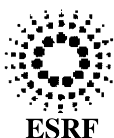
<table>
<tr><td>
ESRF</td><td>Experiment title:
The High Resolution Structure of an RNA Fragment</td><td>Experiment number:
LS645</td></tr>
<tr><td>Beamline:
BM14</td><td>Date of experiment:
from: 7th May 1997 to: 9th May 1997</td><td>Date of report:
27/1/98</td></tr>
<tr><td>Shifts:
3</td><td>Local contact(s):
Gordon Leonard</td><td>Received at ESRF:
20 FEV. 1998</td></tr>
</table>

Names and affiliations of applicants (* indicates experimentalists):

Dr. W.N. Hunter, Dr. M.R. Peterson*, D.R. Hall[1]*, Dr. G.A. Leonard[2]* ‘Department of Biochemistry, University of Dundee, Dundee DDl 3WN, Scotland, U.K. [2]ESRF, BP 220, F-38043 Grenoble Cedex 9, France.

Report:

Molecular replacement calculations using a medium resolution (d_{min} = 2Å) data set collected from a single-crystal of r(CGCGAAAUUUGCGC) at station PX9.5 did not result in a solution of the crystal and molecular structure of the RNA fragment. These crystals have a rhombohedral unit cell with space group R32 and dimensions a=b=40Å, c=216Å. The aim of our experiment on BM14 was therefore two-fold. (1) To solve the structure of the RNA fragment using MAD data collected at wavelengths around the Br K-absorption edge from crystals of a brominated derivative of the RNA fragment. (2) To collect data from native crystals to as high a resolution as possible for structure refinement purposes.

To begin our experiment an EXAFS scan of the Br K-absorption edge was performed on a crystal containing the ‘brominated’ RNA. Disconcertingly, the scan showed no increase in fluorescence and was indicative that the crystals did NOT contain the brominated RNA. However, given our, at the time, relative inexperience in measuring absorption edges at BM14 it was decided to collect a data set from this crystal using a wavelength on the high energy side of the absorption edge. The rationale for this was that a single-wavelength experiment at such a wavelength would result in an anomalous difference Patterson with peaks indicating the positions of any bromine atoms present in the crystal. Data for this experiment was collected at λ=0.9049Å. Sixty degrees of data were collected in each of two passes, the first to a high resolution limit of 1.8Å (20 3° oscillations, 40secs/°), with the second, with much shorter exposure time, to a high resolution limit of 2.6Å to allow the

……………

the measurement of those reflections which were overloaded on the detector (a MAR image plate) in the first pass (15 4° oscillations, 8.5secs/°). On line processing and scaling (R_{svm}=5.8%, completeness 97.5%, multiplicity 4.59) of this data revealed a further surprise. Not only did the crystals appear not to contain any bromine, they also had a different unit cell dimensions and space group to those used for data collection in Daresbury (space group P321, cell dimensions a=b=39.79Å, c=30.09Å). Moreover, an anomalous difference Patterson calculated using data in the range 10.0-2.0Å was exceptionally flat (the highest peak not lying on a symmetry element on the w=0 Harker section is just 1.5 times the r.m.s. value of the entire Patterson) another indication that the crystal did not contain bromine.

To completely eliminate the possibility that the crystals contained bromine a second data set was collected from crystals of the 'native' RNA. This was collected in the same manner to that described above but to a high resolution limit of 1.7Å. Here R_{svm} = 5.4%, completeness = 98.9%, multiplicity = 4.52 with unit cell dimensions and space group as for the 'brominated' crystals. A Patterson map calculated using isomorphous differences between the two data sets was also exceptionally flat (highest peak not on a symmetry element on w=0 Harker section 1.5 times the r.m.s. value of map) again indicating no bromine in either of the crystals and this time also suggesting the two crystals were of an identical material. It has recently been reported that during the high temperature deprotection step of solid phase-synthesized nucleic acids halogenated pyrimidine bases are involved in substitution reactions with the concentrated ammonia used to remove the protecting groups. This results in the loss of the halogen atom and the formation of 5-aminopyrimidines. This process is likely to explain the lack of brominated bases in our 'brominated' crystals.

Subsequent Molecular Replacement calculations using the 'native' data set described above and a variety of search models revealed yet another surprise. The crystals do not contain the 13-mer which was originally synthesized, but rather have an asymmetric unit consisting of 5.5 base-pairs! Two of these asymmetric units stack along the c-axis of the unit cell and mean the crystal contains either intact RNA 11-mer duplexes or 5.5 base-pair mini duplexes which stack to form a pseudo-continuous helix. Both chemical considerations and the behaviour of R_{free} during refinement indicate the latter to be the real situation. It was hoped that refinement of the structure would, as is usual in the case of nucleic acid structures at 1.7Å resolution, reveal unequivocally the base composition of these 5.5 base-pair mini-duplexes which may have allowed us to provide an explanation for what has happened in the synthesis/crystallisation process to modify the originally synthesized RNA 13-mers. However, although the current value of R_{work} is 0.29 (R_{free} = 0.30) for all data in the range 20.0-1.75Å, unequivocal assignment of the base composition is difficult as it is clear from the electron density that there are two, mutually exclusive, orientations of the 5.5 base-pair mini duplexes in the asymmetric unit. Given that the overhanging base in the mini-duplexes is almost certainly a thymine it would appears that the crystals contain r(CGCGA).r(UUCGCG) helices. Quite how this has arisen is not yet clear.