INSIGHTS INTO MOLECULAR PLASTICITY OF CHOLINE BINDING PROTEINS (PNEUMOCOCCAL SURFACE PROTEINS) BY SAXS

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Phosphocholine moieties decorating the pneumococcal surface are used as a docking station for a family of modular proteins, the so-called choline binding proteins or CBPs. Choline recognition is essential for CBPs function and may also be a determinant for their quaternary structure. There is little knowledge about modular arrangement or oligomeric structures in this family. Therefore, we have used the small angle x-ray scattering (SAXS) technique combined with analytical ultracentrifugation in order to model the three dimensional envelope of two highly different CBPs: the phage encoded Cpl-1 lysozyme and the pneumococcal phosphorylcholine esterase Pce. Both enzymes have an N-terminal catalytic module and a C-terminal choline-binding module (CBM) that attaches them to the bacterial surface and comprises six and ten sequence repeats in Cpl-1 and Pce, respectively. SAXS experiments have shown an inherent conformational plasticity in Cpl-1 that accounts for the different relative position of these regions in the solution and crystal structures. Dimerization of Cpl-1 upon choline binding has been also visualised for the first time, and monomer-monomer interactions take place through the first CBR where a non-canonical choline binding-site has now been identified. This mode of association seems to be independent of the absence or presence of the Cpl-1 catalytic module and reveals that the arrangement of the monomers differs from that previously found in the isolated CBM dimer of pneumococcal LytA amidase. In contrast, Pce displays the same modular disposition in the solution and crystal structures, and remains almost invariant upon choline binding. The present results suggest that protein dimerization and duplication of CBRs may be alternative but not equivalent ways of improving cell wall recognition by CBPs, since they provide different interaction geometries for choline residues present in (lipo)teichoic acids.

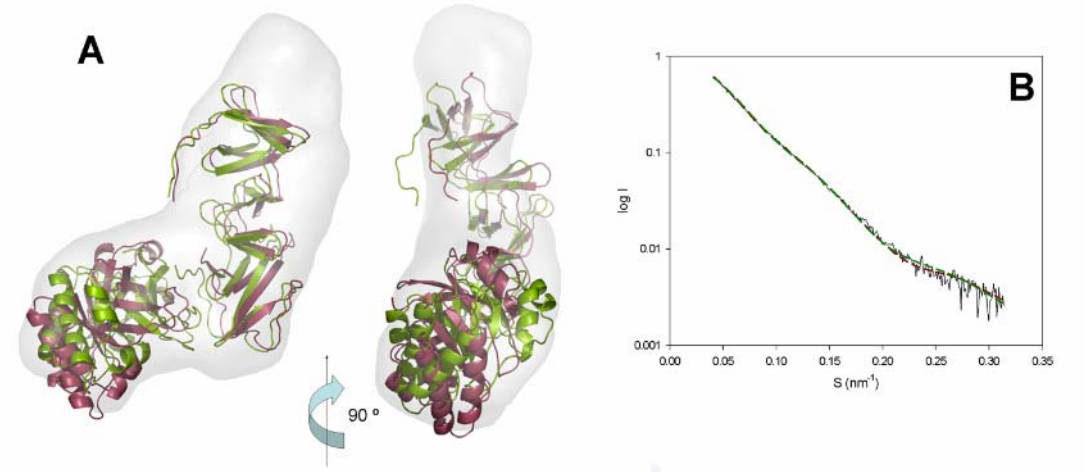

Figure.- Low resolution envelope for the monomer of Cpl-1 in solution without choline and SAXS scattering profiles. **A**. Best fittings of the proposed solution structures of Cpl-1 into the SAXS-derived low resolution envelope. The red structure was generated by rigid-body modelling using MASSHA[35], while the green structure was manually generated as described in the text. **B**. Experimental Small Angle X-Ray Scattering profile of Cpl-1 monomer (black continuous line) protein in comparison with the theoretical curves generated for the rigid body model (red discontinuous line) and manually generated model (green discontinuous line) using CRYSOL.