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Yessotoxin induces the accumulation of altered E-cadherin dimers that are not part of adhesive structures in intact cells

Giuseppe Ronzitti, Gian Paolo Rossini *

Dipartimento di Scienze Biomediche, Università di Modena e Reggio Emilia, Via Campi 287, I-41100 Modena, Italy

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Abstract

We have studied the alteration induced by yessotoxin in the E-cadherin–catenin system of epithelial cells by stabilizing the protein–protein interactions in oligomers, through the introduction of covalent bonds between subunits *in vitro* and *in vivo*. The E-cadherin–catenin complexes that we have stabilized by crosslinking comprise multiple forms of dimeric, trimeric, tetrameric and hexameric complexes, with different subunit compositions. A 1-day treatment of MCF-7 cells with yessotoxin resulted in an increase in cellular levels of the complexes including a 100 kDa fragment of E-cadherin ($ECRA_{100}$), with a relative increase in cellular E-cadherin $\cdot ECRA_{100}$ heterodimers, as opposed to the E-cadherin homodimer that represents the core structure of the E-cadherin–catenin system of adhesive structures in normal cells. The high MW oligomers of cell adhesive structures, in turn, were not appreciably altered by cell treatment with yessotoxin. Most of these oligomers partitioned in a fraction that cannot be solubilized by non-ionic detergents after crosslinking of intact cells. Yessotoxin treatment did not significantly alter the levels of $ECRA_{100}$ in the Triton X-100 resistant fraction of plasma membrane, but increased the relative abundance of $ECRA_{100}$ in the Triton X-100 soluble pool of crosslinked cells. We have concluded that cell exposure to yessotoxin leads to increased cellular contents of E-cadherin $\cdot ECRA_{100}$ heterodimers that are not participating to cell adhesive structures but are located in other membranous fractions of intact cells.
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Yessotoxin (YTX) is a sulphated polyether compound that was originally found in scallops, and was capable to cause death when administered i.p. at very low doses into mice (Murata et al., 1987). Cultured cells are targets of YTX that has been shown to induce an array of responses, depending on the dose and the cell line (reviewed in Rossini et al., 2006). Cell adhesion is particularly sensitive to YTX, as very low concentrations of the toxin (10^{-10} to 10^{-9} M) are sufficient to cause cell detachment from culture dishes (Ogino et al., 1997; Pierotti et al., 2003; Ronzitti et al., 2004).

Cadherins represent a superfamily of calcium-dependent trans-membrane glycoproteins that are responsible for cell–cell adhesion and play key biological roles in proper development of the embryo and in tissue integrity in the adult (Nollet et al., 2000; Takeichi, 1990; Tepass et al., 2000). Cell–cell adhesion is based on the homophilic binding between identical cadherin molecules projecting on the extracellular side of adjacent cells (Hirano et al., 1987; Koch et al., 1999; Miyatani et al., 1989). The adhesive structures are then stabilized

* Corresponding author. Tel.: +39 059 205 5388;
fax: +39 059 205 5410.
E-mail address: rossini.gianpaolo@unimore.it (G.P. Rossini).

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and gain mechanical strength by the association of cadherin molecules with intracellular proteins (Kobielak and Fuchs, 2004; Ozawa et al., 1989). The general model of supramolecular structures containing cadherins in intact cells is based on the results obtained with E-cadherin that is specifically expressed in epithelia (Nollet et al., 2000). The major intracellular proteins found associated with E-cadherin include β- and γ-catenin (plakoglobin) (Hinck et al., 1994; Ozawa and Kemler, 1992; Ozawa et al., 1989), interacting with the carboxy-terminal portion of the intracellular domain of E-cadherin (Aghib and McCrea, 1995; Nagafuchi and Takeichi, 1989; Ozawa et al., 1989; Reynolds et al., 1994), the Src substrate p120 that binds the juxtamembrane intracellular portion of E-cadherin (Aghib and McCrea, 1995; Reynolds et al., 1994; Yap et al., 1998), and α-catenin that does not take direct contact with E-cadherin, but interacts with β-catenin and plakoglobin (Oyama et al., 1994). Based on those findings, the proposed core structure of adhesive complexes involves the interaction of dimeric E-cadherins and bridging of cadherin molecules to the actin cytoskeleton through β-catenin (or plakoglobin) and α-catenin (Kobielak and Fuchs, 2004).

YTX alters the E-cadherin system in epithelial cells, where it induces the accumulation of a 100 kDa fragment ($ECRA_{100}$), devoid of the intracellular carboxy terminal domain of the molecule that is involved in the binding of catenins (Pierotti et al., 2003; Ronzitti et al., 2004). In previous studies we have shown that the accumulation of $ECRA_{100}$ is attained within the first day of YTX treatment in cultured MCF-7 cells, but disruption of the E-cadherin–catenin system is detectable only after prolonged cell exposure to the toxin (Ronzitti et al., 2004). More recent findings, however, have shown that YTX does not actually increase E-cadherin degradation, but slows down the complete disposal of $ECRA_{100}$ (Callegari and Rossini, 2008), leading to increased cellular levels of this intermediate proteolytic product.

In those studies we have not directly analyzed the supramolecular structures of the E-cadherin–catenin complexes, and we could not characterize the changes induced by YTX in our experimental systems.

We have then set out an investigation to analyze the effects of YTX on supramolecular structures of the E-cadherin system with reference to altered cell–cell adhesive structures, and have studied the E-cadherin–catenin complexes by a more direct approach, using chemical crosslinking under cell-free conditions and in intact cells. By this procedure, covalent bonds are introduced between the proteins associated in the complexes, stabilizing existing oligomers and preventing the rearrangement of their subunits that may occur during sample preparation and analysis. Here we show that YTX increases the cellular content of altered E-cadherin dimers that partition in the Triton X-100-soluble pool of the cellular protein that should not participate to adhesive structures of intact epithelial cells, providing a molecular explanation of the observation that cell–cell adhesion is not severely affected by accumulation of $ECRA_{100}$ *per se*.

1. Materials and methods

1.1. Materials

Yessotoxin was obtained from the Institute of Environmental Science and Research Limited (Lower Hutt, New Zealand) and from Patrizia Ciminiello (Università di Napoli, Napoli, Italy). Disuccinimidylsuberate (DSS) and dithiobis(succinimidyl propionate) (DTSP) were from Pierce (Erembodegem, Belgium). Anti-E-cadherin antibodies (clone HECD-1) were purchased from Alexis Corporation (San Diego, CA, USA). The anti-α-catenin, anti-β-catenin, anti-plakoglobin and anti-p120 antibodies were obtained from Transduction Laboratories (Lexington, KY, USA). Peroxidase-linked anti-mouse Ig antibody, and the enhanced chemiluminescence (ECL) detection reagents were from GE Healthcare (Milano, Italy). The prestained molecular mass markers and the crosslinked phosphorylase b markers for SDS-PAGE were obtained from Sigma (Milano, Italy). The nitrocellulose membrane Protran BA83 was obtained from Schleicher and Schuell (Brentford, UK).

1.2. Cell culture conditions and preparation of cell extracts

MCF-7 cells were obtained from the European Collection of Animal Cell Cultures (ECACC No. 86012803 CB No. 2705), and were grown in 5% carbon dioxide in air at 37 °C, in 90 mm diameter Petri dishes, with a culture medium composed of Dulbecco's modified Eagle medium, containing 1% non-essential amino acids and 10% foetal calf serum, as previously described (Ronzitti et al., 2004).

Stock solutions (1 μM) of YTX were prepared by dissolving the compounds in absolute ethanol, and were stored in glass vials protected from light at −20 °C. If not stated otherwise, cell treatments were carried out using dishes near confluency, by addition of 1 nM YTX and incubations for 24 h at 37 °C. Parallel dishes received the addition of absolute ethanol (control samples).

The preparation of cell extracts was carried out at 2 °C. Cells from culture dishes were washed once with 20 mM phosphate buffer, pH 7.4, containing 0.15 M NaCl (PBS), harvested with PBS and transferred to centrifuge tubes. The cell suspensions were washed twice with PBS and recovered by centrifugation for 8 min at 800 × *g*. The cell pellets were lysed with

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