Characterization of an i-Motif Forming Sequence from the Promoter Region of ALOX5

Ying Xia, Mark Searcey & Zoë A. E. Waller

School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ

University of East Anglia

Abstract

i-Motifs are DNA secondary structures that form in cytosine rich sequences, consisted of four strands, stabilized by hemi-protonated cytosine–cytosine base pairs[1]. Figure 2 shows structure of i-motif. A number of i-motif forming sequences have been characterized previously[2], including measurement of their transitional pHs (pH_T, the pH at which the structure is 50% folded), melting temperatures (T_m, the temperature at which the structure is 50% unfolded on heating), annealing temperatures (T_a, the temperature at which structure is 50% folded on cooling). Here we describe the characterization of the i-motif forming sequence from the promoter region of ALOX5, the gene encoding the enzyme arachidonate 5-lipoxygenase[3]. This gene is a current target for pharmaceutical intervention in a number of diseases, including Leukemia[4][5]. We are particularly interested in the ***ALOX5*** i-motif, because it has a similar sequence to other i-motifs previously characterized from the promoter regions of DAP and MSMO1 (Figure 1)[2]. This poster will describe the characterization of ***ALOX5*** and comparison with ***DAP*** and ***MSMO1***. i-Motifs were assessed by circular dichroism (CD) and UV spectroscopy. We found that increasing C-tract repeats, increases thermal stability, but also complexity in the structures possible.

Figure 1 : Sequences used in this study

ALOX5	$(CCCCCG)_5CCCCC$	n=6
DAP	$(CCCCCG)_4CCCCC$	n=5
MSMO1	$(CCCCCG)_3CCCCC$	n=4

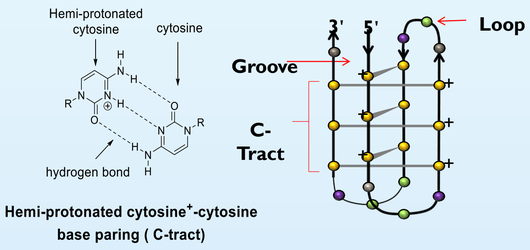
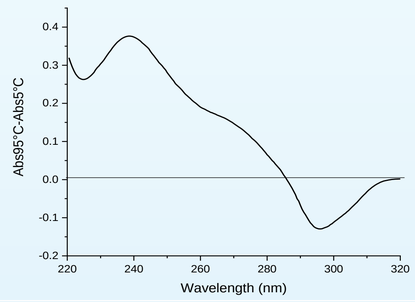


Figure 2 : Structures of C-C base pairs and hTelo i-motif DNA

Biophysical Techniques

- CD spectroscopy is used to measure transitional pH of oligonucleotide and melting temperature
- UV spectroscopy is used to measure melting temperature & annealing temperature and thermal difference spectrum

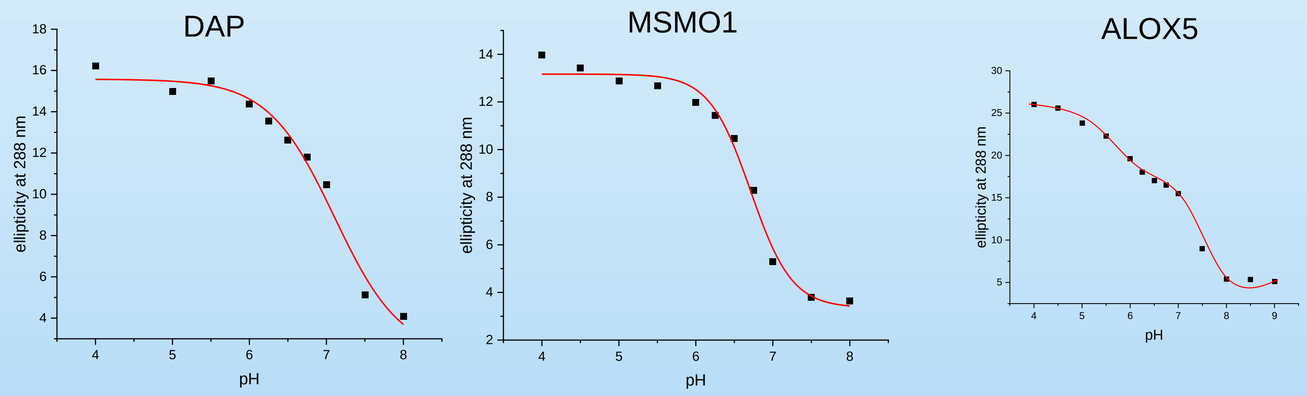
Thermal difference spectrum of ALOX5



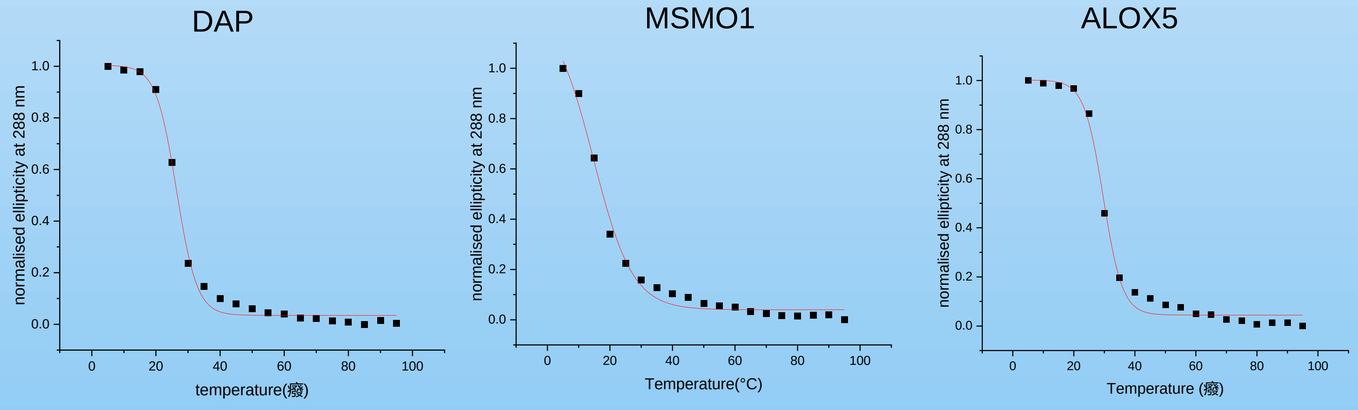
The positive peak is at 240 nm, the negative peak is at 295 nm, It means formation of i-motif is occurring.

BLAST				
ALOX5	$(CCCCCG)_5CCCCC$	nothing	$(GGGGGC)_5GGGGG$	nothing
DAP	$(CCCCCG)_4CCCCC$	Setaria viridis cultivar hits	$(GGGGGC)_4GGGGG$	Setaria viridis hits
MSMO1	$(CCCCCG)_3CCCCC$	Setaria viridis hits	$(GGGGGC)_3GGGGG$	Diutina rugosa hits

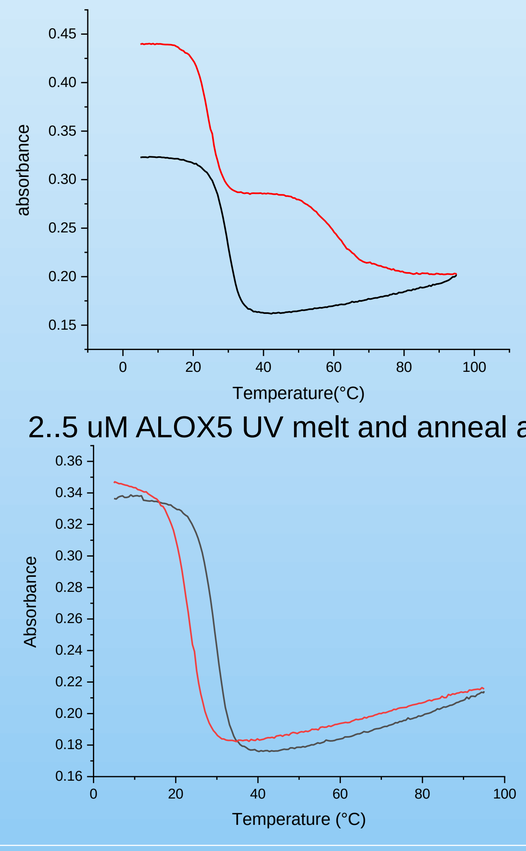
CD Titrations at Different pHs



CD Melt at pH 7.0

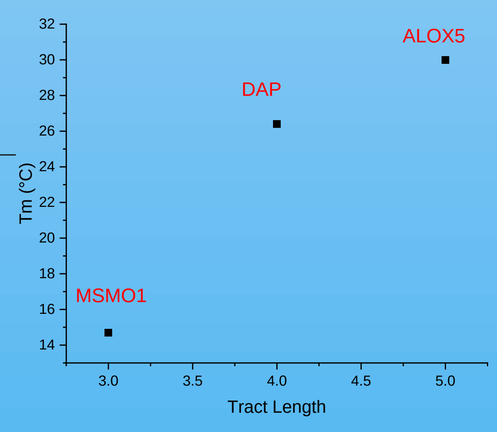


2.5 uM ALOX5 UV melt and anneal at pH7.0



2..5 uM ALOX5 UV melt and anneal at pH7.0 repeat

Name	Tm (pH 7.0) Measured by UV	Ta (pH 7.0) Measured by UV	Tm (pH 7.0) Measured by CD	Transitional pH	sequence	sequence length	number of (CCCCCG) repeats
ALOX5	28.7±0.2	22.5 ± 0.2	29.97 ± 0.23	6.95	$(CCCCCG)_5CCC\text{-}CC$	35	5
DAP	24.7 ± 0.5	22.0 ± 0.4	26.4 ± 0.36	7.1	$(CCCCCG)_4CCC\text{-}CC$	29	4
MSMO1	16.6 ± 0.5	15.9 ± 0.4	14.7 ± 1.6	6.71	$(CCCCCG)_3CCC\text{-}CC$	23	3



Conclusion and future work

- Through current results of transitional pHs and *Tms* and *Tas*, we found the more C-tract repeats in sequence, the more stable in thermal stability.
- Future work will continue to complete UV absorbance experiment to get annealing temperature of *ALOX5* and compare results with *DAP* and *MSMO1*.

References:

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