MS05-1-1 Crystallization of Alzheimer DNA Promoter Sequences from Amyloid Precursor Gene with Thioflavin T and Other Fluorescent Markers
#MS05-1-1

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Abstract

The object of the present work is the detection of binding interactions and co-crystallization of Alzheimer's DNA promoter sequences of the amyloid precursor (APP) gene with Thioflavin T (ThT), new ThT analogues and other fluorescent markers.

Thioflavin T fluorescence is often used as a diagnostic of amyloid structure, but it is not completely specific for amyloid [1]. Depending on the particular protein and experimental conditions, Thioflavin T may or may not undergo a spectroscopic change upon binding to precursor monomers, small oligomers, and unaggregated material with high β-sheet content or even alpha helix-rich proteins. Conversely, some amyloid fibrils do not affect Thioflavin T fluorescence, raising the prospect of false negative results [1]. However, the in depth analysis of the binding of ThT to DNA can significantly contribute to shed light on the optical properties of ThT and their possible dependence on the hydrophobic local characteristics of the binding pocket. This information can be of use in the improvement of the biomedical/biological applications of ThT or similar sensors.

The variety DNA oligonucleotide sequences from the promoter region of the APP gene containing 5'-CAGCTG-3' (S1) sector [2] and/or 5'-AATGAGGTGGAGAATGT-3' (S2) part [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 $[Co(NH_3)_6]^{3+}$ and polyamines (Spermine). As found in the process of optimization of the crystallization conditions, for the tested sequences, crystal growth was observed only in the conditions featuring Spermine. Crystals were grown by the "hanging drop" method and 1.5µl (2mM) ligand was added to 1.5 µl DNA (2mM) for a 3 µl total drop volume (room temperature) and the drop was equilibrated against 50% MPD. Crystals suitable for single crystal X-ray analysis, formed within a month (Fig. 1).

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 with Thioflavin T and other fluorescent markers, represents a cutting-edge research. Chemical synthesis of homologous Thioflavin T molecules was performed. Thioflavin T analogues were prepared by a two-step procedure consisting of a condensation reaction to obtain Schiff bases and subsequent quaternization with CH_3I or $(CH_3)_2SO_4$. The reactions were performed both in solution and solid phase.

It is expected that single crystal samples of the co-crystallized Alzheimer DNA Promoter Sequences from Amyloid Precursor Gene to be structurally determined on the basis of single crystal X-ray analysis. The structural determination of the ThT binding site will contribute to the most accurate determination of the sequence specificity of this DNA marker or the exclusion of such affinity for particular bases from the DNA sequence.

References

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Obtained crystals