The enzyme subunit SubA of Shiga Toxin-Producing *E. coli* strains demonstrates comparable intracellular transport and cytotoxic activity as the holotoxin SubAB

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Shiga toxin-producing *Escherichia coli* (STEC) strains are bacterial pathogens, which are mainly found in the gastrointestinal tract of farm and wildlife animals. The bacteria can be transmitted to humans via food and causes diarrheal and extraintestinal diseases. In addition to the Shiga toxin, some STEC strains also produce a second toxin, namely the subtilase cytotoxin (SubAB) [1]. SubAB belongs to the family of AB_5 toxins, consisting of an enzymatically active A subunit (SubA) and separate B subunits (SubB), which mediate cell binding and uptake of SubAB. Inside the host cell, SubAB is transported via a retrograde pathway into the endoplasmic reticulum (ER), where it cleaves the glucose-regulated protein GRP78. SubAB-induced GRP78 cleavage activates a cellular stress response eventually resulting in cell death [1]. We previously demonstrated that SubA, without SubB, binds and intoxicates HeLa cells [2], whereas the cellular and molecular mechanisms behind this remained unclear. In the present study, we found that even in the absence of SubB, SubA is internalized into cells, reaches the endoplasmic reticulum (ER) and cleaves the chaperone GRP78. Furthermore, SubA-induced GRP78 cleavage in cells is prevented by the pre-treatment of cells with brefeldin A (BFA), which inhibits the transport of protein toxins into the ER. Obviously, SubA contains a yet undescribed ER localization signal, predicted by the software scanProsite™ as a SEEL motif at the C-terminus. Hence, a SubA mutant, lacking the SEEL motif ($SubA_{\Delta C344}$), was generated, which exhibited no cytotoxicity alone, but only together with SubB, when tested on HeLa and HCT116 cells. Fluorescence microscopy revealed that $SubA_{\Delta C344}$ is generally taken up into cells, but further investigations regarding substrate cleavage have shown that $SubA_{\Delta C344}$-induced GRP78 cleavage was delayed when compared to wildtype SubA. We therefore assume that the SEEL motif plays a crucial role in guiding SubA into the ER [3]. Furthermore, we confirmed our findings in the human intestinal mini-gut organoid system. Our results not only contribute to a better understanding of the mode of action of the subtilase cytotoxin, but also raise the question whether a B component-independent cytotoxic effect of the A component is also true for other bacterial AB_5 toxins.

Literature

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3. Sessler K et al. (2021); Arch. Toxicol. (in press)