ESRF

ESRF	**Experiment title:** Structural modifications of Pseudomonas aeruginosa exotoxin A upon trans-membran translocation	**Experiment number**: SC 1047
Beamline: ID02	**Date of experiment**: from: 6/11/2002 to: 8/11/2002	**Date of report**: 29/08/2003
Shifts: 6	**Local contact(s)**: Stéphanie Finet	*Received at ESRF:*

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Report:

1. *Pseudomonas aeruginosa* exotoxin A

Pseudomonas aeruginosa is an opportunistic pathogen responsible for numerous infections. Antibiotic abuse, leading to the outbreak of multiresistant bacteria and the intrinsic resistance of this pathogen to antibiotics, led to a multiplication of infections by *P. aeruginosa*. This Gram-negative bacteria secretes a number of virulence factors such as degradation enzymes and toxins including exotoxin A which is a major virulence factor and could be used for immunisation against *P. aeruginosa*. The mechanisms of exotoxin A translocation across the two bacterial membranes and the eukaryotic membrane is performed using a typical type II pathway with three different stages. The unfolding of the protein is required for the translocation from the cytoplasm to the periplasm through the inner membrane. Several mutants of exotoxin A devoid of different disulphide bonds have been studied by SAXS in order to see their importance on the folding of the protein when it should pass the membrane.

The experiments carried out on ID02 have shown a slight aggregation of the mutants. However, we could anyway see that the radius of gyration of the different mutants compared to that of exotoxin wild type was not significantly altered by removal of the corresponding disulphide bonds. This suggest that the protein needs a further unfolding to cross the membrane.

2. *Natively unfolded nucleoprotein of Measles Virus*

The Measles virus is an important human pathogen responsible for 1 million deaths per year in the world, especially in the developing countries and up to now there is no therapeutic treatment. We have therefore undertaken a study of the replicative complex of this virus. The replicative complex of the Measles virus is made of the nucleoprotein (N), which encapsidates the single-strand RNA genome to form a helical nucleocapsid, and by the RNA-dependent RNA polymerase which acts in association with the phosphoprotein (P). A computational analysis of the Nucleoprotein N indicates that the C-terminal moiety of N, N_{TAIL}, is natively unfolded. Moreover, circular dichroism experiments performed on N_{TAIL} showed that there is no secondary structure. We have therfore carried out SAXS experiments on N_{TAIL} in order to measure its degree of compaction and to determine whether it was a random coil or not.

The radius of gyration of N_{TAIL} extrapolated at zero concentration (guinier plots on figure 1) was 27.5 ± 0.7 Å and the deduced Molecular mass (M_w) was 15,250 Da, which is in perfect agreement with the expected M_w for a monomeric form (15,300 Da). The expected R_g of N_{TAIL} according to its M_w would be 15 Å for a globular protein and around 35-38Å for a denatured, fully unfolded protein. Therefore, the observed R_g indicated that N_{TAIL} is not globular. However, the protein is more compact than a random coil, suggesting that it possess some residual structure.

The Kratky plot of N_{TAIL} displays a bump at $q \approx 0.08 Å^{-1}$ followed by a plateau for $q > 0.15$ $Å^{-1}$ (see Fig. 1). The absence of a maximum clearly indicates that N_{TAIL} is not globular and does not posses a tightly packed core. However, the observed bump may be indicative as well of some residual structure. We determined the distance distribution function, deduced from the scattering intensities of N_{TAIL}, which had a bell-shape, with a maximum dimension D_{max} of 120-130 Å. This value, while being indicative of an extended, non globular conformation, is lower than expected for a random coil.

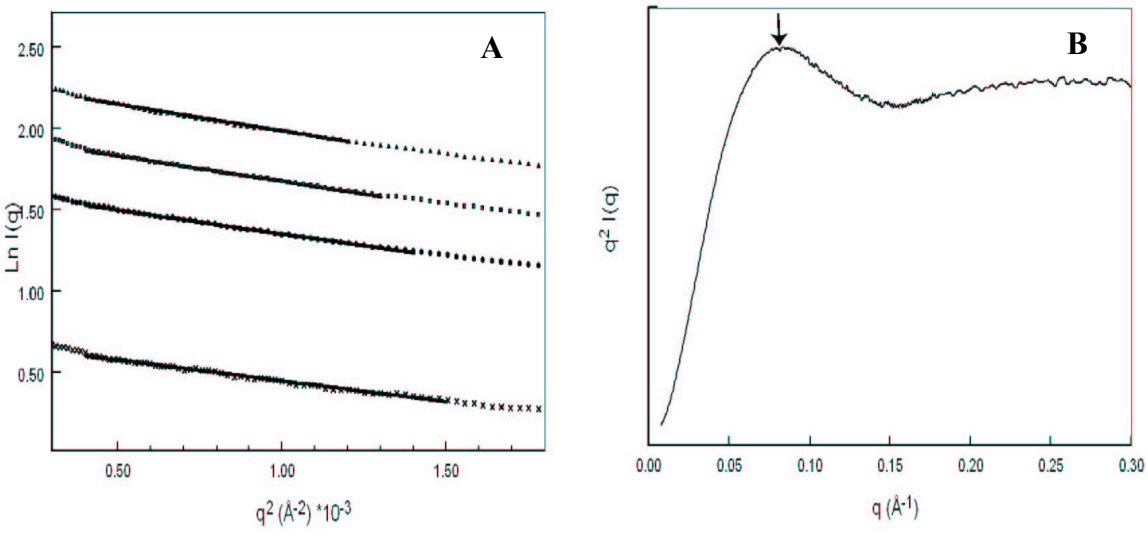

Fig. 1. Small Angle X-Ray scattering experiments on N_{TAIL}. (A) Guinier plot of N_{TAIL} in 10 mM pH 8 Tris, 5 mM EDTA, 10% glycerol, at different protein concentrations: full triangles, 9 mg/ml; empty squares, 6.7 mg/ml; full circles, 4.5 mg/ml; crosses, 1.8 mg/ml. The slope of the straight lines (shown in thick) gives the value of R_g. The regression lines were fitted to the data within $qR_g<1.0$. **(B)** Kratky plot of the scattered intensity of N_{TAIL} at 9 mg/ml. The arrow indicates the bump at $q \approx 0.08 Å^{-1}$.

All these results, together with the data obtained by circular dichroism indicate that N_{TAIL} does not behave as a random coil but adopts a typical non globular, premolten globule conformation in solution. N_{TAIL} probably displays residual structures that would initiate the induced folding when in presence of its physiological partner.

These results have lead to the publication an article :

S. Longhi, **V. Receveur-Bréchot**, D.Karlin, K. Johansson, H. Darbon, D. Bhella, R.Yeo, S. Finet and B.Canard (2003) The C-terminal domain of the measles virus nucleoprotein is intrinsically disordered and folds upon binding to the C-terminal moiety of the phosphoprotein, *J. Biol. Chem*, **278**, 18638-18648.