Structural analysis of *i*-Motif formation at the human *MEST* promoter region

Aaron J. Stevens[1*], Martin A. Kennedy[1]

[1]*Department of Pathology, University of Otago, Christchurch, New Zealand.*

*To whom correspondence should be addressed, Department of Pathology, University of Otago, Christchurch, P.O. Box 4345, Christchurch, New Zealand. Email: aaron.stevens@otago.ac.nz, phone: +643 364 0530

INTRODUCTION

Cytosine rich DNA can potentially adopt *i*-motif structures with hemiprotonated C-C^+ base pairs at an acidic pH. Circular dichroism (CD) spectroscopy was used to investigate the potential for *i*-motif structure to form on the DNA (C-rich) strand complementary to the G4 *MEST* regions. Due to the requirement of high protonation for the formation of C:C bonds, structure was analysed over a pH gradient from 5.5 – 8.0 in sodium phosphate (NaPi) buffer. This technique has been extensively used for the investigation of *i*-motif structure where formation is represented by a peak at 285-290 nm, and a trough at 250 nm. This can be discerned from linear DNA which is represented by a trough at 240 nm and a peak at 275 nm [1, 2]. To investigate potential *i*-motif formation, analyses were then repeated in 1 x PCR buffer and the results compared.

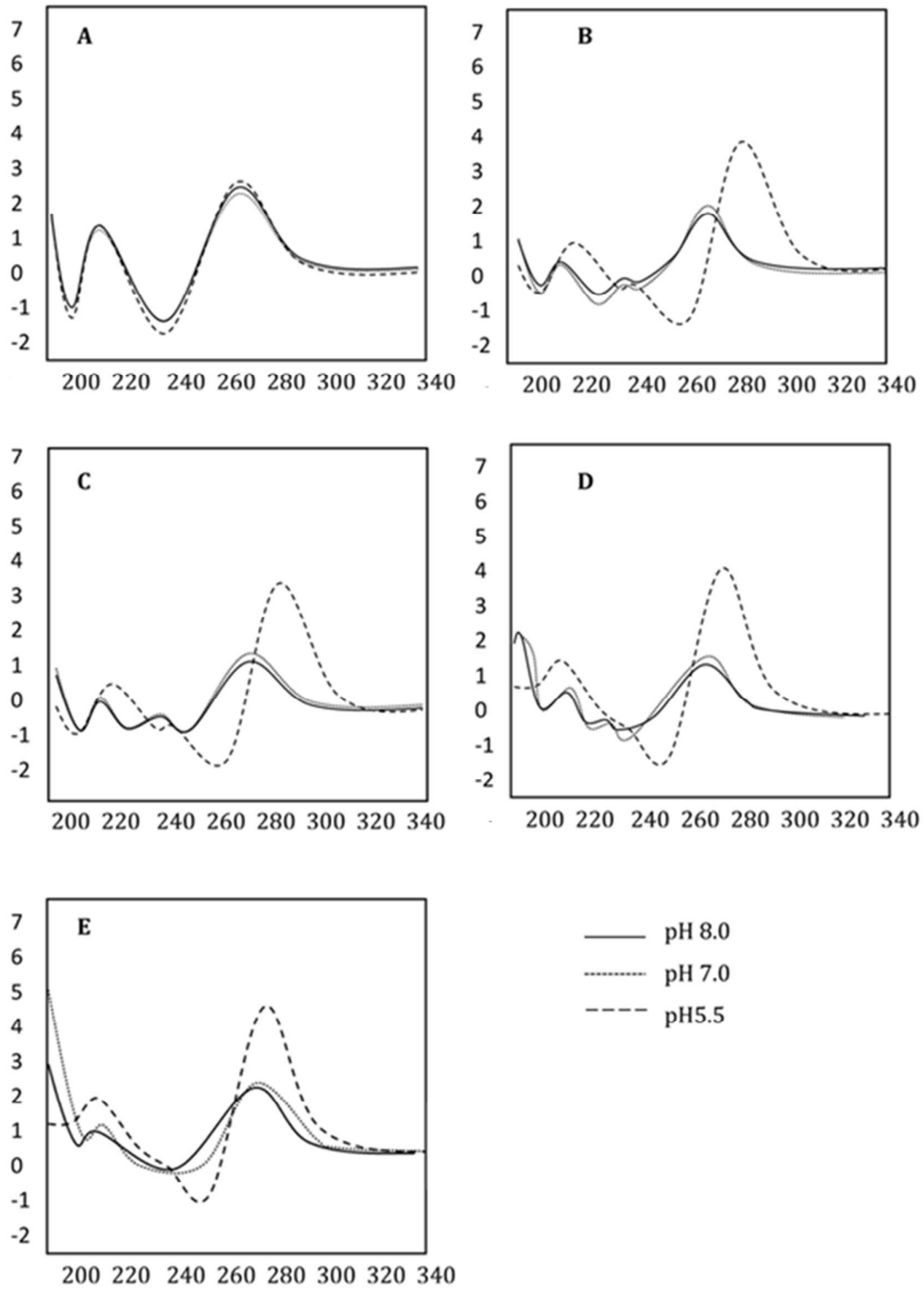
EXPERIMENTAL PROCEDURES

CD spectra were gathered for the complementary oligonucleotide of each G4 *MEST* motif. In the following descriptions, "G4" was substituted with "IM" in the oligonucleotide name, and the oligonucleotides are complementary to their G4 counterpart in Table 1 (main text). Oligomers were purchased from Integrated DNA Technologies (IDT Pte. Ltd., Singapore). Experiments were carried out in 10 mM sodium phosphate (NaPi) buffer with 50 mM KCl, or Tris-based PCR buffer (Roche, Mannheim, Germany) containing 1.5 mM $MgCl_2$. 6 μM of oligonucleotide was heated at 95°C for 10 minutes then cooled slowly to room temperature (22°C) overnight, for CD analyses the following day.

CD measurements and CD melting studies were performed on a J-815 CD Spectrometer (Jasco Analytical Instruments, MD, USA), with a 1 mm path length quartz cuvette. Sample temperature was regulated with a Peltier controller. CD spectra were collected across 350 nm to 220 nm in 1 nm increments and the reported spectra corresponded to the average of at least three scans.

RESULTS

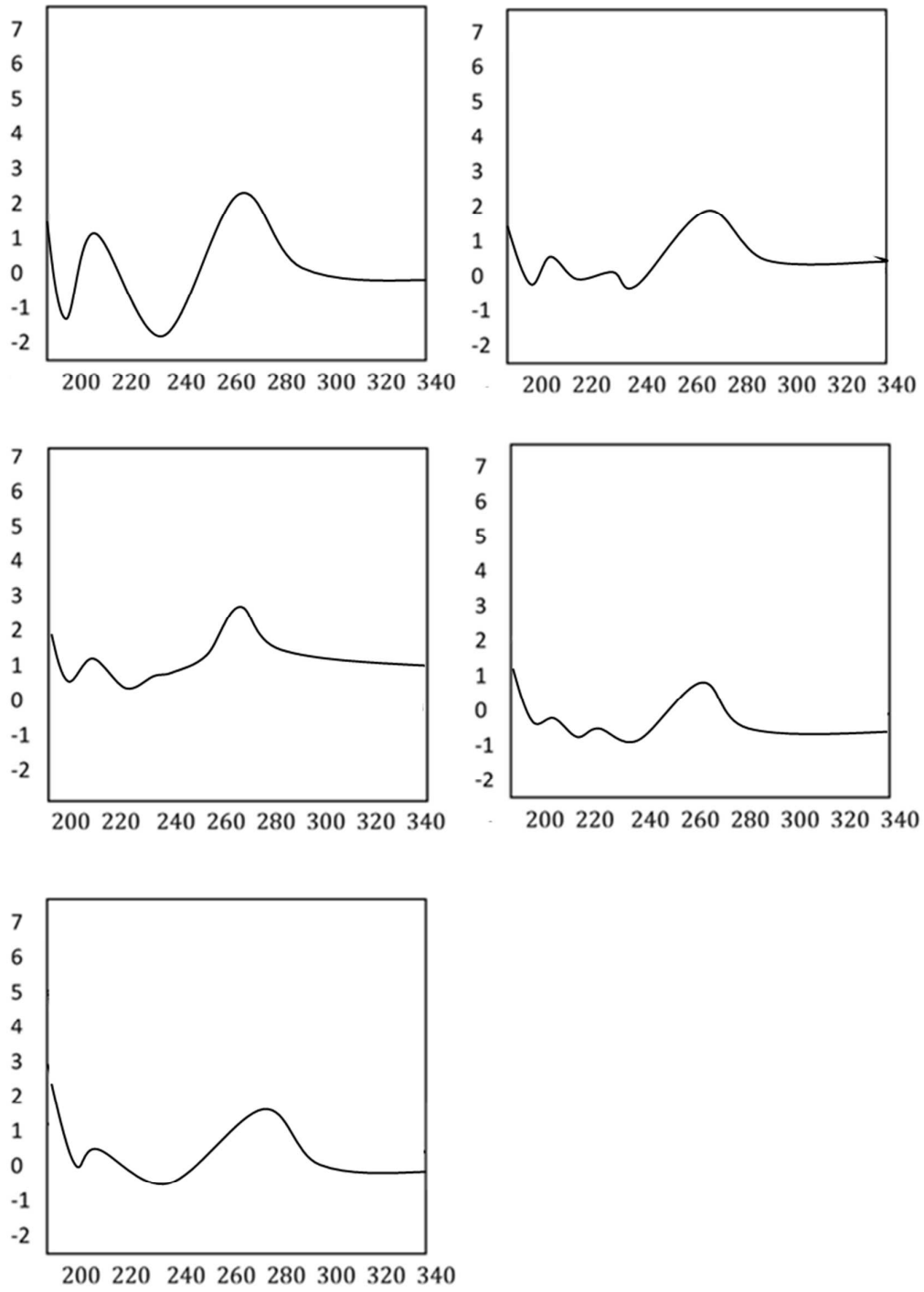
The spectral profile of IMMEST1L demonstrated oligomers do not form *i*-motif structure and exist in a linear, deprotonated state as represented by the formation of a single spectroscopically active species [1, 2] (Supplementary Figure 1). This observation was consistent for IMMEST1LM (data not shown). Analysis of the remaining four oligomers, IMMEST2, IMMEST2M, IMMEST3 and IMMEST3M revealed the formation of two spectroscopically active structures. At pH 7.0 or greater, CD spectra had a maxima at 275 nm and a trough at 240 nm, indicating linear DNA oligonucleotide. At pH 5.5 the maxima shifted to a peak at 285-290 nm with a trough at 250 nm and, which is characteristic of an *i*-motif structure [3-6].



Supplementary Figure 1. CD spectra on putative i-motif oligonucleotides in NaPi (50 mM KCL)

A: IMMEST1L; B: IMMEST2; C: IMMEST3; D: IMMEST2M; E: IMMEST3M. Solid line represents pH 8.0, dotted line represent pH 7.0 and dashed line represents pH 5.5. Molar ellipticity ($x10^5$ $deg.cm^2.dmol^{-1}$) is on the vertical axes and wavelength (nm) on the horizontal axis.

The e*ffect of PCR buffer on i-motif formation.* To investigate *i*-motif formation at conditions relevant to PCR, spectral profiles were analysed in PCR buffer (Roche™), containing 1.5 mM $MgCl_2$. All oligomer sequences demonstrated the presence of a single spectroscopically active structure between 25°C and 95°C, however only the results at 25°C are presented (Supplementary Figure 4). This structure had a maxima at 275 nm and a minima at 245 nm and is not indicative of *i*-motif formation (Supplementary Figure 2).



Supplementary Figure 2. CD spectra of i-motif oligonucleotides in PCR buffer.
A: IMMEST1L; B: IMMEST2; C: IMMEST3; D: IMMEST2M; E: IMMEST3M. Analysis performed in PCR at 25°C. Molar ellipticity ($x10^5$ $deg.cm^2.dmol^{-1}$) is on the vertical axes and wavelength (nm) on the horizontal axis.

CONCLUSION

This analysis demonstrated that *i*-motif structural formation is unlikely to occur at conditions relevant to PCR amplification. From this it can be concluded that *i*-motif structure is unlikely to be a factor in polymerase arrest, or contribute towards allelic drop-out during genotyping of the human *MEST* promoter.

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