Phospholipase A activity of adenylate cyclase toxin?

Jiri Masin[a], Radim Osicka[a], Ladislav Bumba[a], and Peter Sebo[a,1]

González-Bullón et al. (1) detected a phospholipase A (PLA) activity in the preparations of adenylate cyclase (AC) toxin (ACT) purified from recombinant *Escherichia coli*. The authors conclude that the PLA activity is intrinsic to the toxin protein and facilitates translocation of its AC enzyme domain across the target cell membrane. However, controls excluding a quite different and much simpler interpretation of the presented results are not given. All results of the study of González-Bullón et al. (1) can, indeed, be explained by: (*i*) a varying extent of contamination of the ACT and ACT-S606A toxin preparations by the calcium-dependent PLA enzyme from the outer membrane of the producing *E. coli* cells (see www.uniprot.org/uniprot/P0A921 and references therein); (*ii*) degradation and loss of RTX domain function of ACT-D1097A protein; and (*iii*) inappropriate handling of methyl arachidonyl fluorophosphonate (MAFP)-treated ACT that ablated its cell-penetrating capacity. The presented data then directly contradict the conclusions made by González-Bullón et al.: (*i*) the presumable noncatalytic PLA mutant ACT-S606A protein exhibited the lowest PLA activity, while exhibiting substantial (~50% of intact toxin) capacity to penetrate J774A.1 cells and elevate cytosolic cAMP. In contrast, (*ii*) the preparation of the other presumable PLA mutant, ACT-D1079A, exhibited higher PLA1 and PLA2 activities than ACT-S606A, but was inactive as an AC toxin (see, for example, figures 4–8 and table S1 of ref. 1). Hence, the PLA activity of ACT preparations had no relation to the capacity of the ACT proteins to deliver the AC enzyme domain across cellular membrane and produce cAMP in target cells. Moreover, the used ACT-D1097A protein was degraded into a smaller protein that migrated faster on SDS/PAGE than ACT and ACT-S606A proteins (see, for example, figures S6 and S8 of ref. 1). Because ACT-D1097A exhibited a full activity of its N-terminal AC enzyme, its degradation occurred in the C-terminal RTX domain that is essential for ACT binding to the toxin receptor CD11b/CD18 (2–4). This would explain why ACT-D1097A lacked the cAMP-elevating activity on J774A.1 cells and was unable to lyze erythrocytes. Second, chemical inhibition of PLA activity by overnight incubation of ACT with the MAFP reagent covalently modifying Ser residues, followed by overnight dialysis in buffer lacking chaotropic concentrations of urea, was used. This did not cause loss of the AC enzyme activity in solution, but dialysis is known to cause self-association of toxin molecules and a substantial loss of ACT capacity to bind and penetrate target cell membranes (5, 6). This finding would explain why no cAMP-elevating and cytotoxic capacity of ACT-MAFP was observed. The modest, if any, effect of the S606A substitution on toxin activities on cells (see, for example, figures 5–8 and table S1 of ref. 1) then goes well with a previous report that insertion of a VYT tripeptide between residues 606 and 607 has no impact on toxin activity of ACT (7). Finally, incubation of J774A.1 cells with 20 nM (3.5 μg/mL) ACT leads to rapid ATP depletion in cells within minutes (8) and this is known to provoke arachidonate release by cellular enzymes (9). Hence, numerous other reasons than a PLA activity of ACT easily explain all of the data reported by González-Bullón et al. (1).

1 González-Bullón D, Uribe KB, Martín C, Ostolaza H (2017) Phospholipase A activity of adenylate cyclase toxin mediates translocation of its adenylate cyclase domain. *Proc Natl Acad Sci USA* 114:E6784–E6793.

2 Guermonprez P, et al. (2001) The adenylate cyclase toxin of *Bordetella pertussis* binds to target cells via the alpha(M)beta(2) integrin (CD11b/CD18). *J Exp Med* 193:1035–1044.

3 Osicka R, et al. (2015) *Bordetella* adenylate cyclase toxin is a unique ligand of the integrin complement receptor 3. *eLife* 4:e10766.

4 Wang X, et al. (2017) Fine epitope mapping of two antibodies neutralizing the *Bordetella* adenylate cyclase toxin. *Biochemistry* 56:1324–1336.

5 Rogel A, Meller R, Hanski E (1991) Adenylate cyclase toxin from *Bordetella pertussis*. The relationship between induction of cAMP and hemolysis. *J Biol Chem* 266:3154–3161.

[a]Institute of Microbiology, Czech Aacademy of Sciences, 142 20 Prague 4, Czech Republic

Author contributions: L.B. analyzed data; and J.M., R.O., L.B., and P.S. wrote the paper.

The authors declare no conflict of interest.

[1]To whom correspondence should be addressed. Email: sebo@biomed.cas.cz.

Published online February 21, 2018.

6 Rogel A, et al. (1989) *Bordetella pertussis* adenylate cyclase: purification and characterization of the toxic form of the enzyme. *EMBO J* 8:2755–2760.
7 Osicka R, et al. (2000) Delivery of CD8(+) T-cell epitopes into major histocompatibility complex class I antigen presentation pathway by *Bordetella pertussis* adenylate cyclase: Delineation of cell invasive structures and permissive insertion sites. *Infect Immun* 68:247–256.
8 Basler M, Masin J, Osicka R, Sebo P (2006) Pore-forming and enzymatic activities of *Bordetella pertussis* adenylate cyclase toxin synergize in promoting lysis of monocytes. *Infect Immun* 74:2207–2214.
9 Chien KR, et al. (1985) Release of arachidonate from membrane phospholipids in cultured neonatal rat myocardial cells during adenosine triphosphate depletion. Correlation with the progression of cell injury. *J Clin Invest* 75:1770–1780.