from the UK Biotechnology and Biological Sciences Research Council to NJG.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febselet.2005.06.009.

References


