Experimental Report

Introduction

The pestivirus genus belongs to the *Flaviviridae* family and comprises important veterinary pathogens like classical swine fever and bovine viral diarrhea viruses. The classical swine fever virus (CSFV) causes a severe disease in pigs that results in high morbidity and mortality. The RNA genome contains a single open reading frame that codes for a large polyprotein precursor that is processed both co- and postranslationally by a combination of viral and cellular proteolytic enzymes. The NS3 protein is a multifunctional enzyme, acting both as protease and RNA helicase. Its N-terminal domain, which associates with an NS4A cofactor, possesses a serine protease activity involved in maturation of the precursor. The C-terminal domain has nucleoside triphosphatase (NTPase) and helicase activities. The tridimensional structural of the helicase domain of the protein NS3 of CSFV was determined with a diffraction limit of 2.8 Å. The protein crystallized in a monoclinic space group P21 and the phases were obtained from a selenomethionine derivative. As expected, the structure of this domain is similar to that of its counterparts from the flavivirus and hepacivirus genera of this viral family, which were previously determined. It is a triangular-shaped molecule with three structural domains of about 150 amino acids each, separated by clefts. Domains 1 and 2 form the catalytic core of the molecule and have similar RecA-like folds. Domain 3 is distinctive to helicases of *Flaviviridae*, and varies most in structure among the different genera. Interestingly, two different conformations were found among the four molecules that form the asymmetric unit with the major difference observed in domain 2. We described this transition using the two lowest frequency normal modes. However, since such movement has never been previously characterized we would like to perform small-angle x-ray scattering in solution.

Results

The Guinier plot of the sample eluted from the SE-HPLC system is shown in Fig. 1A inset. It yields a radius of gyration value of 27.5 ± 03 Å. From the intensity extrapolated at the origin I(0) an estimate of 57.5 kDa was derived, in good agreement with the calculated from the amino acid sequence which is 55.9 kDa, confirming that the solution was free from inter-particle interactions (Swing Beamline

Soleil). This curve was combined with the curve obtained at high concentration, which provide high angle information, so as to obtain a composite pattern spanning the whole spectrum (Fig. 1A). The data was used to calculate the pair distance distribution function p(r) using the program Gnom. The maximum diameter is 90 Å and a value of 27.2 Å is derived for the radius of gyration, very close to the Guinier determination (Fig. 1B). Scattering patterns were calculated from the atomic coordinates of the two crystal structures designated A for the open conformation and B for the closed conformation using the program Crysol (Fig. 1C). Superposition of the calculated scattering patterns to the experimental curve reveal clear deviations, most conspicuously in the region of the shoulder around q= 0.16 A^{-1} (see inset to Fig. 1C). This result indicates that the conformation in solution of the NS3hel is different from both structures found in the crystal. Assuming that in solution both conformations are in equilibrium, then the scattering pattern should be a linear combination of the two scattering curves weighted by their respective fractional concentrations. A good fit to the data was indeed obtained using 65% of the open conformation and 35% of the closed one (red curve in Fig. 1C). Following on the NMA performed on the crystal structures, we calculated perturbed conformations using combinations of the same two normal modes and obtained a fit to the data equivalent to that using directly the two crystal structures (data not shown). This suggests that the scattering pattern cannot discriminate between the two descriptions of conformer population in solution. However, it confirms that NS3 helicase is most likely to explore a conformational space in solution possible through movements of domain 2 around the flexible linkers to the other two domains.

Figure 1