Food-Borne Toxin Sensitivity

Subtilase cytotoxin (SubAB) is an AB5 toxin produced by Shiga toxigenic E. coli (STEC) bacteria. AB5 toxins bind to cognate glycan receptors on target cells via their pentameric B subunits and inflict cellular damage through their A subunit. SubAB causes gastrointestinal disease in humans and hemolytic uremic syndrome in mice through SubA-mediated cleavage of the endoplasmic reticulum chaperone BiP/GRP78.

B subunit binding is key to host susceptibility of AB5 toxins. Therefore, Byres et al. [(2008) Nature 456, 648] investigated the binding specificity of SubAB. They found the strongest binding affinity for glycans terminating in N-glycolylneuraminic acid (Neu5Gc) and then used X-ray crystallography to identify the binding site for Neu5Gc on SubB. The data revealed the critical determinants of the binding specificity, namely, interactions of a hydroxyl group on Neu5Gc with Tyr78 and Met10 on SubB. The absence of this hydroxyl group on N-acetylneuraminic acid (Neu5Ac) explains why this sugar binds poorly to SubB. Byres et al. went on to confirm their crystallographic results with mutational studies, showing that alteration of key amino acids, such as Tyr78, reduced binding of Neu5Gc-related glycoproteins to SubAB and decreased its cytotoxicity.

Byres et al. point out that humans lack the enzyme cytidine monophosphate-N-acetylneuraminic acid hydroxylase (Cmah), which is required to synthesize CMP-Neu5Gc from CMP-Neu5Ac. Studies of Cmah-null mice demonstrated the absence of Neu5Gc from cell surface glycoproteins, and SubAB bound poorly to kidney tissue from these mice as compared to wild-type mice. Similarly, SubAB bound poorly to human cells in culture, but binding could be increased by incubating the cells with Neu5Gc, which became incorporated into cell surface glycoproteins. In fact, Byres et al. point out that Neu5Gc can be found in human tissues, presumably as the result of incorporation of the exogenous sugar from dietary sources.

Cmah-null mice demonstrated an increased susceptibility to SubAB toxin in vivo, which was attributed to the absence of Neu5Gc-containing proteins in the serum that could compete for toxin binding. Byres et al. argue that human susceptibility to the toxin may be similarly exacerbated by a lack of protective serum proteins. Thus, despite the absence of the primary binding glycan for SubAB toxin entry, humans are susceptible to its cytotoxic effects, largely due to the tissue incorporation of Neu5Gc from dietary sources coupled with the absence of protective glycoproteins in body fluids. Ironically, the major dietary sources of Neu5Gc, red meat and dairy products, are also the foods most likely to be contaminated with STEC, so exposure and sensitization to SubAB come via the same mechanism. • Carol A. Rouzer

Endogenous AHR Ligand

The aryl hydrocarbon receptor (AHR) is well-known as a transcription factor by which lipophilic xenobiotic hydrocarbons activate the expression of metabolizing enzymes. However, the AHR plays a role in processes such as immunity, reproduction, and development, suggesting that there must be endogenous ligands that modulate its activity. New studies by Wincent et al. [(2009) J. Biol. Chem. 284, 2690] provide support for the possible importance of 6-formylindolo[3,2-b]carbazole (FICZ) as a natural AHR ligand in humans.

FICZ was formed in vitro by irradiation with visible light in the presence of riboflavin. Incubation of FICZ with human liver S9 fractions led to the identification of seven new metabolites. Antibody and inhibitor studies showed that the AHR-dependent CYP1 class of P450 enzymes was primarily responsible for FICZ metabolism. Hydroxylated metabolites of FICZ were rapidly conjugated by four sulfotransferases, suggesting that these would be the primary metabolites in humans. FICZ and two closely related indolo[3,2-b]carbazoles were strong AHR activators, exceeding the potency of TCDD, the commonly used xenobiotic reference AHR ligand. Two FICZ metabolites also showed strong AHR activation. LC-MS analysis of seven human urine samples revealed FICZ-derived sulfated metabolites in all samples with positive identification of 8-SO$_4^{-}$-FICZ in two cases. The evidence that FICZ exists in vivo, likely by irradiation of Trp, combined with its efficiency as an AHR activator and substrate for AHR-dependent metabolism strongly supports its role as a natural AHR ligand. • Carol A. Rouzer

**His-Mediated Oxidant Sensing**

Key to a cell’s capacity to protect itself against oxidative stressors is its ability to regulate the expression of protective enzymes in response to increased intracellular or environmental oxidants. In many cases, oxidant sensing depends on proteins bearing reversibly oxidizable cysteine residues. The PerR protein of the bacterium *B. subtilis* uses a different oxidant sensing mechanism. This protein consists of a DNA binding domain and a C-terminal regulatory domain that contains two metal binding sites. The Zn(Cys)_3 structural site is critical for protein dimerization, whereas binding of Fe^{2+} or Mn^{2+} to the regulatory site is required for DNA binding. Prior studies have shown that reaction of PerR-Zn-Fe with H_2O_2 leads to Fe^{2+}-mediated oxidation of His37 or His91, both of which are found in the regulatory metal binding site. The result is release of the Fe^{2+} and dissociation of bound DNA. This reaction does not occur in the Mn^{2+}-bound protein. To better understand the mechanism of PerR-Zn-Fe regulation, Traoré et al. [(2009) Nat. Chem. Biol. 5, 53] have now fully characterized the structure of the oxidized PerR protein.

After developing a method to isolate PerR-Zn-ox, the singly oxygenated PerR protein bearing no metal at the regulatory site, Traoré et al. used MALDI-TOF analysis to show that 55 and 25% of His37- and His91-containing peptides were oxygenated, respectively. HPLC-MS/MS analysis identified the oxidized amino acid as 2-oxo-His and allowed quantification that was consistent with the MALDI-TOF data. X-ray diffraction analysis of crystallized PerR-Zn-ox verified 2-oxo-His at the 37 position, but oxidation at His91 could not be confirmed.

**A Cellular Look at Toxicity**

We routinely evaluate the effects of toxic substances on whole cell populations without considering the likelihood of cell-to-cell variability. Now, Cohen et al. [(2008) Science 322, 1511] take toxicology to a new level by exploring the effects of the cytotoxic topoisomerase-1 (TOPO1) poison camptothecin (CPT) on the expression of over 1000 proteins in individual cells. They used “CD tagging”, a retrovirus-based approach, to insert a red fluorescent tag (mCherry) into the genome of H1299 lung carcinoma cells. A clone of these cells expressed red fluorescence uniformly in the cytosol and more intensely in the nucleus. From these cells, Cohen et al. generated a library of over 1000 new clones, each bearing an enhanced yellow fluorescent protein (eYFP) tag in a different protein. Approximately 80% of the eYFP-labeled proteins were characterized, and control studies confirmed that the eYFP tag did not affect protein dynamics.

Cohen et al. used the mCherry tag to evaluate cell location and morphology and the eYFP tag to assess the location and quantity of the individual proteins. Following camptothecin (10 µM) treatment, they monitored the cells microscopically for 48 h, collecting several movies for over 200 consecutive frames at 10–40 cells per frame. During that time, cells ceased dividing and lost motility (by ∼10 h) and showed morphological changes associated with death (15% by 36 h).

The eYFP results showed distinct patterns of change for different groups of proteins, with about 76% decreasing and 7% increasing over time. A small number of proteins demonstrated a change in localization. Among the first to respond was the CPT target TOP1, which decreased in amount (within 1 h) and then translocated from nucleus to cytoplasm (over the next 5 h). Patterns were noted for groups of functionally related proteins. For example, decreases in cytoskeletal proteins correlated temporally with loss of motility, and the appearance of DNA damage repair and apoptosis-related proteins occurred late in the response.

A small number of proteins were unique in that their changes varied widely from cell to cell. For two of these, the RNA-helicase DDX5 and replication factor RFC1, increasing levels correlated with cell survival. Indeed, the investigators showed that knockdown of DDX5 by RNAi increased cell sensitivity to camptothecin cytotoxicity. These exciting results demonstrate the power of the use of CD tagging to identify key determinants of cell survival during the response to a toxic insult. • Carol A. Rouzer

**Spotlight**

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