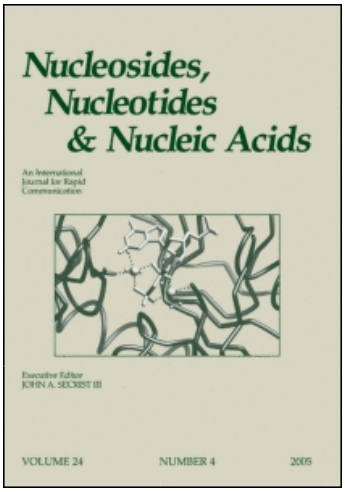

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PROMOTION OF PYRIMIDINE MOTIF TRIPLEX FORMATION BY MORPHOLINO MODIFICATION OF TRIPLEX-FORMING OLIGONUCLEOTIDE: KINETIC AND THERMODYNAMIC STUDIES

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INTRODUCTION

Triplex DNA has attracted considerable interest because of its possible bilogical function in vivo and its wide variety of potential applications, such as regulation of gene expression. A triplex is formed through the sequence-specific interaction of a single-stranded homopurine or homopyrimidine triplex-forming oligonucleotide (TFO) with the major groove of homopurine-homopyrimidine stretch in duplex DNA. In the pyrimidine motif triplex, a homopyrimidine TFO binds parallel to the homopurine strand of the target duplex by Hoogsteen hydrogen bonding to form T · A:T and C^+· G:C triplets.[1] Extreme instability of the pyrimidine motif triplex at physiological pH severely limits its utility for artificial control of gene expression in vivo. Stabilization of the pyrimidine motif triplex at neutral pH is, therefore, of great importance to improve its therapeutic potential. Previous study has shown that morpholino (MOR) backbone modification of TFO (Figure 1) increased the thermal stability of the pyrimidine motif triplex at neutral pH.[2] In the present study, we have examined the kinetic and thermodynamic effects of the MOR backbone modification of TFO on the pyrimidine motif triplex formation at neutral pH.

RESULTS AND DISCUSSION

Table 1 summarizes the kinetic parameters of the triplex formation between a 15-mer TFO [Pyr15T: 5′-CTCTTCTTTTCTTTC-3′ or Pyr15MOR: 5′-*CTCTTC-TTTTCTTTC*-3′ (MOR-modified positions are underlined)] and a 23-bp target duplex [Pur23A · Pyr23T: 5′-GCGCGAGAAGAAAAGAAAGCCGG-3′/3′-CGCGCTCTTCTTTTCTTTCGGCC-5′] at 25°C and pH 6.8, obtained from

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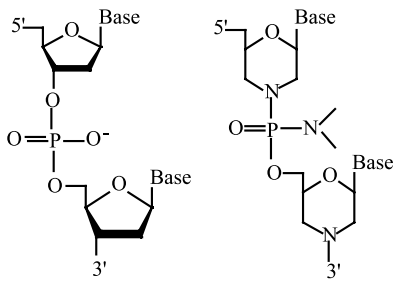

Phosphodiester (PO) Morpholino (MOR)

FIGURE 1 Structural formulas of oligonucleotides.

interaction analysis system (IAsys).[3] The magnitude of K_a calculated from k_{assoc}/k_{dissoc} for Pyr15MOR was about 60 times larger than that for Pyr15T. Thus, the MOR backbone modification of TFO increased the K_a for the pyrimidine motif triplex formation at neutral pH. The k_{assoc} increased 35 times by the MOR backbone modification of TFO. In contrast, when the k_{dissoc} was compared, only 1.7 times smaller k_{dissoc} was obtained by the MOR backbone modification of TFO. Thus, the larger K_a by the MOR backbone modification of TFO resulted mainly from the increase in k_{assoc} rather than the decrease in k_{dissoc}. Acceleration of the association of the target duplex and TFO is the major effect of the MOR backbone modification to increase K_a.

Table 2 summarizes the thermodynamic parameters of the same triplex formation at 25°C, obtained from isothermal titration calorimetry (ITC).[4] Because the K_a for Pyr15T at pH 6.8 was below the lowest detection limit of ITC, we were unable to obtain the thermodynamic parameters for Pyr15T at pH 6.8. The detectable K_a for Pyr15T at pH 5.3 was significantly larger than the unobserved K_a for Pyr15T at pH 6.8. The pH dependence of the K_a for Pyr15T is consistent with the previously reported result that neutral pH is unfavorable for pyrimidine motif triplex formation involving $C^+ \cdot$ GC triads.[1] The larger K_a for Pyr15MOR at pH 6.8 than that for Pyr15T at pH 6.8 is consistent with the results in Table 1.

The K_a and ΔG for Pyr15MOR at pH 6.8 were similar in magnitude to that observed for Pyr15T at pH 5.3. However, the ingredients of ΔG, that is, ΔH and ΔS, were obviously different from each other. The magnitudes of the negative ΔH and ΔS for Pyr15MOR at pH 6.8 were smaller than those observed for Pyr15T at pH

TABLE 1 Kinetic Parameters for the Triplex Formation Between a 15-mer TFO (Pyr15T or Pyr15MOR) and a 23-bp Target Duplex (Pur23A·Pyr23T) at 25°C and pH 6.8[a], Obtained from IAsys

TFO	k_{assoc} ($M^{-1}s^{-1}$)	k_{assoc} (relative)	k_{dissoc} (s^{-1})	k_{dissoc} (relative)	K_a (M^{-1})	K_a (relative)
Pyr15T	1.57×10	1	2.12×10^{-3}	1	7.41×10^{3}	1
Pyr15MOR	5.44×10^{2}	34.6	1.22×10^{-3}	0.58	4.46×10^{5}	60.2

[a]10 mM sodium cacodylate-cacodylic acid and 200 mM sodium chloride (pH 6.8).

TABLE 2 Thermodynamic Parameters for the Triplex Formation Between a 15-mer TFO (Pyr15T or Pyr15MOR) and a 23-bp Target Duplex (Pur23A·Pyr23T) at 25°C, Obtained from ITC

TFO	pH	K_a (M^{-1})	ΔG (kcal mol^{-1})	ΔH (kcal mol^{-1})	ΔS (cal mol^{-1} K^{-1})
Pyr15T	5.3[a]	2.66×10^6	−8.76	−73.7	−218
Pyr15T	6.8[b]	N. D.	N. D.	N. D.	N. D.
Pyr15MOR	6.8[b]	1.02×10^6	−8.20	−55.1	−157

N.D.: Not determined.
[a]10 mM sodium cacodylate-cacodylic acid and 200 mM sodium chloride (pH 5.3).
[b]10 mM sodium cacodylate-cacodylic acid and 200 mM sodium chloride (pH 6.8).

5.3. The hydrogen bonding and the base stacking involved in the triplex formation are usually considered to be the major sources of the negative ΔH upon the triplex formation.[4] Thus, the difference in ΔH between Pyr15MOR at pH 6.8 and Pyr15T at pH 5.3 (Table 2) suggests that the hydrogen bonding and/or the base stacking of the triplex with the MOR-modified TFO may be significantly different from those with the corresponding unmodified TFO. On the other hand, the negative ΔS upon the triplex formation is mainly contributed by a negative conformational entropy change due to the conformational restraint of TFO involved in the triplex formation.[4] Therefore, the smaller magnitude of the negative ΔS for Pyr15MOR at pH 6.8 than that for Pyr15T at pH 5.3 (Table 2) suggests that the MOR-modified TFO in the free state may be more rigid than the corresponding unmodified TFO. The more rigidity of the MOR-modified TFO in the free state causes the smaller entropic loss upon the triplex formation, which leads to the increase in the K_a at neutral pH.

We conclude that the MOR backbone modification of TFO could be a key modification and may lead to progress in therapeutic applications of the antigene strategy in vivo.

REFERENCES

1. Soyfer, V.N.; Potaman, V.N. *Triple-Helical Nucleic Acids*. Springer-Verlag: New York, 1996.
2. Lacroix, L.; Arimondo, P.B.; Takasugi, M.; Helene, C.; Mergny, J.-L. Pyrimidine morpholino oligonucleotides form a stable triple helix in the absence of magnesium ions. Biochem. Biophys. Res. Commun. **2000**, *270*, 363–369.
3. Torigoe, H.; Ferdous, A.; Watanabe, H.; Akaike, T.; Maruyama, A. Poly (L-lysine)-*graft*-dextran copolymer promotes pyrimidine motif triplex DNA formation at physiological pH: thermodynamic and kinetic studies. J. Biol. Chem. **1999**, *274*, 6161–6167.
4. Kamiya, M.; Torigoe, H.; Shindo, H.; Sarai, A. Temperature dependence and sequence specificity of DNA triplex formation: an analysis using isothermal titration calorimetry. J. Am. Chem. Soc. **1996**, *118*, 4532–4538.