Structural studies of antigenic determinants recognized by a protective monoclonal antibody in view of developping a vaccine against shigellosis

Muriel Delepierre

François Theillet (1), Clément(1) M-J, Catherine Simenel(1), Ada Prochnicka-Chalufour (1), Laurence Mulard (2), Antoine Fortuné (4), Anne Imberty (4) and Armelle Phalipon (3)

Nuclear Magnetic Resonance of Biomolecules Unit, Institut Pasteur
28 rue du Docteur Roux, 75724, Paris, France
(murield@pasteur.fr)

[1]Unité de RMN des Biomolécules, URA CNRS 218 5,
[2]Unité de Chimie Organique, URA CNRS 2128,
[3]Unité de Pathogénie Microbienne Moléculaire, INSERM U389, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris Cedex 15, France,
[4]CERMAV-CNRS (affiliated to Université Joseph Fourier), 38041 G renoble BP53, Cedex 09, France

Shigella, an invasive enterobacterium, is the causative agent of human dysentery or shigellosis. It raises a serious health problem, especially in those regions of the Third World where bad sanitary conditions and malnutrition prevail. Development of a vaccine against shigellosis is one of the priorities of the WHO. There are four different species of the bacterial agent causing shigellosis, each of them possessing many serotypes which are defined by the structure of the O antigen (Ag-O), the main target of the immune response protecting against Shigella and that differ mainly by the position of the glucosyl redisue (E).

Interactions between pentasaccharide corresponding to the repeating unit of both Shigella flexneri 5a and 2a and peptides that mimic the pentasaccharide with protective antibodies, IgA and IgG, were investigated by NMR and molecular modeling. First the average structures of the free ligands were established and then their conformations in the bound form were investigated using transferred NOE (TrNOE) experiments. The pentasaccharide structures in the bound form is similar to the average structure in the free form. Similarly, the peptides both free and bound to the antibody adopt a turn conformation. In order to map the epitopes at the molecular level, contacts between ligands and antibodies were evidenced using saturation transfer difference (STD) experiments with selective saturation of the antibody resonances. Finally, NMR derived pentasccharide and peptide models coupled to STD informations were used to generate antibody:pentasaccharide and antibody:peptide complexes. Interactions of pentasaccharides with protective antibodies show that the glucose residue remains in close contact with the antibodies and thus is part of the epitope. The peptide side-chains of residues forming the turn, contact the antibodies residues in the Ab-peptide complexes.

References

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Characterisation of the structure and dynamics of the unfolded state by NMR and optical spectroscopy

Sanne M. Nabuurs

Carlo P.M. van Mierlo

Laboratory of biochemistry, Wageningen UR
Postbus 9101, 6700 HB, Wageningen, Netherlands
(sanne.nabuurs@wur.nl)

The folding of proteins has been studied intensively during the past decades and includes the 179-residue *Azotobacter vinelandii* flavodoxin. The topology of flavodoxin is characterised by a five-stranded parallel β-sheet surrounded by α-helices. An intermediate is populated during denaturant-induced equilibrium unfolding of flavodoxin. The intermediate has molten globule-like characteristics, which as demontrated by fluorescence and CD spectroscopy. The population of the intermediate depends on the temperature, at certain denaturant concentrations the population is up to 80% at 35ºC. To further examine flavodoxin folding at residue-level, NMR spectroscopy is used. Since protein folding starts with unfolded polypeptides, flavodoxin, unfolded with guanine hydrochloride, will be structurally and dynamically characterised by heteronuclear NMR spectroscopy. Resonances of the unfolded protein need to be assigned and chemical shift deviations from random coil values can will reveal residual secondary structure. In addition ^{15}N relaxation studies will lead to a better view of the unfolded protein. Once the unfolded state is assigned, the folding of the molten globule-like intermediate of flavodoxin can be followed at the residue-level by NMR spectroscopy.