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Poster presentation

Cytidylyl cyclase activity of bacterial exotoxins

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3',5'-Cyclic adenosine monophosphate (cAMP) and 3',5'-cyclic guanosine monophosphate (cGMP) are key second messengers for a wide variety of mammalian cellular processes. The natural occurrence of a third cyclic nucleotide, 3',5'-cyclic cytidine monophosphate (cCMP) had been discussed very controversially 30–35 years ago [1-3], but later, cCMP was identified unambiguously in various mammalian tissues [4] indicating cCMP to be a novel second messenger with potential importance in regulation of cell growth, proliferation, tissue development and modulation of immune responses [5]. However, the precise identity of the cCMP-forming enzyme in mammalian cells is still unclear. Here, for the first time, we provide evidence for cytidylyl cyclase (CC) activity of purified bacterial exotoxins.

Bacillus anthracis and *Bordetella pertussis*, the causative bacteria of anthrax disease and whooping cough, respectively, secrete the adenylyl cyclase(AC) toxins edema factor (EF) and CyaA, weakening immune responses through massive cAMP production and, thereby, promoting the pathogenesis of the infections [6]. We found that both toxins also possess cytidylyl cyclase activity, resulting in the conversion of CTP to cCMP.

In our CC activity assay, the radioactively labeled substrate [α-^{32}P]CTP is converted to [^{32}P]cCMP which is quantified by liquid scintillation. Upon incubation of the toxins with [α-^{32}P]CTP, [^{32}P]cCMP is produced in a linear manner over time. As [^{32}P]cCMP production depends on the endogenous toxin activator protein calmodulin, as physiological pH promotes the reaction and as potent AC inhibitors show high potency on EF and CyaA, catalysis results from specific CC activity. Michaelis-Menten kinetics of EF CC activity yielded K_m = 13 ± 3 μM and V_{max} = 8 ± 1 s^{-1}.

When CTP consumption and cCMP formation were monitored by HPLC, 100 μM CTP were converted to cCMP by 20 nM EF within 1 h. In the presence of heat-inactivated enzyme, no cCMP was formed. The identity of cCMP was confirmed by co-eluting standard cCMP in HPLC experiments and by mass spectrometry methods. Based on these findings and the fact that cCMP inhibits host immune responses [7,8], we propose that the bacterial exotoxins edema factor (EF) and CyaA generate cCMP, resulting in increased infection severity. The molecular targets of cCMP remain to be determined.

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