Critical Interactions at the Dimer Interface of κ-Bungarotoxin, a Neuronal Nicotinic Acetylcholine Receptor Antagonist†

Gregory A. Grant,* Regina Al-Rabiee, Xiao Lan Xu, and Yueping Zhang

Departments of Medicine and Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, Missouri 63110

Received December 4, 1996; Revised Manuscript Received January 23, 1997⊗

ABSTRACT: The α- and κ-neurotoxins are polypeptide antagonists of nicotinic acetylcholine receptors derived from snake venom. They are structurally very similar but differ in their specificity for receptor subtype and in their native aggregation state. While the α-neurotoxins are monomeric, the κ-neurotoxins occur as homodimers. The crystal structure shows that there is a correlation in the distance between essential arginine residues in the κ-bungarotoxin dimer and the distance between the acetylcholine binding sites in the pentameric receptor. This has lead to an investigation of the critical interactions at the dimer interface of κ-bungarotoxin. Mutations of residues that the crystal structure indicates participate in dimer interaction were found to fall into two general groups: those that do not affect the dimerization state or activity of κ-bungarotoxin as single mutants, and those that interfere with it to such an extent that the protein is no longer able to fold properly. In general, those residues that fall into the latter group are found to be invariant in κ-neurotoxins and not found in α-neurotoxins. The results suggest that the extent of both the main chain−main chain β-sheet hydrogen bond interaction and van der Waals interactions between Phe 49 and Ile 20 are required for dimer formation. These studies provide a basis for understanding why the κ-neurotoxins readily dimerize in solution and the α-neurotoxins do not and also suggest that there is a possible interrelationship between dimer formation and protein folding in κ-bungarotoxin.

The α- and κ-neurotoxins comprise a large family of snake venom derived nicotinic acetylcholine receptor antagonists (Endo & Tamiya, 1991). In general, the α-neurotoxins display selectivity for the mammalian muscle end-plate receptor and fish electric organ receptor while the κ-neurotoxins display selectivity for receptors of neuronal origin (McGehee & Role, 1995). The α- and κ-neurotoxins are structurally similar proteins, both being composed of three main chain loops containing four or five disulfide bonds (Endo & Tamiya, 1991; Dewan et al., 1994; Falkenstein et al., 1996). In all of these structures, the two strands of the central loop and one strand of the third loop form a three-stranded antiparallel β-sheet. One striking element of structure that distinguishes the κ-neurotoxins is the fact that they exist as dimers in solution, whereas the α-neurotoxins are monomeric (Chiappinelli & Lee, 1985; Oswald et al., 1991; Fiordalisi & Grant, 1995). Dimer formation in κ-bungarotoxin (Dewan et al., 1994) results in an extension of the three-stranded β-sheet of the monomer to a six-stranded β-sheet in the dimer.

It has long been a subject of discussion as to whether or not the dimeric nature of κ-bungarotoxin plays a role in the differential specificity of the toxin. The potential relevance of this question has increased with a recent observation from the crystal structure which shows that the guanidine groups of the essential arginine residues found in the turn region of the central loops of the toxin dimer are nearly identical in distance from each other as are the acetylcholine binding sites in the pentameric receptor (Herz et al., 1989; Unwin, 1993; Dewan et al., 1994). This raises the possibility that κ-bungarotoxin, because it is a dimer, may interact simultaneously with each of the two acetylcholine binding sites on a single receptor and possibly, by spanning the channel, physically block ion flow. While this scenario may explain why the monomeric α-neurotoxins do not block the neuronal receptor, it leaves open the question of why the κ-neurotoxins do not significantly block the muscle end-plate receptor, unless of course their dimeric nature interferes with binding or access to the binding site.

In order to begin to address the significance of the dimer interface in the activity of the κ-neurotoxins, this investigation presents a systematic study of the contribution of specific amino acid residues found at the interface. Moreover, this investigation provides information on the nature and stability of the interface interactions and establishes a basis on which to explain why the α-neurotoxins do not dimerize in solution.

MATERIALS AND METHODS

Native and mutant κ-bungarotoxins were expressed in *Pichea pastoris* and isolated as previously described (Fiordalisi et al., 1996). Multiple colonies from a single yeast transformation were screened for toxin aggregation characteristics by growing 5 mL cultures inoculated with each colony and isolating the immunoreactive material on Waters C-18 reverse phase Sep-Pak cartridges using a protocol similar to that for the reverse phase column used in large-scale purification (Fiordalisi et al., 1996). Mutagenesis was performed either by the cassette method or by PCR (Cor-

† Supported by a grant from the National Institutes of Health (GM 50624).

* Author to whom correspondence should be addressed at the Department of Molecular Biology and Pharmacology, Box 8103, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110. FAX: 314-362-4698. E-mail: ggrant@pharmdec.wustl.edu.

⊗ Abstract published in *Advance ACS Abstracts,* March 1, 1997.

S0006-2960(96)02968-6 CCC: $14.00

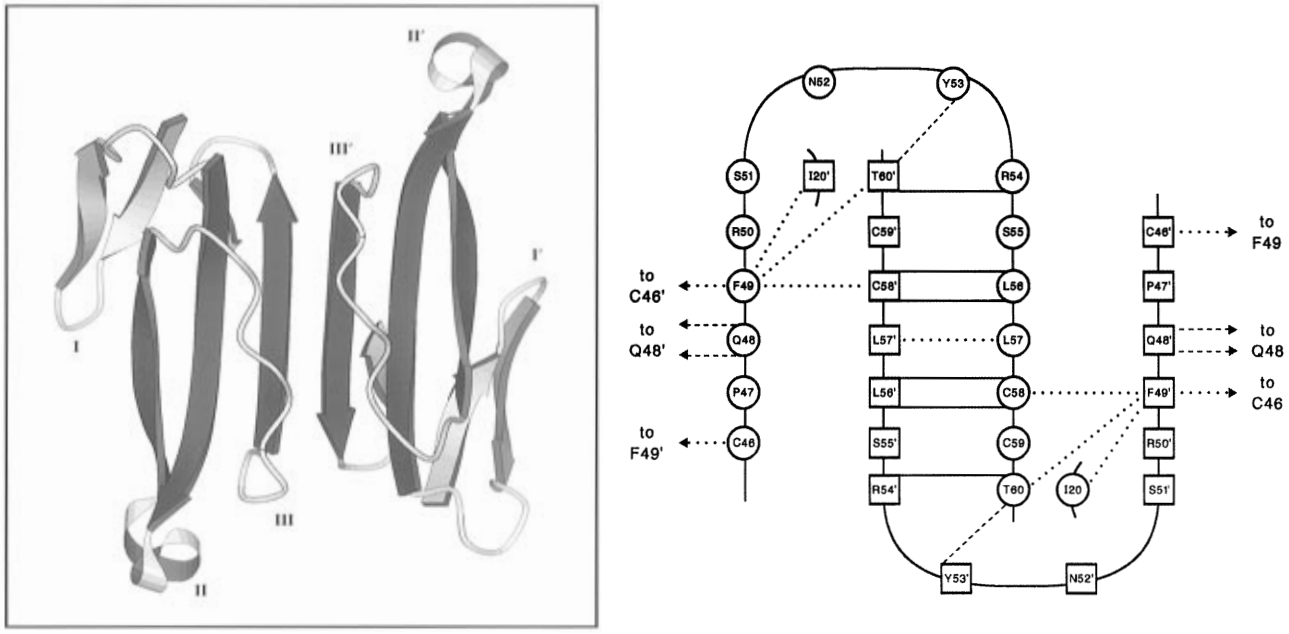

FIGURE 1: Dimeric structure of κ-bungarotoxin and the interactions across the dimer interface. The panel on the left shows a ribbon diagram of the structure of κ-bungarotoxin from crystal coordinates (produced with MOLSCRIPT; Kraulis, 1991). β-Sheet structure is indicated by broad arrows. The loops mentioned in the text are designated with Roman numerals. A prime (′) distinguishes one subunit from the other. The panel on the right shows a schematic diagram indicating the dimer interactions. The strands from R54 to T60, depicted in the center of the diagram, correspond to the two β-sheet strands of loop III and loop III′. The outside strands, from C46 to S51, correspond to the non-β-sheet strands of loop III (and III′). Main chain−main chain hydrogen bonds are depicted as solid lines, hydrogen bonds involving at least one side chain are depicted with dashed lines, and van der Waals interactions are depicted with dotted lines.

mack, 1991). All PCR reagents were obtained from Perkin-Elmer, and restriction fragments were isolated from agarose gels with the Gene Clean kit from Bio 101. All mutations were confirmed by Sanger dideoxy sequencing which was performed with the Sequenase version 2.0 sequencing kit from United States Biochemical. All mutations were visualized using the BIOPOLYMER program of SYBYL from Tripos Inc. (St. Louis, MO) using the crystal structure coordinates on an IRIS molecular graphics system. Energy minimization simulations were performed with MAXIMIN 2 utilizing the tripos force field.

Toxin proteins were characterized by SDS−polyacrylamide gel electrophoresis and Western blot analysis with rabbit anti-κ-bungarotoxin antisera (Fiordalisi et al., 1992). Circular dichroism analysis was performed on a Jasco J-600 spectropolarimeter. Secondary structure was qualitatively assessed by a comparison to CD spectra of proteins with known content and to that of native venom-derived κ-bungarotoxin. The β-sheet structure of κ-bungarotoxin is characterized by a spectrum that has a single minimum between 210 and 225 nm and a stronger positive maximum between 190 and 200 nm (Venyaminov & Yang, 1996; Chiappinelli et al., 1988). HPLC-based gel filtration chromatography was performed with a 7.5 mm × 60 cm TSK G2000SW column pumped isocratically at 0.5 mL/min with 50 mM sodium phosphate, pH 6.5, 100 mM sodium chloride. The column was calibrated with Bio-Rad gel filtration standard mixture 151-1901 containing standards of 1.35, 17, 44, 158, and 670 kDa. In addition, the column was calibrated with venom-derived α-bungarotoxin from Biotoxins Inc. (St. Cloud, FL) and recombinant κ-bungarotoxin produced in *P. pastoris*. Protein concentration was determined with a Waters Aminoquant amino acid analysis system. Biological activity was assayed using a chick ciliary ganglia system as previously described (Chiappinelli et al., 1990; Fiordalisi et al., 1991).

RESULTS

The crystal structure of κ-bungarotoxin (Dewan et al., 1994) indicates a number of molecular interactions at the interface of the dimer. These interactions, which are depicted in Figure 1, consist of main chain−main chain, main chain−side chain, and side chain−side chain hydrogen bonds as well as several van der Waals interactions. Arg 54 is a conserved cationic residue insertion found just before the turn of the third loop in κ-neurotoxins that is not present in the α-neurotoxins. Because this residue is deleted in the α-neurotoxins, their shortened polypeptide strand decreases the potential for main chain hydrogen bonds across the interface from six to four. Of the residues whose side chains interact across the dimer, Phe 49, Leu 57, and Ile 20 are strictly conserved in κ-neurotoxins and not found in α-neurotoxins (see Figure 2). Thr 60 is not conserved in κ-neurotoxins, and while Gln 48 is not usually found in the α-neurotoxins, it is not strictly conserved in the κ-neurotoxins either.

Mutagenesis of Interface Residues. A series of mutations, based on the above observations in the crystal structure, were made to explore the role of these residues at the dimer interface of κ-bungarotoxin. The mutations tested are listed in Table 1 along with their characteristics. In all but one case, residue side chains were converted to alanine side chains. ΔR54 indicates a deletion where the arginine residue at position 54 was completely removed, resulting in a polypeptide chain shortened by one amino acid as is found in the α-neurotoxins.

All of the mutants introduced into the gene for κ-bungarotoxin produced immunoreactive material when expressed

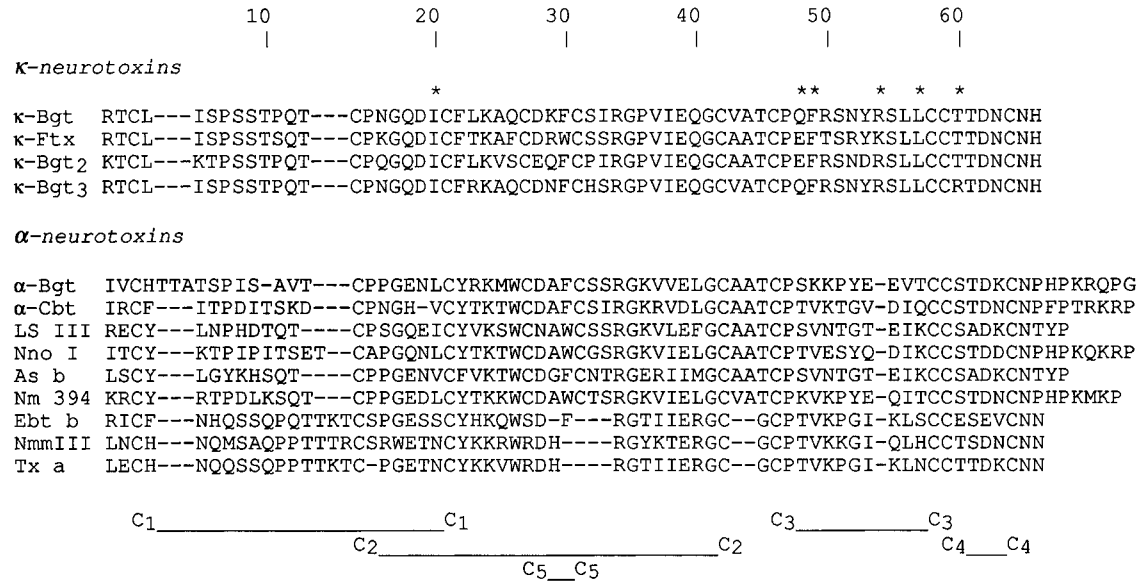

FIGURE 2: Sequence alignment of the four known κ-neurotoxins and a few representative α-neurotoxins. Numbering is according to the sequence of κ-bungarotoxin. Asterisks show the locations of the mutated residues, and the conserved disulfide linkage pattern is shown below the sequences. κ-Bungarotoxin from *Bungarus multicinctus*, Taiwan (κ-Bgt), κ-flavitoxin from *Bungarus flaviceps* (κ-Ftx), and κ-bungarotoxins 2 and 3 from *Bungarus multicinctus*, China (κ-Bgt_2 and κ-Bgt_3), are κ-neurotoxins. α-Bungarotoxin from *Bungarus multicinctus* (α-Bgt), α-cobratoxin from *Naja naja siamensis* (α-Cbt), toxin LS III from *Laticauda semifasciata* (LS III), neurotoxin I from *Naja naja oxiana* (Nno I), toxin b from *Astrotia stokesii* (As b), toxin 3.9.4 from *Naja melanoleuca* (Nm 394), erabutoxin b from *Laticauda semifasciata* (Ebt b), toxin III from *Naja mossambica mossambica* (NmmIII), and toxin a from *Naja nigricollis* (Tx a) are α-neurotoxins.

Table 1: Properties of Native and Mutant κ-Bungarotoxins

κ-Bgt	MW, SDS gel predominant species	MW, gel filtration	association state[a]	CD spectra, fold	active[b]
native	∼7000	∼14000	dimer	β-sheet	yes
R54A	∼7000	∼14000	dimer	β-sheet	yes
T60A	∼7000	∼14000	dimer	β-sheet	yes
Q48A	∼7000	∼14000	dimer	β-sheet	yes
L57A	∼7000	∼14000	dimer	β-sheet	yes
ΔR54	disperse[c]	disperse[d]	aggregate	random	nd[e]
Q48A, F49A	disperse	disperse	aggregate	random	nd
F49A	disperse	disperse	aggregate	random	nd
I20A, T60A	disperse	disperse	aggregate	random	nd
I20A	disperse	disperse	aggregate	random	nd

[a] The SDS gel and gel filtration data indicate that the dimer is noncovalent. The aggregated state consists of a disperse population of higher molecular weight disulfide-linked multimers. [b] Produces a complete block in the chick ciliary ganglia at 300 nM dimer. [c] The predominant species is a broad band migrating from a position corresponding to approximately 14 000 daltons to the top of the gel. [d] The majority of protein elutes as a broad peak with material at elution positions corresponding to between 7000 and 160 000 daltons. [e] Not determined.

in *P. pastoris*. After initial isolation by chromatography on a C-18 reverse phase column, the immunoreactive material was analyzed by SDS−polyacrylamide gel electrophoresis and Western blotting. Multiple colonies from each mutant transformation were screened to assure uniformity of the characteristics of the expressed product for a particular mutation. Additional characterization of the expressed product from a single colony was performed by gel filtration chromatography, circular dichroism spectroscopy, and, when appropriate, a bioassay for activity using chick ciliary ganglia as previously described. This combination of techniques allowed the determination of the approximate size of the dissociated monomeric species, the approximate size of the associated state under nondenaturing conditions, if any, the presence of secondary structure, and the ability of the product to inhibit nerve impulse propagation in ganglia.

Table 1 demonstrates that the mutants fall into two groups: those that express and fold into a structure basically indistinguishable from native κ-bungarotoxin, and those that express but fail to fold properly. Representative data from each technique, which is summarized in Table 1, are shown in Figures 3−5.

The first group consists of those mutants which exist predominantly as a monomer of approximately 7 000 daltons in the dissociated state (Figure 3) and comigrate with unmutated κ-bungarotoxin. Some higher molecular weight immunoreactive material is seen, but it is present in far less quantity than in the second group (see below). This first group also exist as dimers of approximately 14 000 daltons in solution under nondenaturing conditions (Figure 4), yield CD spectra indicative of β-sheet structure and essentially identical to that of native κ-bungarotoxin (Figure 5), and possess full activity in the ganglion assay. Native κ-bungarotoxin displays an IC_{50} in this assay of approximately 100 nM dimer and complete block after 60 min between 200 and 300 nM dimer (Fiordalisi et al., 1994). Single concentration assays were performed which demonstrated approximately equivalent activity. In other words, this group is essentially indistinguishable from native κ-bungarotoxin at the level of resolution of these analyses. The second group consists of a diffuse population of immunoreactive protein that migrates as a broad zone of higher molecular weight species that predominantly ranges from approximately 14 000 daltons and larger in the dissociated state (Figure 3). Since the protein is expressed as a 69 residue polypeptide chain, this pattern indicates the presence of higher molecular weight covalent aggregates. These aggregates can be partially reversed by treatment with DTT (not shown), indicating that they are formed by intermolecular disulfide cross-linking. In addition, the protein products in this group display a range

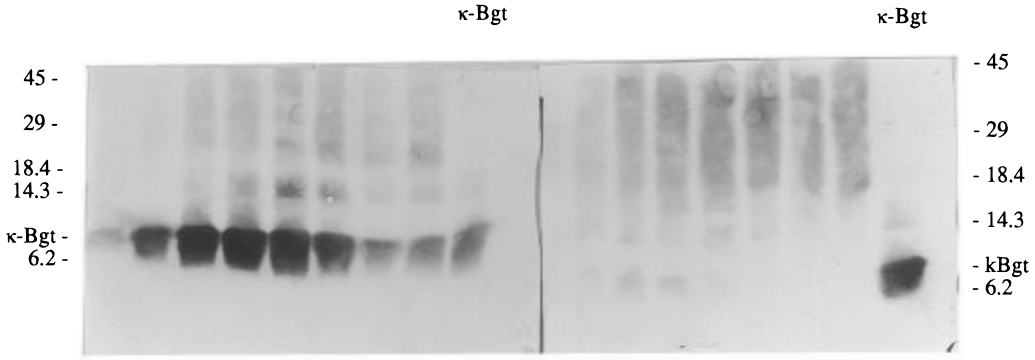

FIGURE 3: Western blots of SDS gels of the immunoreactive protein products from successive fractions of the reverse phase column. Two mutant constructs, L57A (left panel) and I20A (right panel), are shown as being representative of the two groups discussed in the text. The lane at the far right of each panel, labeled κ-Bgt, contains a nonmutated κ-bungarotoxin standard. The proteins were run on a 15% acrylamide gel so that a molecular mass standard of approximately 43 000 daltons runs just into the top of the separation gel. Standards are ovalbumin (43 000), carbonic anhydrase (29 000), β-lactoglobulin (18 400), lysozyme (14 300), and bovine trypsin inhibitor (6200).

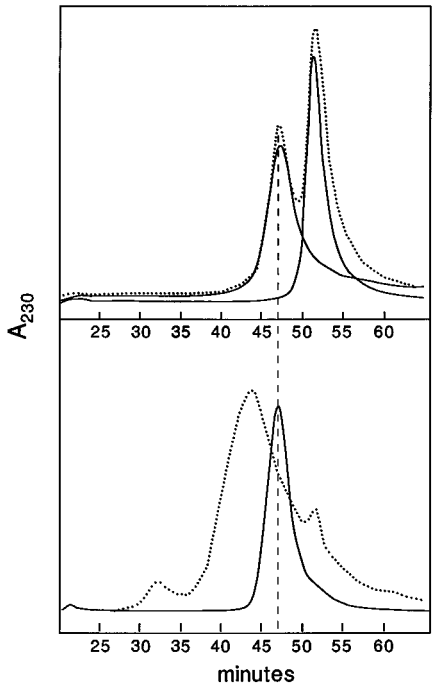

FIGURE 4: Gel filtration profiles of native and mutant neurotoxins. The top panel shows the elution positions of α- and κ-bungarotoxin. α-Bungarotoxin, a monomer of approximately 8000 daltons, elutes at approximately 52 min, and κ-bungarotoxin, a dimer of approximately 15 200 daltons, elutes at approximately 47 min. The solid line is the absorption of each protein run separately, and the dotted line shows the separation when both are run together as a mixture. The bottom panel shows the elution profiles of the R54A and F49A mutant proteins. The peak shown as a solid line eluting at 47 min and corresponding to the elution position of κ-bungarotoxin is R54A. The broadly eluting material shown with a dotted line is F49A. All members of each respective group discussed in the text show nearly identical elution behavior within their group.

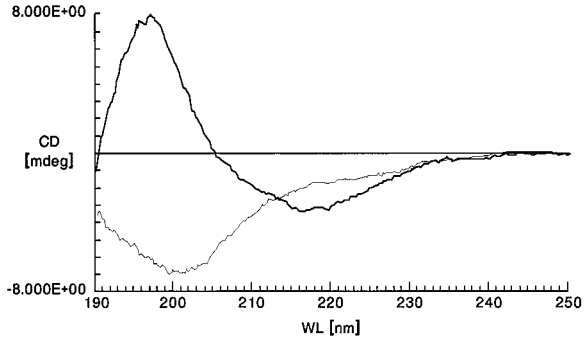

FIGURE 5: Circular dichroism spectra of T60A and F49A. The spectrum shown as a thicker line and with an optimum at approximately 198 nm is T60A. Its spectrum is virtually identical to that of κ-bungarotoxin and indicates the presence of predominantly β-sheet structure. The spectrum shown as the thinner line and with a minimum at approximately 200 nm is F49A. Its spectrum is very similar in appearance to that of a polypeptide that is predominantly random coil. All members of each respective group discussed in the text show nearly identical spectra within their group.

of size in solution consistent with the molecular weight distribution of the SDS gels (Figure 4) and display CD spectra suggestive of a random conformation (Figure 5). Activity assays were not performed on this group because past experience has indicated that toxin protein displaying these physical properties is always inactive. Some material which migrates at the approximate position of active toxin (approximately 7 000 daltons on SDS gels and approximately 14 000 daltons on gel filtration) is evident in this group of proteins. However, when isolated, this material displays CD spectra indicative of a random conformation (i.e., absence of β-sheet) and remains monomeric under nondenaturing conditions.

DISCUSSION

The group of mutants that consist of R54A, T60A, Q48A, and L57A all yield products that are biologically active and form dimers in solution that display the characteristic β-sheet conformation found in native κ-bungarotoxin. Thus, although the crystal structure indicates that these residues probably contribute to the interaction across the dimer interface, mutation of any one by itself is insufficient to disrupt the dimer. Moreover, the cationic and hydrophobic side chains of Arg 54 and Leu 57, respectively, although conserved in the κ-neurotoxins, do not appear to be necessary for conformation or activity. The side chains of Thr 60 and Gln 48 are not conserved in the κ-neurotoxins and also are not required.

The group of mutants that consist of ΔR54, F49A, and I20A, on the other hand, appear to promote a significant disruption in the protein structure. The disruption caused by these mutants results in the apparent lack of ability of the protein to fold into an active structure, even as a monomer.

The removal of Arg 54 shortens the β-sheet-forming strand of loop III of the protein. Molecular graphics simulation

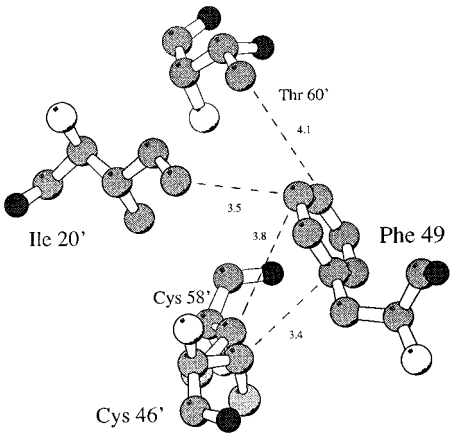

FIGURE 6: Representation of the major hydrophobic interactions at the dimer interface. The figure is produced from the crystal coordinates with Molscript (Kraulis, 1991), and the distances are shown in angstroms. The prime (′) designates residues from the adjacent subunit. Carbon atoms are in light gray, oxygen atoms are in dark gray, and nitrogen atoms are unshaded.

and energy minimization analysis of this excision product indicate that the resulting main chain carbonyl and amide groups, now consisting of Tyr 53-CO and Thr 60-NH, would be approximately 4−4.7 Å away from each other as opposed to 3.3 Å in the native Arg 54-CO and Thr 60-NH interaction. Thus, shortening of the strand has the potential of reducing the hydrogen bonds in the strand from six to four. Additional disruption of the Thr 60−Tyr 53 interaction may also occur.

Phe 49 from one subunit and Ile 20, Thr 60, and the disulfide of Cys 46 and Cys58 from the other subunit appear to interact by van der Waals contact (Figure 6). Because of the 180° symmetry of the two subunits, there are two pairs of these groups present at the interface. Mutation of the cysteine residues was not attempted because of its potential for disrupting the structure in its own right. However, of the remaining residues, the interaction between Phe 49 and Ile 20 appears to be critical. The requirement for Phe 49 is not surprising since it is the only residue contributed by one of the subunits, but on the other subunit, Ile 20′ appears to provide the critical interaction. Inspection of the α- and κ-neurotoxin sequence indicates that both Phe 49 and Ile 20 are absolutely invariant in κ-neurotoxins. Conversely, Phe 49 is never found in α-neurotoxins, and the position corresponding to Ile 20 is occupied by a variety of different residues in the α-neurotoxins.

These results suggest that the extent of both the main chain−main chain β-sheet hydrogen bond interaction and the van der Waals interactions between Phe 49 and Ile 20 are required for dimer formation since disruption of either one appears to result in an inability to produce active protein. Furthermore, the observation that a properly folded monomer was never produced suggests that there is a possible interrelationship between dimer formation and protein folding in κ-bungarotoxin. In other words, these data raise the possibility that initial interactions at these regions of two polypeptide chains may act as a nucleation site for folding of the remainder of the molecular dimer.

In this regard, κ-bungarotoxin displays certain similarities to the Arc protein recently described by Milla and Sauer (1995). Arc is a 53 residue, homodimeric, DNA binding protein whose dimerization is mediated by hydrophobic interactions at the dimer interface, particularly between two residues, Ile 37 and Val 41′ (and Val 41 and Ile 37′). Of the 64 permutations constructed for these residues, only the wild-type residue combination was recovered as fully active protein when expressed in *Escherichia coli*. Moreover, only seven additional conservative mutations were stable enough to be expressed and then were only partially active. The remainder of the mutants attempted showed no detectable intracellular expression. The authors suggested that this may be due to rapid intracellular degradation of the unstable conformations. All of the genetic constructs of κ-bungarotoxin were expressed as isolatable material in the *P. pastoris* expression system. The stability of these products to degradation may be due to the fact that they tend to form aggregates due to incorrect, intermolecular disulfide formation. Nevertheless, the dependence on a limited number of hydrophobic interactions at the dimer interface for proper folding is strikingly similar. Milla and Sauer concluded that their data were consistent with a model in which the hydrophobic residues are involved in loose interactions formed in the transition state but also participate in the tight packing of the core which occurs later in folding. This conclusion is consistent with the earlier observation that the rate-determining step for Arc refolding involves dimerization (Milla & Sauer, 1994). The dimer interface of κ-bungarotoxin may provide a similar situation. In this case, disruption of the folding process brought on by certain mutations appears to result in the trapping of the unfolded or partially folded polypeptide in the form of disulfide-linked aggregates rather than the unstable, rapidly degraded monomeric polypeptide chains in the case of Arc.

These data are also consistent with the observations of Kelly and colleagues (Graciani et al., 1994) that hydrophobic groups play a significant role in drawing peptide strands together to form β-sheets. In fact, they demonstrated that hydrogen bonding in the presence of a hydrophobic nucleator can hold β-sheet strands together that have no additional side chain hydrophobic interactions. In the case of κ-bungarotoxin, both the hydrophobic interaction and the β-sheet hydrogen bonds appear to be necessary for a stable dimer. The association at the level of the β-sheet is stabilized by the hydrophobic contact, but the hydrophobic contact is not strong enough in itself without a minimal number of hydrogen bonds at the interface.

These studies provide a basis for understanding why the κ-neurotoxins readily dimerize in solution and the α-neurotoxins do not. Moreover, they suggest that the dimerization may play a role in the folding of the κ-neurotoxins. That raises the question of why the monomeric α-neurotoxins, which are structurally quite similar to the κ-neurotoxins in their mature form, fold in the absence of a similar interface interaction. One possibility would be that the two fold with different mechanisms. For example, the core interactions in the α-neurotoxins may be stronger than in the κ-neurotoxins such that monomeric intermediates along the folding pathway are sufficiently stable to proceed to the mature form. Weaker core interactions in the κ-neurotoxins may not be sufficient and require additional stabilization brought about by the intermolecular contacts. Another possibility would be that the two fold with similar mechanisms, that is, by nucleation involving the same general areas, but the interface interaction in the final folded state of the α-neurotoxins is not strong enough to maintain the molecules in a dimeric state. In this regard, it is curious to note that the α-neuro-

toxins pack into the crystalline state with a similar 2-fold symmetry to the κ-bungarotoxin dimer involving contact along the third loops of the adjacent monomers.

If the dimer is the active unit of the κ-neurotoxins, it suggests that they may inhibit receptor function by a different mechanism than the α-neurotoxins. This is consistent with several studies (McLane et al., 1990a,b) that have indicated that the two classes of toxins interact with different residues on the α-subunit of their receptors. It is too early to speculate whether this will have significant implications in the use of these toxins as neuronal receptor probes or as models for developing inhibitors with different receptor specificity, but additional studies are planned to address these questions.

ACKNOWLEDGMENT

We thank Mr. Roger Summers for excellent technical assisitance and Dr. Vincent Chiappinelli and Ms. Kathleen Wolf for performing the chick ciliary ganglia assays.

REFERENCES

Chiappinelli, V. A., & Lee, J. C. (1985) *J. Biol. Chem. 260*, 6182−6186.

Chiappinelli, V. A., Dryer, S. E., Sorenson, E. M., Wolf, K. M., Grant, G. A., Chen, S.-J., Nooney, J. M., Lambert, J. J., & Hider, R. C. (1988) in *Nicotinic Acetylcholine Receptors in the Nervous System* (Clementi, F., Ed.) pp 15−29, Springer-Verlag, Berlin.

Chiappinelli, V. A., Wolf, K. M., Grant, G. A., & Chen, S.-J. (1990) *Brain Res. 509*, 237−248.

Cormack, B. (1991) in *Current Protocols in Molecular Biology* (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., & Struhl, K., Eds.) pp 8.5.1−8.5.9, John Wiley and Sons, New York.

Dewan, J. C., Grant, G. A., & Sacchettini, J. C. (1994) *Biochemistry 33*, 13147−13154.

Endo, T., & Tamiya, N. (1991) in *Snake Toxins* (Harvey, A. L., Ed.) pp 165−222, Pergamon Press, New York.

Falkenstein, R. J., Pena, C., & Biscoglio de Jimenez Bonino, M. J. (1996) *Int. J. Pept. Protein Res. 47*, 167−176.

Fiordalisi, J. J., & Grant, G. A. (1994) in *Techniques in Protein Chemistry V* (Crabb, J. W., Ed.) pp 269−274, Academic Press, New York.

Fiordalisi, J. J., Fetter, C. H., TenHarmsel, A., Gigowski, R., Chiappinelli, V. A., & Grant, G. A. (1991) *Biochemistry 30*, 10337−10343.

Fiordalisi, J. J., Al-Raibee, R., Chiappinelli, V. A., & Grant, G. A. (1994) *Biochemistry 33*, 3872−3877.

Fiordalisi, J. J., James, P. L., Zhang, Y., & Grant, G. A. (1996) *Toxicon 34*, 213−224.

Graciani, N. R., Tsang, K. Y., McCutcheon, S. L., & Kelly, J. W. (1994) *Bioorg. Med. Chem. 2*, 999−1006.

Herz, J. M., Johnson, D. A., & Taylor, P. (1989) *J. Biol. Chem. 264*, 12439−12448.

Kraulis, P. J. (1991) *J. Appl. Crystallogr. 24*, 946−950.

McGehee, D. S., & Role, L. W. (1995) *Annu. Rev. Physiol. 57*, 521−546.

McLane, K. E., Tang, F., & Conti-Tronconi, B. M. (1990a) *J. Biol. Chem. 265*, 1537−1544.

McLane, K. E., Wu, X., & Conti-Tronconi, B. M. (1990b) *J. Biol. Chem. 265*, 9816−9824.

Milla, M. E., & Sauer, R. T. (1994) *Biochemistry 33*, 1125−1133.

Milla, M. E., & Sauer, R. T. (1995) *Biochemistry 34*, 3344−3351.

Oswald, R. E., Sutcliffe, M. J., Bamberger, M., Loring, R. H., Braswell, E., & Dobson, C. M. (1991) *Biochemistry 30*, 4901−4909.

Unwin, N. (1993) *J. Mol. Biol. 229*, 1101−1124.

Venyaminov, S. Y., & Yang, J. T. (1996) in *Circular Dichroism and the Conformational Analysis of Biomolecules* (Fasman, G. D., Ed.) pp 69−108, Plenum Press, New York.

BI9629687