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Commentary

Comment to Knoop et al. (1990) FEBS Letters 267, 9–12, Toxin B of *Clostridium difficile* does not have enolase activity

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Clostridium difficile, recognized as a major cause of antibiotic-associated pseudomembranous enterocolitis, produces two toxins: toxin A and B. Toxin B is an extremely potent cytotoxin (for review see [1]). The toxin B gene has been sequenced [2,3]. Knoop et al. [4] showed that toxin B has enolase activity as measured by degradation of 2-phosphoglycerate. Here we show that toxin B does not have enolase activity.

Amino acid sequences of three tryptic peptides obtained from presumed toxin B showed homology to α-enolase of the rat and enolase of *Saccharomyces cerevisiae* [5]. These sequences were compared with the amino acid sequence of toxin B deduced from the DNA sequence and showed no homology. The tryptic peptides were obtained from a protein with a relative molecular mass of 52 kDa under denaturing conditions. The Toxin B preparation (MW 163 kDa on SDS-PAGE) showed enolase activity [12]. However, the expected molecular weight is 270 kDa [3].

These discrepancies led us to study the presence of enolase activity in culture supernatants of toxigenic and nontoxigenic strains of *C. difficile* (obtained from the American Type Culture Collection (ATCC 9689, 43593, 43594, and 43596 through 43603)). Culture supernatants from six *C. difficile* strains which produced toxin and contained the genes for both toxin A and B, and five strains which possessed neither the toxin A nor the toxin B gene and were not toxigenic [6], were assayed for enolase activity. Both toxigenic and nontoxigenic strains of *C. difficile* produced enolase activity in the culture supernatant.

Partial purification of culture supernatant over DEAE-Sepharose CL-6B showed co-elution of cytotoxicity and enolase activity. However, enolase activity was also demonstrated in the fraction containing unbound proteins. When culture supernatants of nontoxigenic strains were used enolase activity was only recovered in the fraction containing unbound proteins.

Further purification of pooled fractions containing cytotoxicity on a Sephacryl S300 gel filtration column also showed co-elution of toxicity and enolase activity. This is in agreement with literature where it has been reported that enolase activity copurifies with toxin B in a wide variety of purification methods including $(NH_4)_2SO_4$ precipitation, Biogel A5m gelfiltration, phenylboronate column chromatography, ultracentrifugation [4], FPLC anion exchange [7], and DEAE-Sepharose ion-exchange chromatography. Neutralization of cytotoxicity by *C. sordellii* antitoxin did not result in inhibition of enolase activity. However, a goat antiserum against *C. difficile* filtrate abolished enolase activity [4], but when the enolase activity and toxin B are two closely linked entities as suggested by purification of culture supernatants over DEAE-Sepharose, it is to be expected that the antiserum contained neutralizing antibodies directed against *C. difficile* enolase activity and toxin B.

From these data we conclude that enolase activity is not an integral part of the toxin B gene product, but toxin B and enolase form a stable complex.

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