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A region of the 75 kDa neurotrophin receptor homologous to the death domains of TNFR-I and Fas

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Abstract Members of the NTR/TNFR family mediate apoptosis in many tissues, yet sequence homology has not been detected in their intracellular domains except for a 'death domain' in TNFR-I and Fas. Here, a region of the 75 kDa neurotrophin receptor (NTR) has been aligned with this apoptosis-inducing motif. Peptides at the carboxyl terminus of each domain potentially form amphiphilic helices, one of which (in NTR) resembles mastoparan, a G-protein activating peptide. Molecular models of three death-region peptides suggest that observed sequence similarities reflect a common structure, perhaps capable of undergoing an induced coil to helix transition.

Key words: Neurotrophin receptor; NGF receptor; Molecular modeling; Apoptosis; Alpha-helix; Mastoparan

1. Introduction

Receptors of the NTR/TNFR family function during development and in adult tissues by regulating apoptotic cell death [1,2]. All possess extracellular domains composed of repeating units that fold into a characteristic structure [3,4]. However, except for an 'apoptosis motif' in TNFR-I (55 kDa) and Fas/Apo-I, neither sequence nor structural similarity has been detected in the intracellular domains of these molecules [5–7].

Various signaling mechanisms have been proposed for members of the family. Although direct substrate phosphorylation has been ruled out by the absence of catalytic domains, interactions with cytoplasmic signaling molecules apparently link activated receptors to signaling cascades [8]. The established function of the neurotrophin receptor (NTR) is to modulate the affinity and activity of tyrosine kinases that promote neuronal survival [9]. It was recently shown, however, to induce apoptotic cell death much like that stimulated by TNFR-I and Fas [10,11]. Unlike TNFR-I and Fas, cell death induced by NTR is reversed rather than caused by ligand binding. This function is consistent with observations that its contribution to the survival response can best be measured at high ratios of NTR to co-expressed trk receptors, which also bind neurotrophins [9]. These reports prompted a re-examination of the intracellular domain of NTR for possible similarities to TNFR-I and Fas, which may signal through a related mechanism, i.e. sphingomyelinase activation [12–14].

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Abbreviations: NTR, 75 kDa neurotrophin receptor; TNFR, tumor necrosis factor receptor; GDP, guanosine diphosphate; G-protein, heterotrimeric guanine nucleotide-binding regulatory protein.

Here, primary sequence alignment was used to locate a death domain in the intracellular portion of the neurotrophin receptor. To explore structural implications of this homology, putative structures were modeled using computer-aided molecular design and visualization tools. Peptides in the death-mediating region appear to be structurally similar to peptides that undergo a transition from coil to helix, and which activate heterotrimeric G-proteins.

2. Experimental

2.1. Alignment and analysis of secondary structure

Amino acid sequences having the most complete annotation were obtained from databases as follows: SwissProtein; human fas antigen – P25445, chicken NTR – P18519, and rat TNFR-I – P22934. PIR; mouse fas antigen – A46484 and human TNFR-I – A34899. GenBank; rat fas antigen – D26112, human NTR – M14764, rat NTR – X05137 and mouse TNFR-I – M59378. Sequences and measurements of biological activity were obtained for insect venom peptides as follows: MP, MP-X, MP-A′, MP-T, Mas7, Mas9 and Mas19 [15]; MP14 and MP15 [16]; melittin [17]. Matrix calculations were performed by DNA Strider version 1.2 (Christian Marck, CEN-Saclay, France).

Initial alignments were prepared using the algorithm of Altschul [18]. Multiple alignments were optimized by hand using SeqVu version 1.0.1 (James Gardner, Garvan Institute of Medical Research, Sydney, Australia), based on sidechain chemistry and rational placement of gaps. The methods of Garnier et al. and Chou and Fasman, as modified by Nishikawa [19], were to evaluate the probability that a sequence could adopt a helical structure. Helical wheels were drawn as described by Schiffer and Edmundson [20].

2.2. Model building

Hypothetical structures were assembled SYBYL version 6.1 (Tripos Associates, St. Louis, MO) on Silicon Graphics workstations. Free amino and carboxylate groups were left at the ends. Van der Waals conflicts resulting from sidechain substitutions were resolved by rotamer library and molecular mechanics functions in SYBYL. Energy minimization was carried out using the AMBER all-atom force field, a distance-dependent dielectric constant of 4.0 R and a non-bonded atom cutoff of 10 Å. Computer graphics were prepared with MIDASPlus [21] and displayed using the conic delegate [22].

3. Results

3.1. Identification of death-domain homology in NTR

Because widely-used alignment algorithms had not detected similarity in the intracellular domains of the NTR/TNFR family, representative sequences were evaluated with a matrix procedure. This method looks for matches (amino acid identities) within a sliding window. The test is relatively insensitive to gaps, and can be adjusted to detect low levels of amino acid identity. Comparisons of NTR with TNFR-I and Fas intracellular domains detected matches in the previously-identified death domain at approximately 27% sequence identity.

Using short, matched sequences from the matrix and homologs from additional species, residues 330 to 390 of NTR were

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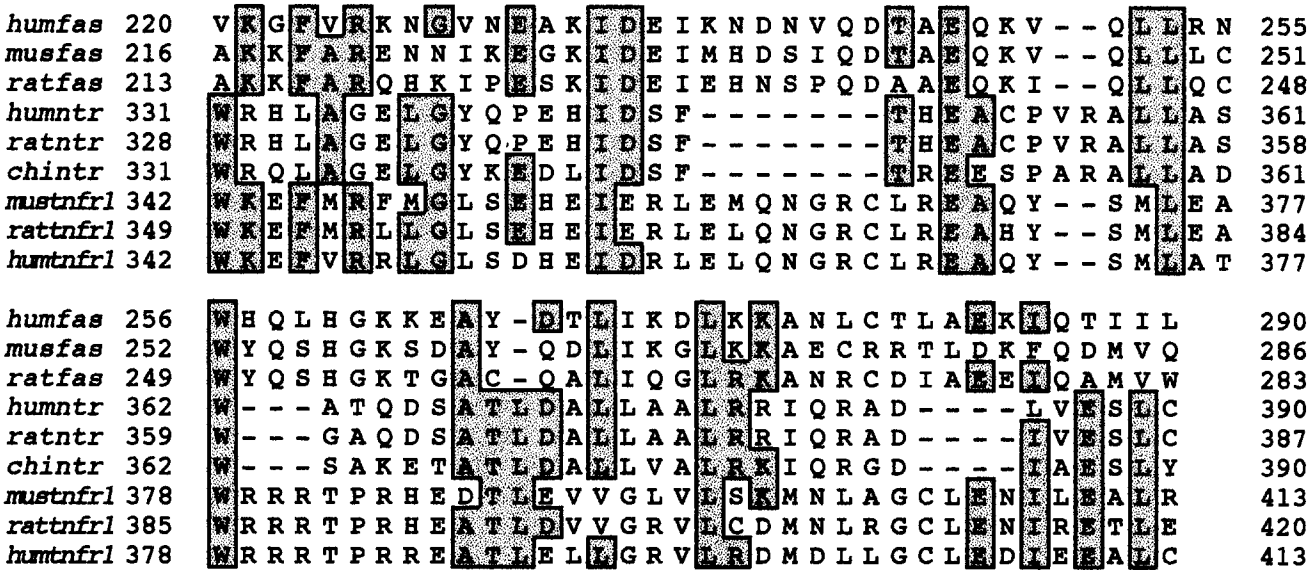

Fig. 1. Multiple alignment of the death domain of NTR with those of TNFR-I and Fas. Sequence identities at the 50% level are boxed and shaded. Residues are numbered from the amino terminus of each receptor.

aligned with the death regions of TNFR-I and Fas (Fig. 1). The alignment begins near the amino terminus of the apoptosis domain identified by Itoh et al. [5], and extends through the region probed by alanine-scanning mutagenesis of TNFR-I [6]. The arrangement of the domain suggests that it may have arisen as a duplication of approximately 38 codons [23]. While NTR and TNFR-I share higher sequence identity (32.8%), TNFR-I and Fas (25.4% identity) are more closely related with respect to the placement of gaps and insertions. Several structural motifs appear to be anchored by highly-conserved residues. The functions of Glu-369, Trp-378 and Ile-408 were tested by mutation to Ala in TNFR-I [6]. Substitution of these residues, conserved in the homology with NTR, blocked transmission of apoptotic signals.

3.2. Potential for formation of helical structure

Unexpected patterns of sequence gaps and amino acid identities were investigated first by evaluating secondary-structure propensities in the aligned domain. Two algorithms [19] independently predicted alpha-helical conformations for most residues of the nine sequences aligned in Fig. 1. For the human homologs of Fas, TNFR-I and NTR, regions are plotted in Fig. 2A in which both methods predict alpha helices. Fas and TNFR-I show helical potential in the amino-terminal half of the alignment. These segments may engage in self-association as a consequence of activation by ligand, since deletions that eliminate dimerization and block signaling have been mapped to this region [24,25].

Common to all three receptors is a potential helix in the carboxyl-terminal half of the aligned region (Fig. 2A). This sequence in NTR was previously identified as a homolog of the wasp venom peptide, mastoparan [26]. To determine whether the sequences of TNFR-I and Fas might also encode mastoparan-like helices, peptides from the shared part of this segment were analyzed by display in helical-wheel plots (Fig. 2B). The arrangement of sidechains strongly suggests the possibility of forming amphiphilic structures having one hydrophobic surface and one electrostatically-charged surface. Potential of this region to form an amphiphilic helix has been recognized for NTR and TNFR-I [26,27], but not for Fas.

3.3. Sequences of three receptors can be modeled as stable, low-energy helices

Alignment (Fig. 1) and secondary-structure prediction (Fig. 2) pointed to potential structural similarities among the three receptors. To visualize these structures, hypothetical models were constructed using molecular design software. Residues 245–284 of human Fas (40 monomers) were modeled with alpha-helical phi and psi angles, then energy minimized using molecular mechanics. The AMBER force field was used to estimate the total energy of the model after minimization, calculated to be −188.7 kcal/mol. Models representing human NTR residues 349–384 (36 monomers) and human TNFR-I residues 367–407 (41 monomers) were similarly prepared. These minimized respectively to −172.9 kcal/mol and −223.5 kcal/mol.

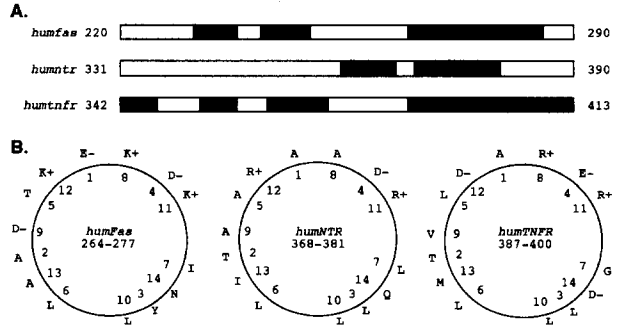

Fig. 2. Predictions of secondary structure in the region of homology. (A) Aligned sequences are represented as open bars. Consensus segments of alpha helix are plotted in black. (B) Helical wheel representations of the carboxyl-terminal peptides. These show the distribution of amino acid sidechains as seen looking down the axis of the helix from the amino-terminal end.

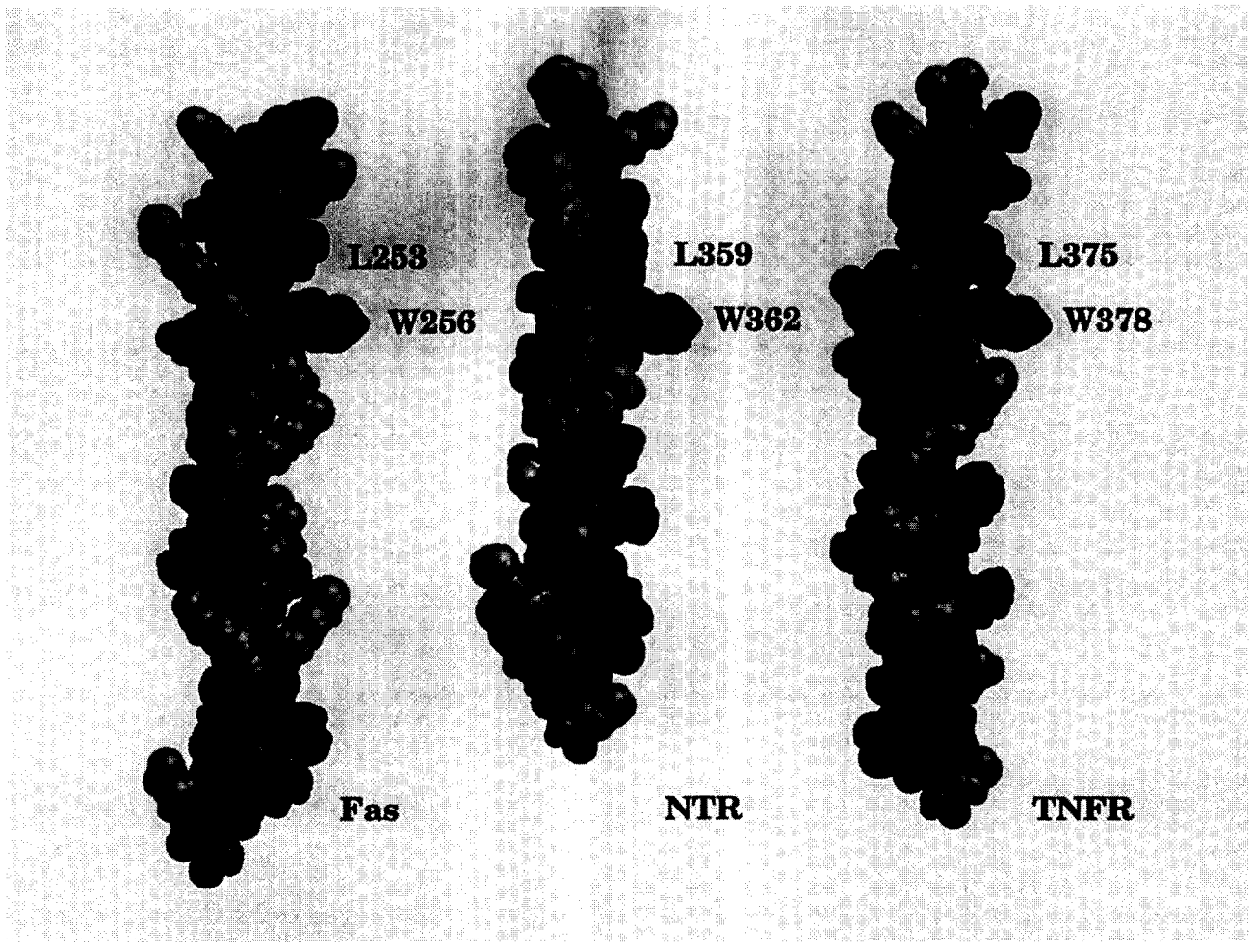

Fig. 3. Three-dimensional models of homologous regions of human Fas, NTR and TNFR-I rotated to show positions of conserved Leu and Trp residues. Atoms are displayed in space-filling representation with sidechains color coded as follows: polar, dark gray; charged, light gray; hydrophobic, black.

Low energies achieved for the models indicate stabilization by extensive hydrogen bonding and excellent van der Waals interactions. The structures are completely theoretical, since neither solvent molecules nor dissolved ions were considered in the calculations. Views of these models are shown in Figs. 3 and 4.

The three-dimensional models start at the end of the first gap in NTR (see Fig. 1), which corresponds to the C-terminal boundary (TNFR-I Leu-367) of the postulated dimerization motif [25]. Fig. 3 shows the prominent feature created near the amino terminus of the helix by sidechains of conserved Leu and Trp residues. The opposite sides of these structures appear different from each other, consistent with experimental results showing that mutation of Trp-378 in human TNFR-I abrogated induction of cell death, but mutation of Arg-379 had no detectable effect [6].

When rotated to display a predicted helical region near its C-terminus, each receptor model presents a striking hydrophobic face (Fig. 4). The aromatic ring of Tyr-266 in Fas appears to substitute for conserved leucines in NTR and TNFR-I, orienting to contribute part of a non-polar surface. A basic sidechain following the last conserved Leu seems to occupy a similar position in each structure (not labeled, coming out of the plane of the figure. See also 2B, where a positively-charged sidechain is indicated at position 11 of each helical wheel). The three receptor models demonstrate structural similarities, despite the low level of sequence identity.

3.4. Potential amphiphilic helices resemble a modeled structure of mastoparan

Naturally-occurring mastoparans and synthetic derivatives have been intensively studied as potent activators of heterotrimeric G proteins [15]. These peptides reportedly assemble into biologically-active helices upon interaction with phospholipid membranes or in the presence of chaotropic salts [17,28]. To investigate resemblances between modeled receptors and mastoparan, a three-dimensional representation was constructed for the fourteen-residue peptide. The mastoparan model relaxed to a total energy of −45.5 kcal/mol, and closely matched the membrane-bound structure of mastoparan-X obtained by 2D-NMR [28]. Like MP-X, modeled mastoparan (Fig. 5) forms an amphiphilic helix in which five aliphatic sidechains contribute to a non-polar surface [28]. This surface has several features in common with the non-polar surfaces of the receptor models (Fig. 4). The basic surface of mastoparan

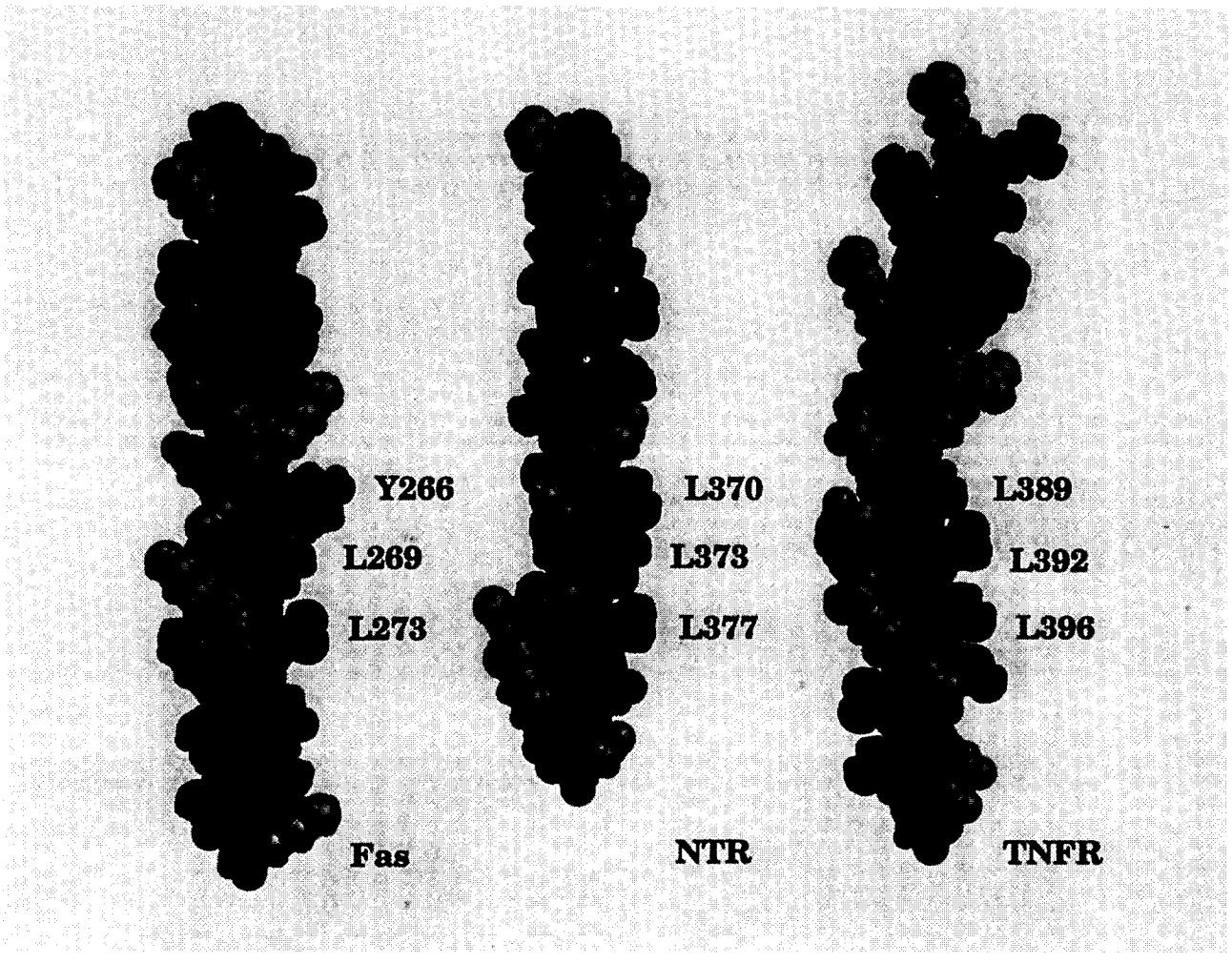

Fig. 4. Models rotated to display amphiphilic helices in the lower half of each structure. Conserved hydrophobic residues are labeled. Color coding is identical to Fig. 3.

likewise looks like the polar surfaces of the modeled receptors. For example, Lys-11 is oriented similarly to the basic sidechain at the bottom of each receptor's hydrophobic face. Modeling thus shows that the death-domain peptides could mimic the mastoparan structure were they to adopt a helical conformation.

Mastoparans, biologically-active analogs and bee-venom peptide (melittin) undergo a conformational transition from coil to helix under specific conditions [17,28]. Amino acid substitutions in these peptides can produce molecules that neither stimulate G-proteins nor form helices [15,17,28]. To evaluate chemical similarities at critical positions, death-domain sequences containing putative amphiphilic helices were aligned with biologically-active mastoparans and melittin (Fig. 6). The three apoptosis-motif peptides show strict conservation of aliphatic residues at mastoparan positions 1, 6, 10 and 13. All other positions display substitutions comparable to at least one active molecule, except for the fourteenth residue, which is an amide or acid in the receptor peptides and aliphatic in the insect peptides. Chemical similarity between active mastoparans and segments of TNFR-I, Fas and NTR suggests these death-domain peptides may be able to undergo an induced coil to helix transition.

4. Discussion

Standard methods for detecting homology have consistently failed to find sequence similarities in intracellular domains of the NTR/TNFR family. This has led to the suggestion that these receptors may be chimeras [7]. An alternative possibility is that each member has undergone extensive evolution from a common ancestor in the process of adapting its structure for specific, non-catalytic functions. Shown here are significant evolutionary and structural relationships in the intracellular domains of the neurotrophin receptor, Fas antigen and the 55 kDa TNF receptor. The death domain in NTR appears to be a robust homolog, since the percent identity with TNFR-I is higher than between TNFR-I and Fas, and because several residues conserved in the alignment are known to be critical for function [6].

Death-domain signaling has been difficult to unravel, owing to its novel structure and complex regulation. While protein phosphorylation may be important, experiments have not clarified whether kinases and phosphatases are primarily used for signaling or for regulation. In identifying a mastoparan-like peptide in the C-terminal half of each apoptosis domain, the present study expands the list of potential mechanisms. If con-

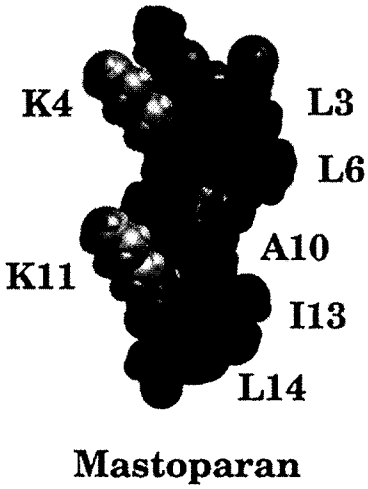

Fig. 5. Hypothetical model of mastoparan. This structure has a free carboxylate at the C-terminus, differing slightly from mastoparans and melittin, which are amidated. Color coding of sidechains is the same as in Fig. 3.

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MP          1  ---INLKALAALAKKIL-  14
MP-X        1  ---INWKGLAAMAKKLL-  14
MP-A        1  ---IKWKAILDAVKKVL-  14
MP-T        1  ---INLKAIAAFAKKLL-  14
humfas    262  KKEA-YDTLIKDLKKANL  278
humntr    365  QDSATLDALLAALRRIQR  382
humtnr1   384  RREATLELLGRVLRDMDL  401
MP14        1  ---INLGALAALAKKIL-  14
MP15        1  ---INLKALAALAKGIL-  14
Mas7        1  ---INLKALAALAKALL-  14
Mas9        1  ---INLKALAALAAKLL-  14
Mas19       1  ---INLAALAALAKKLL-  14
melittin    1  GIGAVLKVLTTGLPALIS  18
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Fig. 6. Multiple alignment of insect venom peptides and synthetic analogs with potential amphiphilic helix residues of human Fas, TNFR-I and NTR. Residues chemically homologous to the mastoparan sequence are boxed (e.g. aliphatic, aromatic, amidated, hydroxylated, basic, acidic).

formational change from coil to helix were induced by receptor activation, such a peptide would act as a switch. A peptide representing NTR residues 367–379 has been shown by circular dichroism spectroscopy to assume a helical conformation in non-polar solvent [27]. The potentially amphiphilic peptides of TNFR-I and Fas should also be tested for ability to undergo the coil to helix transition. NTR, when activated, has been shown to stimulate Gi proteins in isolated membranes [29]. It may be worth exploring the pertussis-toxin sensitivity of TNFR-I and Fas signaling.

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