May 1983

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NOTES

Toxicity of Bacillus thuringiensis var. israelensis Crystals to Aedes aegypti Larvae: Carbonate Reversal

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Received 28 December 1982/Accepted 10 February 1983

The toxicity of purified Bacillus thuringiensis var. israelensis crystals to larvae of Aedes aegypti could be reversed 100-fold by levels of K2CO3 as low as 0.15%.

The crystal-forming bacterium Bacillus thuringiensis var. israelensis is toxic to the larval stage of many mosquitoes and black flies. However, very little is known regarding the mode of action of the B. thuringiensis var. israelensis toxin. One approach to this question involves identification of physical or chemical factors which will counteract an observed toxicity. To this end, we screened 20 common inorganic salts at both 0.1 and 0.5% (wt/vol) to determine whether their presence reversed the toxicity of B. thuringiensis var. israelensis crystals purified on NaBr gradients (1). The crystal-containing salt solutions were bioassayed on Aedes aegypti larvae. The crystal concentrations at which 50% of the larvae were killed (LC50 values) were determined after 4 h as described previously (K. W. Nickerson and D. J. Schnell, J. Invertebr. Pathol., in press). Seventeen of the salts, CaCl2, Ca3(PO4)2, FeNH4(SO4)2·12H2O, K2B4O7·4H2O, KCl, K2HPO4·3H2O, KI, KNO3, KSCN, MgCl2·6H2O, MgSO4·7H2O, MnCl2·4H2O, NaBr, NaCl, NaF, Na2S2O3·5H2O, and (NH4)2SO4, did not reverse toxicity. The LC50 values were still ≤1 ng/ml. No attempt was made to detect enhanced toxicity.

In contrast, three salts, BaCO3, K2CO3, and MgCO3, did exhibit significant reversal. Of these, K2CO3 was chosen for further study because BaCO3 and MgCO3 are virtually insoluble in water. K2CO3 can undergo two ionization reactions (8) and, consequently, different carbonate species will be present depending on the pH chosen. We wanted to determine which of them is responsible for the observed reversal of toxicity. Figure 1 depicts the pH dependence of the B. thuringiensis var. israelensis LC50 values in the presence of 0.5% K2CO3. The LC50 values were strongly pH dependent; the carbonate reversal increased 30-fold as the pH was lowered from 8.0 to 6.0. Moreover, the pH dependence curve in Fig. 1 is identical in both shape and position to the demarcation line between H2CO3 and HCO3− in the carbonic acid equilibrium (8). Evidently it is the nonionized K2CO3 which accomplishes toxicity reversal.

Once the pH optimum for carbonate reversal had been determined (Fig. 1), it was then possible to construct a dose-response curve. The B. thuringiensis var. israelensis LC50 values at pH 5.5 in the presence of increasing levels of K2CO3 are presented in Fig. 2. As observed previously (Nickerson and Schnell, in press), the unsupplemented B. thuringiensis var. israelensis crystals gave an LC50 value of 1 ng/ml for A. aegypti larvae. However, this value increased rapidly with increasing K2CO3 until it reached a plateau of ca. 100 ng/ml at 0.14% K2CO3. Thus, a 100-fold reversal of toxicity is achieved with K2CO3.

However, four trivial explanations of carbonate reversal must be eliminated before it can be concluded that the phenomenon is actually operative in the larval gut and that it is related to the mode of action of the B. thuringiensis var. israelensis crystals. (i) It is not primarily a pH effect. pH 5.5 in the absence of carbonate did not achieve reversal in either a buffered (0.5% KH2PO4) or unbuffered (0.5% NH4Cl or K2SO4) test solution. These solutions were monitored throughout the bioassay to ensure the maintenance of pH 5.5. (ii) It is not a crystal solubilization phenomenon. The B. thuringiensis var. israelensis crystals would, of course, be solubilized if exposed to the pH 10.5 to 11 of fresh K2CO3 solutions (3), and solubilized crystal preparations are generally found to be at least 1,000 times less toxic than intact crystals (3). However, the pH of the K2CO3 solutions employed was adjusted with HCl both before and after B. thuringiensis var. israelensis crystals were added. Additionally, the crystals could be harvested from 0.5% K2CO3 (pH 5.5 to 8.0) by centrifugation and suspended in distilled water.
with full retention of their toxicity; i.e., LC50 = 1 ng/ml. (iii) It is not due to protein carbamate formation. Alkaline carbonate buffers are known to convert the \( \epsilon \)-NH\(_2\) of lysine residues to the negatively charged carbamate (6). However, these protein carbamates are only formed under alkaline conditions, and they readily dissociate in mild acid (6). (iv) It is not a feeding inhibition phenomenon. Such a concern is reasonable since gaseous CO\(_2\) is known to narcotize A. aegypti larvae (4). However, a quantitative particle consumption assay with radioactive cells of Enterobacter cloacae (Table 1) indicated that in the presence of K\(_2\)CO\(_3\) at pH 5.5 the A. aegypti larvae actually experienced feeding stimulation rather than feeding inhibition.

Thus, we are left with the probability that carbonate exerts its toxicity reversal in the larval gut. This deduction has several implications with regard to the mode of action of the toxin. (i) Ca\(^{2+}\) ions did not induce toxicity reversal. It is well known (2) that external Ca\(^{2+}\) antagonizes insecticidal pyrethroid- and dichloro-diphenyl-trichloro-ethane-induced toxicity to nerves; consequently, it is unlikely that the B. thuringiensis var. israelensis toxin has a similar mode of action. (ii) External nonionized carbonate would undoubtedly shift the equilibrium of any carbonic acid preexisting in the larval gut. Such a shift could affect the overall larval gut pH, as well as influence the extent of protein carbamate formation on the toxin once it is ingested into the gut. (iii) A 100-fold toxicity reversal by carbonate is consistent with the suggestion (7; Nickerson and Schnell, in press) that both the Lepidoptera-active and mosquito-active toxins of B. thuringiensis act as ionophores, with the distinction that the Lepidoptera-active toxins influence cation transport whereas the mosquito-active toxins influence anion transport. More precise conclusions must await further data on the ionic composition of the larval gut in A. aegypti and the active mechanism (5) by which its highly alkaline pH is maintained.

Regardless of its ultimate mechanism, however, the mere existence of carbonate reversal
should have a profound influence on the reproducibility of data wherein the *B. thuringiensis* var. *israelensis* crystals were solubilized in carbonate buffers. Additionally, the possible presence of both soluble and insoluble carbonates must be considered in any further studies on the field efficacy and pH dependence of *B. thuringiensis* var. *israelensis*.

We thank Vance Kramer for his expert technical assistance. K.W.N. is a National Institutes of Health Research Career Development Awardee (AI 00327-TMP).

**LITERATURE CITED**


