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The interaction of neurotrophins with the p75^{NTR} common neurotrophin receptor: A comprehensive molecular modeling study

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Abstract

Neurotrophins are a family of proteins with pleiotropic effects mediated by two distinct receptor types, namely the Trk family, and the common neurotrophin receptor p75^{NTR}. Binding of four mammalian neurotrophins, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5), to p75^{NTR} is studied by molecular modeling based on X-ray structures of the neurotrophins and the extracellular domain of p55^{TNFR}, a homologue of p75^{NTR}. The model of neurotrophin/receptor interactions suggests that the receptor binding domains of neurotrophins (loops I and IV) are geometrically and electrostatically complementary to a putative binding site of p75^{NTR}, formed by the second and part of the third cysteine-rich domains. Geometric match of neurotrophin/receptor binding domains in the complexes, as characterized by shape complementarity statistic S_c, is comparable to known protein/protein complexes. All charged residues within the loops I and IV of the neurotrophins, previously determined as being critical for p75^{NTR} binding, directly participate in receptor binding in the framework of the model. Principal residues of the binding site of p75^{NTR} include Asp47, Lys56, Asp75, Asp76, Asp88, and Glu89. The additional involvement of Arg80 and Glu53 is specific for NGF and BDNF, respectively, and Glu73 participates in binding with NT-3 and NT-4/5. Neurotrophins are likely to induce similar, but not identical, conformational changes within the p75^{NTR} binding site.

Keywords: Alzheimer's disease; cysteine-rich domain; molecular modeling; molecular recognition; NGF; protein docking

The neurotrophins represent a family of structurally and functionally related proteins conventionally considered to play a crucial role in the development, survival, and maintenance of sympathetic and sensory neurons (Purves, 1988; Barde, 1989; Snider & Johnson, 1989; Thoenen, 1991; Korsching, 1993; Persson & Ibáñez, 1993; Davies, 1994; Ibáñez, 1995). In addition to these effects, neurotrophins are now recognized to influence a broad range of biological phenomena, including apoptotic cell death (Rabizadeh et al., 1993; Casaccia-Bonnefil et al., 1996; Frade et al., 1996; Van der Zee et al., 1996; Frade & Barde, 1998), immune cell function (Levi-Montalcini et al., 1996), and cell migration (Herrmann et al., 1993; Anton et al., 1994; Wang et al., 1998). The mammalian neurotrophin family consists of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5) (Barde, 1989; Berkemeier et al., 1991; Barbacid, 1993; Bradshaw et al., 1994; Chao, 1994). Each neurotrophin is capable of binding independently with the common neurotrophin receptor p75^{NTR} and a specific Trk receptor kinase, thereby activating signal transduction cascades (Chao, 1992a, 1992b; Kaplan & Stephens, 1994; Chao & Hempstead, 1995; Ibáñez, 1995; Rydén & Ibáñez, 1996). Currently, three classes of Trk receptors are recognized, such that TrkA selectively binds to NGF, TrkB to BDNF and NT-4/5, and TrkC to NT-3 (Barbacid, 1994; Ibáñez, 1995).

The common neurotrophin receptor p75^{NTR} is a transmembrane glycoprotein structurally related to type I (p55^{TNFR}) and type II (p75^{TNFR}) tumor necrosis factor receptors (TNFR-1 and TNFR-2, respectively), FAS (CD95), CD-40 and CD27 receptors (Meakin & Shooter, 1992; Myers et al., 1994; Rydén & Ibáñez, 1996; Naismith & Sprang, 1998). Because all neurotrophins bind to p75^{NTR} with similar affinity (Rodriguez-Tébar et al., 1990, 1992; Hallböök et al., 1991; Ibáñez, 1995), neurotrophin specificity is convention-

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ally thought to result from the binding selectivity of Trk receptors (which are differentially expressed in neuronal populations; Ibáñez, 1995). However, considerable experimental data on neurotrophin activity reveal important functional aspects of $p75^{NTR}$ (Heldin et al., 1989; Jing et al., 1992; Herrmann et al., 1993; Barker & Shooter, 1994; Dobrowsky et al., 1994; Matsumoto et al., 1995; Marchetti et al., 1996; Washiyama et al., 1996; Dechant & Barde, 1997; Rydén et al., 1997; Ross et al., 1998). The common neurotrophin receptor enhances Trk-mediated functions and increases binding specificity of Trk receptors (Barker & Shooter, 1994; Mahadeo et al., 1994; Chao & Hempstead, 1995; Rydén & Ibáñez, 1996). In addition, $p75^{NTR}$ possesses unique, Trk-independent signalling properties that involve apoptosis (Rabizadeh et al., 1993; Barrett & Bartlett, 1994; Casaccia-Bonnefil et al., 1996; Frade et al., 1996; Van der Zee et al., 1996; Frade & Barde, 1998), ceramide production through activation of the sphingomyelin cycle (Dobrowsky et al., 1994), and activation of the NFκB transcription factor (Carter et al., 1996). Recently, $p75^{NTR}$ has also been demonstrated to participate in human melanoma progression (Herrmann et al., 1993; Marchetti et al., 1996). While all neurotrophins bind $p75^{NTR}$, biological effects are dependent on neurotrophin species (Frade et al., 1996; Marchetti et al., 1996; Dechant & Barde, 1997).

The results of site-directed mutagenesis suggest that the $p75^{NTR}$ binding domains within neurotrophins consist of juxtaposed positively charged residues located in the two adjacent hairpin loops I (residues 23–35) and IV (residues 93–98) (Fig. 1B; Ibáñez et al., 1992; Ibáñez, 1994, 1995; Rydén et al., 1995; Rydén & Ibáñez, 1996). In NGF, residues Lys32, Lys34, and Lys95 have been found to participate in $p75^{NTR}$ binding, with Lys32 making the strongest contact. In addition, a role for Asp30, Glu35, Arg103, Arg100, Lys88, Trp21, and Ile31 in $p75^{NTR}$ binding and biological activity has been demonstrated (Drinkwater et al., 1991; Ibáñez et al., 1992; Ibáñez, 1994). Two residues of variable region I of NT-3, namely Arg31 and His33, have been found to be essential for binding to $p75^{NTR}$, whereas corresponding residues Arg34 and Arg36 mediate binding of NT-4/5. In contrast to NGF, a positively charged residue in loop IV is not critical for binding of NT-3 or NT-4/5 to $p75^{NTR}$ (Rydén et al., 1995). In BDNF, however, only residues of loop IV, Lys95, Lys96, and Arg97, bind to $p75^{NTR}$ and compensate for the lack of positively charged residues in loop I (Rydén et al., 1995).

$p75^{NTR}$ belongs to a family of cell surface proteins that share a common four-repeat cysteine-rich domain (CRD) in the extracellular unit (Yan & Chao, 1991; Baldwin & Shooter, 1994). X-ray crystallographic studies on a related protein (the soluble extracellular domain of TNFR-1) revealed that CRDs fold independently from each other and that three conserved disulfide bonds maintain a specific geometry for each CRD (Banner et al., 1993), consisting of two modular units (usually loops A1 and B2, Fig. 1A; Naismith et al., 1996; Naismith & Sprang, 1998). Little is known about the $p75^{NTR}$ functional epitope and particularly about residues directly

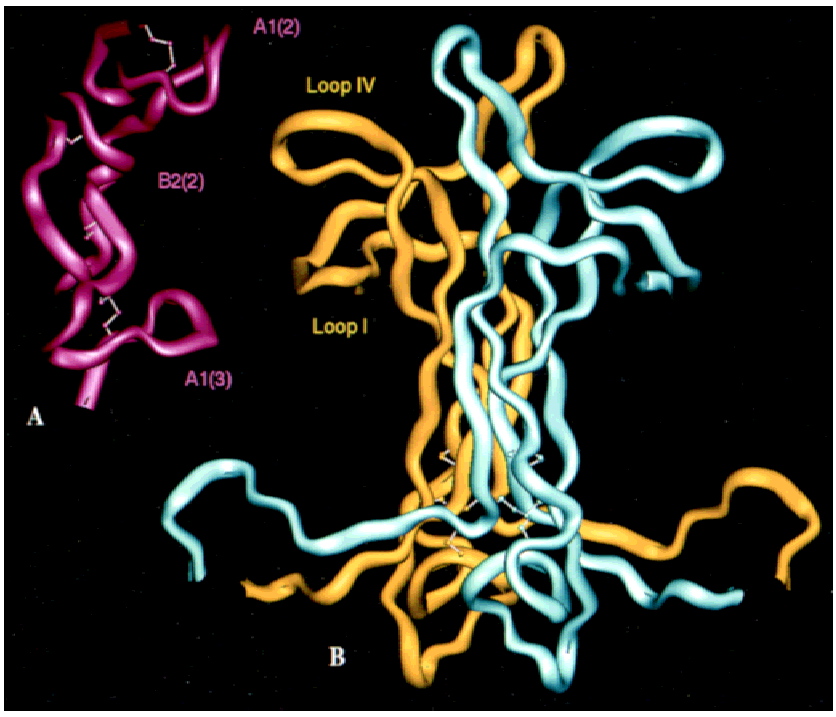

Fig. 1. Geometric compatibility of the $p75^{NTR}$ receptor binding domain of NGF to a putative binding site of $p75^{NTR}$. **A:** Generalized geometry of a putative binding site of $p75^{NTR}$, which consists of three modules [A1(2) and B2(2) of the second CRD; and A1(3) of the third CRD] based on the X-ray structure of the extracellular domain of $p55^{TNFR}$ (Banner et al., 1993). According to the classification of modular units of CRDs suggested by Naismith and Sprang (1998), A-modules have 12–17 residues and are C-shaped, whereas B-modules have 21–24 residues and are S-shaped; the following digit (1 or 2) designate the number of disulfide bonds within the module; the number in the parenthesis denotes the number of the CRD domain within the peptide. **B:** The X-ray backbone geometry of mouse NGF (McDonald et al., 1991; Shamovsky et al., 1996, 1998). Yellow and green polypeptide chains denote different protomers. The $p75^{NTR}$ binding determinant (loops I and IV) is denoted. Conserved disulfide bonds are shown in ball-and-stick mode.

participating in molecular recognition processes. All four repeats of $p75^{NTR}$ are required for binding, with the second CRD being the most important (Baldwin & Shooter, 1995). Because of the established importance of positively charged residues in neurotrophins for binding to $p75^{NTR}$ and considering the overall negative charge of the $p75^{NTR}$ extracellular domain (Ibáñez et al., 1992; Ibáñez, 1994; Rydén et al., 1995), negatively charged residues within the $p75^{NTR}$ binding site are expected to be critical for molecular recognition processes (Ibáñez et al., 1992; Ibáñez, 1994, 1995). Nevertheless, no key negatively charged residue of $p75^{NTR}$ has been identified to date, and only the neutral residue Ser50 has been demonstrated to be critical for binding to NGF (Baldwin & Shooter, 1995). This discrepancy may be a consequence of insufficient experimental data.

A detailed description of neurotrophin/receptor interfaces is required to design specific NGF antagonists that could selectively prevent undesirable pro-apoptotic signaling of $p75^{NTR}$ upon NGF binding without affecting other functions of NGF or the biological activity of other neurotrophins. Elucidation of three-dimensional structures of the neurotrophin-receptor complexes by NMR is not currently feasible because of the large size of these dimeric proteins. Furthermore, transition metal ions commonly used in X-ray crystallography (McDonald et al., 1991; Holland et al., 1994; Bax et al., 1997) cause conformational alterations in neurotrophins that prevent their receptor binding (Ross et al., 1997), suggesting that useful crystal structures of the complexes may not presently be obtained. Therefore, determination of the geometry of these complexes and identification of the residues involved in ligand–receptor binding are highly relevant problems for the application of theoretical methods. Although molecular modeling of NGF/$p75^{NTR}$ binding has been reported (Chapman & Kuntz, 1995), results of that study suggesting that the second CRD is not involved in receptor binding are not consistent with recent experimental observations (Baldwin & Shooter, 1995). In the present study we report results of molecular dynamics computations of the binding domains of NGF, BDNF, NT-3, and NT-4/5 juxtaposed with a putative binding site of $p75^{NTR}$, which includes the second CRD. These calculations reveal three-dimensional structures of the neurotrophins bound to the $p75^{NTR}$ binding site and demonstrate their geometric and electrostatic complementarity. The shape complementarity statistic S_c has been calculated for the neurotrophin/receptor interfaces and found to be consistent with published values for interfaces of natural protein/protein complexes (Lawrence & Colman, 1993). All neurotrophins are predicted to induce similar conformational changes in the $p75^{NTR}$ binding site, generally located in the linker regions between sequential modular units within the CRDs.

Results and discussion

Putative binding domains

Figure 1A illustrates the general shape of both units of the second CRD and the first unit of the third CRD of human $p75^{NTR}$ as revealed by crystallographic studies of the homologous $p55^{TNFR}$ protein (Banner et al., 1993; Naismith & Sprang, 1998). Figure 2 presents the sequence of this fragment and its alignment with those of the other peptides in this family (Johnson et al., 1986; Radeke et al., 1987; Large et al., 1989) using the conserved pattern of cysteine residues (Chapman & Kuntz, 1995). The size and shape of the concave region of $p75^{NTR}$ are complementary with the convex

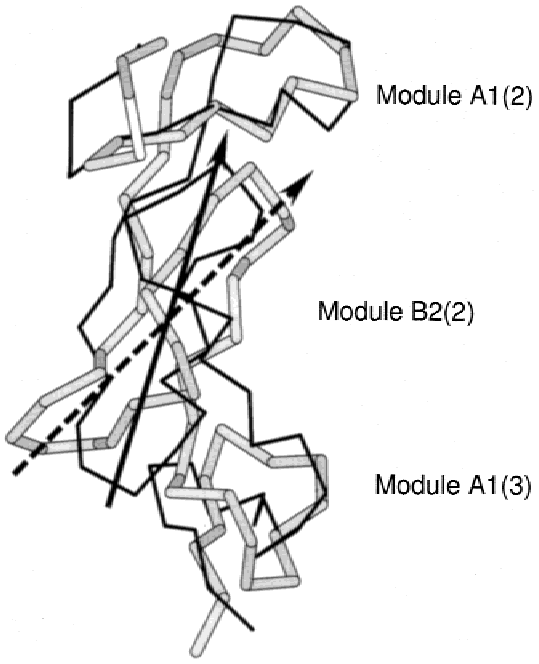

Fig. 2. Sequence alignment of a putative binding site for neurotrophins of $p75^{NTR}$ with the corresponding region of homologous human $p55^{TNFR}$. Alignment was done using the conserved pattern of cysteine residues. Distinct modular units A1 and B2 of CRDs separated by linker regions as defined by Naismith and Sprang (1998) are shown. Charged residues critical for binding to the neurotrophins according to the present model are shaded.

binding determinant of NGF (Fig. 1). Accordingly, the 58-amino acid fragment of human $p75^{NTR}$, including the second and part of the third CRDs (Fig. 2; from Cys39 to Ala96), has been considered as a putative binding site for neurotrophins.

Binding epitopes of the complexes

The most stable structures of the ligand–receptor complexes under study obtained by molecular dynamics computations are illustrated in Figure 3. Table 1 presents net values of the electrostatic and van der Waals terms of the ligand–receptor interaction energy. Tables 1 and 2 list individual residues of the neurotrophins and $p75^{NTR}$ that significantly contribute to the intermolecular electrostatic interaction energy. The highlighted residues represent the functional epitopes of the ligands (Table 1) and the receptor (Table 2), whereas the other listed residues, although being located within the binding interfaces, do not participate in major ligand–receptor electrostatic interactions in the particular complex. Significant contribution of a particular residue to the electrostatic interaction energy indicates its participation in specific electrostatic ligand–receptor interactions. Most of those residues demonstrate attractive interactions with their counterparts except for three negatively charged neurotrophin residues (Asp30, Asp93, Asp105 of NGF and corresponding residues of other neurotrophins; Table 1) and one positively charged residue of $p75^{NTR}$ (Lys56; Table 2). The reason for significant repulsive interactions between the four specified residues and their counterparts is that their charges are of the same sign,

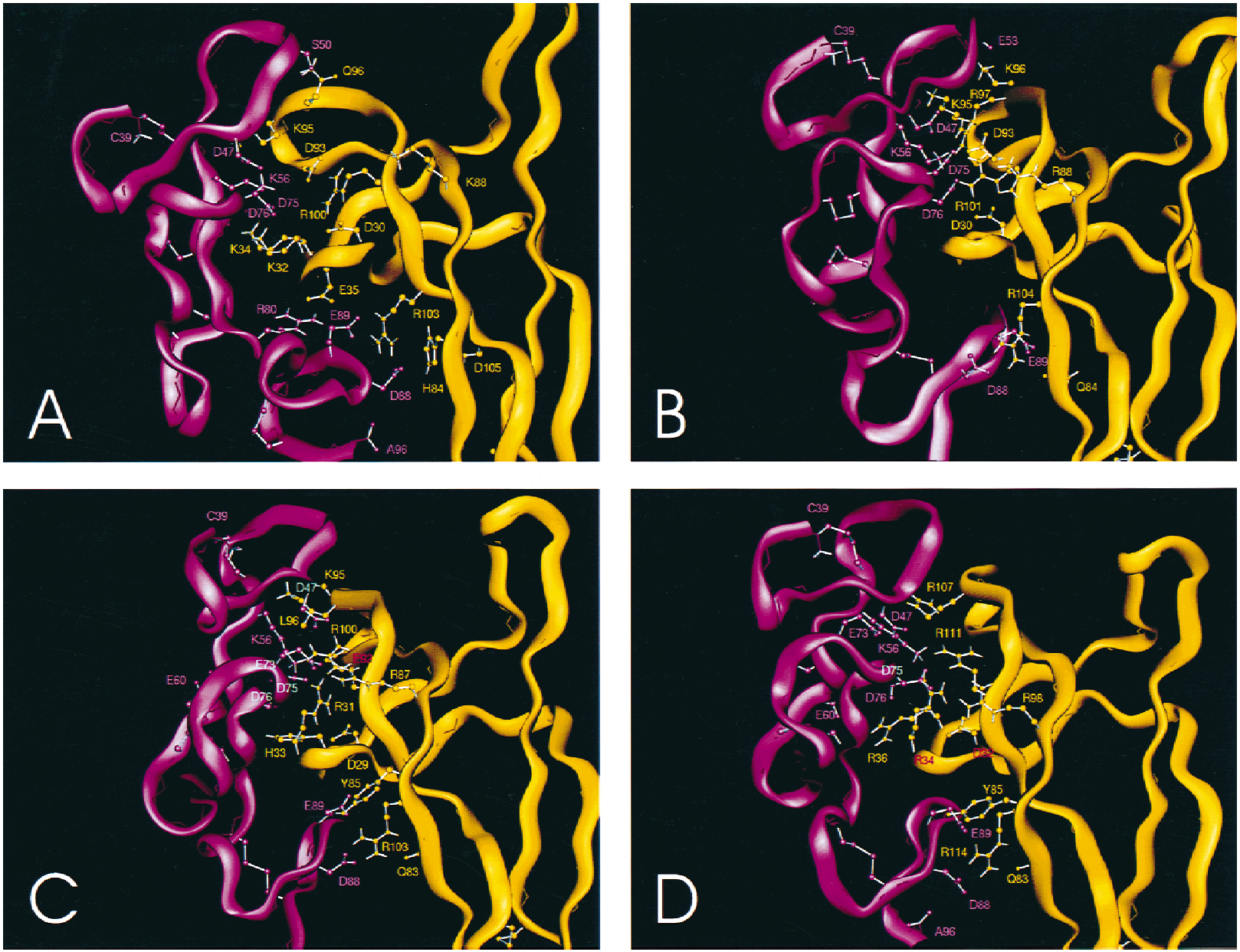

Fig. 3. The most stable structures of the complexes of the neurotrophins with the common neurotrophin receptor $p75^{NTR}$: (**A**) with NGF; (**B**) with BDNF; (**C**) with NT-3; (**D**) with NT-4/5. The binding domains of the neurotrophins are shown in yellow, the $p75^{NTR}$ binding site in purple. Only residues important for discussion are denoted.

Table 1. *Contributions of individual residues of the neurotrophins to the intermolecular interaction energy in their complexes with the common neurotrophin receptor p75^{NTR} (kcal/mol)*[a]

NGF			BDNF			NT-3			NT-4/5		
Residue	EL	VDW	Residue	EL	VDW	Residue	EL	VDW	Residue	EL	VDW
D-30	4.9	−1.0	**D-30**	6.2	−1.8	**D-29**	5.7	−2.3	**D-32**	5.3	−1.8
K-32	−20.2	−5.7	S-32	0.1	−4.5	**R-31**	−11.6	−7.6	**R-34**	−11.0	−10.4
K-34	−16.8	−6.3	G-34	−0.5	−3.1	**H-33**	−7.8	−6.9	**R-36**	−12.4	−11.8
E-35	−2.3	−3.0	T-35	0.4	−1.4	Q-34	0.1	−1.1	D-37	0.1	−1.8
H-84	−7.8	−1.3	Q-84	0.3	−2.4	Q-83	0.1	−0.7	Q-83	0.1	−1.2
F-86	−0.1	−0.1	Y-86	−0.1	−0.5	Y-85	−0.8	−1.2	**Y-96**	−2.3	0.0
K-88	−2.1	−0.1	**R-88**	−3.2	−0.2	**R-87**	−14.6	0.6	**R-98**	−4.2	−0.2
D-93	2.6	−1.4	**D-93**	3.4	−2.2	**E-92**	3.8	−2.3	D-103	0.4	−1.4
K-95	−18.2	−7.8	**K-95**	−20.7	−7.6	N-94	0.0	−0.2	Q-105	−0.1	−0.2
Q-96	−0.1	−5.2	**K-96**	−14.5	2.0	**K-95**	−12.0	−4.0	G-106	−0.2	−0.6
A-97	0.0	−0.2	**R-97**	−19.8	−5.7	L-96	0.0	−7.6	**R-107**	−9.2	−10.2
R-100	−3.5	−0.6	**R-101**	−14.8	−1.1	**R-100**	−20.1	2.4	**R-111**	−13.7	−1.7
R-103	−16.9	0.0	**R-104**	−16.1	−1.6	**R-103**	−14.2	−0.8	**R-114**	−13.7	−1.8
D-105	2.8	−0.2	**D-106**	2.1	−0.2	D-105	1.5	−0.1	D-116	1.1	−0.1
Total	−77.5	−22.6		−77.4	−25.3		−70.8	−28.3		−61.2	−34.2
	100%	51%		96%	54%		98%	52%		103%	57%
Net	−77.5	−44.7		−80.3	−46.7		−72.5	−54.4		−59.4	−59.7

[a]EL and VDW denote electrostatic and van der Waals terms of the ligand–receptor interaction energy, respectively. Dielectric constant $\epsilon = 4$ is used. The ±2 kcal/mol cutoff of the intermolecular electrostatic interaction energy has been used to identify the functional epitopes (boldface). "Total" electrostatic and van der Waals contributions of these functional epitopes into the "net" values of the intermolecular interaction energy calculated over the whole binding interface are presented. Residue numbering corresponds to the general alignment of neurotrophins (Bradshaw et al., 1994).

although all of them participate in local attractive ionic interactions within the complexes (Fig. 3).

Significant contributions of electrostatic interactions to the ligand–receptor interaction energy (Table 1) arise primarily from opposite net charges of the binding domains of neurotrophins (positively charged) and the common neurotrophin receptor (negatively charged), which is consistent with published predictions (Rydén et al., 1995). Data presented in Tables 1 and 2 demonstrate that, on

Table 2. *Contributions of individual residues of p75^{NTR} into the intermolecular interaction energy in their complexes with neurotrophins (kcal/mol)*[a]

NGF			BDNF			NT-3			NT-4/5		
Residue	EL	VDW	Residue	EL	VDW	Residue	EL	VDW	Residue	EL	VDW
S-46	−3.4	0.8	S-46	−1.9	−0.9	S-46	0.0	−0.4	S-46	0.1	−0.2
D-47	−9.9	−1.9	**D-47**	−16.8	0.1	**D-47**	−10.6	−2.2	**D-47**	−6.4	−3.5
E-53	−0.8	−0.3	**E-53**	−13.9	1.6	E-53	−1.6	−0.2	E-53	−0.9	−0.1
P-54	−2.6	−2.9	**P-54**	−3.4	−2.5	**P-54**	−3.6	−0.3	P-54	−0.6	−0.5
C-55	−3.1	−1.1	**C-55**	−2.1	−1.3	**C-55**	−3.6	−0.7	**C-55**	−3.6	0.8
K-56	7.7	−4.9	**K-56**	3.3	−4.8	**K-56**	4.5	−4.5	**K-56**	1.7	−5.2
P-57	−3.5	−1.5	P-57	−0.3	−0.5	P-57	0.1	−0.6	P-57	0.0	−0.2
E-60	0.5	−0.8	E-60	−0.8	−0.1	**E-60**	−2.3	−0.2	**E-60**	−1.8	−0.2
E-73	−1.0	−0.1	**E-73**	−2.4	−0.2	**E-73**	−13.6	−0.5	**E-73**	−2.9	−0.3
D-75	−13.2	0.2	**D-75**	−19.3	0.4	**D-75**	−16.5	−0.4	**D-75**	−14.1	−3.4
D-76	−18.1	−0.4	**D-76**	−5.6	−8.4	**D-76**	−8.4	−3.7	**D-76**	−12.6	−6.9
R-80	−6.6	−4.8	R-80	0.7	−2.1	R-80	1.5	−3.5	R-80	0.7	−4.0
D-88	−10.9	−3.1	**D-88**	−9.9	−0.4	**D-88**	−9.6	0.3	**D-88**	−8.2	−1.1
E-89	−9.6	−0.1	**E-89**	−6.3	−6.2	**E-89**	−6.8	−8.8	**E-89**	−8.7	−4.6
Total	−73.2	−15.7		−76.4	−24.0		−70.4	−23.3		−56.5	−27.8
	94%	35%		95%	51%		97%	43%		95%	47%

[a]See footnote to Table 1.

average, the interaction between the functional epitopes is responsible for 97% of intermolecular electrostatic energy but only 49% of van der Waals interaction energy. Consequently, residues participating in electrostatic ligand–receptor interactions represent only part of a larger binding interface in which a significant van der Waals contribution arises from the large number of weak contacts, which in turn is possible only as a consequence of the geometric complementarity of the binding domains. Interaction energies of the complexes (Tables 1, 2) cannot be directly compared with experimentally obtained values without taking into account solvation energies; nevertheless, based on van der Waals interaction energy, the geometric complementarity in the NGF/p75^{NTR} complex is lower than in the others (Table 1). This is consistent with its fast rate of dissociation observed for this complex (Ibáñez, 1995; Dechant & Barde, 1997). However, the geometric factor alone cannot itself explain the slowest rate of dissociation observed for the BDNF/p75^{NTR} complex (Ibáñez, 1995). Thus, stability of the complex arises from a combination of geometric and electrostatic complementarities of the binding interfaces, consistent with generalizations described by Clackson and Wells (1995). A specific feature of the neurotrophin/p75^{NTR} binding interfaces is that they do not include substantially hydrophobic residues, so they do not possess a hydrophobic core, which has been found in many other protein–protein complexes (Clackson & Wells, 1995; Livnah et al., 1996). The predominant interactions within the binding domains of the complexes under study are of salt-bridge nature. This agrees well with a recently proposed model of the CD40L/CD40 interaction in which the interface of the complex is primarily composed of charged residues (Singh et al., 1998), which suggests that ionic contacts may play a major role in molecular recognition processes involving cysteine-rich domains.

Specificity of binding of the neurotrophins to p75^{NTR} is known to be determined by charged residues within the binding domains (Rydén et al., 1995). As seen in Figure 3, they create three-dimensional networks in which individual residues are in contact with more than one residue of the opposite charge. Therefore, a missing charge can destroy not only one bond, but the entire network, i.e., the electrostatic complementarity of the binding interfaces (which is consistent with the experimentally observed sensitivity of binding to mutations of charged residues; Ibáñez et al., 1992; Rydén et al., 1995; Rydén & Ibáñez, 1996). Both ligand and receptor provide the network with positive and negative charges (Tables 1, 2; Fig. 3), even though negative charges dominate in the receptor and positive in the ligands. This particular spatial arrangement of charge increases specificity of binding and decreases electrostatic repulsions within the same binding domains. For example, in all the complexes, the positively charged Lys56 residue of the receptor forms intramolecular salt-bridges with negatively charged residues Asp75 and Asp76, thereby occupying one site of their coordination, such that only the opposite sides of the latter residues remain available for intermolecular ionic interactions (Fig. 3). Negatively charged residue Asp93 of BDNF bridges two positively charged residues Arg97 and Arg101, such that they are both able to interact with the juxtapositioned amino acids Asp75 and Asp76 of the receptor (Fig. 3B).

Despite the differences in the binding interfaces of the complexes, binding patterns of the p75^{NTR} receptor are similar. All three modules of the receptor docking sites [A1(2), B2(2), and A1(3)] participate in binding (Fig. 3). The principal residues of module A1(2) are Asp47 and Lys56, those of module B2(2) are Asp75 and Asp76, whereas those of module A1(3) are Asp88 and

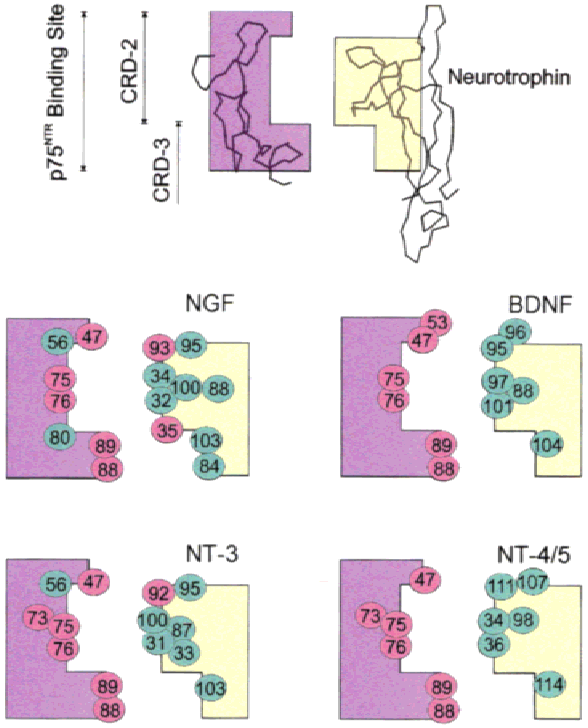

Fig. 4. Schematic representation of principal ionic interactions within the neurotrophin/p75^{NTR} complexes. Purple moiety denotes a putative p75^{NTR} binding site made of the second (CRD-2) and beginning of the third (CRD-3) cysteine-rich domains. Yellow moieties denote the binding determinants of neurotrophins (NGF, BDNF, NT-3, and NT-4/5) made of hairpin loops I and IV. Principal charged residues of the complexes are illustrated (basic, blue; acidic, red).

Glu89. As seen in Table 2, module B2(2) forms the strongest contacts. In addition to the principal pattern of electrostatic interactions, the p75^{NTR} binding site possesses specific residues for ionic interactions with particular neurotrophins (Table 2; Fig. 3). Thus, Arg80 interacts only with Glu35 of NGF; residue Glu53 binds only to Lys96 of BDNF, whereas Glu73 is involved only in binding with NT-3 and NT-4/5. Simplified general shapes of the binding domains of neurotrophins and a putative binding site of the neurotrophin receptor with principal charged residues participating in ionic ligand–receptor interactions as obtained by the present molecular dynamics simulations (Tables 1, 2; Fig. 3) are illustrated in Figure 4.

In addition to a major role of electrostatic interactions in binding interfaces of p75^{NTR}, TNFR-1 (Banner et al., 1993), and CD40 (Singh et al., 1998), there is also a striking similarity of location and modular organization of the receptor sites. Although topology of the ligands (neurotrophins vs. TNFβ and CD40L, which belong to the TNF gene family; Farrah & Smith, 1992) is very different, corresponding receptor binding sites consist of the same modular units, namely units A and B of the second CRD and the A unit of the third CRD (Figs. 2, 4).

Explanation for the observed structure–function relationships

Site-directed mutagenesis studies on the p75^{NTR} binding site have suggested that mutation of any one of residues Asp75, Asp76, or

Glu89 by Ala does not affect NGF binding, whereas mutation of Ser50, which does not participate in principal ionic interactions, results in abolition of binding (Baldwin & Shooter, 1995). Although both adjacent residues Asp75 and Asp76 are involved in ionic interactions in the B2(2) region with positively charged residues of neurotrophins, the presence of one of them may be sufficient to retain binding affinity. Likewise, the functional role of the missing residue Glu89 in module A1(3) might be fulfilled by adjacent Asp88. Residue Ser50 of $p75^{NTR}$ is predicted to be hydrogen bonded to Gln96 of NGF (Fig. 3A), and its mutation to Asn, Ala, or Thr may create steric hindrances within the NGF/$p75^{NTR}$ interface. The present model suggests that Ser50 is not important for binding of any other neurotrophin to $p75^{NTR}$ (Fig. 3B–D). More detailed mutational studies are required to identify the key negatively charged residues of the binding site.

In contrast to the highly conserved $p75^{NTR}$ binding pattern, neurotrophins have only two common residues within their binding determinants that directly interact with the receptor site, Arg100 and Arg103. Contribution of Arg103 is significant in all the complexes, while Arg100 seems to be less important for NGF/$p75^{NTR}$ binding.

As seen in Table 1, the NGF residues that are important for binding to $p75^{NTR}$ in the framework of the present model are Lys32, Lys34, Lys95, Arg103, His84, Arg100, and Glu35, all of which form specific ionic contacts with the charged residues of $p75^{NTR}$ (Figs. 3A, 4). Involvement of Lys32, Lys34, and Lys95 in $p75^{NTR}$ binding has been well established experimentally (Ibáñez et al., 1992). The most important residues of the NGF binding domain, Lys32 and Lys34 (Ibáñez et al., 1992), are predicted to bind to the module B2(2) residues of $p75^{NTR}$ (Fig. 3A). Residue Lys88 is not directly involved in binding to $p75^{NTR}$, whereas residues Arg103 and His84 likely make direct ionic contacts with the receptor binding site. Importance of the latter residue has not been previously suggested in context of $p75^{NTR}$ binding. The role of Asp30, Glu35, Asp93, and Arg100 may be secondary, because their contributions are relatively small. Hydrophobic residues Trp21 and Ile31, which have been suggested to be important for receptor recognition, are not directly involved in ligand–receptor interactions, but instead participate in hydrophobic interactions within NGF.

In the model of BDNF binding, all three positively charged residues of loop IV, Lys95, Lys96, and Arg97 (Rydén et al., 1995) interact with negatively charged residues of the $p75^{NTR}$ binding site, namely Asp47, Glu53, and Asp75, respectively (Figs. 3B, 4). In addition, residues Arg88 and Arg101 play a more important role than corresponding residues Lys88 and Arg100 in NGF (Table 1); they bind to the module B2(2) residues Asp75 and Asp76 of $p75^{NTR}$, thereby compensating for the absence of positively charged residues within loop I. The increase of role of residue Arg88 in BDNF and other neurotrophins with respect to that of Lys88 in NGF (Table 1) is permitted by a longer side chain of Arg, which results in decreasing separations between opposite charges of ligand and receptor (Fig. 3). Because of missing residue His84, only the positively charged residue Arg104 binds to the module A1(3) residues Asp88 and Glu89 of $p75^{NTR}$.

In NT-3, residues Arg31, His33, Arg100, and Arg87 interact with the module B2(2) residues of the receptor Glu73, Asp75, and Asp76 (Figs. 3C, 4). In addition, residue Arg100 interacts with Asp47 of module A1(2) of the receptor binding site. Residue Lys95 of loop IV does not form any salt-bridge contact with the receptor, consistent with its lack of importance in NT-3 binding (Rydén et al., 1995). Thus, the present results agree well with the established role of Arg31 and His33 in NT-3 (Rydén et al., 1995; Rydén & Ibáñez, 1996) and predict the increased importance of residues Arg87 and Arg100 in NT-3 for $p75^{NTR}$ binding (Table 1). The binding pattern of NT-4/5 is similar to that of NT-3 (Figs. 3C,D, 4). The increased electrostatic contribution of Arg36 with respect to the corresponding NT-3 residue His33 (Table 1) is caused by the longer side chain of the former, which forms an additional salt-bridge contact with distant residue Glu60 of the receptor site (Fig. 3D).

Shape complementarity of neurotrophin/receptor binding domains

The shape complementarity statistic values and the total areas buried in the interfaces of the obtained complexes of neurotrophins with $p75^{NTR}$ are presented in Table 3. Corresponding parameters for the experimentally determined TNFβ/TNFR-1 interface are also tabulated. As seen, shape complementarity of binding domains at the TNFβ/TNFR-1 interface (0.62) is consistent with those of other protein/protein complexes deposited in the Brookhaven Protein Data Bank (PDB) (with S_c values ranging from 0.63 to 0.76; Lawrence & Colman, 1993). Shape complementarity at the interfaces of the computationally obtained neurotrophin/receptor complexes (ranging from 0.68 to 0.76) thus appears to be as good as in other protein/protein complexes. The area of the NGF/$p75^{NTR}$ interface (W) is significantly smaller than in the other neurotrophin/receptor complexes, which could account for the lowest value of the van der Waals interaction energy (Table 1).

Induced conformational changes in $p75^{NTR}$

Binding of neurotrophins is predicted to induce distinct conformational changes in the $p75^{NTR}$ binding site, in agreement with predictions made by Bothwell (1995). Conformational motion within modules A1(2), B2(2), and A1(3) is constrained by four disulfide bonds (Fig. 2); therefore, the most significant changes occur in the linker regions connecting those units consistent with predictions (Naismith et al., 1996; Naismith & Sprang, 1998). Figure 5 illustrates the superimposed equilibrium geometries of the free and the

Table 3. *Parameters of ligand/receptor interfaces*

Interface	S_c[a]	W[b] (Å^2)
Computationally generated complexes		
NGF/$p75^{NTR}$	0.68	467
BDNF/$p75^{NTR}$	0.72	554
NT-3/$p75^{NTR}$	0.68	685
NT-4/5/$p75^{NTR}$	0.76	637
Crystallographically resolved complex		
Human tumor necrosis factor-β/$p55^{TNFR}$ complex (1TNR)[c]	0.62	423

[a] The shape complementarity median statistic S_c values are calculated according to Lawrence and Colman (1993).

[b] Total area W buried in the interface of the complex after omitting the 1.5 Å band from the periphery of the interface as described by Lawrence and Colman (1993).

[c] The four-letter code assigned by the Brookhaven Protein Data Bank.

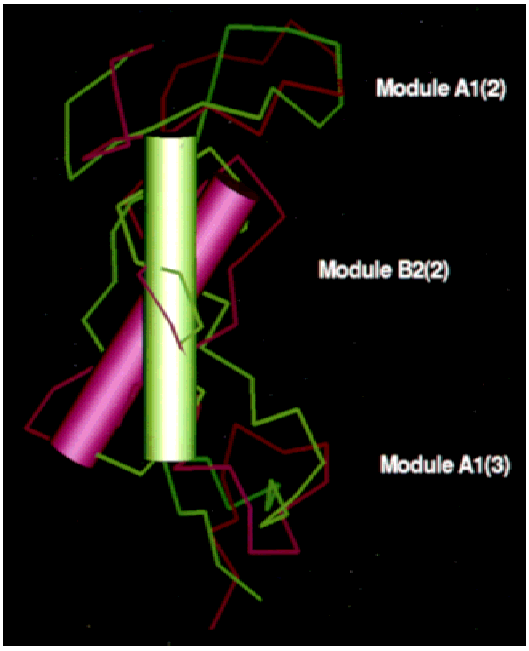

Fig. 5. Conformational changes within the p75^{NTR} binding site upon BDNF binding. Green and purple cylinders represent overall orientations of module B2(2) in free and bound p75^{NTR} binding site, respectively.

BDNF-complexed p75^{NTR} binding site. The superimposition is performed by a least-squares minimization technique using the α-carbon reference atoms of modules A1(2) and A1(3). As illustrated, the overall orientation of module B2(2) with respect to the two flanking units is considerably inclined upon binding. Induced conformational changes within p75^{NTR} binding domain are consistent with the model of overlapping hinges (Naismith & Sprang, 1998) in which connecting (linker) regions permit the structure on either side to make considerable rigid body movements. Because of striking similarities of the binding patterns of different neurotrophins to p75^{NTR}, similar, but not identical, conformational alterations take place in the receptor binding site in the complexes under investigation. This result is consistent with the observed changes in circular dichroism (CD) spectra upon binding of the neurotrophins to the extracellular domain of p75^{NTR} (Timm et al., 1994) and further suggests that the observed effects are mainly associated with specific conformational alterations within the receptor. The magnitude of the CD changes correlates with the area of the binding interface (*W*; Table 3), i.e., the largest and the smallest spectral changes have been observed for NT-3 and NGF, respectively (Timm et al., 1994). Consequently, spectral changes increase with the increase of the protein–protein contact area. According to the model, the driving force for the conformational transitions arises from the salt-bridge interactions of the module B2(2) residues in the receptor binding site, Asp75 and Asp76, with positively charged residues of the neurotrophins; spatial positions of the flanking A1(2) and A1(3) modules are fixed by ionic interactions of their residues Asp47, Lys56, Asp88, and Glu89 with corresponding residues in the neurotrophins (Figs. 3, 4). Thus, structural variability of neurotrophins does not preclude them from inducing similar conformational changes within the binding site of the common neurotrophin receptor. Further investigation is required to distinguish conformational changes within p75^{NTR} induced by different neurotrophins.

Structural variability of the binding domains

The predicted binding pattern of neurotrophins to p75^{NTR} is verified by comparison of the sequences of the identified binding domains in different species. If the predicted binding pattern is accurate, the principal residues of both counterparts should be conserved throughout all species. The presented sequences of the p75^{NTR} binding site from different species (Fig. 2) demonstrate that the residues comprising the binding epitope, namely Asp47, Lys56, Asp75, Asp76, Asp88, and Glu89, are absolutely conserved in p75^{NTR}. As far as neurotrophins are concerned, because of observed structural variability of loops I and IV in neurotrophins (Rydén et al., 1995), conservation of the principal residues of their binding determinants may be established only within the same neurotrophin. The most recent and comprehensive sequence alignment of neurotrophins (Bradshaw et al., 1994) indicates that the identified functional epitopes of the neurotrophins (Table 1) are highly conserved within the same neurotrophins even though there are some exceptions, often represented by the substitution by a similar amino acid. Arg100 and Arg103 are the only two residues of the binding epitopes that are absolutely conserved throughout all neurotrophins (Bradshaw et al., 1994). Other residues within the functional epitopes maintain specificity of binding of different neurotrophins. The present model also permits understanding the conservation of residues that do not directly participate in receptor binding. For example, Gly33 is absolutely conserved in all neurotrophins yet is not involved in any specific interactions with the receptor binding site. However, within the present model, its conservation can be understood because it is located very closely to module A1(3) of the receptor and forms direct van der Waals contacts with it (Fig. 3), such that any other residue will induce sterical hindrances that would destroy the ligand–receptor geometric complementarity.

Methods

Molecular modeling of the neurotrophin/receptor interactions

Molecular modeling of the ligand–receptor complexes has been performed to demonstrate the stereochemical fit of the binding domains of neurotrophins to a single putative binding site within p75^{NTR} rather than to comprehensively explore the conformational spaces. Geometric features of the complexes of single protomers of neurotrophins with the p75^{NTR} binding site have been obtained by multiple independent molecular dynamics runs of 200–300 ps at constant temperatures between 300 and 380 K, followed by local energy refinements. The QUANTA-97 molecular modeling program (Molecular Simulations Inc., San Diego, California) with the united-atom CHARMM potential energy terms (Brooks et al., 1983) has been used. Solvent has not been included in the molecular dynamics calculations because the binding interfaces within the neurotrophin/p75^{NTR} complexes are not expected to involve solvent molecules.

Initial geometries of individual components of the complexes were based upon the X-ray crystallographic coordinates of NGF (McDonald et al., 1991), BDNF and NT-3 (from the BDNF/NT-3 heterodimer; Robinson et al., 1995), and the p55^{TNFR} extracellular

domain (Banner et al., 1993; Chapman & Kuntz, 1995), which is homologous to $p75^{NTR}$. Geometry of NT-3 monomer derived from the BDNF/NT-3 heterodimer has recently been shown to be very similar to that from the NT-3 homodimer, particularly in the receptor binding regions (Butte et al., 1998). Geometry of the NT-4/5 monomer was constructed from the X-ray structure of NT-3 (Robinson et al., 1995). Because of remarkable geometric similarity of all the neurotrophins that reveals their overall high rigidity (Butte et al., 1998), the molecular motions of the neurotrophins were restricted by imposing the following atom constraints. First, the neurotrophin peptide backbones were fixed at their crystallographic coordinates. Second, conformations of the amino acid side chains of the neurotrophins were restricted except for those located within or in the vicinity of loops I and IV (the $p75^{NTR}$ binding determinant). Specifically, amino acid side-chain motions were allowed for residues 23–35 and 93–98 (NGF numbering) as well as for eight additional neighboring residues, namely His84 of NGF (Gln in other neurotrophins), Phe86 (Tyr in other neurotrophins), Lys88 (Arg in other neurotrophins), Trp99, Arg100, Phe101 (Trp in NT-3 and NT-4/5), Arg103, and Asp105. The flexible amino and carboxyl termini of the neurotrophins were excluded to avoid nonrelevant hindrances for receptor docking. The artefactual charges on the terminal amino and carboxyl groups of the binding site arising from truncation of the flanking peptide bonds in $p75^{NTR}$ and the neurotrophins were neglected.

Evaluation of shape complementarity at neurotrophin/receptor interfaces

Shape complementarity is well known to be an important feature of protein/protein interfaces in natural complexes and can distinguish them from mismatching artificial associations (Lawrence & Colman, 1993). The shape complementarity median statistic S_c at the interfaces of the predicted neurotrophin/receptor complexes was calculated with the algorithm of Lawrence and Colman (1993) to demonstrate their geometric match. This parameter characterizes the correlation of shapes of two close surfaces. Interfaces with $S_c = 1$ precisely mesh, whereas $S_c = 0$ indicates the complete mismatch of their topographies. This particular parameter has three valuable properties, namely (1) it measures complementarity of shape rather then surface separation; (2) it is relatively insensitive to atomic coordinate errors; (3) it reflects the complementarity only of those parts of the two surfaces that are in association (Lawrence & Colman, 1993). Solvent-accessible molecular surfaces of the protein molecules under study (the union of contact and re-entrant surfaces consisting of convex, saddle, and concave portions as defined by Richards, 1977) were constructed using a series of discrete points (Connolly, 1983) generated with a surface sampling of 16 dots/Å^2. The ligand/receptor interfaces (defined as molecular surfaces of ligand and receptor buried from the solvent by their interaction) were obtained using the three-dimensional structures of the neurotrophin/receptor complexes obtained. A solvent molecule was represented by a sphere of radius 1.4 Å (Richards, 1977). The set of atomic van der Waals radii inherent in the CHARMM united-atom force field was used (Brooks et al., 1983). The shape complementarity statistic (S_c) and the area (W) of the ligand/receptor interfaces were calculated after the exclusion of those portions of the interfaces, which lie within 1.5 Å from the solvent-accessible surfaces of the complexes, as suggested by Lawrence and Colman (1993). Because numerical values of atomic radii and algorithmic details of our method may be different from those used by Lawrence and Colman (1993), we have validated the reliability of our algorithm by evaluating the parameters for two protein/protein interfaces tabulated by Lawrence and Colman (1993). The N9 neuraminidase subunit interface (Brookhaven PDB code 1NCA) is found to be characterized by $S_c = 0.71$ and $W =$ 1,945 Å^2 (published values are 0.71 and 1,947 Å^2, respectively), while those parameters for the tern N9 neuraminidase-NC41 Fab complex interface are $S_c = 0.64$ and $W =$ 1,149 Å^2 (published values are 0.63 and 1,212 Å^2, respectively). This is in agreement with the predicted relative insensitivity of the S_c statistic to the atomic radii used (Lawrence & Colman, 1993) and demonstrates the ability of our particular interpretation of the algorithm to reproduce published data. All calculations were performed on a 200 MHz Indigo-2 Silicon Graphics Workstation.

Supporting information

Full atomic coordinates (in PDB format) of the complexes are available in the WWW site: http://www.chem.queens.ca/faculty/weaver/weaverpub.html.

Note added in proof

Reference to Van der Zee et al. (1996) is being retracted.

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