MECHANISM OF ACTIVATION OF A MAJOR TOXIN INVOLVED IN WHOOPING COUGH

The CyaA protein is a toxin produced by Bordetella pertussis, the causative agent of whooping cough. It interacts with calmodulin, an interaction that leads to CyaA activation. Scientists from the Institut Pasteur and the CNRS coupled SAXS and SR-CD studies performed at SOLEIL with hydrogen-deuteron exchange monitored by mass spectrometry (HDX-MS) in an integrative structural biology approach. The combined results allowed them to elucidate the relationship between the structure and activation of CyaA. The mechanism involves calmodulin binding to an unstructured region of CyaA that folds upon binding. The resulting structural rearrangement of the catalytic domain of CyaA allows its activation.

Adenylate cyclase toxin (CyaA) is a major virulence factor of Bordetella pertussis, the causative agent of whooping cough [1]. This disease, which can affect people of all ages, is particularly severe and potentially fatal for infants and vulnerable individuals. The CyaA toxin contributes to the early stages of bacterial colonization of the lungs of people infected by B. pertussis. CyaA is synthesized and secreted by the bacterium in an inactive form. The CyaA toxin employs a unique mechanism to access the cytosol in our cells (the liquid phase inside cells). The catalytic domain is directly transported via the plasma membrane of target cells through a process known as membrane translocation.

HOW DOES CALMODULIN ACTIVATE CyaA?

Calmodulin is a highly conserved eukaryotic protein that interacts with a wide variety of other proteins. It controls their activities in response to variations in concentrations of intracellular calcium. After invading the cytosol and attaching to calmodulin, the catalytic domain of CyaA is activated by calmodulin to synthesize huge quantities of cAMP, a molecule used in low concentrations as an intracellular messenger. But the large quantities produced by CyaA impair cell physiology and ultimately lead to cell death. The activation of CyaA by calmodulin – an act of molecular piracy – has been known for some time, but the molecular activation mechanism remained a mystery.

Researchers from the Institut Pasteur and the I2BC used an integrative structural biology approach combining several biophysical techniques to characterize the structural changes in the CyaA catalytic domain and in calmodulin when they interact.

2 Structural models of the catalytic domain of CyaA isolated and activated by calmodulin. Left: structural model of the catalytic domain (AC) of isolated, inactive CyaA and below the resulting adjustment of its calculated scattering pattern (red curve) against experimental scattering data (black dots). The regions folded into helices and sheets are represented in green, while the regions characterized by structural disorder are represented as a black line. Structural disorder prevents catalytic activity. Right: the active enzyme complex, formed by the catalytic domain of CyaA (in colour) and calmodulin (in grey) and below the resulting adjustment of its calculated scattering pattern (blue curve) against experimental data (black dots). The stabilization of the catalytic domain induced by calmodulin binding is represented by the colour gradient red, yellow, green and blue. The red and yellow regions correspond to the maximum stabilization (observed with HDX-MS). The flexible catalytic loop is indicated by a red star. The structural models were obtained using experimental SAXS data recorded on SWING while incorporating results from HDX-MS and SR-CD experiments. 2