Poster

Co-crystallization of DNA Sequences, from the Promoter Regions of Genes Responsible for the Development of Degenerative Diseases, with Fluorescent Markers and Ligands

Hristina I. Sbirkova-Dimitrova [1], R. Rusev[1], Boris L. Shivachev 1, Christo Gerginov[1]

1 Institute of Mineralogy and Crystallography "Acad. Ivan Kostov", Bulgarian Academy of Sciences, Acad. G. Bonchev str., bl. 107, 1113 Sofia, Bulgaria

The object of the present work is the detection of binding interactions and co-crystallization of palindromic DNA sequences from promoter regions of genes responsible for the development of neurodegenerative diseases with fluorescent markers and new/modified ligands.

Neurodegenerative diseases such as Parkinson's (PD) and Alzheimer's disease (AD) are progressive diseases that lead to serious disabilities and impair the quality of life of patients, which in turn leads to ever higher individual and societal costs [1]. There is no reliable treatment, so patients rely only on symptomatic treatment. Current therapies unfortunately have no effect on the progression of these diseases, moreover, they lose efficacy over time and their adverse effects increase the severity of the disease [2]. For these reasons, the development of new and safe compounds is of leading importance.

The variety DNA oligonucleotide sequences from promoter regions of genes responsible for the development of neurodegenerative disease [3] were crystallized by the vapour diffusion method. The crystallization conditions contained cacodylate buffer (pH 6.5-7.5), alcohol (2-propanol or methylpentanediol (MPD)), cations (Mg^{2}+, Ba^{2+}, Zn^{2+}), cobalt hexamine $[Co(NH3)6]^{3+}$ and polyamines (Spermine). Crystals were grown by the "hanging drop" method and 1.5µl (2mM) ligand (3 µl total drop volume) was added to 1.5 µl DNA (2mM) at room temperature equilibrated against 50% MPD. Crystals suitable for single crystal X-ray analysis, formed within a month.

The improvement of the optimal conditions for crystallization and co-crystallization of selected sequences from the promoter region of the APP gene as well as their subsequent co-crystallization include markers such as Thioflavin T, Hoechst, DAPI, TO, Berenil and some new homologous Thioflavin T molecules (RR1 and XRB)[4]. Single crystal samples of the co-crystallized DNA sequence d(CGTGAATTCACG) with the Hoechst 33342 (HT1) marker were structurally determined. The experiments were performed at low temperature (130 K). The structure including HT1 marker is solved by single crystal X-ray analysis, in (19) $P2_12_12_1$ space group, at a
1.9 °A resolution. HT1 lies in the minor groove at the centre of the B-DNA fragment, positioned over the A-T base pairs, with the ends approaching the G4-C21 and the G16-C9 base pairs. It is bound to the DNA by hydrogen interactions. HT1 inserts itself at the central AATT site (Fig. 1), displacing the ordered spine waters.

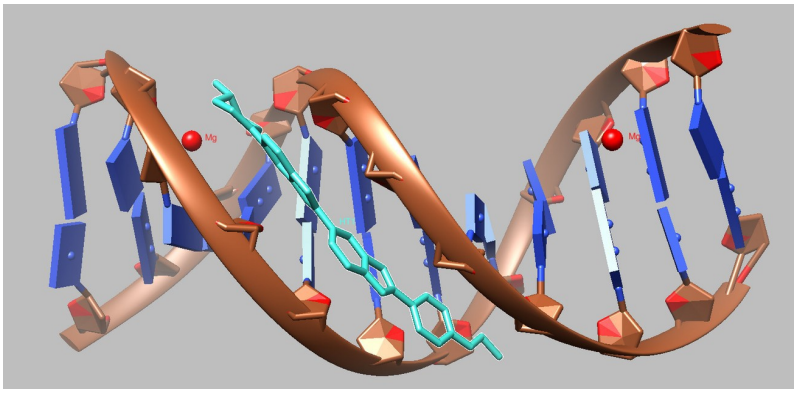

Figure 1. Crystal structure of a DNA sequence d(CGTGAATTCACG) with HT1.

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