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Putative cytoplasmic amphiphilic domains in the nerve growth factor/tumour necrosis factor receptor superfamily

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Abstract

Potential α-helical regions in cytoplasmic domains of the NGF/TNF receptor superfamily were searched to identify amphiphilic sequences favouring association with membrane surfaces, analogous to the predicted secondary structure of mastoparan (MP). Similar to MP, NGFR (rat, chick, human), human TNFR-1, and human 4-1BB have domains with putative surface membrane associating sequences. The circular dichroism spectra of mastoparan and a peptide homologous to the putative amphiphilic domain of NGFR were identical in an aqueous milieu, and both adopted an α-helical conformation in trifluoroethanol.

Keywords: Helix; Membrane association; Receptor domain; Neurotrophin

1. Introduction

The nerve growth factor (NGF) [1] or tumour necrosis factor (TNF) [2] receptor superfamily has been so-designated because of homologous regions in extracellular domains containing cysteine repeat domains (CRD's) and single transmembrane spanning sequences [1,2]. In addition, some family members share other similarities; most of the receptor intracellular domains do not contain descriptive motifs for signal transduction, but some have PEST sequences and truncated forms suggesting that receptor fragments are shed. To date, this superfamily includes $gp75^{NGFR}$ (NGFR) [3], tumour necrosis factor receptor-1 (TNFR-1) [4,5], TNFR-2 [6], Fas [7], CD-30 [8], 4-1BB [9], CD-40 [10], and OX-40 [11]. The low-affinity NGF receptor $gp75^{NGFR}$/NGFR is the common receptor for the neurotrophins that also bind to the trk family of receptors.

The purposes of the present study were first, to predict regions of α-helical secondary structure in the intracellular (cytoplasmic) domains of the NGF/TNF receptor superfamily; secondly, to identify within these regions amphiphilic segments designated by the hydrophobic moment [12,13] that have been implicated in membrane association and intracellular signalling [14,15]; thirdly, to optimize geometrically and conformationally the amphiphilic segments using empirical force field energy refinements; and fourthly, to determine the distance and angular relationships between charged groups of the hydrophilic faces of the amphiphilic α-helices, and to compare these relationships with those of MP, a homologous cationic amphiphilic tetradecapeptide that stimulates nucleotide exchange on G-proteins by mimicking the actions of liganded receptor [14]. Finally, using a synthetic peptide homologue of the amphiphilic domain of NGFR, the circular dichroism spectra of this peptide and MP were compared in aqueous and non-polar environments.

2. Materials and methods

2.1. Computational studies

The PRISM method [16–18], a secondary structure prediction algorithm, was used to predict α-helical regions in the deduced intracellular sequences of the NGF/TNF

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receptor superfamily. Maximum length of α-helical structure was set at 35 residues [17], and the most probable sequence was used. All identified α-helices were systematically searched for amphiphilicity appropriate for a membrane associating sequence using the method of Eisenberg et al. [12,13] which calculates the mean hydrophobic moment (μ_H) for eleven amino acid segments of sequences.

To define the geometric properties of the hydrophilic faces of the amphiphilic helices, conserved amphiphilic sequences were conformationally optimized using the AMBER force field equation implemented in the BIOGRAF molecular modelling system [19]. Empirical force field calculations were performed with a dielectric constant of 4. This value was employed to assist in simulating a membrane environment in these computer-assisted conformational predictions. Resulting optimized structures were compared to the cationic amphiphilic tetradecapeptide mastoparan (MP). Calculations were performed on an IBM RS/6000 550 RISC graphics workstation using an AIX operating system.

To ensure viability as membrane-associating sequences, the steric properties of the lipophilic faces of the amphiphilic helices were defined by partition coefficients [20] and Van der Waals volume calculations [21] carried out on the lipophilic side chains of amphiphilic sequences.

2.2. Peptide synthesis

A peptide homologous to rat NGFR 367-379 (designated R3) was custom synthesized on solid phase using Fast-moc chemistry by the Core Facility for Protein/DNA Chemistry (Department of Biochemistry, Queen's Univer-

Table 1
α-Helical sections of MP and intracellular regions of members of the NGFR superfamily

MP residues 1 - 14

*i***nlkalaalakki***l*

NGFR residues 245 - 396

*krwnsckqnkqgansrpvnqtpppegeklhsdsgisvd***sqslhdqqthtqtas***gqalkgdgnlysslpltkreev ekllngdtwrhlagelgyq***pehidsftheacpvrallasw***gaqdsatldallaalrriqradiveslc sest***atspv*

TNFR-I residues 193 - 415

*myryqrwksklysivcgkstpekegelegtttkplapnpsfsptpgftptlgfspvpsstktssstytpgdcpnfaap rrevappyqgadpilatalasdpipnplqkwedsahkpqsldtddpatlyavvenvp***plrwkefvrrl***glsdhei drlelqn***grc***lreaqysmlatwrrrt***prreatldllgrvlrdmdllgcledieealc***gpaalppaps llr*

TNFR-II residues 286 - 461

*q***vkkkplclqreak***vphlpadkargtqgpeqqhllitapsssssslessasaldrrapt***rnqpqapgveasg agearastgssdsspgghgtqvnvtcivnvcsssdhssqcssqasstmgdtdsspsespkdeqvpfs***keecafrsq letpetllgstee***kplplgvpdagmkps*

CD30 residues 408 - 595

*h***rracrkrirqklh***lcypvqtsqpklelvdsrprrsstqlrsgasvtepvaeerglms***qplmetchsvgaay les***lplqdaspaggpssprdlpeprvstehtnnkiekiyimkadtvivgtvkaelpegrglagpae***peleeelea dh***tphypeqetepplgs***csdvmlsveee***gkedplptaasgk*

Fas residues 175 - 319

*k***rkevqktcrkhrkenqgs***hesptlnpetvainlsdvd***lskyittiagv***mt***lsqvkgfvrkn***gvn***eaki deiknd***nvqd***taeqkvqllrnwhqlhgkkeaydtlikdlkkanlc***tlaekiqtiilkditsdsensnfr neiqslv*

CD40 residues 216 - 277

kkvakkptnkaphpkqepqeinfpddlpgsntaapvqetlhgcqpvtqedgkesrisvqerq

4-1BB residues 212 - 256

*k***wirkkfphifkqpfkkttgaaqeedacs***crcpqeeeggggggyel*

OX-40 residues 236 - 271

*rkawrspntpkpcwgnsfrtpiq***eeqtdthftlaki**

Table 2
Surface membrane associating sequences in the NGF receptor superfamily

	α-helical sequence	μH	Hn	surface membrane sequence
MP				
1-14	*in* **lkalaalakkil**	.58	.23	+
NGFR				
rat:				
361-391	*aqdsat* **ldallaalrriqra** *diveslcsest*	.76	-.18	+
human:				
364-394	*tqdsat* **ldallaalrriqra** *dlveslcsest*	.76	-.18	+
chick:				
362-392	*aketat* **ldallvalrkiqrg** *diaeslysest*	.69	-.06	+
TNFR-I				
372-402	*prr* **eatldllgrvlrdmdll** *gcledieealc*	.81	-.04	+
4-IBB				
212-240	*kwir* **kkfphifkqpfk** *kttgaaqeedacs*	.65	-.05	+

Highlighted areas are sequences that gave maximum mean hydrophobic moments of idealized α-helices (μ_H). Calculations were based on 11 amino acid sequences as per Eisenberg et al. [12,13] using the formula:

$$\mu_H = \left(\left[\sum_{n=1}^{N} H_n \sin \delta_n \right]^2 + \left[\sum_{n=1}^{N} H_n \cos \delta_n \right]^2 \right)^{1/2}$$

Boxed sequences are the full length of the membrane-associating helical area. H_n = mean hydrophobicity at μ_H.

sity). Prior to use, the synthetic product was purified by reverse phase high performance liquid chromatography on a Pep-S column (Pharmacia), and the amino acid composition and sequence were verified. Mastoparan was purchased from Sigma.

2.3. Circular dichroism spectroscopy

CD spectra were obtained using a Jasco-J600A spectropolarimeter with a 1 cm path length quartz sample cell. Spectra depicted were the average of 8 scan acquisitions at room temperature (22 ± 1°C). Peptide concentrations were 100 μM, and chain length was normalized by expressing the mean residue molar ellipticity (Θ) in deg cm^2 dmol^{-1}.

3. Results

3.1. Characteristics of amphiphilic domains

Using the PRISM program, α-helical sequences were detected in MP and seven of eight members of the NGF receptor superfamily (Table 1). Analysis of μ_H of α-

Table 3
Homology of amino acids in potential membrane surface associating sequence regions

			X H X H C H H H X H H C C H X X
MP		1 - 14	I N L K A L A A L A K K I L
NGFR	rat	364 - 380	S A T L D A L L A A L R R I Q R A
	human	367 - 383	S A T L D A L L A A L R R I Q R A
	chick	365 - 381	T A T L D A L L V A L R K I Q R G
TNFR-I		375 - 391	E A T L D L L G R V L R D M D L L
4-1BB		216 - 227	K K F P H I F K Q P F K

H = hydrophobic amino acid, C = charged, X = no homology.

Table 4
Van der Waals volume and partition coefficient values for potential membrane surface associating sequences

	Peptide sequence	Van der Waals volume (Å^3) [a]	Partition coefficient [b]
MP	**lkalaalakkil**	250.2	5.50
NGFR			
rat & human	**ldallaalrriqra**	250.2	5.50
chick	**ldallvalrki**	250.2	5.50
TNFR-1	**atldllgrvlrdmdll**	317.7	6.73
4-1BB	**kkfphifkqpfk**	353.2	6.64

Side chains of residues used in calculations are highlighted.
[a] Van der Waals volume fragmental constants are from Motoc and Marshall [21].
[b] Lipophilicity constants are from Tayar et al. [20].

Table 5
Lowest energy conformations for MP 3–14, NGFR 367–379 and TNFR-1 378–390

Peptide	ϕ	ψ	ω	χ_1	χ_2	χ_3	χ_4	χ_5	χ_6
MP									
L		−58.7	−178.4	−166.9	167.9	−70.7			
K	−62.9	−41.4	179.5	44.3	67.1	60.1	65.7		
A	−60.7	−43.6	−178.1						
L	−64.9	−39.1	178.8	56.2	82.0	−157.0			
A	−61.3	−43.0	−179.7						
A	−60.9	−43.4	−178.2						
L	−64.6	−39.4	178.8	56.6	82.4	−156.6			
A	−60.7	−43.6	−177.5						
K	−67.0	−36.7	179.7	55.9	63.3	56.3	65.1		
K	−67.8	−35.8	178.6	55.0	64.6	59.7	70.9		
I	−65.1	−39.0	179.0	49.6	−72.3	81.4			
L	−61.9			56.8	78.5	−160.4			
NGFR									
L		−59.3	−176.8	−166.2	168.2	−70.3			
D	−67.8	−35.9	177.4	89.1	109.5	−68.2			
A	−62.0	−42.2	−178.7						
L	−64.9	−39.1	180.0	56.3	82.7	−156.4			
L	−64.7	−39.3	178.8	58.9	82.4	−156.7			
A	−61.4	−42.8	179.9						
A	−60.3	−44.0	−177.4						
L	−66.1	−37.8	179.7	55.6	82.4	−156.5			
R	−65.5	−38.4	−179.1	52.4	67.3	55.1	75.1	−173.1	6.5
R	−68.8	−34.4	178.3	60.1	71.0	53.9	85.2	−175.4	4.3
I	−66.5	−37.3	−178.7	50.2	−71.8	80.5			
Q	−72.0	−30.6	176.4	53.0	69.0	20.5	−156.4		
R	−66.5			71.0	70.4	49.2	90.0	−172.7	7.6
TNFR-1									
L		−58.7	−176.8	−166.2	167.8	−70.7			
D	−69.1	−34.3	176.9	98.7	77.6	−100.9			
L	−62.1	−42.2	−178.6	57.2	88.8	−150.2			
L	−64.8	−39.3	176.7	57.0	82.8	−156.3			
G	−57.0	−46.6	−176.6						
R	−65.5	−38.2	179.7	62.0	67.6	52.3	87.7	169.9	−10.3
v	−64.6	−39.3	−179.1	63.3	−173.9				
L	−66.8	−36.9	179.5	55.8	82.5	−156.3			
R	−66.7	−36.9	179.0	52.1	70.8	55.8	58.7	−170.4	10.3
D	−64.0	−40.5	−178.7	32.1	82.4	−99.3			
M	−67.6	−36.0	179.4	56.9	72.7	134.8			
D	−68.3	−35.3	177.1	61.7	77.4	−103.4			
L	−61.9			56.2	79.4	−159.5			

helices revealed that the three species of NGFR, TNFR-1, and 4-1BB had amphiphilic conformations similar to MP and suitable for membrane surface association as depicted by boxed sequences in Table 2. Rat and human domains of NGFR showed identical primary sequences and secondary structure characteristics as defined by μ_H. The primary sequence in the amphiphilic domain of chick NGFR 368–381 was highly homologous to that of rat NGFR 367–380 and human NGFR 370–383, and μ_H calculations were compatible with membrane surface association.

Both TNFR-1 375–391 and 4-1BB 216–227 were calculated to have μ_H values compatible with membrane surface association, and TNFR-1 375–391 showed significant primary sequence homology with NGFR 367–380 (Table 3).

Partition coefficients and Van der Waals volume calculations for lipophilic faces of the α-helices of MP and NGFR were identical and less than those of TNFR-1 and 4-1BB. Lipophilicity, as quantified by partition coefficient and volume indices, suggested equal tendencies of MP and NGFR 367–379 to orient towards the membrane on the side of the α-helix (Table 4). Compared to NGFR 367–379, the lipophilic faces of TNFR-1 376–390 and 4-1BB 216–227 had greater tendencies to insert into the membrane and also would be expected to occupy larger areas based on their volumes. These data lend further support to the suggestion that these cytoplasmic domains of the three receptors have the potential to associate with the surface of a membrane.

Because of primary sequence homology in the amphiphilic domains of NGFR and TNFR-1, predictive analyses of the secondary structure relationships between these receptors and their similarity to MP were undertaken. Minimization of amphiphilic regions was carried out using the AMBER force field equation where the amino acid side chains were free to find their most stable conformations. Dihedral angles defining the conformations following minimization are provided for MP, NGFR and TNFR-1 (Table 5), and Fig. 1 depicts the predicted conformations.

3.2. Relationships of hydrophilic residues in amphiphilic domains

Relationships between charged groups in their lowest energy conformations within the amphiphilic structures likely describe the specificity of their interactions with intracellular proteins [14,22]. To address quantitatively the specificity of these putative interactions, predicted distance

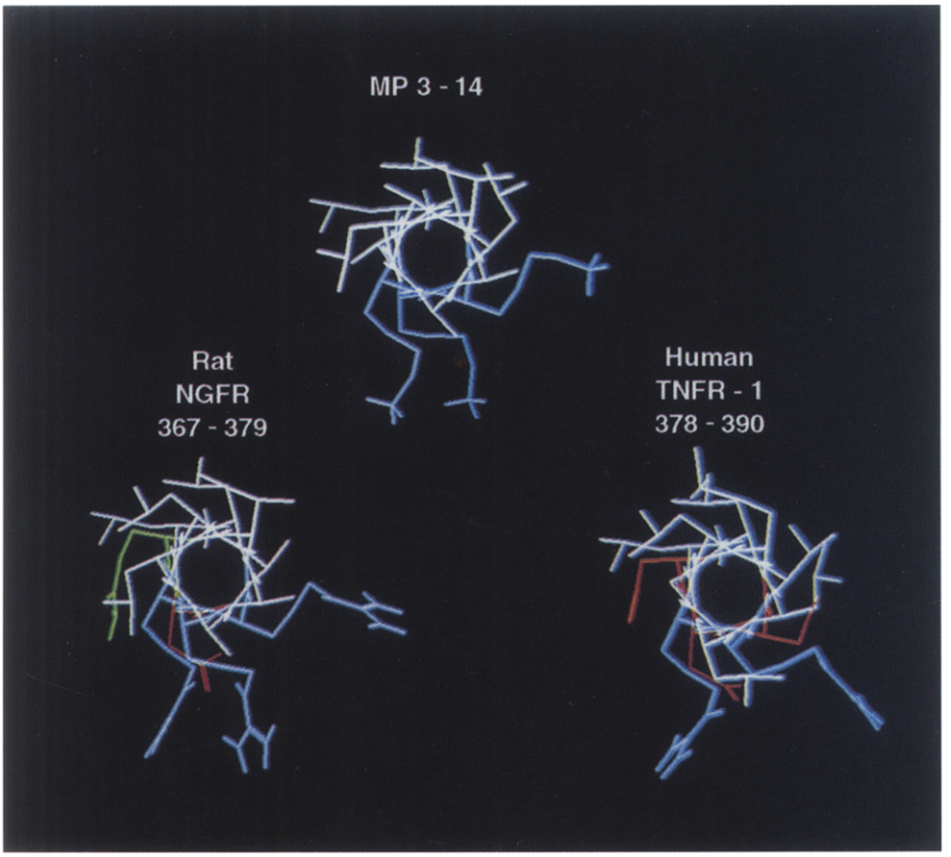

Fig. 1. Amphiphilicity of computer-simulated peptide motifs of MP 3–14, NGFR 367–379, and TNFR–1 378–390 viewed parallel to the long axis of the α-helices. The program BIOGRAF was used to construct identified membrane surface associating regions with minimization carried out using an AMBER force field. The α-helices were constrained, and the amino acid side chains were free to find their most stable conformations. Blue = basic residues; red = acidic; white = hydrophobic; green = ambivalent.

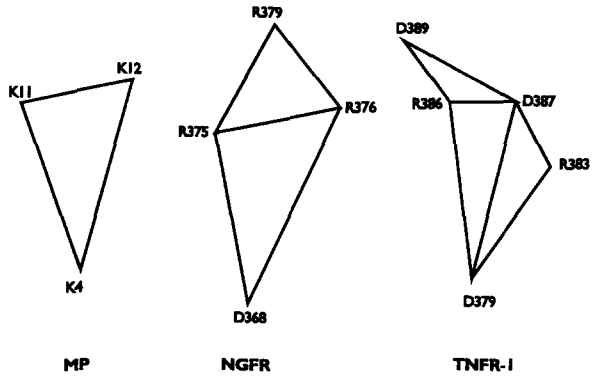

Fig. 2. Display of computed distance and angular relationships of charged groups of MP 3–14, rat NGFR 367–379, and human TNFR-1 378–390 derived from Table VI. Distances are in Å and angular relationships are in degrees. The reference points for charged groups are nitrogens of amino groups of lysine side chains, carbons of guanidino groups of arginines, and carbons of carboxyl groups of aspartic acid side chains.

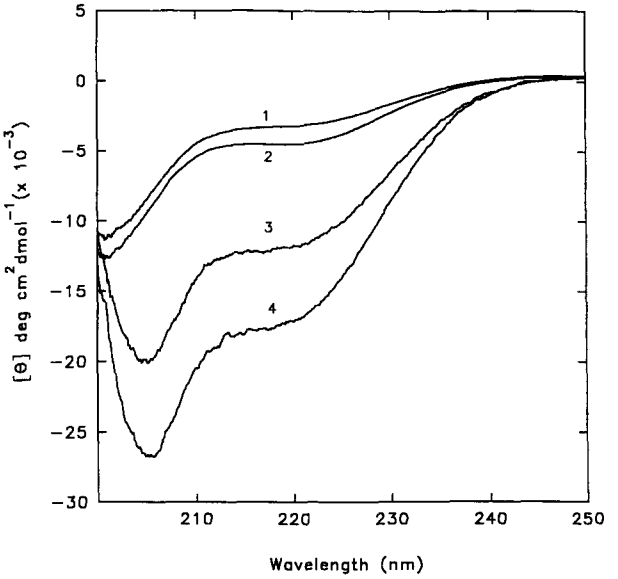

Fig. 3. CD spectra of peptide R3 (1,3) and MP (2,4) in aqueous milieu (1,2), and in trifluoroethanol (3,4). Legend: 1 = R-3 in H_2O, 2 = MP in H_2O, 3 = R-3 in TFE, 4 = MP in TFE.

and angular relationships of charged groups within MP (K4, K11, K12), NGFR (D368, R375, R376, R379), and TNFR-1 (D379, R383, R386, D387, D389) were calculated (Table 6) and are depicted in Fig. 2.

Angles subtended by the three lysines (K) of MP were similar to those subtended by D368, R375, and R376 in NGFR, and to those subtended by D379, R386, and D387 in TNFR-1. These observations might suggest that if MP, NGFR, and TNFR-1 share functional properties, the molecular basis of similarities resides with the relative positions of these three charged groups in each amphiphilic domain. The molecular basis of an element of functional molecular specificity may also reside within relationships between these three charged residues, since MP possesses three cationic groups, NGFR contains 1 anionic and 2 cationic residues, and TNFR-1 has 2 anionic and 1 cationic groups displaying similar distance and angular relationships. However, the amphiphilic domains of NGFR and TNFR-1 contain other charged residues that likely confer higher degrees of functional specificity that could distinguish biological effects. Angles subtended by R375, R376, and R379 in NGFR were similar to those subtended by R386, D387, and D389 in TNFR-1 but, whereas 3 cationic groups formed the relationship in

Table 6
Distances (Å) and angular relationships (degrees) of charged groups of MP 3–14, NGFR 367–379 and TNFR-1 378–390

Distances			Angles		
peptide	amino acids	distance	peptide	amino acids	angle
MP	K4-K11	12.13	MP	K4-K11-K2	85.88
NGFR	D368-R375	11.69	NGFR	D368-R375-R376	90.50
TNFR-1	D379-R386	11.45	TNFR-1	D379-R386-D387	74.55
MP	K11-K12	9.53	MP	K11-K12-K4	54.39
NGFR	R375-R376	9.16	NGFR	R375-R376-D368	51.59
TNFR-1	R386-D387	6.55	TNFR-1	R386-D387-D379	72.41
MP	K12-K4	14.88	MP	K12-K4-K11	39.73
NGFR	R376-D368	14.92	NGFR	R376-D368-R375	37.91
TNFR-1	D387-D379	11.58	TNFR-1	D387-D379-R386	33.04
NGFR	R375-R379	7.79	NGFR	R375-R379-R376	68.64
TNFR-1	R386-D389	6.98	TNFR-1	R386-D389-D387	53.10
NGFR	R379-R376	8.44	NGFR	R379-R376-R375	52.34
TNFR-1	D389-D387	7.62	TNFR-1	D389-D387-R386	58.46
			NGFR	R376-R375-R379	59.02
			TNFR-1	D387-R386-D389	68.44
TNFR-1	D379-R383	8.53	TNFR-1	D379-D387-R383	41.60
	R383-D387	4.96		D387-R383-D379	115.70
				R383-D379-D387	22.70

Reference points for charged groups are nitrogens of amino groups of lysine sidechains, carbons of guanidino group of arginines, and carbons of carboxyl groups of aspartic acid side chains.

NGFR, 1 cationic and 2 anionic groups subtended similar angles in TNFR-1. TNFR-1 differed further from NGFR in that the cationic residue (R) at position 383 had no homologue in NGFR.

3.3. Circular dichroism spectroscopy

The CD spectra of both MP and a peptide (R3) homologous to NGFR 367–379 in water and in trifluoroethanol (TFE) are depicted in Fig. 3. The spectra of MP in the two solvents indicated that this peptide adopted an α-helical conformation in TFE, from a less ordered structure in the aqueous milieu. These data are comparable to those reported by Higashijima et al. [14], and Higashijima et al. [15] using water and either phosphatidylcholine or methanol, respectively. The same observations were apparent for R3, which exhibited an disordered conformation in the aqueous environment, with a transition to an α-helical structure in the nonpolar TFE, as indicated by the strong negative band at 208 nm and the shoulder in the 215–225 nm region of the spectrum.

4. Discussion

The present studies extend earlier observations of Feinstein and Larhammar [23] with NGFR and predict that within the NGF/TNF receptor superfamily three receptors have consensus secondary amphiphilic structures that, like MP, have the potential to associate with membranes and to interact with binding sites on α-subunits of G-proteins and other cytoplasmic proteins [22]. CD spectroscopy confirmed the computational conformational data that predicted α-helical conformations of MP and a peptide homologous to the amphiphilic domain of NGFR in a nonpolar milieu.

As demonstrated by Higashijima et al. [14], the biological properties of MP are likely related to its amphiphilicity whereby non-polar groups promote an association with the cytoplasmic surface of the plasma membrane phospholipid bilayer, and charged groups face the cytoplasm where they can dock with appropriate binding sites on α-subunits of G-proteins. Those properties describing the secondary structure of MP that would permit surface membrane association — μ_H, partition coefficient and Van der Waals volume — are shared by domains of NGFR, TNFR-1, and 4-1BB.

The interaction of cationic groups on the cytoplasmic face of MP and α-subunits of G-proteins are likely involved in initiating guanine nucleotide exchange, and in this way MP mimics liganded receptor [14]. Implied is the understanding that those α-subunits interacting with MP and amphiphilic domains of receptors have binding sites that likely interact with charged groups that face the cytoplasm. These binding sites are not found exclusively on α-subunits of G-proteins. Traub et al. [22] have used affinity techniques with antibody to peptide sequences of amphiphilic binding domains on α-subunits of G-systems involved with the noradrenergic receptor to purify an intracellular protein that, with a molecular mass of 100 kDa, is larger than any described α-subunit. This observation suggests that amphiphilic domains of receptors may be descriptive motifs involved in signalling via a variety of intracellular proteins.

Observations on the distance and angular relationships between hydrophilic groups in the amphiphilic domains of NGFR and TNFR-1, when compared to those of MP, begin to suggest how specificity of response in ligand-induced receptor-mediated activation of intracellular proteins might be mediated if these motifs are involved in signalling. Within their respective amphiphilic domains the numbers of charged residues, their types, and calculated distance and, in particular, angular relationships between them may begin to describe signalling specificity at the receptor level.

Some data have emerged to suggest that amphiphilic domains of TNFR-1 and NGFR may be involved in signal transduction, but to date, little is known of the biological properties of the 4-1BB receptor. Yanaga et al. [24] have observed that TNF-α stimulates prostaglandin E2 production and de novo synthesis of cyclooxygenase via a pertussis-sensitive G-system in an osteoblast-like cell line. Evidence has also been provided that TNFR-1 may be coupled to a pertussis-sensitive G-protein in membrane fractions of HL-60 cells [25].

More direct evidence to suggest that the amphiphilic domain of NGFR 367–379 is involved with members of the trk receptor family in neurotrophin-mediated neurite growth promotion by responsive cells is required to establish a functional role mediated by this descriptive motif of the common neurotrophin receptor.

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