Application for beam time at ESRF – Experimental Method

Structure of AT-*rich oligonucleotides*

Code 30 01 672

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Aims of the experimental and scientific background

For the last 25 years many oligonucleotide structures have been determined by x-ray crystallography (1). All of them have simple unit cells, which are stabilized by end-to-end interactions of a few types. The latter interactions are limited and have only allowed the study of certain classes of sequences (2). For example there are only two structures known (3,4) for DNA oligonucleotides which only contain A·T pairs. Such sequences give rise to more complicated, large, unit cells. Synchrotron radiation is essential for the analysis of these structures. In fact, Subirana's group in Barcelona has recently studied the structures of $(AT)_5$, $(AT)_6$ and $CG(AT)_4$ crystals. All of them have large, pseudohexagonal unit cells with a = b = 28 Å and c = 220 - 650 Å depending on the oligonucleotide. Resolutions up to 6 Å have been obtained thus far. For an adequate analysis of such structures we should use crystals which have been oriented with their long c axis approximately parallel to the spindle axis. For this reason we request access to the BM30A line.

We should stress that very little is known about AT- rich sequences. We recently found (4) that they form Hoogsteen base pairs instead of the standard Watson-Crick base pairs, and they may constitute of helical molecules with 12 base pairs/turn, instead of the standard 10 base pairs/turn.

AT rich sequences have very specific non-coding functions in the cell nuclei, such as attachment to the nuclear scaffold (5) or centromere structure (6). Therefore we think it has a significant biological interest to study the conformation of AT rich oligonucleotides.

Experimental method

Oligonucleotides rich in AT sequences have been obtained in an automatic synthesizer and have been purified by gel filtration and reverse phase HPLC. Crystallization has been done by the vapour diffusion method. Crystals have been frozen by flash cooling and are kept in liquid nitrogen. Crystals of $CG(AT)_4$, $(AT)_5$ and $(AT)_6$ are now available. Subirana's group investigators have recently collected data at the BM30A and IDI4.3 beamlines (Project MX187), but they only got a resolution of about 6 Å. We expect to obtain a higher resolution with smaller crystals. We also need to avoid the overlap of spots by using oriented crystals. The crystals are very anisotropic, with unit cells:

$(AT)_5$ 26.4, 26.4, 501Å // 90º, 90º, 120º, probably P21
$(AT)_6$ 26.6, 26.6, 229Å // 90º, 90º, 120º, probably P21

Both cells are pseudo hexagonal.

Results expected

We expect to have improved the quality of the crystals, so we hope to obtain better diffraction data in order to be able to solve the structure of several oligonucleotides with AT rich sequences. We are carrying out crystallization trials with other sequences. Also we are trying to crystallize $(AT)_5$ and $(AT)_6$ with HMG peptides which contain the recognition sequence for AT-rich DNA.

References

(1) Dickerson, R.E. (2001) In *International Tables for Crystallography, Volume F:* Crystallography of Biological Macromolecules, Rossmann, M.G. & Arnold, E. (eds.), Kluwer Academic, Dordrecht, pp. 588-622.

(2) Subirana, J.A. (2003) DNA discoveries through crystallography. *Nature* **423**, 683.

(3) Viswamitra, M.A., Kennard, O., Jones, P.G., Sheldrick, G.M., Salisbury, S., Falvello, L. and Shakked, Z. (1978) DNA double helical fragment at atomic rsolution. *Nature* **273**, 687-688.

(4) Abrescia, N.G.A., Thompson, A., Huynh-Dinh, T. and Subirana, J.A. (2002) *Proc. Natl. Acad. Sci. USA* **99**, 2806-2811.

(5) Liebich, I., Bode, J., Reuter, I. and Wingender, E. (2002) Evaluation of sequences motifs found in caffold/matrix-attached regions (S/MARs). *Nucleic Acids Res.*, **30**, 3433-3442.

(6) Choo, K.H.A. (1997) In *The centromere*. Oxford University Press, Oxford.