Mutations at Domain II, Loop 3, of *Bacillus thuringiensis* CryIAb and CryIAb δ-Endotoxins Suggest Loop 3 Is Involved in Initial Binding to Lepidopteran Midguts*

(Received for publication, April 1, 1996, and in revised form, June 6, 1996)

Francis Rajamohan‡, Syed-Rehan A. Hussain‡, Jeffrey A. Cotrell‡, Fred Gould¶, and Donald H. Dean||

From the ‡Department of Biochemistry, ¶Molecular, Cellular and Developmental Biology Program, The Ohio State University, Columbus, Ohio 43210 and the ||Department of Entomology, North Carolina State University, Raleigh, North Carolina 27695

Alanine substitutions of loop 3 residues, 438SGF-SN5443, of CryIAb toxin were constructed to study the functional role of these residues in receptor binding and toxicity to *Manduca sexta* and *Heliothis virescens*. Experiments with trypsin and insect gut juice enzyme digests of mutant toxins showed that these mutations did not produce any gross structural changes to the toxin molecule. Bioassay data showed that mutant G439A (alanine substitution of residue Gly439) and F440A significantly reduced toxicity toward *M. sexta* and *H. virescens*. In contrast, mutants S438A, S441A, N442A, and S443A were similar or only marginally less toxic (2–3 times) to the insects compared to the wild-type toxin. Binding studies with brush border membrane vesicles prepared from *M. sexta* and *H. virescens* midgut membranes revealed that the loss of toxicity of mutants G439A and F440A was attributable to substantially reduced initial binding. Consistent with the initial binding, mutants G439A and F440A showed 3.5 times less binding to *M. sexta* and *H. virescens* brush border membrane vesicles, although the off-rate of bound toxins was not affected. The role of hydrophobic residue, Phe440, is distinctly different from our previous observation that alanine substitution of Phe372 at loop 2 of CryIAb did not affect initial binding but reduced irreversible association of the toxin to the receptor or membrane toward *M. sexta* (Rajamohan, F., Alcantara, E., Lee, M. K., Chen, X. J., and Dean, D. H. (1995) *J. Bacteriol.* 177, 2276–2282). Likewise, deletion of relatively hydrophobic CryIAb loop 3 residues, 440AAGA443 (D3a), resulted in reduced toxicity to *Bombus mori* (>62 times less) and *M. sexta* (28 times less). The loss of toxicity was correlated with reduced initial binding to midgut vesicles prepared from these insects. However, alanine substitution of residues 435L543G499 (A3a), contiguous to loop 3, altered neither toxicity nor receptor binding toward *B. mori* or *M. sexta*. These results suggest that the loop 3 residues of CryIAb and CryIAb toxins establish hydrophobic interactions with the receptor molecule, and mutations at these hydrophobic residues affect initial binding.

The insecticidal crystal proteins (ICPs or δ-endotoxins) produced by *Bacillus thuringiensis* are of great scientific interest because of their potency and specificity against a wide range of agronomically important insect pests and vectors of human diseases (1). The bacteria express the protein during the late growth phase as a protoxin (120–140 kDa for CryI type toxins), which accumulates in the cell as crystals of various shapes (2). Upon ingestion of the crystals, the protoxin is solubilized and activated into a 60–65-kDa protease-resistant toxin by the proteolytic enzymes present in the larval midgut. The activated toxin binds to specific receptors (toxin-binding proteins) located on the midgut brush border membrane of the columnar cells (3, 4). Binding of toxin to the receptor generates ion channels across the midgut apical membrane, leading to death of the cells (5–7) and finally of the larvae.

The x-ray crystal structure of a lepidopteran active δ-endotoxin, CryIAb, has been recently determined by Grochulski et al. (8). This structure supports the three domain structure of CryIIIA, a coleopteran active toxin, determined by Li et al. (9). In summary, domain I is composed of seven α-helices, which may be involved in the membrane spanning activity of the toxin. Mutations in domain I of Cry type toxins can inhibit toxicity and channel forming activity (10, 11). Domain II has three antiparallel β-sheets, connected to each other with surface-exposed loops of different lengths, oriented in parallel with the helical bundle of domain I. These loops are attractive candidates for a role in receptor recognition and binding. Domain III, a bundle of β-sheets, has been reported to be involved in ion-channel activity (12), receptor binding (13), and structural stability (14). CryIAb toxin is believed to have a similar structure as CryIAb, since it shares about 89% amino acid sequence identity with CryIAb toxin (8).

In many cases, in vitro binding studies using 125I-labeled toxins and midgut brush border membrane vesicles (BBMV)1 isolated from susceptible and resistant insect larvae have shown a direct correlation between insect toxicity and binding (15, 16). Recent studies on binding kinetics suggest a two-step process (reversible and irreversible) for Cry toxins with several lepidopteran insects (17, 18). Interestingly, a direct correlation between toxicity and the rate constant for irreversibly bound toxin has been observed (17). Recent progress on the identification and purification of insect midgut toxin-binding (receptor) molecules suggests 120- and 210-kDa proteins from *Manduca sexta* as binding proteins for CryIAc and CryIAb toxins, respectively (19, 20). In gypsy moth BBMV, CryIAb, and CryIAb toxins bind to a 210-kDa protein and CryIAc binds to a 120-kDa amino peptidase-N (13, 21).

The domain II loop residues of Cry toxins have been targeted

*This work was supported by Grant RO1 29092 from the National Institutes of Health (to D. H. D.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

|| To whom correspondence should be addressed: Tel.: 614-292-8829; Fax: 614-292-3206; E-mail: dean.10@osu.edu.

1 The abbreviations used are: BBMV, brush border membrane vesicle; PAGE, polyacrylamide gel electrophoresis.
by site-directed mutagenesis and membrane binding assays to investigate the molecular basis for the action of δ-endotoxins. Wu and Dean (22) mutated the loop residues of CryIIA and observed that loops 1 and 3 are involved in receptor binding. Recent studies by Rajamohan et al. (18, 23) on CryIAb showed that loop 2 residues, RRHP70, are involved in initial receptor binding, while residues Phe177 and Gly174 of the same loop are largely involved in irreversible binding of the toxin to *M. sexta*. In earlier reports, deletion of a portion of CryIAb loop 2 (residues 365–371) removed nearly all toxicity and initial binding to *Bombbyx mori* (24), while mutations in loop 1 showed no effect on initial binding (25). These studies establish the significance of domain II loop residues in receptor binding. In the present communication we target another loop in domain II, loop 3, of CryIAb and CryIAb toxins and analyze its functional role in receptor binding and toxicity. We demonstrate that deletion or alanine substitution of loop 3 amino acids, especially affecting hydrophobic residues, of CryIAb and CryIAb toxins significantly affect the initial binding ability to CryIAa and CryIAb toxins and analyze its functional role in communication we target another loop in domain II, loop 3, of CryIAb and CryIAb toxins and analyze its functional role in communication.

In earlier reports, deletion of a portion of CryIAb loop 2 (residues 365–371) removed nearly all toxicity and initial binding to *Bombbyx mori* (24), while mutations in loop 1 showed no effect on initial binding (25). These studies establish the significance of domain II loop residues in receptor binding. In the present communication we target another loop in domain II, loop 3, of CryIAb and CryIAb toxins and analyze its functional role in receptor binding and toxicity. We demonstrate that deletion or alanine substitution of loop 3 amino acids, especially affecting hydrophobic residues, of CryIAb and CryIAb toxins significantly affect the initial binding ability to CryIAa and CryIAb toxins and analyze its functional role in communication.

**MATERIALS AND METHODS**

Site-directed Mutagenesis—The oligonucleotides used for site-directed mutagenesis were kindly provided by Dr. Takashi Yamamoto, Sandoz Agro Inc., Palo Alto, CA. A uracil-containing template of cryIAb and cryIAb genes was obtained by transforming Escherichia coli C636 (Bio-Rad) with pMOS1313 (26) and pSB333B (18), respectively. The site-directed mutagenesis procedure followed the manufacturer’s manual (Muta-Gene M13 in vitro mutagenesis kit; Bio-Rad). DNA sequencing was carried out by the method of Sanger et al. (27) following the manufacturer’s (U. S. Biochemical Corp.) instructions. Fine chemicals and restriction enzymes were purchased from Boehringer Mannheim.

Expression and Purification of Toxin—Mutant and wild-type δ-endotoxins were expressed in *E. coli* MV1190 and were purified as described previously (18). The purified crystal protein was solubilized in crystal solubilization buffer (50 mM Na2CO3, pH 9.5, 10 mM dithiothreitol) at 37°C for 3 h. Activation of the solubilized protein was carried out by treating with 2% (by mass) trypsin (Sigma) at 37°C for 5 h and was analyzed by sodium dodecyl sulfate-10% polyacrylamide gel electrophoresis (PAGE).

Protease Digestions and Western Blotting—Protein concentrations of toxins and BBMV were determined with the Coomassie protein assay reagent (Pierce) using bovine serum albumin as a standard.

Membrane Protease Digestion—Insect gut enzyme digestion of mutant and wild-type proteins was performed by incubating the toxin with freshly prepared gut enzymes at 37°C for 3 h as described before (18). The final digested products were separated on SDS-10% PAGE, transferred onto polyvinylidene difluoride membrane (Bio-Rad) treated with anti-CryIAb or CryIAb serum and the blot was processed and developed as described previously (18).

Toxin Induction—Twenty μg of trypsin-activated toxin were labeled using 1 μCi of 125I (Dupont) and one IODOBED (Pierce) following the manufacturer’s directions. Free iodine was separated from the toxin using a preparative Bio-Gel G-5 column (Pierce) as described previously (18). The specific activities of labeled CryIAs, A1a, D3a, CryIAb, S438A, G439A, F440A, S441A, N442A and S443A were 1.9, 1.9, 1.7, 2.1, 1.9, 2.3, 1.4, 1.5, 1.8, and 1.6 μC/mg, respectively.

Preparation of BBMV—Insect midguts were prepared as described by Rajamohan et al. (32). The amplifying equipment for voltage clamp consisted of a D.C. 1000 volt (current clamp), an A-310 Accupulser (World Precision Instruments), and a strip chart recorder (Kipp and Zonen). The voltage clamp analysis was performed as described by Rajamohan et al. (23). After stabilization of the membrane, trypsin-activated toxin was injected into the lumen side of the gut (final concentration, 50 ng/ml). The Ip (inhibition of short-circuit current) was tracked with a recorder, and data were collected with the MacLab data acquisition system. Each individual experiment was repeated at least three times, and the mean values were plotted using the CA-CRICKET Graph III application program.

Results

**Alignment and Construction of Mutants**—The alignment of domain II, loop 3, residues of CryIAb toxin (8), with CryIAb toxins was expressed in amounts comparable to toxin inclusion bodies purified from wild-type and mutants were solubilized and analyzed on SDS-10% PAGE. All the mutant proteins were expressed in amounts comparable to wild-type (Figs. 2B and 3A). Each also yielded a 60-kDa stable, trypsin-resistant toxin core upon activation with trypsin (Figs. 2A and 3B).

To investigate the stability of trypsin-activated toxins with insect gut proteases, the toxins were treated further with gut juice collected from target insects. Western blot analysis showed that all CryIAb (Fig. 2C), CryIAb (Fig. 3). The mutant proteins yielded stable 60-kDa toxin, similar to wild-type, upon treatment with *M. sexta* (Figs. 2C and 3D), *H. virescens* (Fig. 2D), and *B. mori* (Fig. 3C), gut juice.
Toxicity and Binding of CryIAb Mutants to M. sexta—The LC_{50}, K_{corn} and P_{max} values of CryIAb and mutants S438A, G439A, F440A, S441A, N442A, and S443A toward M. sexta were analyzed, and the values are reported in Table I. Mutants G439A and F440A reduced the toxicity (100 and 20 times less, respectively) to M. sexta. The LC_{50} values of mutants S438A, S441A, N442A, S443A, and wild-type were 45.5, 10.8, 37.3, and 43.6, and 9.7 ng/cm^2, respectively. These mutants were similar or up to 4 times less potent when compared to the wild-type toxin. The K_{corn} of mutants G439A and F440A was 12 and 9 times, respectively, higher than that of CryIAb (Table I). The heterologous binding curves showed that mutant toxins G439A and F440A competed for the binding of labeled wild-type toxin with reduced binding affinity compared to CryIAb, S438A, S441A, N442A, and S443A toxins (Fig. 4A). Dissociation binding assays with CryIAb, G439A, and F440A labeled toxins showed that about 85–90% of the BBMV-bound toxins were irreversibly associated with the vesicles. However, the total amount of toxin that irreversibly bound to the BBMV was significantly different between the wild-type and the mutants. While 38 ng/mg BBMV of CryIAb toxin was irreversibly associated with the vesicles, only 18 and 15 ng/mg BBMV of F440A and G439A toxins, respectively, were irreversibly bound to M. sexta vesicles (Fig. 5).

Response of M. sexta Midgut to CryIAb Mutant Toxins—The voltage clamp experiment measures the active transport of ions across the midgut cells from the hemolymph side to the lumen side. The inhibition of short-circuit current (I_{sc}) illustrates the depolarization of the midgut membrane due to the channel forming activity of Cry toxin. Our experiments showed that the slope of I_{sc} inhibition of CryIAb, S438A, S441A, N442A, and S443A toxins were between –94 to –97 μA/cm^2/min (Table I), whereas the slope for F440A was –60.3 μA/cm^2/min. We were unable to calculate the slope of mutant G439A because of insufficient inhibition of I_{sc} by the mutant toxin at this concentration (Fig. 6).

Binding and Toxicity of CryIAb Mutants to H. virescens—The biological activity of CryIAb and mutant toxins to H. virescens were compared and reported in Table II. The LC_{50} of S438A, S441A, N442A, and S443A showed that these mutants were only 2–4 times less toxic (LC_{50} 3.6, 1.6, 1.2, and 2.2 μg/ml diet, respectively) than the wild-type (LC_{50} 0.82 μg/ml diet). In contrast, G439A lost most of its toxicity (insufficient mortality at 15 μg/ml concentration to calculate the exact LC_{50}), and F440A reduced the toxicity 15 times compared to wild-type (Table I). The K_{corn} estimated by homologous competition assays for wild-type, S438A, S441A, N442A, and S443A toxins were between 3.17 and 5.9 nM (Table II), whereas G439A and F440A were 6–7 times higher (22.33 and 19.97 nM, respectively). In heterologous competition binding studies CryIAb, S438A, S441A, N442A, and S443A toxins competed for binding with higher affinity to H. virescens BBMV (Fig. 4B). Mutant toxins G439A and F440A competed with reduced binding affinity (the binding curves were shifted to the right) for the binding sites of labeled wild-type toxin as shown in Fig. 4B. The dissociation binding data with H. virescens were similar to that of M. sexta reported here, and CryIAb toxin bound 3.7 times more than the mutants G439A and F440A (data not shown).

Insect Bioassay and Binding of CryIAb Mutants—The toxic-
Mutations in Loop 3 of CryIAa and IAb δ-Endotoxins

25223

Although CryIAa and CryIAb share 89% overall amino acid sequence identity and bind to the same receptor (210 kDa) in the gypsy moth, loop 3 residues of domain II are significantly different (Fig. 1). In this study, loop 3 residues, D386SGFSNS443, of CryIAb toxin were individually replaced with alanine and tested for toxicity to M. sexta and H. virescens. Our bioassay

The binding affinity (K<sub>com</sub>) and binding site concentrations (B<sub>max</sub>) of CryIAa and mutant toxins to midgut vesicles prepared from B. mori and M. sexta were calculated by homologous competition binding assays, and the results were shown in Table III. The K<sub>com</sub> and B<sub>max</sub> value of the mutant A3a was comparable with CryIAa for both the insects, whereas the K<sub>com</sub> value of D3a was about 15 and 9 times higher than CryIAa, for M. sexta and B. mori, respectively, representing reduced binding affinity (Table III). When the wild-type toxin was labeled with <sup>125</sup>I and put into competition with nonlabeled wild-type or mutant toxins, CryIAa and A3a displayed higher affinity binding to B. mori and M. sexta (Fig. 7, A and B), whereas the D3a curve was shifted to the right compared with that of CryIAa or A3a to both insect BBMV (Fig. 7, A and B).

**DISCUSSION**

Elucidation of the mechanism of interaction between the δ-endotoxin and insect midgut receptor(s) is critical to the rational design of improved insecticidal toxins with broader insect specificity and higher larvicidal potency. The insect specificity determining region of CryI type toxins has been located primarily in domain II for several lepidopterans (26, 33, 34). The three-dimensional structure of CryIAa suggests that the apex of domain II is composed of three solvent-exposed loops comprising residues 310–313 (loop 1), 367–379 (loop 2), and 438–446 (loop 3). The striking dissimilarity between CryIAa and CryIAb toxins in amino acid sequences in loops 2 and 3 inspired us to investigate the role of these residues in insect specificity, toxicity, and receptor binding.

**Fig. 3.** Coomassie blue-stained SDS-10% PAGE of CryIAa mutants, comparing the yield of protoxins (A) trypsin-activated toxins (B), Western blot analysis of the stability of wild-type and mutant proteins after digestion with B. mori (C) and M. sexta (D) gut juice. Lane 1, molecular mass markers. Masses of the protein markers (in kilodaltons) are shown on the left; lane 2, CryIAa; lane 3, A3a; and lane 4, D3a.

**Fig. 4.** Binding of <sup>125</sup>I-labeled (1 nM) CryIAb toxin in the presence of increasing concentrations of nonlabeled CryIAb, S438A, G439A, F440A, S441A, N442A, and S443A toxins to M. sexta (A) and H. virescens BBMV (B). Binding is expressed as a percentage of the total amount bound upon incubation with labeled toxin alone. On M. sexta and H. virescens vesicles, the amount is 2400 ± 80 cpm and 2250 ± 55 cpm, respectively for CryIAb.

| Compounds | K<sub>com</sub> (nM) | B<sub>max</sub> (pmol/mg) | I<sub>sc</sub> inhibition rate (µA/cm²/min) |
|-----------|------------------|------------------------|----------------------------------------|
| CryIAa    | 9.7 (6.2–9.3)    | 4.05 ± 0.4             | –97.4 ± 5.6                             |
| S438A     | 45.5 (37–61)     | 5.25 ± 0.2             | –93.1 ± 4.9                             |
| G439A     | 1000 (920–1300)  | 9.05 ± 1.1             | –60.3 ± 4.1                             |
| F440A     | 190 (155–222)    | 8.11 ± 1.7             | –94.1 ± 5.2                             |
| N442A     | 37.3 (26–51)     | 4.99 ± 0.8             | –93.9 ± 8.9                             |
| S443A     | 43.6 (36–53)     | 5.79 ± 0.7             | –94.3 ± 3.1                             |

<sup>a</sup> LC<sub>50</sub>, 50% lethal concentration, 95% confidence intervals are given in parentheses.

<sup>b</sup> K<sub>com</sub>, dissociation constant (determined from homologous competition binding). Each value is the mean of three individual experiments.

<sup>c</sup> B<sub>max</sub>, binding site concentration (determined from homologous competition binding). Each value is the mean of three experiments.

<sup>d</sup> Each value if the mean of three experiments.

<sup>e</sup> Undetectable.
data show that the mutants G439A (alanine replacement of Gly439) and F440A have substantially reduced toxicity to M. sexta (100 and 20 times, respectively) and H. virescens (>15 times). The other mutants (S438A, S441A, N442A, and S443A) only marginally affected toxicity. Evidence such as 1) expression of mutant toxins at levels comparable to wild-type (Fig. 2B), 2) stability of the mutant toxins (60 kDa) upon digestion with trypsin (Fig. 2A), and 3) processing wild-type and mutant toxins alike into 60-kDa toxin by insect gut enzyme digestion (Fig. 2, C and D) suggest that the loss of toxicity was not caused by instability of mutant toxins in the insect midgut. It was noticed that the digestion of mutant toxins, especially G439A, with M. sexta and H. virescens gut juice generated a few minor peptides in addition to the stable 60-kDa toxin, suggesting that G439A might be slightly more susceptible to insect gut pro-

FIG. 5. Dissociation of bound 125I-labeled toxins from M. sexta BBMV. M. sexta BBMVs (200 μg/ml) were incubated with 1 nM 125I-labeled CryIAb, G439A, and F440A toxins for 1 h (association reaction). After the association reaction, 100 nM corresponding nonlabeled toxins were added to the test samples, and incubation was continued (post-binding incubation). Binding is expressed as nanograms of toxin bound/mg of BBMV.

FIG. 6. Inhibition of I_{sc} across M. sexta midgut. A total of 50 ng/ml CryIAb, S438A, G439A, F440A, S441A, N442A, and S443A toxin were injected in separate experiments into the lumen side of the chamber, and the drop in I_{sc} was measured. The I_{sc} measured before the addition of the toxin is considered as 100%.

Mutations in Loop 3 of CryIAa and IAb δ-Endotoxins
indirectly affect binding affinity. Phe at loop 3 plays a critical role in toxicity and receptor binding, similar to the role in loop 2 (18), but its effect on binding is significantly different in the two loops. Alanine substitution of Phe" at CryIAb loop 2 did not have any effect on initial receptor binding, but extensively disrupted the irreversible association of the toxin to the BBMV and dramatically reduced (400 times less) the toxicity to M. sexta (18). In contrast, alanine substitution of Phe" (a stronger hydrophobic residue than alanine) in the loop 3 affected the initial binding of the toxin to the same insect. This may suggest that Phe plays functionally distinct roles (initial binding and irreversible binding to M. sexta) when located at different loops of CryIAb toxin. Furthermore, alanine substitution of two positively charged residues at loop 2 (368RR369) eliminates the initial binding of the toxin almost completely with an 15 times to M. sexta, which are located between β10 and loop 3, they might not be completely exposed for the interaction with the receptor. On the contrary, D3a reduced the toxicity >68 times to B. mori and 28 times to M. sexta (Table III). Binding experiments with insect midgut vesicles showed that the deletion of relatively hydrophobic loop residues 440AAGA443 (D3a) disrupted binding affinity (\( K_{\text{on}} \)) by 15 times to B. mori and 9 times to M. sexta (Table III). Hence, it is reasonable to speculate that the reduced potency of D3a to both insects is attributable to the reduced initial binding affinity. These experiments suggest that the loop 3 residues (440−443) of CryIAa contain important binding determinants to B. mori and M. sexta receptor(s). Considering the lack of any active side chain among the loop 3 residues, 440AAGA443, the stretch of alanines might provide hydrophobicity, and their deletion could remove a hydrophobic interaction. Consequently, the studies with CryIAa also support our earlier proposal that the hydrophobic residues of loop 3 are important for initial receptor binding. We have previously reported that a deletion of charged and hydrophobic loop 2 residues (465LYRHI371) of CryIAa resulted in substantial loss of initial binding and toxicity to B. mori (24). It is obvious from these studies that in both CryIAa and CryIAb
Mutations in Loop 3 of CryIAa and IAb δ-Endotoxins

toxins charge (loop 2) and hydrophobicity (loops 2 and 3) play a key role in initial receptor binding.

Our experiments do not exclude the possibility that the toxins (CryIAa and CryIAb) bind to two different binding sites on the same receptor, one with higher binding affinity and the other with lower affinity. In that case, the loop 3 mutations selectively affected a higher affinity site that is important for toxicity. These results provide evidence that the receptor binding residues of these toxins are physically scattered, rather than clustered in a confined region of the toxin. Recent studies with domain swapping experiments suggest that domain III, in addition to domain II, is involved in insect specificity and receptor recognition (15, 36). As a result, these loops are excellent targets for genetic redesigning of novel toxins with diverse specificity by exchanging the residues or chain lengths of the active site without affecting the structural frame work of the toxin.

Acknowledgments—We thank Daniel R. Zeigler, Mi K. Lee, Oscar Alzata, and S. J. Wu for their critical evaluation and Dr. Takashi Yamamoto (Sandoz Agro Inc.) for preparing the mutagenic oligonucleotides. We are grateful to Dr. David Stetson (Department of Zoology, The Ohio State University) for the use of the voltage clamp apparatus.

REFERENCES
1. Feitelson, J. S., Payne, J., and Kim, L. (1992) Bio/Technology 10, 271–275
2. Yamamoto, T., and Powell, G. K. (1993) in Advanced Engineered Pesticides (Kim, L., ed) pp. 3–42, Marcel Dekker, New York
3. Hofmann, C., Luthy, P., Hutter, R., and Piska, V. (1988) Eur. J. Biochem. 173, 85–91
4. Lee, M. K., Milne, R. E., Ge, A. Z., and Dean, D. H. (1994) J. Biol. Chem. 269, 3115–3121
5. Knowles, B. H., and Ellar, D. J. (1987) Biochim. Biophys. Acta 924, 509–518
6. Woltersberger, M. G. (1989) Arch. Insect Biochem. Physiol. 12, 267–277
7. Schwartz, J.-L., Garneau, L., Masson, L., and Brousseau, R. (1991) Biochim. Biophys. Acta 1065, 250–260
8. Grochulski, P., Masson, L., Boriiova, S., Puszat-Carey, M., Schwartz, J.-L., Brousseau, R., and Cogler, M. (1995) J. Mol. Biol. 254, 447–464
9. Li, J., Carroll, J., and Ellar, D. J. (1991) Nature 353, 815–821
10. Wu, D., and Aronson, A. I. (1992) J. Biol. Chem. 267, 2311–2317
11. Chen, X. J., Curtiss, A., Alcantara, E., and Dean, D. H. (1995) J. Biol. Chem. 270, 6412–6419
12. Chen, X. J., Lee, M. K., and Dean, D. H. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 9041–9045
13. Lee, M. K., Young, B. A., and Dean, D. H. (1995) Biochim. Biophys. Res. Commun. 216, 306–312
14. Nishimoto, T., Yoshihue, H., Ibara, K., Sakai, H., and Komano, T. (1994) FEBS Lett. 348, 249–254
15. Van Rie, J., McGaughey, W. H., Johnson, D. E., Barnett, B. D., and Van mellaert, H. (1990) Science 247, 72–74
16. Ferré, J., Real, M. D., Van Rie, J., Jansens, S., and Pefersen, M. (1991) Proc. Natl Acad. Sci. U. S. A. 88, 5119–5123
17. Liang, Y., Patel, S. S., and Dean, D. H. (1995) J. Biol. Chem. 270, 24719–24724
18. Rajamohan, F., Alcantara, E., Lee, M. K., Chen, X.-J., and Dean, D. H. (1995) J. Bacteriol. 177, 2276–2282
19. Sangadala, S., Walters, F. S., English, L. H., and Adang, M. J. (1994) J. Biol. Chem. 269, 10088–10092
20. Vadlamudi, R. K., Ji, T. H., and Bulla, L. A., Jr. (1991) J. Biol. Chem. 266, 12334–12340
21. Lee, M. K., and Dean, D. H. (1996) Biochem. Biophys. Res. Commun., 220, 575–580
22. Wu, S. J., and Dean, D. H. (1996) J. Mol. Biol. 255, 628–640
23. Rajamohan, F., Cotrill, J. A., Gould, F., and Dean, D. H. (1996) J. Biol. Chem. 271, 2390–2396
24. Lu, H., Rajamohan, F., and Dean, D. H. (1994) J. Bacteriol. 176, 5554–5559
25. Kwak I. S., Lu, H., and Dean, D. H. (1995) Mem. Inst. Oswaldo Cruz Rio J. 90, 75–79
26. Ge, A. Z., Shivarova, N. I., and Dean, D. H. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 1754–1758
27. Sanger, F. A., Nicklen, A., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
28. Woltersberger, M. G., Luthy, P., Mauro, A., Parenti, P., Sacchi, V. F., Giordana, B., and Hanetz, G. M. (1987) Comp. Biochem. Biophysiol. 86A, 301–308
29. Raymond, M. (1985) Entomol. Mer. Parasitol. 22, 117–121
30. Gould, F., Anderson, A., Reynolds, A., Bumgarner, L., and More, W. (1995) J. Econ. Entomol. 88, 1545–1559
31. Munson, P. J., and Rodbard, D. (1980) Anal. Biochem. 107, 220–239
32. Harvey, W. R., Crawford, D. N., and Spach, D. D. (1996) Methods Enzymol. 235, 599–608
33. Masson, L., Mazza, A., Gringorten, J. L., Baines, D., Anelunias, V., and Brousseau, R. (1994) Mol. Microbiol. 14, 851–860
34. Schnepf, H. E., Tomczak, K., Ortega, J. P. and Whiteley, H. R. (1990) J. Biol. Chem. 265, 20925–20930
35. Ramachandra, G. N., and Sassiekharan, V. (1968) Adv. Prot. Chem. 28, 263–437
36. Bosch, D., Schipper, B., van der Kleij, H., de Maagd, R. A., and Stekema, W. J. (1994) Bio/Technology 12, 915–919