

Cloning and sequencing of cDNAs encoding the two subunits of Crotoxin

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Crotoxin (1) is a heterodimeric neurotoxin which blocks acetylcholine release from nerve endings. One subunit called CA (2), is acidic, non-toxic, has no enzymatic activity and comprises three peptides cross-linked by seven disulfide bridges. The other subunit designated as CB (2), is basic, toxic, has phospholipase A2 (PLA2) activity and is composed of a single chain of 122 amino acids. A cDNA library was prepared from mRNA extracted from one venom gland of *Crotalus durissus terrificus*. The cDNA sequences encoding CA and CB precursors are presented below on the right and left, respectively. Both open reading frames encode a signal peptide (underlined) of 16 residues followed by a polypeptide chain of 122 residues. The signal peptides of CA and CB are identical. The sequence of CB chain differs from that reported by Aird *et al.* (3) by a single amino acid change at position 65 (Arg is replaced by Pro). The sequence of CA chain contains three peptides analogous to those previously described for mature CA by Aird *et al.*, (4) and three additional connecting peptides, emphasized by individually underlined residues. The presence of additional fragments in the chain of CA precursor is indicative of post-translational events which interestingly, do not happen in CB.

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AGAGGAGCAGAGGGAGCCCTGCTGAGTGTGAATCTTTGGCATTTTCCCTGGCTGGCTTC 59
TCCTTCTGATCCTTGGCTACAGGTTATCTTGACTTACAAACCGTTTGTATTAGTGACCGT 118
TCTAAGGGCCATTTCCAGACTTTTCCACGCGGGGCGATACAGGGCTGTCTGATTC 177
CCAGGCTCTGGATTGAGGAGG ATG AGG GCT CTC TGG ATA GTG GCC GTG 224
      M R A L N I V A V
TTG CTG GTG GGC CTC GAG GGG CAC CTG CTG CAA TTC AAC AAG ATG 269
L L V G V E G H L L Q T F N K M
ATC AAG TTT GAG ACA AGG AAA AAC GCT ATT CCC TTC TAT GCC TTT 314
I K F E T R K N A I P F Y A F
TAC GGC TGC TAC TGT GGC TGG GGG GGC GCA GGC CGG CCA AAG GAC 359
Y G C Y C G W G G R G R P K D
GCC ACT GAC CGC TGC TGC TTT GTG CAT GAC TGC TGT TAC GGA AAA 404
A T D R C C F V H D C C Y G K
CTG GCC AAG TGC AAC ACC AAA TGG GAC ATC TAT CCC TAC AGC TTG 449
L A K C N T K W D I Y P Y S L
AAG AGT GGG TAT ATC ACC TGC GAA AAG GGC ACC TGG TGC GAG GAA 494
K S G Y I T C G K G T W C E E
CAG ATT TGT GAG TGC GAC AGG GTC GCG GCA GAA TGC CTC AGA AGG 539
Q I C E C D R V A A E C L R R
AGT CTG AGC ACG TAC AAG TAT GGA TAT ATG TTT TAC CCG GAC TCT 584
S M L S T Y K Y G Y M F Y P D S
CGT TGC AGG GGG CCT TCA GAG ACA TGC TAA GTCTCTGCAGGCATGCA. 631
R C R G P S E T C end
    
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AGAGGAGCAGAGGGAGCCCTGCTGAGTGTGAATCTTTGGCATTTTCCCTGGCTGGCTTC 59
TCCTTCTGATCCTTGGCTACAGGTTATCTTGACTTACAAACCGTTTGTATTAGTGACCGT 118
TCTAAGGGCCATTTCCAGACTTTTCCACGCGGGGCGATACAGGGCTGTCTGATTC 177
CCAGGCTCTGGATTGAGGAGG ATG AGG GCT CTC TGG ATA GTG GCC GTG 225
      M R A L N I V A V
TTG CTG GTG GGC CTC GAG GGG ACC CTG GTG GAA TTT GAG ACG TTG 270
L L V G V E G S L V E E T L
ATG ATG AAA ATT GGG GGG AGA AGT GGT ATT TGG TAC TAC AGC TCT 315
M M K I A G B S G I S Y Y S S
TAC GGA TGC TAC TGT GGC GGG GGG GGC CAA GGC TGG CCA CAG GAC 360
Y G C Y C G A G G Q G W P Q D
GCC AGC GAC CGC TGC TGC TTT GAG CAC GAC TGC TGT TAT GCA AAA 405
A S D R C C F E H D C C Y A K
CTG ACT GGC TGC GAC CCA ACA GAC GTC TAC ACC TAC AGA CAG 450
L T G C D P T T D V Y T Y B Q
GAG GAC GGG GAA ATC TGT TGT GGA GAG GAC GAC CCG TGC GGG ACA 495
E D G E I V C G E D D P C G T
CAG ATT TGT GAG TGC GAC AAG GCC GCA GCA ATC TGC TTC CGA AAT 540
Q I C E C D K A A A I C F R N
AGT ATG GAC ACA TAC GAC TAC AAA TAT TTG CAG TTC TCG CCC GAA 585
S M D T Y X D Y K X L Q F S P E
AAT TGC CAG GGG GAA TCA CAG CCA TGC TAA GTCTCTGCAGGCATGCA 632
N C Q G E S Q P C end
    
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