The intracellular domain of the low affinity p75 nerve growth factor receptor is a death effector domain

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Abstract. The death domain superfamily, comprising the death domain, death effector domain, caspase recruitment domain and pyrin domain subfamilies, is one of the largest classes of protein interaction modules, and plays a particularly critical function in the assembly and activation of apoptotic and inflammatory complexes. Members of the death domain superfamily share a common structural feature, the 6-helical bundle fold. However, individual subfamilies exhibit distinct structural and sequence characteristics. The most distinct feature identified in structural studies is that only the death effector domain contains a charge triad, which is formed by the E/D-RxDL motif. However, using sequence alignment and structural comparison, in the present study we found that the p75-NGFR death domain also contains a charge triad. We therefore suggest that the p75-NGFR death domain should be classified as belonging to the death effector domain.

Introduction

The low-affinity nerve growth factor receptor p75-NGFR is a well known receptor for neurotrophins. Neurotrophins are growth factors that stimulate neuronal cells to survive and differentiate, and are critical for the development and maintenance of the vertebrate nervous system (1-4). p75-NGFR is also a member of a representative subgroup of the tumor necrosis factor receptor superfamily, and can lead to apoptosis and modulate cell survival via a conserved cytoplasmic protein interaction module known as the death domain (5,6). This domain, along with the death effector domain (DED), the caspase recruitment domain (CARD) and the pyrin domain (PYD), forms a death domain superfamily, one of the largest protein superfamilies (7-10). Members of its individual subfamilies mediate protein-protein interactions and regulate the assembly and activation of proteins involved in apoptosis and inflammation (10,11). Almost all oligomeric signaling complexes in apoptosis and inflammation contain domains of the death domain superfamily. Members of the death domain superfamily exhibit a similar molecular structure, a 6-helical bundle fold with Greek key topology and an internal pseudo 2-fold symmetry. However, variations exist in terms of the direction and length of the helices and surface properties. These differences are known to affect specificity; typically, only members of the same subfamily may interact.

Genome analysis has identified 32 death domains, 7 DEDs, 28 CARDs and 19 PYDs in the human genome (7). Death domain-containing proteins such as FADD (12), RIP (13) and TRADD (14) have been identified by the yeast two-hybrid system, while p75-NGFR (15), the ankyrins (15,16), Pelle (17), Tube (17) and Myd88 (17) have been identified by sequence alignment techniques. Studies have shown that the p75-NGFR death domain differs from other death domains. First, its structure is significantly different compared to that of other death domains, such as the Fas death domain (18). Second, unlike other death domains, which have the tendency to aggregate by self-association, the p75-NGFR death domain does not self-associate in solution (17). Finally, the p75-NGFR interaction protein does not appear to contain a death domain (19).

Although the topology of DEDs is similar to the conserved 6-helical bundle fold of the death domain superfamily, recent studies indicate a distinct conserved surface feature on DEDs, distinguishing them from other members of the death domain superfamily. This feature is a conserved hydrogen-bonded charge triad revealed by the high resolution structure of MC159 (10,20). The charge triad is highly conserved in most single and tandem DEDs and is not present in other members of the death domain superfamily, suggesting that it is a characteristic feature of DEDs alone.

However, using sequence alignment, we found that the p75-NGFR death domain also contains a charge triad, and that this charge triad is located at the same position as that of the DEDs. We therefore suggest that the p75-NGFR death domain should be classified as a DED.

Materials and methods

Comparison of protein structure. The DALI protein structure comparison databases (http://ekhidna.biocenter.helsinki.fi/dali_server), for the comparison of protein structures in 3D, were utilized for the comparative analysis of the structure of the p75-NGFR death domain. The solution structure of the p75-NGFR death domain (PDB:1NGR) was submitted (21).

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A.

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p75 DD ------GDGGLYSSLPPAKREEVEKLLNGSAGDTWRHLAGELGYQPEHID--SFTHEACPVRAL 56
FADD DED ------MDP-----FLVLLHSVSSSLSSSEELTEL-KFLC-LGRVGKRKLERVQSGLDLFSM 48
Casp-8 DED ----MD------FSRNLYDIGEQLDSEDLASL-KFLS-LDYIPQRKQEPIKDALMLFQR 47
MC159-DED1 ----MSDSKEVPSLPFLRHLLEELDSHEDSLL-LFLC-HDAAPG-----CTTVTQALCS 48
cFLIP-DED1 ----MS-------AEVIHQVEEALDTDEKEML-LFLC-RDVAIDVVP---PNVRDLLDI 43
cFLIP-DED2 -PHLVSD-----YRVLMAEIGEDLDKSDVSSL-IFLM-KDYMGRGKISKEKSFLDLVVE 51
MC159-DED2 -TSFLTR-----YRKLMVCVGEELDSSELRALRLFACNLNPSLSTALSESSRFVELVLA 53

p75 DD      LASWATQDSATLDALLAALRRIQRADLVESLCSESTATSPV------ 97
FADD DED    LLEQNDLEPGHTELLRELLASLRRHDLLR-----RVDDFE------- 83
Casp-8 DED  LQEKRWLEESNLSFLKELLFRINRLDLLITYLNTRKEEMERELQTPG 94
MC159-DED1  LSQQRKLT---LAALVEMLYVLQRMDLLKSRFGLSKEGAEQLLG---- 89
cFLIP-DED1  LRERGKLS---VGDLAELLYRVRRFDLLKRILKMDRKAVETHLLRN- 86
cFLIP-DED2  LEKLNLVAPDQLDLLEKCLKNIHRIDLKTKIQKYKQSVQGAGTSYR- 97
MC159-DED2  LENVGLVSPSSVSVLADMLRTLRRLDLCQQLVEYEQQEQARYRYCY- 99
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B.

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p75 DD ----------GDGGLYSSLPPAKREE------VEKLLNGSAGDTWRH-LAGELGYQPEHID 44
FADD DD -------AP--GFEDLCAAFN----------------VICDNVGKDWRR-LARQLKVSDTKID 37
PIDD DD --RGSEGPRRGAGLSLAPLNLGDAETGFLTQSNLLSVAGRLGLDWPA-VALHLGVSYREVQ 58
RAIDD DD MTDLPAGDRLTGIPSHILNSSPSDR------QINQLAQRLGPEWEP-MVLSLGLSQTDIY 53
Fas DD ----------NLSDVDLSKYIT----------------TIAGVMTLSQVKGFVRKNGVNEAKID 38

p75 DD ------SFTHEACP-VRALLASWAT---QDSATLDALLAALRRIQR---ADLVESLCSESTA 93
FADD DD  SIEDRYPRNLTERVRESLRIWKNTE-KENATVAHLVGALRSCQMNLVADLVQEVQQARDL 96
PIDD DD  RIRHEFRDDLDEQIRHMLFSWAERQAGQPGAVGLLVQALEQSDR---QDVA--------- 106
RAIDD DD RCKANHPHNVQSQVVEAFIRWRQR-FGKQATFQSLHNGLRAVEVD--PSLLLHM------ 104
Fas DD   EIKNDNVQDTAEQKVQLLRNWHQLH-GKKEAYDTLIKDLKKANLCTLAEKIQTIILKDIT 97

p75DD   TSPV---------- 97
FADDDD  QNRSG---------- 101
PIDDDD  ----------
RAIDDDD ----------
FasDD   SDSENSNFRNEIQSLV 113
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Figure 1. Sequence alignment of various death effector domains (DEDs) (A) and death domains (DDs) (B) with the p75-NGFR death domain. Alignments were performed using ClustalW. The conserved E/D-RxDL motif is outlined.

Table I. Structural similarity calculation using DALI.

	PDB ID	Z-score	RMSD	LALI	LSEQ
p75-NGFR DD	1NGR	21.8	0.0	85	85
IRAK4DD	1WH4-A	10.6	1.7	83	127
Pelle DD	1D2Z-A	10.6	2.1	84	102
TNFR1 DD	1ICH	8.5	2.2	76	87
Apaf1 CARD	3YGS	8.3	2.8	77	92
Iceberg CARD	1DGN	7.7	3.0	78	89
FADD DED	1A1Z	7.4	2.7	76	83
Fas DD	1DDF	6.0	2.6	78	127
FADD DD	1FAD	5.8	3.1	79	95

Z-score, strength of structural similarity in standard deviations (only matches above a threshold of Z = 5 are reported); RMSD, positional root mean square deviation of superimposed Cα atoms in angstroms; LALI, total number of equivalanced residues; LSEQ, length of the entire chain of the equivalences structure.

Sequence alignment. The amino acid sequences of each death domain were analyzed using ClustalW (http://www.ebi.ac.uk/Tools/clustalw2/index.html) (22).

Results and Discussion

DALI reveals that p75-NGFR shares as many similarities with the DED as it does with the death domain. Due to its importance in apoptosis and inflammation signaling, the death domain superfamily has been extensively investigated by means of biochemical and structural studies. Notably, these

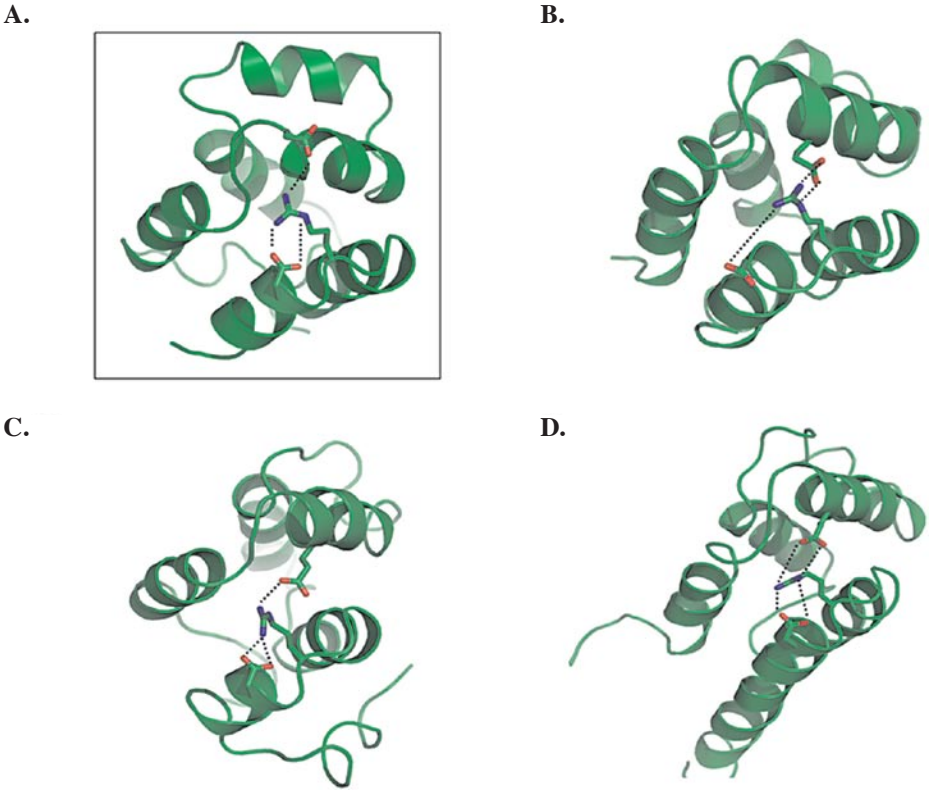

Figure 2. Conserved E/D-RxDL motif on the structure and massive H-bond. (A) p75-NGFR DD. (B) FADD DED, (C) MC159 DED1, (D) MC159 DED2.

studies have revealed that almost all known protein-protein interactions in the superfamily are self-association or homotypic interactions with other members of the same subfamily, though the subfamilies of the death domain share a unifying feature in the form of a 6-helical bundle structural fold. This feature was first revealed by NMR spectroscopy in the Fas death domain (23), FADD DED (24), RAIDD CARD (25) and NALP1 PYD (26), classified by sequence alignment.

In the course of structural studies on the death domain and its complex, we found that the characteristics of the p75-NGFR death domain differ from those of other death domains. Using the DALI databases, 31 candidates were identified possessing a z-score above 2.0 (z-score >3.0, n=17; z-score >5, n=6) (Table I). Based on structural comparison, we realized that the structure of the p75-NGFR death domain most resembles that of the IRAK4 and Pelle death domains. Notably, however, subfamilies such as Afap1 CARD and FADD DED ranked in the top 4 or 6 with a z-score of 8.3 and 7.4, respectively (Table I). This indicates that the structure of the p75-NGFR death domain is as similar to the DED and CARD as it is to the death domain.

p75-NGFR contains the charge triad sequence observed in DEDs. Although all members of the death domain superfamily have a conserved structural fold, individual subfamilies exhibit distinct structural and sequence characteristics not shared by other subfamilies. Though the topology of DEDs is similar to the conserved 6-helical bundle fold of the death domain superfamily, recent studies indicate that there is a distinct conserved surface feature on DEDs, distinguishing them from other members of the death domain superfamily. This feature is a conserved hydrogen-bonded charge triad revealed by the high resolution structure of MC159 (Fig. 1) (10,20). The charge triad is formed by the E/D-RxDL motif and involves the Arg and Asp residues in the RxDL motif in helix H6 and the preceding loop, and an acidic residue in helix H2 (Figs. 1 and 2) (20). Extensive hydrogen bonding interactions are observed among the charged side chains with the Arg residue situated in between the two acidic residues (Fig. 2). These hydrogen bonds likely help to maintain a precise organization of the side chains, which may be functionally important. It is also possible that they play a local structural role in maintaining the conformation of this region of the DEDs. The charge triad is highly conserved in most single and tandem DEDs. Based on sequence alignment, this motif is not present in other members of the death domain superfamily, suggesting that it is a characteristic feature of DEDs alone (Fig. 1). However, using sequence alignment, we found that the p75-NGFR death domain contains a charge triad located at the same position as that of other DEDs (Figs. 1 and 2). Additionally, DED2 was found to be quite similar to the death domain of p75-NGFR (18), suggesting that p75-NGFR may be more DED-like than death domain-like. As a result, we suggest that the p75-NGFR death domain should be classified as a DED.

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