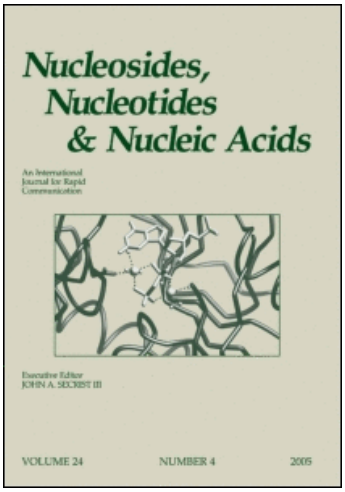

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HYBRIDIZATION PROPERTIES OF THE 5-METHYL-ISOCYTIDINE / ISOGUANOSINE BASE PAIR IN SYNTHETIC OLIGODEOXYNUCLEOTIDES

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ABSTRACT

The hybridization properties of the 5-methyl-isocytidine / isoguanosine base pair in synthetic oligodeoxynucleotides have been investigated. The 5-methyl-isocytidine / isoguanosine base pair has been determined to be isoenergetic with the cytidine / guanosine, and each base can effectively discriminate mismatches.

INTRODUCTION

Incorporation of extra base pairs into synthetic oligodeoxynucleotides can potentially be used to gain additional control over hybridization events. The geometry of the Watson-Crick base pair can accommodate several novel hydrogen-bonding schemes. One new combination, kappa and pi with three hydrogen bonds, has been reported [1]. However, in short duplex DNA the base pair had lower stability than a C-G base pair (35° vs. 42°C), and the complicated synthesis of the required nucleosides make this base pair unattractive for incorporation into large numbers of oligodeoxynucleotides. A new, different, base pair, isoC-isoG (and its variant iso^{Me}C-isoG) with three, unique hydrogen bonds, has been studied [2,3], but the stabilities of isoC-isoG and iso^{Me}C-isoG base pairs in duplex DNA are not known.

The iso^{Me}C-isoG base pair is shown in Figure 1.

We were interested in testing the iso^{Me}C-isoG base pair in duplex DNA to establish its stability and selectivity under controlled conditions. Herein we report the synthesis of oligonucleotides containing d-iso^{Me}C and d-isoG and some hybridization properties of the iso^{Me}C-isoG base pair in short duplex DNA.

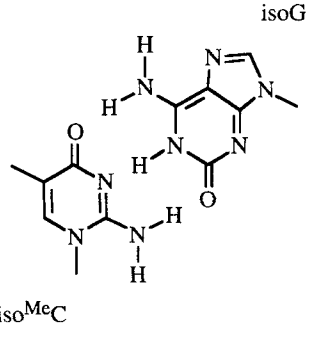

Figure 1.
The iso^{Me}C-isoG base pair.

MATERIALS AND METHODS

The 2'-deoxyribonucleosides of isoguanosine (isoG)[4] and 5-methyl-isocytidine (iso^{Me}C) were synthesized [2,3] and converted into protected phosphoramidite reagents suitable for automated DNA synthesis. Incorporation of d-iso^{Me}C was achieved using 5'-O-DMT-N^2-benzoyl-5-methyl-isocytidine BCE-phosphoramidite [3]. Incorporation of d-isoG was achieved using 5'-O-DMT-O^2-(4-nitrophenylethyl)-N^6-((dimethylamino)methylene)-2'-deoxyisoguanosine BCE-phosphoramidite as monomer block, and O^2-protection was necessary for reasons of solubility of intermediates. Deprotection of d-isoG containing oligomers required treatment with 20% DBU in CH_3CN prior to standard ammonium hydroxide deprotection.

Oligodeoxynucleotides were synthesized on an ABI 380B instrument and after deprotection they were purified by PAGE. Nucleoside composition of d-iso^{Me}C and d-isoG containing oligodeoxynucleotides were determined by snake venom exonuclease or phosphodiesterase I / alkaline phosphatase digestion and identification by HPLC. The analyses in all cases verified the incorporation of nonstandard nucleosides.

Thermal melt analyses were done on a Cary 3E Spectrophotometer in 3x SSC (0.45 M sodium chloride, 0.045 M sodium citrate), pH 7.9. Each of the two oligodeoxynucleotides incubated together was present at approximately 1.5 micromolar concentration. The T_m was calculated as the maximum in a plot of dA_{260}/dT vs. temperature.

RESULTS AND DISCUSSION

Oligomer syntheses using 5'-O-DMT-N^2-benzoyl-5-methyl-isocytidine BCE-phosphoramidite resulted in low yields of target oligomers precluding incorporation of more than a single d-iso^{Me}C residue. It has been determined that the glycosidic bond in isocytidine and related nucleosides is more acid labile relative to cytidine and an explanation for this phenomenon has been advanced [5]. When 3',5'-O-TBDMS$_2$-N^2-benzoyl-2'-deoxy-5-methyl-isocytidine was dissolved in 2.5% trichloroacetic acid in CH_2Cl_2 the compound was completely degraded to yield N^2-benzoyl-5-methyl-isocytosine and 3,5-O-TBDMS$_2$-2-deoxyribose ($t_{1/2}$ was approximately 15 minutes). Protection of the exocyclic amino group as the N,N-di-n-butylformamidine derivative improved the stability of the glycosidic bond towards acid. Ca. 25% degradation of the di-n-butylformamidine derivative was observed in 2.5% trichloroacetic acid in CH_2Cl_2 over 20 hours. A similar stabilizing effect on the glycosidic bond has been observed in 6-amino-2'-O-methylcytidine when going from protection of the two exocyclic amine groups with acyl groups to N,N-di-n-butylformamidine groups [6]. Incorporation of d-iso^{Me}C using 5'-O-DMT-N^2-((di-n-butylamino)methylene)-5-methyl-isocytidine BCE-phosphoramidite improved the oligomer synthesis and allowed the incorporation of multiple d-iso^{Me}C residues.

To determine the effect of an iso^{Me}C-isoG base pair on the stability of a short DNA duplex, it was introduced into several oligodeoxynucleotides using standard chemical DNA synthesis. The oligodeoxynucleotides sequences and the melting temperatures for duplex formation are listed in TABLE 1.

The results shown in TABLE 1 indicate that the iso^{Me}C-isoG base pair is isoenergetic with the natural C-G base pair. The data also show the specificity of the iso^{Me}C-isoG base pair: C-

TABLE 1. Melting temperatures for duplex formation.

```
5'-(Z)CA C X A CTT TCT CC(T)-3'  (15)
3'-(T)GT G Y T GAA AGA GG   -5'  (14)
```

X	Y	Z	T_m [°C]	Comments
C	G	L	60	
isoMeC	isoG	L	60	Biphasic: 45°C, 60°C
isoMeC	G	L	52	isoMeC-G mismatch; broad transition
C	isoG	L	52	C-isoG mismatch; broad transition
T	isoG	-	53	T-isoG mismatch; broad transition
T	G	-	50	T-G mismatch

where L = N^4-(6-aminohexyl)-5-methyl-2'-deoxycytidine, and nucleotides not involved in the base-pairing are indicated in parentheses.

isoG, T-isoG, and isoMeC-G are effective mismatches. The biphasic nature of the melting curve for the isoMeC-isoG base pair may be due to the presence of the enol tautomer, which is theoretically complementary to T [7]. The T-isoG T_m data suggest that the enol tautomer is present at very low concentration in 3x SSC at pH 7.9 or less likely, if present, both keto and enol tautomers form hybrids with a T_m 7-8°C lower than the isoMeC-isoG hybrid.

In summary, we have demonstrated that the isoMeC-isoG base pair is isoenergetic with the C-G base pair and that each base can effectively discriminate mismatches. We are currently extending the investigation to include other mismatches and establish any pH and salt dependency and the kinetics of hybridization. Further we are testing the hybridization properties of oligodeoxynucleotides containing several isoC/isoG base pairs, and the influence of surrounding nucleotides on the stability of the isoC/isoG base pair.

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