Microsymposium

MS072.O01

Structure determination and annotation of serendipitously crystallized proteins

Mathur R Murthy[1]
[1]*Indian Institute Of Science, Bengaluru, India*
E-mail: mbumrn@gmail.com

The structure of several serendipitously crystallized proteins have recently been determined using a large-scale molecular replacement protocol. From the electron density map based sequence, one of these proteins was identified as that of a phosphate binding protein from Stenotrophomonas maltophilia, a multidrug resistant organism, probably involved in phosphate transport across the membrane. Studies were carried out towards structure based functional annotation of two universal stress proteins (USPs), YnaF (UspF) and YdaA (UspE), that are expressed when Salmonella typhimurium encounters unfavorable conditions. It was found that YnaF is a single domain USP while YdaA consists of two tandem USP domains. The N- and C- terminal domains of YdaA are structurally similar in spite of low sequence identity. YnaF was inactive as an ATPase but surprisingly crystallized with a tightly bound ATP and chloride ions at the tetrameric interface. Mutation of residues involved in chloride binding made the protein active as an ATPase. Thus, chloride binding appears to be coupled to inhibition of ATPase activity. This is suggestive of the role of YnaF as an ion sensor mediating cell signaling. YdaA was found to exhibit ATPase activity and an ATP binding motif G-2X-G-9X-G could be identified in its C terminal domain. A large ligand, probably bound to the N-terminal domain, was co-purified with YdaA. The ligand was tentatively identified as UDP-(3-O-(R-3-hydroxymyristoyl))-N-acetylglucosamine, which is an intermediate in the lipid A biosynthetic pathway suggesting that YdaA may provide cellular protection by altering membrane properties during conditions of stress. In an attempt to determine the structure of a mutant of another survival protein (SurE) from Salmonella typhimurium, a seemingly reasonable solution was obtained by molecular replacement (MR) using the wild type SurE structure as the phasing model. Although the crystal was that of glycerol dehydrogenase as demonstrated by the large-scale MR protocol, the initial structure appeared to be reasonable because of the partial similarity in the arrangement of secondary structural elements in the two proteins. Structure elucidation of a protein from M. smegmatis identified as an ACP-thiolase was found to be inactive as a thiolase and its additional domain did not resemble that of the acyl carrier protein. M. smegmatis protein may function as a fatty acyl CoA carrier. These and several other structures determined illustrate the power of X-ray diffraction for elucidating the structure and function of unknown proteins.

Keywords: serendipity, marathomMR, structure