We investigated two colicins which target the murein in the periplasm. Colicin M (Cma) interferes with murein biosynthesis and pesticin (Pst) degrades murein. Both colicins are imported by TonB-dependent outer membrane receptors/transporters. It takes about 8 min after addition to cells until Cma is no longer accessible to proteolytic degradation, inactivation by SDS, or antibodies. Cma becomes trypsin-sensitive in the receptor-bound state. FkpA, a periplasmic chaperone and prolyl cis-trans isomerase is essential for Cma activity. A Cma mutant in Pro176 is inactive. Phe-Pro176 built into a synthetic peptide is cis-trans isomerized by FkpA with a high efficiency. All inactive FkpA mutants carry mutations in the isomerase domain. Cma exported from the cytoplasm into the periplasm by an attached signal sequence also requires FkpA to kill cells. These data suggest that Cma with a compact crystal structure is unfolded during import across the outer membrane and the cytoplasmic membrane and that refolding in the periplasm requires the isomerase and the chaperone activity of FkpA. We determined the crystal structure of Pst which revealed an activity domain fold very similar to T4 lysozymes. Fusion of a T4 lysozyme (gene e product) to the activity and translocation domain of Pst resulted in an active colicin (Pst-T4L) that killed cells. Introduced disulfide bonds prevented the in vivo activity of Pst and Pst-T4L but left the in vitro activity unaffected. It is concluded that Pst unfolds during import across the outer membrane into the periplasm.