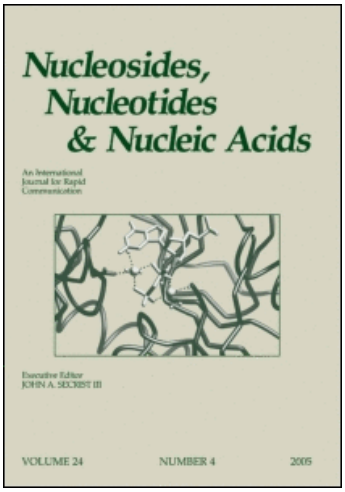

Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Thermodynamic Melting Studies on Oligonucleotide-Peptide Conjugates

C. Frier[a]; J. G. Harrison[a]; S. Balasubramanian[a]
[a] Department of Chemistry, Cambridge University, Cambridge, UK

To cite this Article Frier, C. , Harrison, J. G. and Balasubramanian, S.(1999) 'Thermodynamic Melting Studies on Oligonucleotide-Peptide Conjugates', Nucleosides, Nucleotides and Nucleic Acids, 18: 6, 1477 — 1478
To link to this Article: DOI: 10.1080/07328319908044758
URL: http://dx.doi.org/10.1080/07328319908044758

NUCLEOSIDES & NUCLEOTIDES, 18(6&7), 1477–1478 (1999)

THERMODYNAMIC MELTING STUDIES ON OLIGONUCLEOTIDE-PEPTIDE CONJUGATES

C. Frier, J. G. Harrison and S. Balasubramanian*

Department of Chemistry, Cambridge University, Lensfield Road, Cambridge CB2 1EW, UK.

ABSTRACT : A small library of oligonucleotide-peptide conjugates has been prepared and studied to explore the influence of the various peptide side chain (cationic, anionic or hydrophobic) on the hybridation properties of the DNA.

An important parameter that helps determine the efficacy of a therapeutic oligonucleotide is its binding affinity for its complementary target nucleic acid strand. Peptide-oligonucleotides have been proposed as dual-specific binding agents resulting in improved target binding affinity and specificity over simple oligonucleotides.[1]

We have prepared a library of 51 peptides-oligonucleotide conjugates to explore the influence of the various peptide side chains (cationic, anionic or hydrophobic) on the hybridisation properties of the DNA.

An invariant 8 mer oligonucleotide of sequence 5'-AATGTGAT-3' was coupled to a peptide portion contained a five residue variable region composed of the cationic amino acids lysine, ornithine, histidine and arginine, the hydrophobic amino acid tryptophan, or the anionic amino acid glutamate using alanine and glycine as spacers. A methodology, previously reported, was used to prepare the conjugates from peptides synthetised on solid phase coupled to DNA *via* a thioether linkage.[2] All the conjugates were obtained in >75% yield.

The hybridisation properties of these conjugates to a 16mer of sequence 5'-ATCACATTACACCTAG-3' were studied employing UV melting temperature analysis. We determined the effect of salt concentration (0.1, 0.5 and 1.0 M sodium chloride) on hybridisations (FIG 1). At low salt concentration, the Tm depended principally on the nature and the number of the peptide residues. Increasing the number of cationic residues

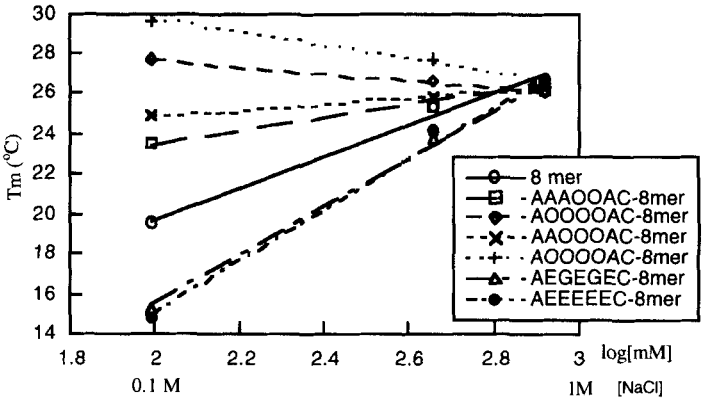

FIG 1 : The melting analysis was carried out using solutions of 2 μM oligonucleotides in 10 mM sodium phosphate (pH 7.0) and 0.1 mM EDTA. The absorbance of the solutions at 260 nm was monitored over a temperature range of 5-80°C at a heating rate of 1°C/ min.

within the peptide segment led to elevated Tm values. Thermodynamic analysis revealed that the origin of this stabilising effect was derived from a more exothermic enthalpy term,with the polyarginine peptide giving the most favourable ΔG_{vH} and the most exothermic ΔH_{vH} (data not shown). Conversely, the presence of anionic peptide seems to destabilise the duplex. The presence of the tryptophan was found to neither stabilise nor destabilise duplex formation (data not shown). Increasing salt concentrations led to decreasing contributions from the peptides to overall stability of the complexes.

We also determined the effect of varying the pH on the stability of the duplexes formed between the pentacationic peptide-oligonucleotides and the 16mer target (data not shown). In the case of the pentalysine, pentaornithine and pentaarginine conjugates, the melting temperature remain largely unchanged as the pH increases from 7.0 to 8.0. At pH 10.0 there was a slight reduction in overall duplex stability, but the stabilising effect of the cationic peptides was still considerable. The polyhistidine conjugate was found to be particularly sensitive to pH changes near neutrality as indicated by a significant rise in Tm from 19.5 °C at pH 8.0 to 28.5 °C at pH 6.0. In general, pH effects correlated well with the pKa values of the ionisable sidechains of the peptide.

REFERENCES

(1) Tung, C. H.; Wang, J. H.; Leibowitz, M. J.; Stein, S. *Bioconjugate Chem.* **1995**, *6*, 292-295. Wagner, R. W.; Matteucci, M. D.; Grant, D.; Huang, T.; Froehler, B. C. *Nature Biotech.* **1996**, *14*, 840-844. Corey, D. R. *J. Am. Chem. Soc.* **1995**, *117*, 9373-9374. Smulevitch, S. V.; Simmons, C. G.; Norton, J. C.; Wise, T. W.; Corey, D. R. *Nature Biotech.* **1996**, *14*, 1700-1704.
(2) Harrison, J. G.; Balasubramanian, S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1041-1046.