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Structure of the Brain-Derived Neurotrophic Factor/Neurotrophin 3 Heterodimer[†,‡]

Robert C. Robinson,[*,§] Czeslaw Radziejewski,[||] David I. Stuart,[§,⊥] and E. Yvonne Jones[§,⊥]

Laboratory of Molecular Biophysics and Oxford Centre for Molecular Sciences, Rex Richards Building, Oxford University, South Parks Road, Oxford OX1 3QU, United Kingdom, and Regeneron Pharmaceuticals Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591

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ABSTRACT: The development and sustenance of specific neuronal populations in the peripheral and central nervous systems are controlled through the binding of neurotrophic factors to high-affinity cell surface receptors. The neurotrophins (nerve growth factor, NGF; brain-derived neurotrophic factor, BDNF; neurotrophin 3, NT3; and neurotrophin 4, NT4) are dimeric molecules which share approximately 50% sequence identity. The crystal structure of the murine NGF homodimer [McDonald et al. (1991) *Nature 354*, 411−414] indicated that the dimer interface corresponds to regions of high sequence conservation throughout the neurotrophin family. This potential compatibility was duly exploited for the production *in vitro* of noncovalent heterodimers between the different neurotrophins [Radziejewski, C., & Robinson, R. C. (1993) *Biochemistry 32*, 13350−13356; Jungbluth et al. (1994) *Eur. J. Biochem. 221*, 677−685]. Here, we report the X-ray structure at 2.3 Å resolution of one such heterodimer, between human BDNF, and human NT3. The NGF, BDNF, and NT3 protomers share the same topology and are structurally equivalent in regions which contribute to the dimer interface in line with the propensity of the neurotrophins to form heterodimers. Analysis of the structure of regions of the BDNF/NT3 heterodimer involved in receptor specificity led us to conclude that heterodimer binding to p75 involves distant binding sites separately located on each protomer of the heterodimer. In contrast, heterodimer interactions with the trk receptors probably utilize hybrid binding sites comprised of residues contributed by both protomers in the heterodimer. The existence of such hybrid binding sites for the trk receptor provides an explanation for the lower activity of the BDNF/NT3 heterodimer in comparison to the homodimers. Finally, we discuss possible functional roles for the heterodimers *in vivo*.

Brain-derived neurotrophic factor (BDNF; Leibrock et al., 1989; Barde et al., 1982; Hofer & Barde, 1988) and neurotrophin 3 (NT3; Maisonpierre et al., 1990; Rosenthal et al., 1990) are two of the four molecules which comprise the neurotrophin family [nerve growth factor (NGF) (Levi-Montalcini & Angeletti, 1968; Thoenen & Barde, 1980); BDNF, NT3, and neurotrophin 4 (NT4) (Ip et al., 1992; Halbook et al., 1991)]. The neurotrophins provide the mechanism by which extracellular control can be exerted over the development and maintenance of neurons. This is achieved by the inhibition of programmed cell death through the binding of neurotrophins to cell surface receptors on specific populations of neurons (Davies, 1994a). Receptor gene localization, neurotrophin knockout, and pharmacological action studies have indicated the biological actions of

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[‡] Atomic coordinates for the BDNF/NT3 heterodimer have been deposited with the Protein Data Bank, Brookhaven National Laboratory (file name 1BDN). Prerelease coordinates may be requested from R.C.R. (e-mail address: BOB@LMB.BIOP.OX.AC.UK).

[*] Author to whom correspondence should be addressed.
[§] Laboratory of Molecular Biophysics.
[||] Regeneron Pharmaceuticals Inc.
[⊥] Oxford Centre for Molecular Sciences.

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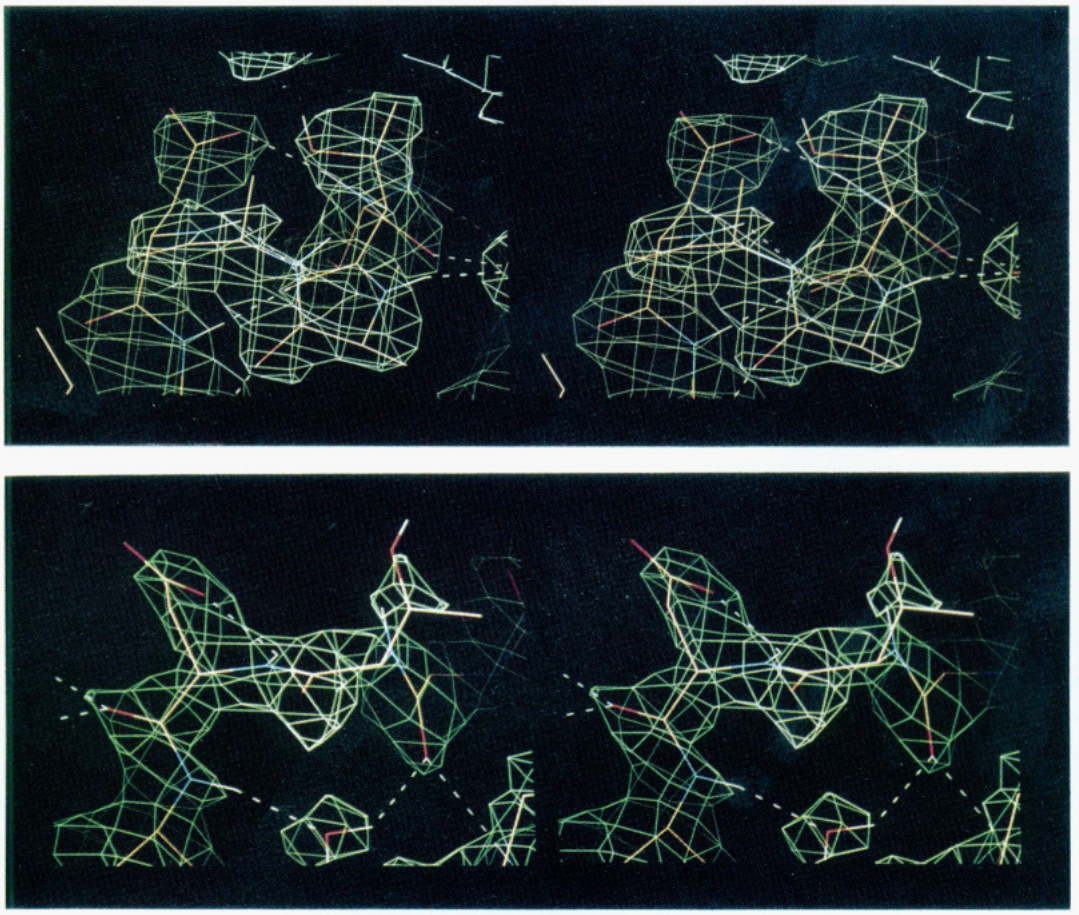


FIGURE 1: (a, top) Portion of the $2|F_o| - |F_c|$ electron density map. Residues Val-21 to Asp-25 of BDNF (which include a two-residue insert, unique to BDNF) are omitted from the phase calculation. (b, bottom) The equivalent region on the NT3 protomer (here residues Val-21 to Asp-23 are omitted from the phase calculation). The 2.3 Å resolution electron density is contoured at 1σ and displayed with the current BDNF/NT3 heterodimer model. The figure was prepared using the program FRODO (Jones, 1985).

the neurotrophins [for review, see Snider (1994)]. Each neurotrophin exerts survival and proliferative effects on various neuron subtypes, overlapping with the other neurotrophins.

The neurotrophins are known to interact with two classes of cell surface receptor. The p75 receptor, a molecule related in structure to the tumor necrosis factor receptor (Mallet & Barclay, 1991; Meakin & Shooter, 1992; Banner et al., 1993), binds to all four neurotrophins, although the biological relevance of this interaction is not completely understood (Lee et al., 1992). The second class of receptor comprises of the trk tyrosine kinases (trkA, trkB, and trkC), molecules that are structurally distinct from the p75 receptor and contain immunoglobulin domains as well as leucine- and cysteine-rich motifs (Snider, 1994; Saltiel & Decker, 1994). These are the functional receptors implicated in neuron growth and survival (Davies, 1994a). NGF exerts its biological effects through binding two molecules of trkA; similarly, BDNF signals through trkB and NT4 through trkB and possibly trkA, while NT3 preferentially binds to trkC but also is able to interact with trkA and trkB [for review, see Davies (1994a)].

All four neurotrophins under physiological conditions exist as tightly associated noncovalent homodimers (Bothwell & Shooter, 1977; Radziejewski et al., 1992), which may be important for the dimerization of receptor subunits in signal transduction. Comparison of the amino acid sequences of the neurotrophins shows that they share approximately 50% identity. The X-ray structure of murine NGF has been elucidated (McDonald et al., 1991). NGF contains β-structure, turn, and random coil with no α-helix. Similar secondary structure content estimations for the other neurotrophins (BDNF, NT3, and NT4) from circulardichroism and Fourier transform infrared spectroscopy have been reported (Radziejewski et al., 1992; Radziejewski & Robinson, 1993; Narhi et al., 1993). The dimer interface in the NGF homodimer consists of hydrophobic residues which are highly conserved throughout the neurotrophin family (McDonald et al., 1991).

These similarities, particularly at the dimer interface, prompted two research groups to produce neurotrophin heterodimers *in vitro* (Radziejewski & Robinson, 1993; Jungbluth et al., 1994). All four neurotrophins readily form heterodimers. The process of protomer exchange was found to be accelerated by the addition of denaturants [urea, guanidine hydrochloride, acetonitrile, or low pH (Radziejewski & Robinson, 1993)]. Heterodimers containing NGF protomers are relatively unstable and show gradual rearrangement into the parent homodimers. Coexpression of BDNF and NT3 in cells using a vaccinia virus expression system also resulted in the formation of the heterodimer (Jungbluth et al., 1994).

The biological relevance of these neurotrophin heterodimers has yet to be fully established; however, their very formation demonstrates the structural similarity of these molecules, particularly at the dimer interface. Initial studies indicate that the BDNF/NT3 heterodimer has biological activity on cells responsive to BDNF and NT3, albeit with

Table 1: Summary of Data Collection and Refinement Statistics

Bragg spacing	reflections[a]	% data[b]	R_{merge}[c]	R_c[d]
20.00−5.00	1008	91.2	0.033	0.147
5.00−4.08	931	94.1	0.036	0.111
4.08−3.54	1096	95.7	0.044	0.150
3.54−3.16	1282	97.0	0.050	0.183
3.16−2.89	1339	94.7	0.066	0.220
2.89−2.67	1468	92.1	0.078	0.251
2.67−2.50	1539	93.2	0.091	0.276
2.50−2.36	1573	91.8	0.117	0.315
2.36−2.30	1455	85.6	0.150	0.324
20.00−2.30	11691	93.4	0.082	0.182

[a] Number of unique reflections. [b] Percentage of theoretically possible data measured. [c] R_{merge} ($\Sigma|I - \langle I\rangle|/\Sigma\langle I\rangle$). [d] R_c ($\Sigma||F_o| - |F_c||/\Sigma|F_o|$).

an reduced activity when compared to a 1:1 mixture of BDNF and NT3 homodimers (Jungbluth et al., 1994). Here we report the X-ray structure of this heterodimer formed by BDNF and NT3 and contrast it to the structure of the NGF homodimer.

EXPERIMENTAL PROCEDURES

Protein Purification and Crystal Growth. Recombinant human BDNF and recombinant human NT3 were supplied by Regeneron Pharmaceuticals Inc. The BDNF/NT3 heterodimer was produced by exposure of the parent homodimers to acid conditions (135 mM HCl, 50 mM NaCl, pH 1.0, 7 h, 20 °C), followed by dialysis against neutral buffer (20 mM Tris.HCl, pH 7.0, 24 h, 4 °C) and purification by mono S cation-exchange chromatography (Radziejewski & Robinson, 1993). The isolated BDNF/NT3 heterodimer yielded crystals suitable for structure determination from a 15 mg/mL protein solution and a reservoir solution of 10% w/v PEG 4000, 10% v/v 2-propanol, and 0.1 M sodium citrate, pH 5.6 at 18 °C. The crystals, which reached full size in 2−3 weeks, were obtained by the sitting drop vapor diffusion method (2 μL of protein solution + 2 μL of reservoir solution) using microbridges (Harlos, 1992).

Data Collection and Processing. Data were collected from three crystals at 18 °C using an 18 cm diameter MAR-research imaging plate system on either an in-house Rigaku RU200 rotating anode (Cu Kα X-rays) with a graphite monochromator and collimating slits set at 0.3 mm × 0.3 mm or at the Synchrotron Radiation Source (SRS) in Daresbury, U.K. (station 9.5), $\lambda = 0.99$ Å, limited by a 0.3-mm collimator. Exposure times were 20 min per 2° oscillation for the in-house collection and 1.5 min per 2° for the synchrotron images. Diffraction data were indexed, integrated, and corrected for polarization and Lorentz effects using the program DENZO (Otwinowski, 1993). Scaling and merging of intensities as well as the conversion to structure factors were carried out using the in-house program 3DSCALE (Stuart et al., 1979).

Molecular Replacement and Refinement. The structure was solved by molecular replacement using the NGF dimer as the search model in the program X-PLOR (Brunger, 1992). The rotation function followed by Patterson correlation refinement in the range 8.0−4.0 Å produced two solutions related by 180° with identical correlation coefficients of 21%, which were twice as large as the next best solution. The translation function, again in the range 8.0−4.0 Å, produced a solution with a correlation coefficient of 35%, which was 7σ above the mean. Simulated annealing refinement in X-PLOR (Brunger, 1992) against 8.0−3.0 Å resolution data (from 2000 to 200 K in 25 K decrements with 50 fs of dynamics at each temperature), with noncrystallographic restraints applied between the halves of the dimer, produced a model with an R value of 29% and an interpretable $2|F_o| - |F_c|$ electron density map in which the BDNF and NT3 protomers were clearly distinguishable. The correct amino acid sequence and a bulk solvent correction (using a solvent level of 0.34 $e^-/Å^3$, a solvent mask smeared by a B factor of 55 $Å^2$, and atomic radii for the protein mask inflated by 0.4 Å) were included, followed by further cycles of refinement (without the 2-fold noncrystallographic restraints) and manual rebuilding facilitated by FRODO (Jones, 1985).

Analysis. The program PROCHECK (Laskowski et al., 1993) was used to assess the final structure. Secondary structure assignments and solvent accessibilities were calculated using the program DSSP (Kabsch & Sander, 1983). Molecular superpositions were carried out using the program SHP (Stuart et al., 1979). Cavities were located using a probe radius of 1.2 Å in the program VOIDOO (Kleywegt & Jones, 1994). A variety of display programs were used to view the final structure: FRODO (Jones, 1985), MOLSCRIPT (Kraulis, 1991), O (Jones et al., 1991), RASTER3D (Merritt & Murphy, 1994), and RIBBONS (Carson, 1991).

RESULTS

Crystals. The heterodimer crystals showed diffraction to a resolution limit of 2.3 Å using synchrotron radiation (SRS Daresbury, Station 9.5). The crystals, typically 1.0 mm × 1.0 mm × 0.2 mm in size, are of space group $C2$ with unit cell dimensions $a = 98.4$ Å, $b = 45.1$ Å, $c = 68.0$ Å, $\alpha = \gamma = 90°$, and $\beta = 118.3°$ and contain one heterodimer in the asymmetric unit with approximately 50% by volume solvent. Table 1 includes the data collection statistics.

Present Model. The present model, which includes 1798 non-hydrogen atoms with 70 ordered water molecules and 1 ordered 2-propanol, has an R_c of 18.2% (refinement statistics are further detailed in Table 1) on all 11 691 reflections in the range 20−2.3 Å, with an rms Δ_{bonds} of 0.015 Å, and an rms Δ_{angles} of 1.89° [symbols as defined by X-PLOR (Brunger, 1992)]. The mean temperature factor is 45.8 $Å^2$ with isotropic B factors restrained such that the rms $\Delta_{Bbonds} = 4.35$ $Å^2$ and the rms $\Delta_{Bangles} = 6.65$ $Å^2$. The model consists of residues 8−116 in BDNF and residues 8−115 in NT3; there was no interpretable electron density beyond these regions. Examples of the electron density with the appropriate residues omitted from the phase calculation are shown in Figure 1. Electron density is good throughout the length of the main chain of BDNF and main-chain temperature factors only rise above 60 $Å^2$ in the mobile termini regions, residues 8−11 and 115−116, and in regions 44−47, 60−66, and 72−75. Electron density is good throughout the majority of the main chain of NT3 although poor in region 42−45 with a break in the main-chain density (1σ level) at Thr-43. NT3 main-chain temperature factors rise above 60 $Å^2$ in the regions corresponding to those which display high temperature factors in BDNF: in termini regions, residues 8−10 and 112−115, as well as in regions 41−48, 58−66, and 71−78. One non-glycine residue, Lys-26 in BDNF, lies in a disallowed region of the Ramachandran plot.

Structural Description and Comparisons. The topology of both BDNF and NT3 protomers is basically that of NGF

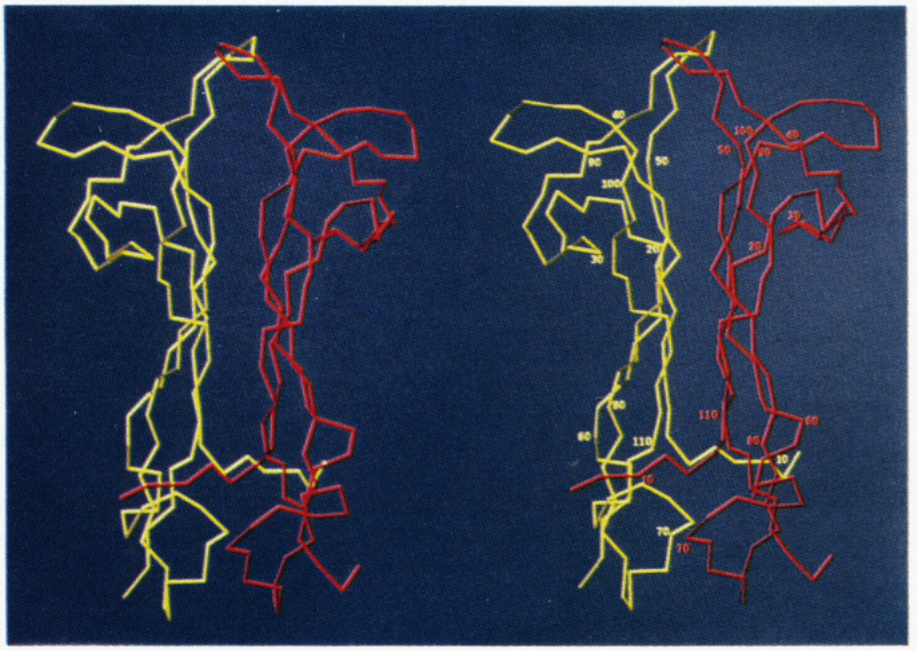


FIGURE 2: Cα stereodiagram with every tenth residue labeled, prepared using the program MOLSCRIPT (Kraulis, 1991). BDNF is shown in red and NT3 is illustrated in yellow.

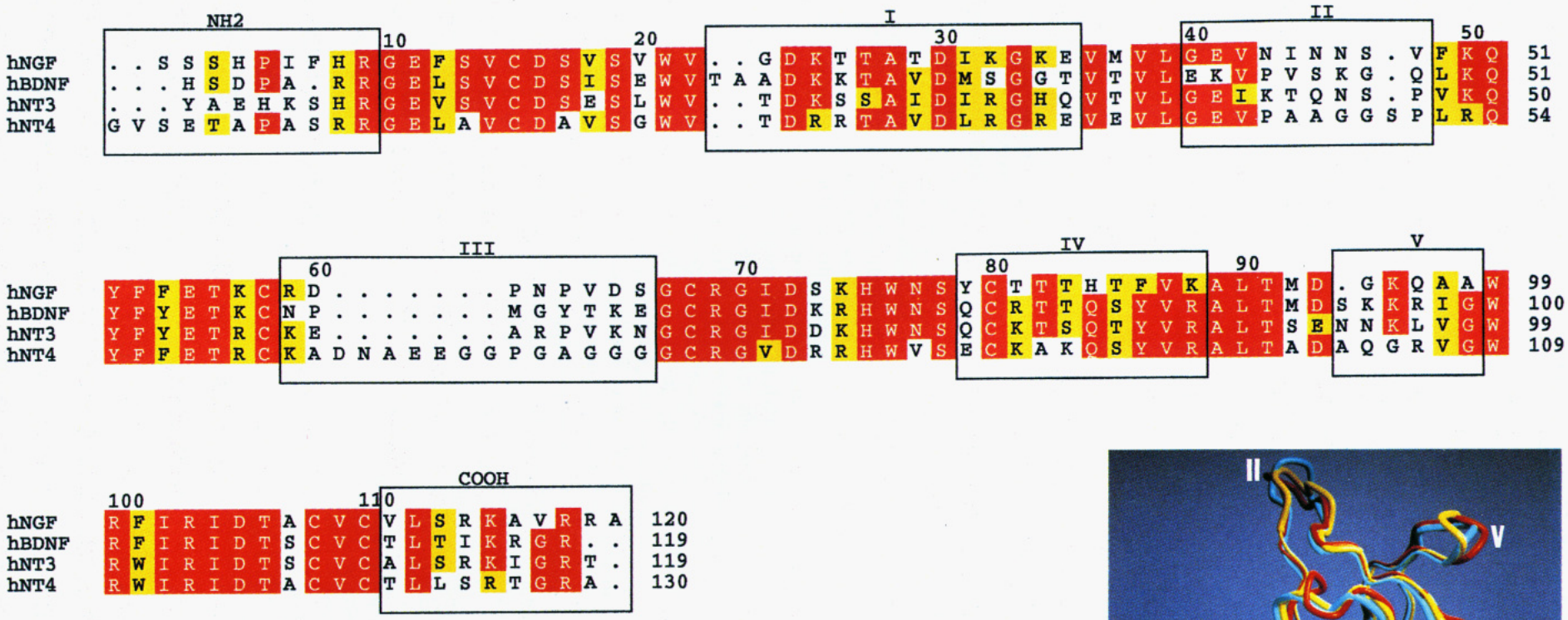
(McDonald et al., 1991), comprising seven β-strands which contribute to three antiparallel pairs of twisted β-strands (Figure 2). This extended structure is locked by a "cystine-knot" of three disulfides (McDonald et al., 1991; McDonald & Hendrickson, 1993). Sequence alignments of the neurotrophins (Figure 3a) reveal that in addition to the N- and C-termini there are five regions of high sequence variability (Ibanez et al., 1993). Only two of these translate into gross structural differences between the NGF, BDNF, and NT3 protomers. Region I (Figure 3) differs in BDNF, when compared to NGF and NT3, accommodating a two amino acid insertion by the formation of a turn of α-helix. Region III, in contrast, contains no sequence insertions or deletions but displays a quite different main-chain conformation in the case of BDNF, which includes a stretch of 3_{10} helix, when compared to NGF and NT3 (although these regions exhibit high crystallographic *B* factors in both BDNF and NT3, the general paths of the polypeptide chains are reliable). This region may be expected to display further variation in the case of NT4 in which there is a seven amino acid insertion. Molecular superpositions show that NT3 is closest in structural similarity to NGF (number of Cα equivalences = 104, rms distance = 0.99 Å) and BDNF is closest to NT3 (number of Cα equivalences = 102, rms distance = 1.07 Å) while BDNF and NGF are the least similar (number of Cα equivalences = 101, rms distance = 1.22 Å).

The perfect 2-fold rotation axis of the NGF homodimer is retained as far as is possible in the BDNF/NT3 heterodimer. The dimer interface is formed, as in NGF (McDonald et al., 1991), by hydrophobic interactions which occur mainly between residues in the β-strands (Figure 2). Superpositions of the BDNF, NT3, and NGF protomers reveal that these hydrophobic residues are structurally conserved, in line with the propensity of the neurotrophins to form heterodimers. The BDNF/NT3 heterodimer contains a closed cavity (Figure 4) at the dimer interface. This cavity is conserved in the NGF dimer, although in this case the cavity has a channel to the exterior of the protein. This difference in the accessibility of the cavities is reflected in the amount of surface area that is classified (by program DSSP; Kabsch & Sander, 1983) as buried on forming the dimers (2343 Å^2 for the NGF dimer in comparison to 3143 Å^2 for the BDNF/NT3 heterodimer).

DISCUSSION

Structural Implications. We have elucidated the structure of the BDNF/NT3 heterodimer by X-ray crystallography using the NGF homodimer (McDonald et al., 1991) as a molecular replacement search model. The BDNF, NT3 and NGF protomers show high structural similarity to each other. Within the context of the heterodimer structure, the BDNF and NT3 protomer structures can be used to form models for the BDNF and NT3 homodimers which show no stereochemical clashes. Hence, we would expect that comparison of the protomer structures for BDNF and NT3 in the heterodimer with their structures in the homodimers would reveal only minor adjustments to residues at the dimer interfaces with the positions of the solvent-exposed residues being unaltered. Thus the structures of the BDNF and NT3 protomers from the heterodimer represent good models for the BDNF and NT3 homodimers in the absence of structures for these molecules.

The heterodimer contains a cavity at the dimer interface (Figure 4) which is occupied by 12 ordered water molecules. This cavity is conserved in NGF, although in the case of NGF the cavity connects to two symmetrical open channels accessible to the exterior of the protein. The mouths of the channels in the BDNF/NT3 heterodimer are obscured due to a substitution of the larger arginine residue (Arg-88, BDNF; Arg-87, NT3) for lysine (Lys-88, NGF). Many of the ordered water molecules in the cavity are conserved between the BDNF/NT3 heterodimer and NGF. This ordering of water molecules in the cavity would presumably present an energy barrier to protein folding, and since the residues lining the cavity (see Figure 4 legend) are mainly hydrophobic and are conserved throughout the neurotrophin family, the cavity may well have a functional role. We



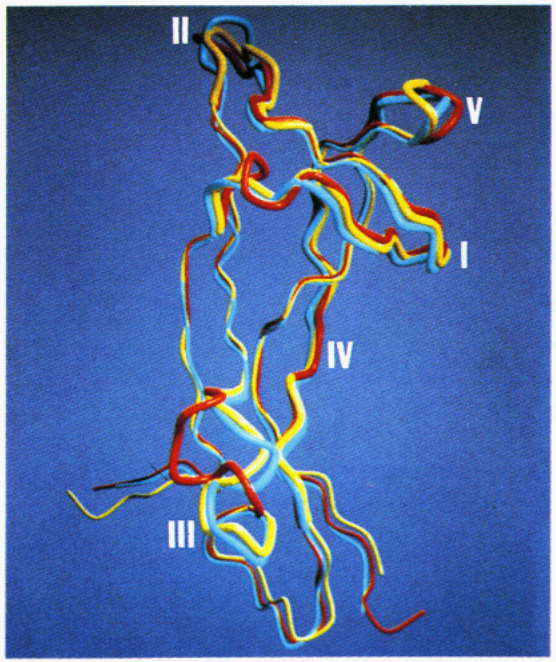


FIGURE 3: (a, top) Sequence alignments of human NGF, BDNF, NT3, and NT4. Conserved residues are painted red and conservative substitutions are shown in yellow. Large open boxes highlight regions of high sequence variance. The figure was prepared using the program ALSCRIPT (Barton, 1993). (b, bottom right) Cα superpositions of the mouse NGF (blue), human BDNF (red), and human NT3 (yellow) protomers. Regions of high sequence variance are represented by the thick Cα trace and referred to by Roman numerals as in Figure 3a. For clarity the orientation is rotated by approximately 90° about the vertical relative to Figure 2. The figure was prepared using the programs MOLSCRIPT (Kraulis, 1991) and RASTER 3D (Merritt & Murphy, 1994).

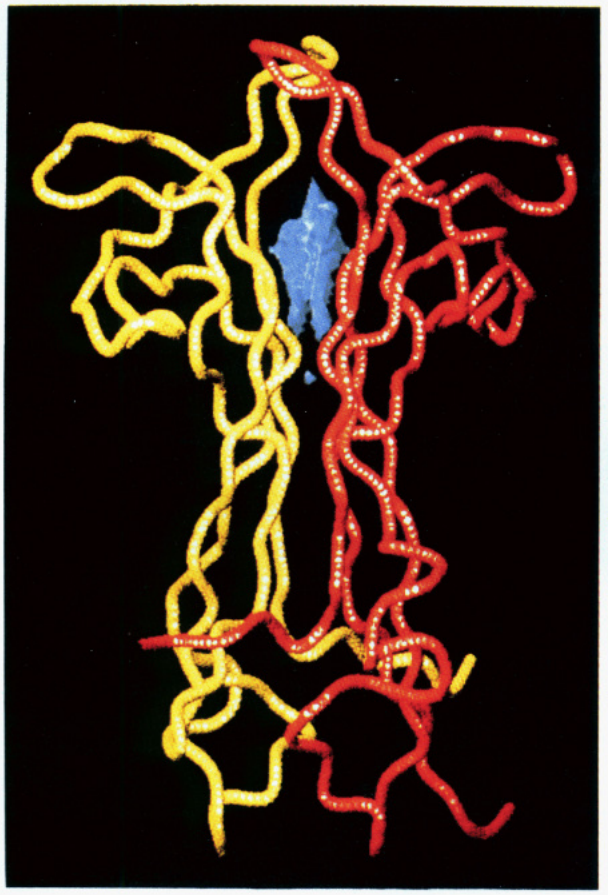


FIGURE 4: Schematic representation of the BDNF/NT3 heterodimer highlighting the cavity at the dimer interface (blue). BDNF is shown in red; NT3 is shown in yellow and the orientation is that used in Figure 2. The figure was prepared using the program O (Jones et al., 1991). Residues lining the cavity are Val-42, Leu-49, Lys-50, Gln-51, Tyr-52, Phe-46, Tyr-47, Val-87, Arg-88, Ala-89, Trp-100, Phe-102, and Ile-105 from BDNF and Ile-41, Gln-50, Tyr-51, Phe-52, Tyr-53, Tyr-85, Val-86, Arg-87, Ala-88, Trp-99, Trp-101, and Ile-104 from NT3.

would propose that the purpose of this cavity may be to facilitate structural change in the neurotrophins on binding their receptors. Such a structural change is consistent with circular dichroism data which show secondary structural changes on binding the neurotrophins to p75 (Timm et al., 1994). Furthermore, cavities have been implicated in functionally important structural reorganizations in other systems, e.g., the capsid of human rhinovirus 16 (Oliveira et al., 1993).

Heterodimers involving NGF are relatively unstable and slowly rearrange into their parent homodimers (Radziejewski et al., 1993). By overlaying the structure of NGF onto the BDNF/NT3 heterodimer, we were able to ascertain whether there may be any clashes expected between the BDNF and NT3 protomers in relation to NGF in a NGF-containing heterodimer. Surprisingly, the only repulsive interaction is between Tyr-79 on NGF and Glu-8 on BDNF (Glu-9 on NT3). This interaction is unlikely to be the cause of the instability, as NGF also has a glutamate at the equivalent sequence position (Glu-10). A small rotation of Glu-10 in NGF when compared to Glu-8 (BDNF) and Glu-9 (NT3) avoids this unfavorable interaction with Tyr-79. Hence there is no obvious structural determinant, from the structures of the NGF homodimer and the BDNF/NT3 heterodimer, to explain the instability of the NGF-containing heterodimers.

Functional Implications. Mutagenesis studies on the neurotrophins have identified residues that are involved in p75 receptor binding. Basic residues in the variable regions I and V on NGF and BDNF (Ibanez et al., 1991, 1992) are implicated in binding this acidic receptor [estimated p*I* 4.4 (Radeke et al., 1987)]. NT3 and NT4 are expected to interact in a similar manner. When related to the heterodimer structure, these residues do indeed represent a conserved region of surface charge which is spatially localized in any one protomer but distant from the equivalent region in the second protomer of the dimer (Figure 5a). Thus the p75 receptor is likely to bind independently to each half of the dimer, allowing the interaction of one heterodimer with two p75 molecules (Grob & Bothwell, 1983) in a fashion similar to that of the parent homodimers.

Mutagenesis data indicate that the trk receptors interact with a more extensive binding site on the neurotrophins than is seen for p75 binding, including residues in variable regions I, II, IV, and V (Ibanez et al., 1991, 1992, 1993). These studies have revealed some overlap in the binding sites on NGF and BDNF between the trk receptors and p75. In particular, Ile-31, Glu-41, Asn-45, Tyr-79, Thr-81, and His-84 and residues 94−98 on NGF are implicated in binding to trkA, while trkB has been shown to bind to residues 45−49 and Gln-84, Lys-96, and Arg-97 on BDNF. The N-terminal region of NGF (residues 3−9), but not BDNF, has also been shown to be important for trk binding although not for activation. The identified residues do not lie in the two major regions of main-chain structural variation. Unlike the case of p75 binding, the residues implicated in trk receptor binding do not segregate into a spatially distinct cluster on each protomer. Regions I and V are in close proximity on one protomer to region II from the second protomer (Ibanez et al., 1992), and hence both protomers are likely to contribute to each receptor binding site (Figure 5b). Clearly, in this case, the heterodimer presents two nonidentical receptor binding sites, each of which displays a different selection of surface residues to those forming the binding sites of the homodimers.

These chimeric binding sites are consistent with BDNF/NT3 heterodimer activity (Jungbluth et al., 1994). The BDNF/NT3 heterodimer has been reported to be active in chick nodose and dorsal root ganglia assays, to the same extent, albeit with approximately a 10-fold lower activity, than a 1:1 mixture of BDNF and NT3 homodimers. Furthermore, the heterodimer displays a 9-fold lower activity on chick neurons from the trigeminal mesencephalic nucleus (neurons which are responsive to both BDNF and NT3) than a 1:1 mixture of BDNF and NT3. Hence, the heterodimer displays partial trk binding characteristics of both protomers.

Other members of the cystine knot superfamily (McDonald & Hendrickson, 1993) are known to form functionally distinct heterodimers that in some cases have been shown to bind to discrete receptors [e.g., platelet-derived growth factor AB (Heidaran et al., 1991), transforming growth factor β 1.2, bone morphogenic proteins, activins, inhibins (Massague, 1990), and chorionic gonadotrophin (Wu et al., 1994; Lapthorn et al., 1994)]. Indeed, in the case of activins, inhibins, and chorionic gonadotrophin, heterodimers play a major functional role. The fact that the neurotrophins have preserved the ability to form both homodimers and heterodimers interchangeably, during a period of divergence of

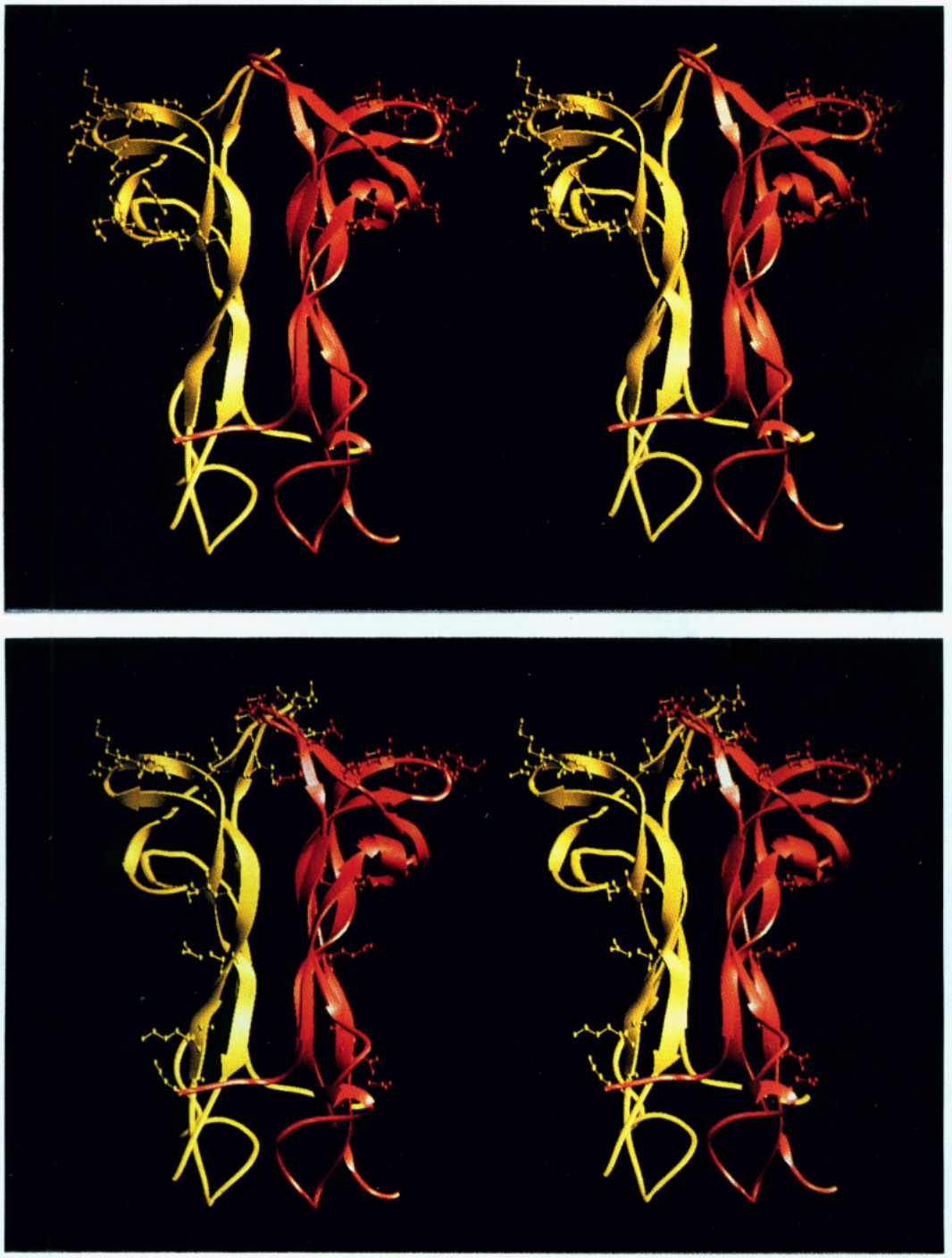


FIGURE 5: Comparison of residues implicated in receptor binding, imposed on a schematic representation of the structure of the BDNF/NT3 heterodimer, where BDNF is shown in red and NT3 is illustrated in yellow. The orientation is that shown in Figure 2. The figure was prepared using the program RIBBONS (Carson, 1991). (a, top) The p75 receptor. Here residues Ile-31, Lys-32, Lys-34, Glu-35, and Lys-95 on NGF and residues 94−98 on BDNF are implicated in binding p75 (Ibanez et al., 1991, 1992). Equivalent residues are highlighted on the heterodimer structure. (b, bottom) The trk receptors. In this case residues Ile-31, Glu-41, Asn-45, Tyr-79, Thr-81, and His 84 and residues 94−98 on NGF are implicated in binding to trkA, while trkB has been shown to bind to residues 45−49, Gln-84, Lys-96, and Arg-97 on BDNF (Ibanez et al., 1991, 1992, 1993). The equivalent residues have been mapped onto the heterodimer structure.

other properties, implies that both homo- and heterodimers could be functionally important *in vivo*. Jungbluth et al. (1994) suggested that the role of the neurotrophin heterodimers is to regulate the output of neurotrophins by forming the less active heterodimers. An extension to this notion is that the heterodimers may provide a mechanism for neurotrophin dominance switching. Overexpression of one neurotrophin in comparison to another by a single cell would reduce the effectiveness of the minor neurotrophin by sequestering it as the less active heterodimer. Precisely, such changes in neurotrophin expression, as well as changes in the neurotrophin requirements of specific neurons for survival, have recently been reported (Davies, 1994b). Alternatively, there may be a subset of neurons which can recognize the message of the heterodimers, possibly through trk heterodimers, providing the neurotrophin heterodimers with distinct roles from the neurotrophin homodimers. Further studies are required to determine whether these "manufactured" neurotrophin heterodimers do indeed have biological relevance.

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