Pore-forming toxins (PFTs) represent the largest family of bacterial protein toxins and constitute important bacterial virulence factors. Their cytolytic function is conducted by introducing pores into the target cell membranes which leads to the lysis of the host cell. Most bacterial toxins are secreted to the extracellular environment in a water-soluble form. A general mechanism of the PFT attack involves three steps: (i) binding of the soluble monomer to the target cell membrane; (ii) oligomerization of the monomer into a prepore structure; (iii) insertion of the transmembrane pore across the target cell membrane. So far, the assembly of most of the known toxins has been found to occur on the lipid bilayer or in the presence of detergents. In our study, we have found that cytolysin A (ClyA) toxin from *E. coli* could assemble in solution in the absence of either bilayer membrane or detergent. Fluorescence and electrophysiological measurements suggest that the oligomers in solution represent an intermediate state in the transformation of the monomer to transmembrane pore. Our data indicates that ClyA may possess an alternative assembly pathway that is distinct from other classic PFTs.