Towards DNA based esterases: uncovering Hoogsteen face regulation of the pK_{aH} of a tethered imidazole functionality by NMR and molecular dynamics

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1. General concept & design

Template sequence = Wild Type

In the development of **esterase** like **DNAzymes**, recent advances in OBCR made the introduction of **amino acid-like side chain functionalities** on the thymine base possible. The hydrophobic and chiral environment of a B-DNA helix is used as a new scaffold for the development of **synthetic enzymes**. Both first generation, single histidine modified systems and second generation, double modified systems are subjected to a systematic study.

2. First glimpse of the pK_{aH} regulating motif

Thermal stability

	T-T mismatch				A-T base pair		
System	WT	T ₆ ^{ImH+}	T ₇ ^{ImH+}	T ₈ ^{ImH+}	WT	T ₈ ^{ImH+} A ₂₁	sT ₂₁ ^{ImH+}
T _m (°C)	48,6 ± 0,5	50,5 ± 0,2	50,1 ± 0,3	54,6 ± 0,1	59,0 ± 0,3	64,1 ± 0,6	59,4 ± 0,2

From melting temperatures:

- No loss of stability due to modification
- Increase in stability is dependent on position of modification: T₈^{ImH+} (+ 5-6°C)

T^{ImH+} pK_{aH} study via ¹H NMR

¹H NMR pH titration: follow ε1 and δ2 of the T^{ImH+}

Specific nOe contacts

- Solely for T₈^{ImH+} there's a clear set of nOe contacts to position n+3 (A₁₁-T₁₈ base pair)
- T₈^{ImH+}A₂₁ and sT₂₁^{ImH+} prove that an AT base pair is tolerated as well as that the motif is robust and sequence-independent

3. Molecular Dynamics

Starting structure

50ns of MD trajectory shows persistent H-bridging in T₈^{ImH+} and close interproton contacts validated in nOe's

4. Application of the motif in double modified systems

Thermal stability

	A-T base pair		
System	WT	T ₆ T ₈ ^{ImH+}	T ₇ T ₈ ^{ImH+}
T _m (°C)	59,0 ± 0,3	65,0 ± 0,4	63,9 ± 0,3
Δ to WT	-	+ 6,0	+ 4,9

- Increase in melting temperature is consistent with presence of the pK_{aH} regulating motif for T₈^{ImH+} in both systems
- Increase in melting temperature is less pronounced with increasing vicinity of the imidazole functionalities

System	pK _{aH}	Δ pK _{aH}
T ₆ T ₈ ^{ImH+}	T ₆ ^{ImH+}	7,82 ± 0,07
	T ₈ ^{ImH+}	8,90 ± 0,05

System	pK _{aH}	Δ pK _{aH}
T ₇ T ₈ ^{ImH+}	T ₇ ^{ImH+}	6,96 ± 0,07
	T ₈ ^{ImH+}	8,70 ± 0,06

- In both systems the T₈^{ImH+} functionality shows an increased pK_{aH} value due to the intact motif
- For the other T^{ImH+} functionalities (T₆ or T₇^{ImH+}) pK_{aH} decreases with increasing proximity due to repulsive positive charge interactions

References

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