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MOLECULAR CONFORMATION OF ERABUTOXIN b; ATOMIC COORDINATES AT 2.5 Å RESOLUTION

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SUMMARY: Atomic coordinates determined from a 2.5 Å electron density map are given for erabutoxin b, a sea snake venom postsynaptic neurotoxin. The principal structural features, anti-parallel β pleated sheet, β bulge and β bends are described. The erabutoxin b structure is discussed as structural prototype of this class of homologous curare-mimetic neurotoxins from both land and sea snakes.

In this communication we provide atomic coordinates for erabutoxin b, a sea snake venom neurotoxin, based on a 2.5 Å electron density map and describe major features of the molecular conformation and stereochemistry of this single chain protein (M_r 6861) considered as prototype for a whole class of neurotoxins. Erabutoxin b, from the sea snake Laticauda semifasciata, is one of nearly 50 homologous neurotoxins from both land (elapid) and sea (hydrophid) snake venoms which bind to the cholinergic receptor at the postsynaptic membrane and block neuromuscular transmission in a nondepolarizing curare-mimetic mode.

The main structural features of this short chain toxin observed at a lower resolution were described earlier, and a preliminary discussion of the distribution of some common invariant and conservatively-substituted+ residues given (1). The significance of the erabutoxin b structure as prototype was recognized and a reactive site region described (1,2). There are two toxin series, short with 60→62 and long with 71→74 residues, with 18 invariant and 7 conservatively-substituted positions in common, which include four invariant disulfide linkages. These latter play a unique and evident struc-

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+Conservative substitution is used here to define conservation of residue type, i.e., Ser/Thr-hydroxyl group, Glu/Asp-carboxyl group, Val/Leu/Arg-hydrophobic group and Tyr/His/Trp-aromatic group.

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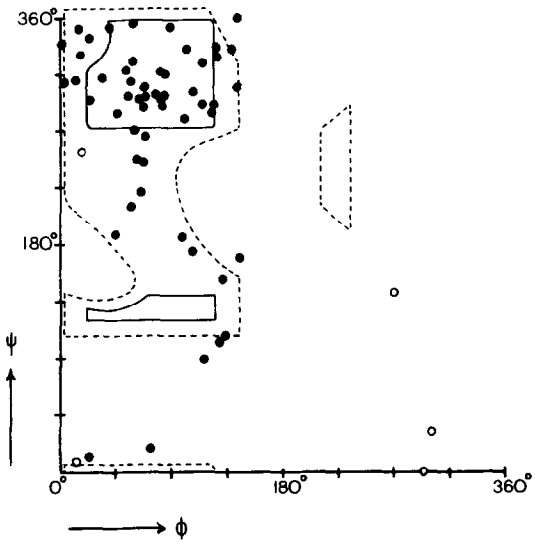

Fig. 1. Ramachandran angle plot for erabutoxin b showing "fully allowed" (——) and "outer limit" (----) boundaries. [○] Gly; [●] Other residues.

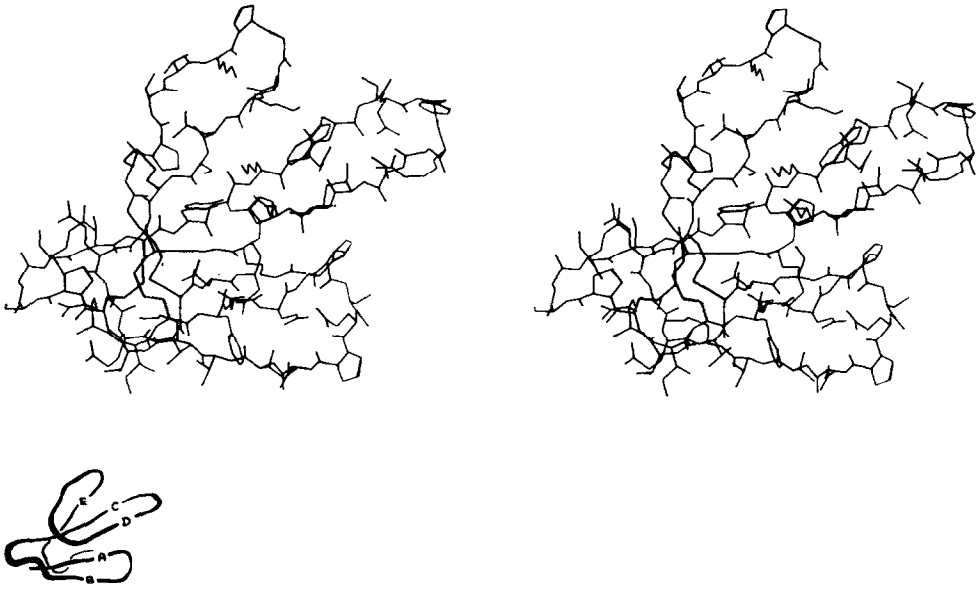

Fig. 2. Stereo drawing of erabutoxin b structure showing all the residues, produced by the GRIP-75 Molecular Graphics System.

tural role; both native conformation and toxicity are lost completely when they are reduced (3).

TABLE 1 Atomic Coordinates for Erabutoxin b.

	10X	10Y	10Z
1 ARG			
N	114	74	93
CA	126	80	100
CB	123	94	102
CG	134	103	97
CD	144	95	87
NE	145	100	74
CZ	135	99	65
NEH2	136	104	53
NEH1	124	92	67
C	129	73	112
O	122	73	122
2 ILE			
N	141	66	111
CA	148	61	123
CB	154	47	120
CG2	157	39	133
CG1	144	38	112
CD1	143	24	117
C	162	65	123
O	171	60	116
3 CSS			
N	164	75	132
CA	177	81	134
CB	174	96	133
SG	170	101	115
C	183	75	147
O	179	65	152
4 PHE			
N	193	82	152
CA	201	78	163
CB	216	79	160
CG	222	66	156
CD1	221	55	164
CE1	227	42	161
CZ	234	41	149
CE2	235	52	140
CD2	229	65	144
C	198	86	176
O	202	98	177
5 ASN			
N	191	80	185
CA	188	85	198
CB	177	76	205
CG	169	84	215
OD1	159	90	212
ND2	173	83	227
C	200	84	208
O	200	87	220
6 GLN			
N	212	79	203
CA	223	76	211
CB	235	70	202
CG	247	73	207
CD	254	71	199
OE1	248	68	190
NE2	267	72	198
C	229	89	218
O	234	98	211
7 HIS			
N	227	90	231
CA	234	100	238
CB	233	97	253
CG	239	107	261
ND1	233	113	272
CE1	242	121	277
NE2	253	121	271
CD2	252	112	261
C	247	102	232
O	253	93	225
8 SER			
N	257	107	232
CA	268	111	225
CB	271	120	232
OG	268	117	246
C	275	98	220
O	272	93	209
9 SER			
N	285	93	228
CA	290	80	224
CB	302	80	217
OG	312	86	225
C	287	69	235
O	295	60	236

	10X	10Y	10Z
10 GLN			
N	277	71	242
CA	270	61	250
CB	257	66	255
CG	244	57	254
CD	230	62	257
OE1	225	72	252
NE2	223	55	266
C	264	51	239
O	263	53	227
11 PRO			
N	264	38	243
CD	265	32	256
CA	262	29	231
CB	261	16	237
CG	263	17	252
C	250	34	224
O	241	40	231
12 GLN			
N	251	29	212
CA	241	33	201
CB	248	28	188
CG	261	34	185
CD	265	33	170
OE1	272	42	165
NE2	262	22	163
C	228	27	203
O	226	14	204
13 THR			
N	218	35	204
CA	203	31	205
CB	199	30	219
CG2	207	20	227
OG1	200	43	226
C	196	41	196
O	201	51	191
14 THR			
N	183	38	193
CA	177	45	182
CB	181	36	170
CG2	173	23	168
OG1	180	44	158
C	161	45	183
O	155	36	190
15 LYS			
N	155	55	177
CA	140	57	178
CB	137	70	184
CG	127	70	195
CD	113	75	191
CE	101	71	200
NZ	88	72	194
C	132	57	165
O	138	62	155
16 THR			
N	121	51	163
CA	112	52	151
CB	106	38	148
CG2	95	35	158
OG1	100	38	135
C	102	62	154
O	97	64	165
17 CSS			
N	99	71	145
CA	92	84	149
CB	101	96	146
SG	120	95	145
C	78	87	144
O	73	79	136
18 PRO			
N	71	97	148
CD	77	107	157
CA	58	100	143
CB	54	113	150
CG	66	117	159
C	55	98	128
O	64	94	121
19 SER			
N	43	96	126
CA	40	91	113
CB	25	91	108
OG	16	93	119
C	48	97	102
O	50	91	91

	10X	10Y	10Z
20 GLY			
N	53	109	102
CA	59	115	90
C	68	127	94
O	65	138	90
21 GLU			
N	79	125	101
CA	90	135	100
CB	94	140	113
CG	99	153	111
CD	104	158	124
OE1	107	171	124
OE2	105	151	134
C	101	130	92
O	107	119	95
22 SER			
N	104	139	83
CA	114	137	72
CB	112	149	62
OG	107	160	69
C	128	137	78
O	138	135	70
23 SER			
N	130	140	90
CA	143	145	94
CB	145	160	93
OG	135	166	101
C	150	143	107
O	145	135	116
24 CSS			
N	161	149	111
CA	168	144	123
CB	172	130	118
SG	159	119	117
C	179	152	131
O	183	162	127
25 TYR			
N	183	146	142
CA	188	154	153
CB	176	161	159
CG	174	161	172
CD1	162	162	178
CE1	162	162	192
CZ	173	162	200
OEH	173	162	213
CE2	186	160	193
CD2	186	160	179
C	202	153	159
O	209	142	159
26 HIS			
N	206	165	163
CA	219	168	169
CB	230	161	161
CG	244	162	166
ND1	253	151	164
CE1	264	155	170
NE2	263	167	175
CD2	251	171	173
C	221	180	177
O	218	192	173
27 LYS			
N	227	178	189
CA	231	188	198
CB	219	195	205
CG	211	185	212
CD	199	191	218
CE	190	181	225
NZ	178	188	231
C	243	184	207
O	246	172	208
28 GLN			
N	249	194	213
CA	261	195	221
CB	271	199	211
CG	283	189	211
CD	294	195	203
OE1	298	189	193
NE2	301	206	207
C	261	207	230
O	254	217	227

	10X	10Y	10Z
29 TRP			
N	268	206	241
CA	268	218	250
CB	254	223	250
CG	243	214	258
CD1	242	214	271
NE1	233	204	274
CE2	228	199	263
CD2	234	205	252
CE3	231	201	240
CZ3	221	191	238
CEH2	216	185	249
CZ2	219	189	262
C	277	216	262
O	282	205	265
30 SER			
N	279	228	267
CA	290	232	276
CB	298	244	271
OG	289	252	262
C	285	236	290
O	289	246	296
31 ASP			
N	275	227	294
CA	270	229	307
CB	259	218	309
CG	259	205	315
OD1	270	198	319
OD2	248	200	317
C	282	222	314
O	292	218	307
32 PHE			
N	285	225	328
CA	298	222	333
CB	298	221	346
CG	285	218	350
CD1	272	220	346
CE1	262	216	354
CZ	264	209	365
CE2	277	207	370
CD2	287	212	362
C	303	208	329
O	311	206	321
33 ARG			
N	297	199	336
CA	301	185	337
CB	288	176	335
CG	282	169	346
CD	267	162	343
NE	260	163	330
CZ	249	171	328
NEH2	243	173	317
NEH1	245	179	338
C	306	180	324
O	311	169	322
34 GLY			
N	305	189	314
CA	312	187	301
C	302	186	289
O	291	192	289
35 THR			
N	307	179	280
CA	301	177	266
CB	310	183	257
CG2	319	174	249
OG1	304	192	248
C	292	164	265
O	297	153	268
36 ILE			
N	281	169	262
CA	269	162	259
CB	257	168	267
CG2	259	184	268
CG1	243	167	258
CD1	240	153	256
C	265	164	244
O	262	175	240
37 ILE			
N	265	153	237
CA	257	155	224
CB	265	151	211
CG2	256	154	199
CG1	278	158	208
CD1	288	156	219
C	244	147	224
O	244	134	226

Atomic coordinates are in Angstroms (x 10) measured along the 3 principal axes a,b and c of the orthorhombic cell (1).

TABLE 1 (continued)

	10X	10Y	10Z
38 GLU			
N	234	154	223
CA	220	148	223
CB	210	156	231
CG	200	146	235
CD	191	153	243
OE1	183	162	237
OE2	189	151	256
C	215	146	208
O	213	155	201
39 ARG			
N	214	133	206
CA	208	128	193
CB	215	114	189
CG	230	114	191
CD	237	108	179
NE	251	106	182
CZ	256	108	194
NEH2	269	106	197
NEH1	249	113	204
C	193	124	196
O	190	119	207
40 GLY			
N	186	128	186
CA	171	126	186
C	165	128	173
O	172	129	163
41 CSS			
N	152	128	172
CA	143	130	160
CB	130	123	158
SG	127	105	162
C	138	144	157
O	131	150	166
42 GLY			
N	141	151	146
CA	134	164	143
C	143	176	144
O	154	176	147
43 CSS			
N	137	188	141
CA	145	200	142
CB	149	206	128
SG	164	215	128
C	141	208	154
O	132	217	152
44 PRO			
N	147	205	165
CD	155	193	166
CA	145	212	177
CB	153	204	187
CG	159	192	180
C	149	227	176
O	161	230	173
45 THR			
N	138	234	177
CA	137	249	176
CB	126	254	185
CG2	121	244	195
OG1	131	265	193
C	150	255	182
O	154	251	193
46 VAL			
N	157	263	175
CA	170	268	179
CB	175	278	168
CG1	189	283	172
CG2	177	271	154
C	169	274	193
O	159	278	198
47 LYS			
N	182	273	199
CA	184	275	213
CB	177	264	220
CG	172	269	232
CD	166	258	239
CE	161	263	252
NZ	156	252	260
C	195	281	216
O	206	277	211
48 PRO			
N	196	293	226
CD	184	298	232
CA	208	301	229
CB	204	312	238
CG	189	310	240
C	220	293	234
O	219	287	245
49 GLY			
N	229	293	225
CA	243	287	227
C	243	272	225
O	253	267	220
50 ILE			
N	232	266	229
CA	230	251	227
CB	218	246	235
CG2	214	256	245
CG1	206	243	226
CD1	207	231	217
C	227	249	212
O	215	251	208
51 LYS			
N	236	244	204
CA	234	244	189
CB	247	247	182
CG	258	240	188
CD	271	243	181
CE	283	236	187
NZ	296	240	181
C	227	232	183
O	224	222	189
52 LEU			
N	225	233	169
CA	216	223	162
CB	204	221	167
CG	193	229	159
CD1	182	220	153
CD2	185	239	167
C	221	222	147
O	219	231	139
53 SER			
N	227	210	144
CA	228	203	131
CB	243	198	130
OG	246	198	116
C	218	192	130
O	215	185	139
54 CSS			
N	210	195	117
CA	198	185	114
CB	184	192	115
SG	179	199	132
C	199	178	101
O	202	183	91
55 CSS			
N	196	165	103
CA	197	155	93
CB	208	147	92
SG	205	134	103
C	186	145	88
O	178	138	96
56 GLU			
N	182	145	76
CA	170	140	69
CB	164	150	59
CG	160	162	65
CD	154	172	55
OE1	149	183	60
OE2	153	170	43
C	173	128	59
O	174	131	46
57 SER			
N	177	117	67
CA	183	106	61
CB	197	110	55
OG	200	103	43
C	185	95	72
O	190	98	83
58 GLU			
N	182	83	68
CA	179	73	79
CB	174	58	73
CG	159	51	77
CD	145	60	77
OE1	146	72	74
OE2	133	55	80
C	189	73	90
O	186	77	101
59 VAL			
N	202	69	89
CA	211	70	100
CB	209	58	108
CG1	208	61	123
CG2	198	49	105
C	222	80	96
O	232	75	90
60 CSS			
N	220	92	100
CA	228	103	95
CB	219	113	86
SG	201	118	91
C	233	111	107
O	237	123	105
61 ASN			
N	231	107	119
CA	230	112	131
CB	220	106	138
CG	205	114	139
OD1	196	111	132
ND2	204	125	146
C	243	109	140
O	242	100	149
62 ASN			
N	254	115	136
CA	266	114	144
CB	275	104	137
CG	268	95	126
OD1	269	96	114
ND2	260	85	131
C	273	128	145
O	266	138	144

Molecular Conformation. Atomic coordinates for the 2.5 Å structure are given in Table 1. The Ramachandran angle plot (4) and a stereo drawing of the structure based on the coordinates are shown in Figs. 1 and 2 respectively. These results were obtained using the GRIP-75 Molecular Graphics System build by the University of North Carolina, Department of Computer Science. Fitting of the molecular model to the electron density map and idealization procedures were employed in an iterative mode to be described elsewhere. In the Ramachandran plot (Fig. 1), apart from the less restrained Gly angles, most residues fall within the outer limit boundary shown and all others are within at least 22° of this region. The three principal molecular lobes may

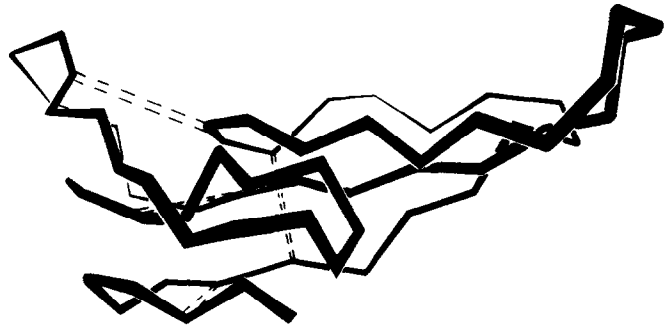

Fig. 3. Erabutoxin b. Freehand sketch of alpha-carbon backbone model. The double broken lines indicate disulfide bridges.

be seen to emerge in Fig. 2 from the central invariant disulfide core. These curved lobes, bounded by Cys3 and Cys17, Cys24 and Cys41 (K*45), and by Cys43 and Cys54 (K49, K61) are formed by the five BADCE strands of the dominant anti-parallel β structure (see inset) as well as by the outer rim segment 43-48 (K49-K55).

Fig. 3 shows the molecular shape in a view which emphasizes the curved shell-like concavity, and shows the protruding curled tongue of the long central DC lobe and the underlying "stand" formed by the C-terminal six-membered ring and 2 residue tail. In the short chain toxin series only the tapered neck region, Cys17-Cys24 (compare Fig. 4), is of variable length; all other molecular segments are invariant in length.

The erabutoxin b molecule is open, much of it only one peptide chain thick. Apart from a very few residues in the region around the disulfide core there are no buried residues or regions of backbone chain inaccessible to solvent. Nonetheless a striking degree of cross-linking maintains molecular stereochemistry. This includes not only hydrogen bonds between main-chain atoms in the β structure and elsewhere, but also numerous other main-chain and side-chain crosslinking interactions. Because of these interactions, the upper concave molecular surface (facing the viewer in Fig. 4) formed by the three

*The sequence enumerations used are that of erabutoxin b and, where different, that of Karlsson (K) which orders all sequence alignments to preserve maximum homology in both short and long chain series.

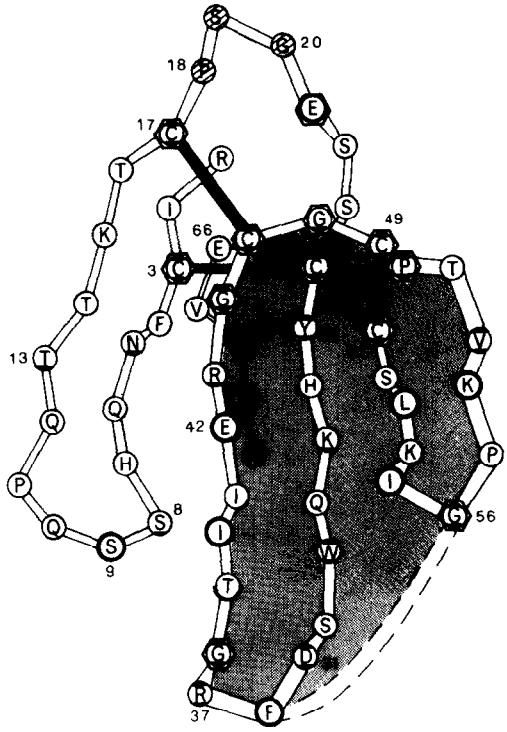

Fig. 4. View (partially schematic) of the erabutoxin b molecule looking into the concavity. The reactive site is shown shaded. One-letter residue code and Karlsson sequence enumeration used.

Invariant position in both long and short chain toxins; in short chain toxins only .
Conservatively substituted position in both long and short chain toxins; in short chain toxins only .
Structural residue position.

lobes is inaccessible even to water molecules from below, an isolation particularly significant for the reactive site region (5,6) shown shaded in the projected view.

Polypeptide Backbone. The barreling curve of the β structure is complex; individual strands both turn and twist. Fig. 5 shows the molecular hydrogen bonding pattern; the three β bends in the β sheet are, respectively, A7→B10 type I (7), C31→D34 (K38) type III (i.e., 3_{10} helix) and rim segment 47(K53)→E50 (K57) type II. There is a "wide" β bulge (8) in the left-hand edge of the sheet which involves Pro11 and Gln12 in strand B and Gln6 in the opposite A strand. The general preference for Pro in the first position has been

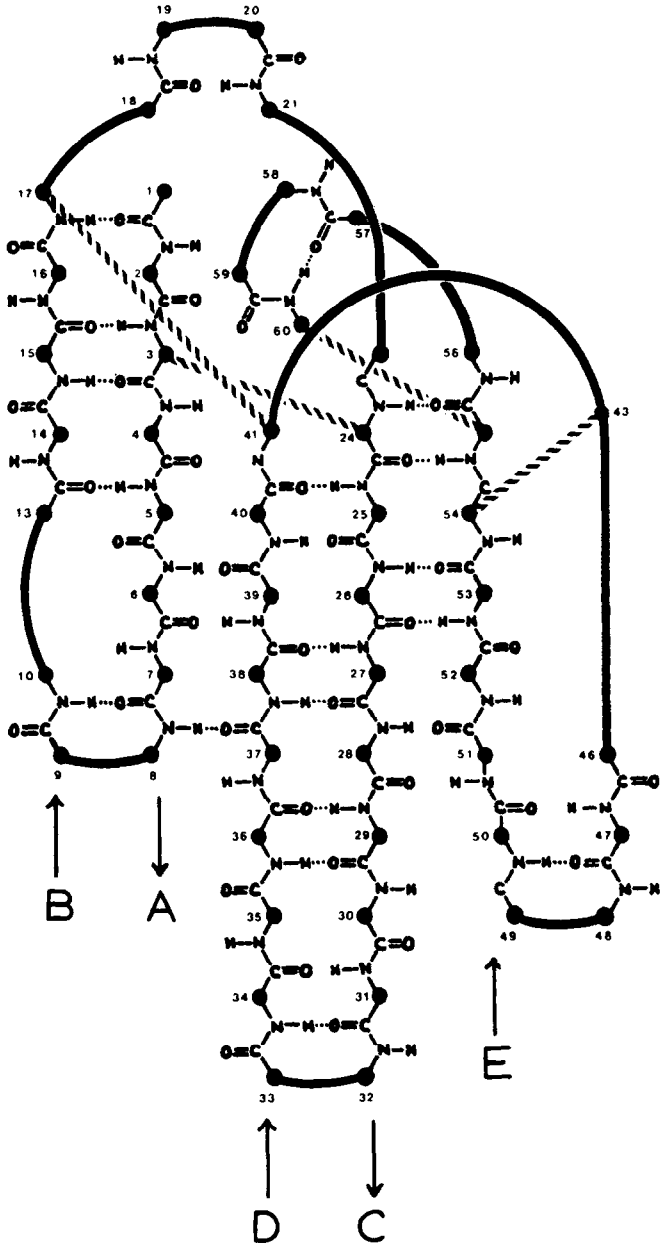

Fig. 5. Hydrogen bonding pattern of erabutoxin b polypeptide chain. The C=O and H-N groups are joined by dotted lines when the O-N distance $\lesssim$ 3.7 Å. Disulfide bridges are shown with striped lines.

noted earlier (8). Strands B and C are joined through the tapered neck region by a "plain +3 connection" which includes a type I β bend 18-21; strands D and E are joined by a right-handed 2_1 cross-over connection (9). The E strand leads into a type I β bend, 57-60 (K64-K68) in the C-terminal closed ring.

The dihedral angles about -S-S of the four disulfide bridges which maintain the β structure, all lie close to 90°. Three linkages are right-handed; that joining Cys17 and Cys41 (K45) is left-handed. The disulfide bridge 43-54 (K49-K61) is Gauche.Gauche.Trans; the other three are Gauche.Gauche. Gauche. These findings agree with the conclusion of the laser Raman solution studies of erabutoxin b (10).

Homologous Side Chain Distribution. The overall sequence homologies of invariant and conservatively substituted positions are further extended in the short series toxins by 6 more invariants and 2 more conservative substitutions. In a broad sense the extent and distribution of all common invariant or type-conserved residues define the principal features of the erabutoxin b structure as short series prototype and long series model. Most of these residues are found within the reactive site (1,2,5,6) region 32——49 ...32 (K33-K56...K33) formed by the two right-hand lobes of the molecule and shown shaded in Fig. 4. In the AB loop of the short-series toxins, the limited number of common residues may imply minor conformational variations; in the tapered neck region the one or two residue deletions found in positions 18,19, or 20 demand conformational variations.

There are residues which evidently play a key structural role; they lie within or are bound to the reactive site and are shown enclosed in open hexagons in Fig. 4. These include four invariant glycines, two in the β bends and two (one a short series invariant) at positions of close approach of two chains. Pro44 (K50) stabilizes the rim segment turn. Two further residues, the type-conserved position Glu21 (Glu/Asp) and the invariant Asn61 (K69) form hydrogen bonds to atoms in neighboring segments of the main chain.

A second group of residues has been designated "functional" because of probable involvement in receptor binding (5,6). With one exception, all point up along Cα-Cβ into the sweeping hollow of the reactive site. The exception Val46 (K52) forms part of the banked wall of this concavity. The functional residues have been sorted into reactive groupings (5,6). Toxicity

is less than normal when some functional residues are chemically modified or are found replaced in certain naturally occurring deviant toxins. Other hydrophobic residues have been designated "functional" on the basis of indirect evidence. We will consider the structure-function relationships and the effect of our recent findings on the details of the earlier model elsewhere.

The designations "structural" and "functional" are in some instances too restrictive; the two roles are not necessarily mutually exclusive. In a reactive grouping, one or more "functional" residues may position the residue which binds directly to receptor. Equally, some "structural" residues may be required for proper local functional expression, as for example, Gly34 (K38) in the acetylcholine mimetic association and binding of Asp31 and Arg33 (K37) proposed earlier (2,5,6,11).

It is a remarkable feature of the neurotoxin homology that functional residue distribution defines, and β structure stereochemistry demands a sequence distribution which involves an interrupted and frequently alternating series of invariant and/or conservative substitutions rather than a continuous segment of identical or near-identical sequence.

The atomic coordinates on which these conclusions are based may be expected to shift somewhat after refinement. Model studies have shown that differences between the long and short series toxins do not need to affect significantly the principal structural features we have described here (5,6).

ACKNOWLEDGEMENTS

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