Abstract

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Doctoral Thesis: **Investigation of the compounds influencing the melting temperature of oligonucleotide probes**

Real-time PCR is widely used method in various research fields like biomedicine, microbiology, veterinary medicine, etc. Quantification of gene expression, allelic discrimination and alteration detection are new and interesting application possibilities. Mismatch discrimination is not optimal when longer probes are used. Melting temperature difference between fully complementary duplex and mismatched duplex is negligible in the case of longer probes.

The short oligodeoxynucleotide (ODN) probes, on the other hand, are suitable for good discrimination of single nucleotide variants. However, these probes suffer from low melting temperatures. Adding minor groove binders (MGB), or other substances, which can strongly interact with DNA such as intercalating dyes or polyamines, can increase the melting temperature of short probes duplexes.

In this work, several series of acridine-4-carboxamide intercalators were synthesized. MGB (Hoechst 33258) and polyamine (spermine) were modified and used for comparison purposes. Stabilisation by these compounds was investigated and structure of acridine stabilisators was optimized to improve the ability to increase thermal stabilisation. The study of large series of acridines revealed that optimal stabilisation was achieved upon decoration of acridine by secondary carboxamide carrying sterically not demanding basic function bound through a two-carbon linker. Several highly active acridines were attached to short probes (13 or 18 bases; designed as a part of HFE gene) by copper-free click chemistry into positions 7 and/or 13 and proved to increase the melting temperate (Tm) of their duplexes by almost 9 °C for the best combination. The acridines interact with both single- and double-stranded DNAs with substantially preferred interaction for the latter. The study of interaction suggested higher affinity of the acridines toward the GC- than AT-rich sequences. Good discrimination of two mutated variants was shown in practical application with HFE gene (wild type, H63D C>G and S65C A>T mutations). Acridine itself can also serve as a fluorophore and also allows discrimination of the matched duplexes from those with point mutations in probes labelled only with acridine.