Abstract

In this doctoral thesis we studied the structural properties of sRNA OxyS and structurally characterized the interaction between OxyS and its mRNA target *fhlA*. OxyS is a 110 nucleotide, stable, *trans*-encoded small RNA found in *E. coli* and is induced by an increased concentration of hydrogen peroxide. OxyS has an important regulatory role in cell stress response, affecting the expression of multiple genes. We individually studied the segments of OxyS which are predicted to adopt stem-loops SL1, SL2 and SL3 and subsequently confirmed their structural integrity in full-length OxyS. Unexpectedly, stem-loop SL4 was identified in the region that was predicted to be unstructured. In the next step we combined SAXS, *de novo* modelling and unbiased molecular dynamics simulations to study the three-dimensional structure of OxyS. The molecular envelope of OxyS demonstrates that OxyS adopts an extended boomerang-like structure with two arms. The superimposition of 3D models onto the molecular envelope shows that SL1 and SL2 constitute the long arm, whereas coaxially stacked SL3 and SL4 constitute the short arm of the molecular envelope. We performed optimisation of the ensemble of OxyS models and demonstrated that OxyS is able to adopt multiple conformations which slightly differ in the relative orientation of the four stem-loops.

We also studied the interaction between OxyS and mRNA *fhlA*. We focused on two short segments of OxyS SL1 and SL3 that base pair with complementary sequences within *fhlA* 5' UTR region. Using NMR spectroscopy we determined the secondary structure of model oligonucleotides fhlA1, SL1Δ17, fhlA41 and SL3, which comprise the sites of OxyS-*fhlA* interaction and confirmed the formation of complexes fhlA1+SL1Δ17 and fhlA41+SL3. We determined that Mg2+ cations were required for the intermolecular base pairing to occur and demonstrated that partial unfolding of fhlA1 and SL3 takes place in their presence. We identified the specific nucleotides involved in the intermolecular base pairing and demonstrated that the interaction does not involve all complementary nucleotides from OxyS and *fhlA*.