Unraveling the mechanism that introduces disulfide bonds in Campylobacter jejuni

The set of proteins present in specific living cell, both eukaryotic and prokaryotic, determines its functioning. Amino acids are the building blocks of proteins and all proteins composed of twenty amino acids. The sequence of amino acids in polypeptide chains (the primary structure) determines their three dimensional structure (the secondary and tertiary structure) which is essential for protein activities. Cysteine – amino acid containing thiol (-SH) group plays a crucial role in protein folding The oxidation reaction between two cysteine thiol groups, even if cysteines are located far apart in the primary protein structure, results in the formation of a disulfide bond. This bond formation is a rate-limiting step in the protein folding process, and it is catalyzed by proteins of the Dsb (disulfide bond) system. A process which is crucial for protein structure stabilization and plays an essential role in the assembly of many virulence factors, takes place in oxidative environments; in the periplasm (the space between cytoplasmic and outer membrane) in gram-negative bacteria cells. A combination of microbiological, biochemical, biophysical and proteomic approaches has yielded a detailed characterization of the Dsb protein network for the model microorganism, Escherichia coli (EcDsb proteins). In general, there are two, mostly antagonistic, metabolic pathways acting in the E. coli periplasm: an oxidation pathway and an isomerization/reduction pathway The first reaction (catalyzed by EcDsbA and EcDsbB) is responsible for the formation of disulfide bonds in the newly synthesized proteins, just after they cross the cytoplasmic membrane. As this process occurs in a non-selective way, a second reaction (driven by EcDsbC and EcDsbD) rearranges improperly introduced disulfide bonds In E. coli, DsbA is converted back to the oxidized form by the inner membrane protein DsbB, which donates electrons to elements of the respiratory chain. DsbC is kept in the reduced form by an integral membrane protein, DsbD, that catalyzes the transfer of electrons from the cytoplasm to the periplasm. Fig.1 presents key elements of the E. coli oxidative protein folding.

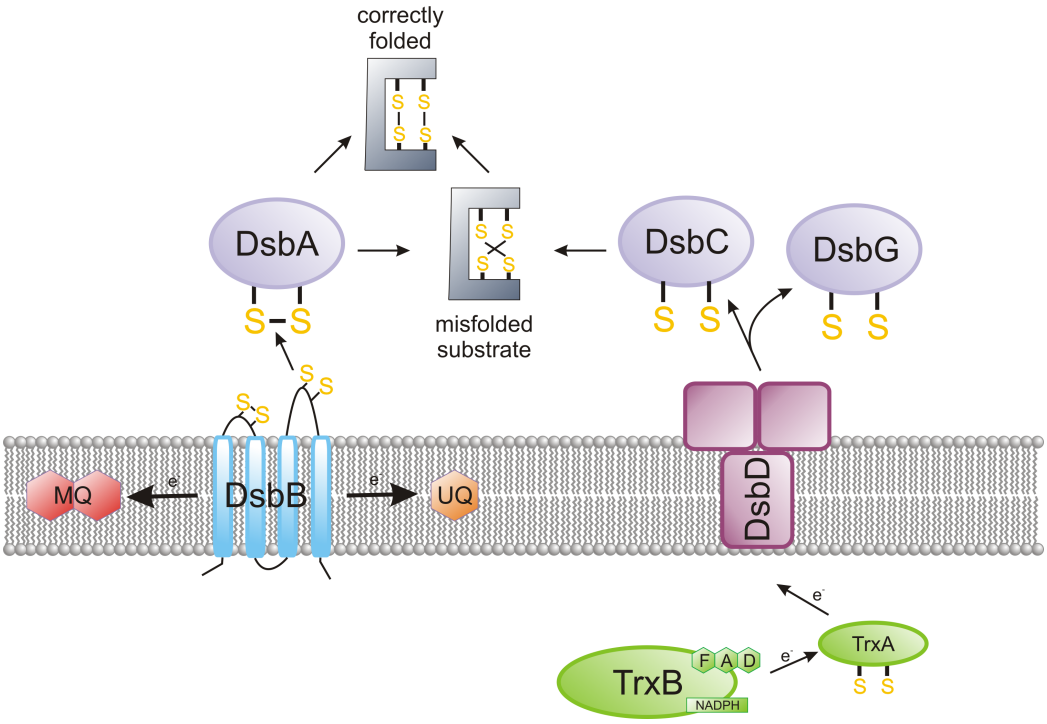

Fig. 1. Model of disulfide – bond formation in the periplasm E. coli [Bocian-Ostrzycka et al. (2015). Front Microbiol 6:570. doi: 10.3389/fmicb.2015.00570]

Recent rapid advances in global analysis of bacteria have thrown light on the enormous diversity among bacterial Dsb systems. The set of Dsb proteins involved in the oxidative pathway, varies, depending on the microorganism. We still do not fully understand the reason for variability of bacterial Dsb systems. For some bacterial species the correct folding of various proteins is ensured by a single pair DsbA/DsbB oxidoreductases, while other species require two or more DsbA or DsbB homologues. The subject of this research project is the Dsb system of Campylobacter jejuni. Campylobacter spp., gramnegative microorganisms, members of the Epsilonproteobacteria, are considered to be one of the emerging pathogens. They are presently recognized as a leading bacterial cause of food-borne illnesses in Europe and the USA, and a major agent of bacterial diarrhoea worldwide. The number of reported confirmed cases of human campylobacteriosis in the EU was 214779 in 2013. Campylobacter infections occasionally lead to the development of autoimmune diseases, such as reactive arthritis and neurological illnesses, of which the most dangerous is Guillain-Barré syndrome (GBS) – an acute inflammation of peripheral nerves. The functioning of the Campylobacter sp. Dsb system has so far been poorly understood. The C. jejuni genome codes for two periplasmic DsbA proteins, and two transmembrane (DsbB and DsbI) oxidoreductases. Additionally it codes for Cj1298, a potentially dimeric Dsb protein with a still-uncharacterized function (oxidase or reductase?). This project is an attempt to elucidate the pathway of disulfide bond formation in C. jejuni, using a combination of biochemical, proteomic, microbiological and biophysical approaches. We propose to: 1) evaluate whether C8J1298 realizes DsbA-like or rather DsbC-like in function by conducting in vivo assays (in E. coli as well as in C. jejuni) that should discriminate between these two activities; 2) analyze the biochemical features of CjDsbAs and C8J1298; 3) solve the structures of CjDsbAs and C8J1298; 3) analyze the influence of DsbAs and C8J1298 on C. jejuni virulence and finally 4) identify the substrates of CjDsbAs and C8J1298. The results should highlight the interplay between C. jejuni Dsb proteins to achieve the final native oxidized states of C. jejuni proteins.

In recent years, the rapid increase in bacterial antimicrobial resistance has become a major public health concern in both developed and developing countries. Increasing incidences of human infection with Campylobacter strains that are resistant to the commonly used antibiotics significantly impairs efforts to combat campylobacteriosis, either by prolonging the duration of therapy or rendering that therapy ineffective. One approach to generate new classes of antibacterials is to target virulence rather than the viability of bacteria. Proteins of the Dsb system, which play a key role in the virulence of many pathogenic gram-negative organisms, represent possible new drug targets. Inhibition of the CjDsbAs or Cj1298 interactions with their protein substrates or their redox partners could constitute a means of blocking the formation of virulence factors. Thus, added knowledge about C. jejuni Dsb protein structures and their activities may facilitate the future discovery of an effective anti-Campylobacter drug. The localization of the Dsb proteins (in the bacterial periplasm) renders them easily accessible to potential small-molecule inhibitors. As CjdsbAs have atypical structural features that potentially are not characteristic of Dsb proteins present in the proteomes of other bacteria, the use of a CjDsb-protein inhibitor should not disturb the balance of the host physiological microflora.
We believe that the project will provide a significant contribution to the knowledge of basic research in such fields as biochemistry and microbiology and, in the future, it may initiate the research aimed at development of a new anti-Campylobacter drugs.