Unusual Amino Acid Determinants of Host Range in the Mtx2 Family of Mosquitocidal Toxins*

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Five different mosquitocidal toxin (mtx2) gene homologs have been cloned from eight Bacillus sphaericus strains. Pairwise comparisons of the predicted amino acid sequences show between four and eight substitutions compared with the prototype Mtx2 from B. sphaericus strain SSII-1. Mtx2 from strain SSII-1 was 7-fold more toxic to Culex mosquito larvae than the Mtx2 homolog from B. sphaericus strain 31-2. Conversely, Mtx2 from strain 31-2 was 100-fold more toxic to Aedes mosquito larvae than Mtx2 from strain SSII-1. Lys224 in Mtx2 was found to be the most important amino acid for toxicity to Culex larvae, and substitution of Lys by threonine abolished the toxicity of Mtx2 from strain SSII-1 to these larvae. In complete contrast, Thr224 was found to be crucial for the toxicity of Mtx2 from strain 31-2 to Aedes larvae, and substitution of Thr by lysine caused a 100-fold drop in toxicity to these larvae. Thus, amino acid 224 in the Mtx2 family of mosquitocidal toxins is an unusual and important determinant of mosquito larvicidal activity and host range.

Bacillus sphaericus is an aerobic, Gram-positive, spore-forming bacterium which is widespread in soil and aquatic environments. Some strains of B. sphaericus produce protein toxins which are lethal to mosquito larvae (1, 2). The best studied mosquitocidal strains of B. sphaericus are divided into a high toxicity group (e.g., 2362, 2297, and IAB59) and a low toxicity group (e.g., SSII-1, 31-2 and Kellen Q) (1). The high toxicity group, but not the low toxicity strains encode 51- and 41.9-kDa proteins, which together form a binary toxin expressed at high levels during sporulation. Most mosquito pathogenic B. sphaericus tested also harbor a 100-kDa toxin gene (mtx2) and a 31.8-kDa toxin gene (mtx2) (3, 4).

Toxin production in the low toxicity strain B. sphaericus SSII-1 begins in the vegetative phase of growth before the onset of sporulation (5, 6). The mtx2 gene of this strain encodes a polypeptide of 292 aa (Mtx2) with a molecular mass of 31.8 kDa, which is detected in the vegetative phase of growth (4). Mtx2 is unrelated to the binary and 100-kDa toxins but has regions of significant homology with the 33-kDa e toxin of Clostridium perfringens and the 31.68-kDa cytoxin of Pseudomonas aeruginosa (4). In this study, we demonstrate that the Mtx2 toxins from six strains of B. sphaericus have few sequence differences, but different strains of B. sphaericus exhibit major differences in larvicidal activities against two species of mosquitoes. The results permitted the design and assay of hybrid toxins and the identification of aa residues in Mtx2 that are unusual determinants of larvicidal activity and mosquito host range.

EXPERIMENTAL PROCEDURES

Materials—Plasmids pTH26 (7), a derivative of plasmid pGEX-1 (8), was used for the initial cloning of the mtx2 genes. Plasmid pTH82 was used for the construction of the mutant mtx2 genes: SSII-1 (T279A); SSII-1 (K224T, T279A); 31-2 (A227F); 31-2 (T224K, A279T); IAB59 (D31H); IAB59 (R50K); 31-2 (P67S); 2297 (T171K); 2362 (K40Q); SSII-1 (S67P); SSII-1 (S371); and SSII-1 (S67P), pTH82, which contains the SSII-1 mtx2 gene derived from genomic DNA, was previously constructed from plasmids pS35Bp2 and pTH81 as follows. Plasmid pS35 (4) was digested with BamHI so as to remove a 3-kb BamHI fragment and self-ligated to give pS35B. Plasmid pS35 (4) was digested with BamHI and SphI, and reduced glutathione was from Sigma. Dried yeast powder was purchased from Life Technologies, Inc.

Bacterial Strains and Media—B. sphaericus SSII-1 was a gift from E. W. Davidson, Arizona State University, Tempe. Strains Kellen Q and 31 were obtained from A. A. Yousif, Virginia Polytechnic Institute and State University, Blacksburg. Strain 1593M was a gift from J. Zulma-jster, C.N.R.S., GIF sur Yvette, and all other B. sphaericus strains were obtained from H. de Barjac, Pasteur Institute, Paris. All B. sphaericus strains were grown either in L-broth or NYSM medium (5).

Cloning, Mutagenesis, and Sequence Analysis—The protein coding regions of mtx2 from eight different strains of B. sphaericus (31-2, IAB59, Kellen Q, 2297, 2362, 1691, 1593M, and 2317) were amplified by PCR from genomic DNA (9) using synthetic oligonucleotides based on the sequence of B. sphaericus SSII-1 mtx2 (4). The sequences of these oligonucleotides were: 5′-CCCCCCCATTCGATGAAAGACCCCAAATCGTCTTTTATAT-3′ (TT119) for the 5′ (upstream) primer, and 5′-CCCCGCTCATCAGTGATCGCTGCTTTGATTTAAAAAGAAATCTTTCAATCATTATTA-3′ (TT120) for the 3′ (downstream) primer. Artificial BamHI, Ncol, CiaI, SphI, and AffII restriction enzyme sites were incorporated into these sequences for cloning purposes.

2 Protein mutations are abbreviated using the following convention; the residue number is preceded by the symbol (in the one-letter code) for the wild-type amino acid and followed by the symbol for the mutant amino acid. Thus D31H denotes a mutation from Asp to His at position 31.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank/EBI Data Bank with accession number(s) U47299, U47300, U47301, U47302, and U41822, which correspond to Mtx2 homologs from B. sphaericus strains 2297, 31-2, 2362, IAB59, and Kellen Q, respectively.

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1 The abbreviations used are mtx2, mosquitocidal toxin type 2 gene; Mtx2, mosquitocidal toxin type 2 protein; aa, amino acid(s); GST, glutathione S-transferase; Lc, lethal concentration of protein or cells theoretically required to kill 50% of mosquito larvae; PCR, polymerase chain reaction; bp, base pair(s); kb, kilobase pair(s).
The 0.8-kb PCR products were digested with NcoI and CiaI, and cloned into the NcoI and CiaI sites of the large (vector) fragment of plasmid pTH26 (7), which was propagated in Escherichia coli DH5α. Another set of recombinants were generated in the same way, and both sets were completely sequenced to ensure mutations were not artefactually introduced into the mtx2 genes during the PCR. Cloning and sequencing were performed as described in Materials and Methods (10). Protein sequences, analysis and alignments were performed using DNAStar software.

Mutagenesis was carried out as described in the Sculptor system in vitro mutagenesis system (Amersham). A 0.8-kb BamHI-SphI restriction fragment of pTH26 containing the appropriate mtx2 gene was subcloned into the BamHI-SphI site of bacteriophage λM13mp19. Recombinants from M13 phage were propagated in E. coli TG1, and single-stranded DNA was purified (10). The mutagenic primers (mutation underlined) were 5'-ATCATGATCATCACAAC-3' (SSI-1-S37I); M3, 5'-CTTCTGGGAGCTATATC-3' (SSI-1-D30H); M5, 5'-ATGACACATGATGAAATAT-3' (IAB59-D30H); M3; and 5'-GATTGATAAGAGAT-3' (IAB59-R59K); M5, and 5'-ACATCCACACACACACGC-3' (2362-4740) were annealed to single-stranded templates of the mtx2 genes cloned in M13mp19, and mutants were identified directly by dideoxynucleotide DNA sequencing. All mutant genes were fully sequenced to ensure unwanted mutations had not arisen. Finally, all mutant genes were cloned from M13mp19 back into pTH82 (7) by inserting the BamHI-SphI gene fragment into the large vector fragment of BamHI-SphI-digested pTH82.

The 31-2 (A279T) and 31-2 (T224K,A279T) mutant mtx2 genes, the 31 mtx2 gene cloned in pTH26 (see above) and the SSI-1 mtx2 genomic DNA cloned in plasmid pTH26 were each digested with Ndel and PstI, and the large (vector) fragments and small (gene) fragments were exchanged, then ligated together. To construct the 31-2 (T224K,A279T) and SSI-1 (K224T,T279A) mutant mtx2 genes, the same strategy was used except that the above plasmids harboring the 31-2 and SSI-1 mtx2 genes were double digested with AccI and PstI.

The construction of genes expressing Mtx2 fusion proteins in E. coli DH5α (in which the N-terminal 15 aa of Mtx2 were removed) was performed as follows. To construct mtx2β from SSI-1 (K224T) and SSI-1 (K224T, T279A), the plasmids containing the SSI-1 (K224T) or SSI-1 (K224T, T279A) mtx2 gene (cloned in pTH26) were digested with Ndel and HindIII, blunt-ended with Klenow, gel-purified, and self-ligated. To construct mtx2β from 31-2 and 31-2 (T224K,A279T), the mtx2 genes cloned in pTH26 were PCR amplified with primer TT120 (see above) and primer 5'-CATGGCAGATTATGCATGAAAATG-3' (W3), and the amplified genes were eluted from an agarose gel, digested with Ndel and CiaI, and cloned in the large vector fragment of NcoI-CiaI-digested pTH26. The construction of mtx2β from SSI-1 has been described (4).

Expression and Purification of Mtx2 Proteins—Synthesis and purification of Mtx2 fusion proteins was carried out essentially by a published procedure (8), except that E. coli DH5α were grown at 30 °C for 2 h after induction of Mtx2 synthesis.

Antibodies and Western Blotting—Polyclonal antibodies against recombinant Mtx2 fusion proteins were raised in a previous study (4) and used to perform Western blotting as described (4) to detect recombinant Mtx2 homologs expressed in recombinant E. coli.

Mosquito Larvicidal (Toxicity) Assays—All plasmids expressing Mtx2 fusion proteins were transformed into E. coli DH5α for larvicidal assay. The toxic activities of different E. coli clones synthesizing the Mtx2 fusion proteins (or purified Mtx2 fusion proteins) (4) were measured on laboratory-reared 1st instar larvae of Culex quinquefasciatus and Aedes aegypti mosquitoes. Recombinant E. coli cultures were harvested, washed once with water, resuspended in 0.1 the original volume with water, and the A670 was measured. The cultures were adjusted to the desired range of concentrations with water and added to 10 1st instar larvae in 1 ml of water with 50 μl of a 100 mg/ml suspension of yeast. Assays using the desired range of concentrations of Mtx2 fusion proteins were performed in a similar manner, except that 50% PBS was used throughout. All assays were performed at least twice, and each concentration of bacteria (or purified Mtx2 fusion protein) was assayed in triplicate. Surviving larvae were counted at 24 h. The LC50 values were calculated as the average mortality observed at 24 h. The mosquito larvae were used in the CA Cricket Graph III program. Mortality of control larvae fed with yeast suspension alone, with 50% PBS, or with E. coli transformed with plasmid vector alone was less than 10% in all assays.

RESULTS

Cloning and Comparison of mtx2 Genes—Using PCR primers based on the sequences at the extremities of the region coding for the mtx2 gene from B. sphaericus SSI-1 (4), 0.8-kb PCR products were generated from the genomic DNA of eight strains of B. sphaericus, namely 31-2, IAB59, Kellen Q, 2297, 2362, 1691, 1593M, and 2317.3. The amplified DNAs were cloned in a plasmid vector, pTH26 (7), and two independent clones of each mtx2 gene were completely sequenced to exclude the possibility of PCR-derived mutations.

The predicted aa sequences of the eight Mtx2 homologs were compared with each other and with the known sequence of B. sphaericus SSI-1 Mtx2, and aa variations were found at 10 positions between aa 31 and aa 279 (Fig. 1). The Mtx2 homologs from B. sphaericus 2362, 1691, 1593M, and 2317.3 were identical to each other (Table I). Overall, there were between four and eight aa substitutions when the Mtx2 sequences from strains 31-2, IAB59, Kellen Q, 2297, and 2362 were compared in a pairwise fashion with the prototype Mtx2 sequence from SSI-1 (Table I). Mtx2 from SSI-1 was unique in having Ser37, Ser40, Lys224, and Thr279, while Phe239 was only found in Mtx2 from Kellen Q, and Thr279 was unique to Mtx2 from strain 2297 (Table I).

Synthesis and Mosquito Larvicidal Activity of Mtx2 Homologs in E. coli—In order to compare the mosquitocidal activities of the six Mtx2 homologs, the mtx2 coding regions were fused in frame to GST (see “Experimental Procedures”). Recombinant E. coli cells were incubated with isopropyl-β-D-thiogalactopyranoside to induce the synthesis of GST-Mtx2, and cell extracts were examined by Western blotting using a polyclonal antiserum to Mtx2 from B. sphaericus SSI-1. All recombinant cells produced a protein of ~58 kDa, the expected size of GST-Mtx2 (Fig. 2). The synthesis in E. coli of mtx2 from B. sphaericus strain 31-2, Kellen Q was lower than that of Mtx2 from strains 31-2, IAB59, 2297, 1593M, and 2317.3. The expression of all fusion proteins did not vary by more than 2.7-fold (Fig. 2).

Intact E. coli cells were fed to larvae of the mosquito C. quinquefasciatus, and larvicidal activities of the various recombinant E. coli clones harboring mtx2 fusion proteins were quantitated (Table II), taking into account the different levels of Mtx2 fusion proteins (Fig. 2 legend). All clones exhibited significant toxicity to C. quinquefasciatus, and E. coli synthesizing Mtx2 from strains SSI-1 and 2362 were the most toxic. E. coli synthesizing Mtx2 from SSI-1 were ~7 to ~14 times more toxic than E. coli synthesizing Mtx2 from strains 31-2, IAB59, 2297, and Kellen Q (Table II). E. coli synthesizing Mtx2 from SSI-1 were ~7 to ~14 times more toxic to C. quinquefasciatus larvae than clones synthesizing Mtx2 from strain 31-2 (Table II).

Surprisingly, the opposite result was obtained when E. coli synthesizing Mtx2 from SSI-1 and 31-2 were assayed against larvae of the mosquito, A. aegypti. Mtx2 from strain 31-2 was about 100-fold more toxic to A. aegypti than Mtx2 from SSI-1 (Table III). SSI-1 and 31-2 Mtx2 differ in four aa positions, namely 37, 67, 224, and 279 (Table I), and any or all of these aa could contribute to the significant differences in the toxicity and mosquito host range of these toxins.

Single or double amino acid substitutions were introduced by
site-directed mutagenesis into selected Mtx2 homologs to test the contribution of individual aa to toxicity and host range. Changes were made to render the Mtx2 proteins more or less like Mtx2 from strain SSII-1 (Table I). None of the single substitutions in Mtx2, including IAB59 (D31H or R50K), 31-2 (P67S), 2297 (T171K), 2362 (K40Q), SSII-1 (S37I), and SSII-1 (S67P) (Table I) had any detectable effect on toxicity toward C. quinquefasciatus larvae (data not shown).

However, substitutions at aa positions 224 and 279 (creating hybrids between SSII-1 and 31-2 Mtx2) significantly affected toxicity to C. quinquefasciatus and A. aegypti larvae. The T279A mutation in SSII-1 Mtx2 resulted in a modest (~5-fold) increase in toxicity to C. quinquefasciatus larvae, whereas the SSII-1 (K224T) mutant and the double mutant SSII-1 (K224T,T279A) were completely nontoxic to these larvae (Table III). The reverse mutations 31-2 (A279T) and 31-2 (T224K,A279T) were generated so that the 31-2 Mtx2 mutants more closely resembled Mtx2 from SSII-1. 31-2 (A279T) was about as toxic to C. quinquefasciatus larvae as the parental 31-2 Mtx2 protein, but 31-2 (T224K,A279T) was at least 50-fold more toxic than 31-2 Mtx2 (Table III) and at least 7-fold more toxic than SSII-1 Mtx2. These results emphasize that K224 is more toxic than S37I (Table III) had any detectable effect on toxicity toward C. quinquefasciatus larvae (data not shown).

Completely opposite results were obtained when the aa 224 and 279 mutants were assayed against A. aegypti larvae. SSII-1 (T279A) was about as toxic to these larvae as the weakly toxic SSII-1 Mtx2 protein, but SSII-1 (K224T) and the double mutant SSII-1 (K224T,T279A) were ~200-fold more toxic than the parental SSII-1 Mtx2. The excellent A. aegypti toxicity of 31-2 Mtx2 was not significantly affected by the A279T mutation, but the double mutant 31-2 (T224K,A279T) was only weakly toxic to A. aegypti due to a drop in larvicidal activity of ~100-fold compared with 31-2 (A279T) (Table III).

Assays against C. quinquefasciatus and A. aegypti larvae were performed with purified Mtx2 fusion proteins in which the N-terminal 15 amino acids containing the putative signal sequence of Mtx2 were deleted from GST-Mtx2 (Fig. 3) to exclude the possibility that the different larvicidal activities of E. coli expressing SSII-1 and 31-2 Mtx2 were due to differences in toxin solubility or stability in the bacteria. Table IV shows that SSII-1 Mtx2 was over 4-fold more toxic than 31-2 Mtx2, and that the 31-2 (T224K,A279T) mutant was about 17-fold more toxic than parental 31-2 Mtx2 to C. quinquefasciatus larvae. Both SSII-1 mutants with the K224T substitution were nontoxic to C. quinquefasciatus (Table IV). Conversely, purified toxins with T224 were the most toxic to A. aegypti, and toxins with K224 (SSII-1- and 31-2 (T224K,A279T)) were much less toxic to these larvae (Table IV). These results mirror the larvicidal activities of these toxins in live E. coli (Table III), suggesting that the different toxicities of the recombinant bacteria are due to variations in the aa sequences of the Mtx2 homologs, particularly in residue 224.

### Table I

| Source of toxin protein (B. sphaericus strain) | LC50 at 24 h | C. quinquefasciatus | A. aegypti |
|-----------------------------------------------|--------------|---------------------|------------|
| SSII-1                                       | 1.08 × 10^6  | 5.34 × 10^6         |            |
| 31-2                                         | 7.77 × 10^6  | 4.20 × 10^6         |            |
| SSII-1 (T279A)                               | 1.93 × 10^6  | 2.10 × 10^6         |            |
| 31-2 (A279T)                                 | 8.71 × 10^6  | 5.75 × 10^6         |            |
| SSII-1 (K224T)                               | >2.00 × 10^6 | 6.57 × 10^6         |            |
| SSII-1 (K224T,T279A)                         | >2.50 × 10^6 | 3.75 × 10^6         |            |
| 31-2 (T224K,A279T)                           | 1.48 × 10^6  | 5.38 × 10^6         |            |

*Variation between replicates within one assay = 10%. Values are means of two or more assays and are normalized taking into account the variation in yield of GST-Mtx2 fusion proteins (Fig. 2).

a Indicates control bacteria lacking mtb2 gene were added.

b ≥2.00 × 10^6 cells/ml is nontoxic.

c ≥2.00 × 10^6 cells/ml is nontoxic.
tive, showing that, although Ala279 is more favorable than Mtx2, but the double mutant SSII-1 (K224T, T279A) was inactive SSII-1 (K224T, T279A) mutant in having Ile37 and Pro67, was overpinpointed Lys224 as an important site in the toxicity of Mtx2 to C. quinquefasciatus larvae. The SSII-1 (T279A) mutant was 5–6-fold more toxic to C. quinquefasciatus larvae than SSII-1 (T224K, T279A) and SSII-1 (K224T, T279A) respectively. The mutations allowed us to design mutagenesis experiments which were crucial in characterizing the extent of the region of the Mtx2 toxin which is toxic to mosquito larvae than others. These observations allowed us to design mutagenesis experiments which pinpointed Lys224 as an important site in the toxicity of Mtx2 to C. quinquefasciatus larvae. The SSII-1 (T279A) mutant was 5–6-fold more toxic to C. quinquefasciatus larvae than SSII-1 Mtx2, but the double mutant SSII-1 (K224T, T279A) was inactive, showing that, although Ala279 is more favorable than Thr279 in the context of SSII-1 Mtx2, Lys224 is an overriding determinant of larval activity. Why did 31-2 Mtx2 exhibit significant toxicity to C. quinquefasciatus despite the absence of Lys224? The highly active 31-2 Mtx2 toxin differs from the inactive SSII-1 (K224T, T279A) mutant in having Ile37 and Pro67, suggesting that one or both of these aa contributes to the toxicity of 31-2 Mtx2 and compensates for the absence of Lys224. This assumption is strengthened by the observation that the 31-2 (T224K, A279T) mutant, which is identical to SSII-1 Mtx2 except for the presence of Ile37 and Pro67, was over 7-fold more toxic to C. quinquefasciatus larvae than SSII-1 Mtx2. Therefore, it was surprising that the single substitutions 31-2 (P67S), SSII-1 (S37I), and SSII-1 (S67P) had no effect on toxicity. Further reciprocal constructs (e.g. 31-2 (I37S), SSII-1 (S37I, K224T), and SSII-1 (S67P, K224T)) are needed to resolve the issue of the relative importance of Ile37 and Pro67 in the context of 31-2 Mtx2.

When E. coli synthesizing Mtx2 from SSII-1, 31-2, and their respective mutants were assayed against A. aegypti larvae, even greater differences in toxicity were observed. However, in complete contrast to the results of larvicidal assays against C. quinquefasciatus, SSII-1 Mtx2 was found to be ~100-fold less toxic to A. aegypti than was 31-2 Mtx2; and surprisingly, the difference was also due to aa position 224. Parental or mutant Mtx2 proteins with Thr224 were always ~50–150-fold more toxic to A. aegypti than their counterparts with Lys224 (Table III).

How can a single aa substitution in a toxin substantially increase toxicity to one species of mosquito and virtually abolish toxicity to another species of mosquito? Although this is a new phenomenon, there are earlier studies on lepidopteran and dipteran toxins which are instructive and allow us to speculate on the possible mechanism of action of aa 224 (11–16). Important genetic determinants of mosquito host range in the binary toxin from B. sphaericus have been localized to aa positions 99 and 104 in the 41.9-kDa subunit, but other aa in the 51.4-kDa subunit also contribute to toxicity (16). This is analogous to aa 224 in Mtx2 playing a major role in mosquito host range and aa 37 and/or 67 playing an accessory role. Several other studies have also concluded that one or a very few aa are major determinants of larvicidal activity and host range in a variety of toxins active against Lepidoptera and/or Diptera (11–15). However, in many cases insect specificity could not easily be attributed to particular aa, as different toxin segments were found to determine specificity by interacting in an undefined manner (11–13). Nevertheless, it is evident that different and sometimes overlapping segments of many toxins carry determinants of specificity for different insects (11–13).

In one interesting study, lle542 of a dual specificity larvicidal protein from Bacillus thuringiensis aizawai ICl was found to be essential for toxicity to A. aegypti larvae, but not to larvae of the caterpillar Pieris brassicae; conversely, the single substitution I568T abolished toxicity to P. brassicae larvae but not to A. aegypti larvae (12). Together, the results suggested that the I568T mutation destroyed proteolytic cleavage activation of the 130 × 10^3 M subunit, protoxin to a known ~55 × 10^3 M, Lepidoptera-active toxin, while the I545P mutation inhibited proteolytic activation at a different site of the protoxin and prevented the formation of a known ~53 × 10^3 M, Diptera-active toxin (12).

Although proteolytic cleavage by larval gut proteases activates many insecticidal toxins (1, 2, 12), it is unclear whether this also occurs in Mtx2 as the mechanism of action of the recently discovered Mtx2 family of mosquitocidal toxins is unknown (4). However, it is worth speculating that K224 in SSII-1 Mtx2 may be a major site of proteolytic cleavage activation by C. quinquefasciatus gut trypsin-like proteases (1, 2), and that other as yet unknown neighboring site(s) in 31-2 Mtx2 (which has Thr224) are exposed for cleavage activation by the compensating aa, lle37 and Pro67. By analogy with the dual specificity larvicidal toxin from B. thuringiensis aizawai ICl, we would have to postulate that in the A. aegypti larval gut a different protease is responsible for cleavage activation of Mtx2 at a distinct site from the one cleaved in the C. quinquefasciatus gut, and that the T224K mutation in 31-2 Mtx2 adversely affects substrate specificity or denies access to the putative A. aegypti protease.

An alternative and perhaps more plausible hypothesis is simply that there are subtle (but vital) aa differences in the C. quinquefasciatus and A. aegypti gut receptors in the domains which interact with position 224 on the surface of Mtx2. For example, a crucial electrostatic interaction might form only with Thr224 in A. aegypti, while in C. quinquefasciatus a similar crucial interaction might form only with Lys224. Position 224 is predicted to lie at the peak of 1 of 11 predicted hydrophilic regions in Mtx2, which is consistent with a surface location. Regardless of the mechanism, it is clear that aa position 224 in the Mtx2 family of mosquitocidal toxins is an important and unusual determinant of toxicity and mosquito host range.

Finally, it is worth noting that some of the mutant Mtx2 toxins were significantly more toxic to C. quinquefasciatus...
larvae than their natural counterparts (SSII-1 (T279A) and 31-2 (T224K,A279T)). This approach may, therefore, be helpful in the design of exceptionally potent Mtx2 toxins.

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