The specific interaction between metal cation and mismatch base pair in duplex RNA

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ABSTRACT

We have already found that mercury (II) cation specifically binds to T:T mismatch base pair in heteroduplex DNA, which increases the melting temperature of heteroduplex DNA involving T:T mismatch base pair by about 4 °C. We have also found that silver (I) cation specifically binds to C:C mismatch base pair in heteroduplex DNA, which increases the melting temperature of heteroduplex DNA involving C:C mismatch base pair by about 4 °C. In the present study, to examine whether the specific interaction between metal cation and mismatch base pair can be also formed in duplex RNA, we investigated the effect of the metal cation on the thermal stability of homoduplex and heteroduplex RNA. Addition of mercury (II) cation increased the melting temperature of heteroduplex RNA containing U:U mismatch base pair by about 6 °C. The thermal stability of homoduplex RNA containing U:A or A:U perfectly matched base pair and heteroduplex RNA containing A:A mismatch base pair was not significantly changed by the addition of mercury (II) cation. On the other hand, addition of silver (I) cation increased the melting temperature of heteroduplex RNA containing C:C mismatch base pair by about 4 °C. The thermal stability of homoduplex RNA containing C:G or G:C perfectly matched base pair and heteroduplex RNA containing G:G mismatch base pair was not significantly changed by the addition of silver (I) cation. We conclude that the specific interaction between metal cation and mismatch base pair can be formed in duplex RNA as in the case of duplex DNA.

INTRODUCTION

The interaction between metal cation and DNA is one of important factors to discuss the stabilization mechanism of DNA. We have already found that mercury (II) cation directly binds to T:T mismatch base pair in heteroduplex DNA with high affinity and specificity, which increases the melting temperature of heteroduplex DNA involving T:T mismatch base pair by about 4 °C.¹,² We have also found that silver (I) cation directly binds to C:C mismatch base pair in heteroduplex DNA with high affinity and specificity, which increases the melting temperature of heteroduplex DNA involving C:C mismatch base pair by about 4 °C.¹,² Here, to examine whether the specific interaction between metal cation and mismatch base pair can be also formed in duplex RNA, we investigated the effect of the metal cation on the thermal stability of homoduplex and heteroduplex RNA. The thermodynamic properties were analyzed by UV melting. We have found that addition of mercury (II) cation increased the melting temperature of heteroduplex RNA containing U:U mismatch base pair by about 6 °C. We have also found that addition of silver (I) cation increased the melting temperature of heteroduplex RNA containing C:C mismatch base pair by about 4 °C.

MATERIALS AND METHODS

We purchased a pair of 25-mer complementary RNA oligonucleotides, INS-F25W-RNA: 5’-gGCCCGCCUGUCWCCACGUAUCACUG-3’ (W=A, U) and INS-R25X-RNA: 5’-gCAGUGAUCUGGGXGACAGGCAAGGC-3’ (X=A, U) from Japan Bio Services Co., Ltd. (Fig. 1). We also purchased another pair of 25-mer complementary RNA oligonucleotides, APM-F25Y-RNA: 5’-tCCAGAUCUCCUCAAAAAAACA-3’ (Y=C, G) and APM-R25Z-RNA: 5’-tUUGUUUUUGAAAGZGCAGAUGUC-3’ (Z=C, G) (Fig. 1). The two pairs of the complementary RNA strands, INS-F25W-RNA:INS-R25X-RNA and APM-F25Y-RNA:APM-R25Z-RNA, were annealed by heating at up to 90 °C, followed by a gradual cooling to room temperature. UV melting experiments were performed on a DU-640 spectrophotometer (Beckman Inc.) equipped with a Peltier type cell holder.

INS-F25W-RNA: 5’-gGCCCGCCUGUCWCCACGUAUCACUG-3’
INS-R25X-RNA: 5’-gCAGUGAUCUGGGXGACAGGCAAGGC-3’
APM-F25Y-RNA: 5’-tCCAGAUCUCCUCAAAAAAACA-3’
APM-R25Z-RNA: 5’-tUUGUUUUUGAAAGZGCAGAUGUC-3’


RESULTS AND DISCUSSION

Table 1 summarizes melting temperatures of a series of duplex RNA, INS-F25W-RNA:INS-R25X-RNA (W:X=

<table>
<thead>
<tr>
<th>W:X</th>
<th>$T_m$ (°C)</th>
<th>$T_m$ (+Hg) (°C)</th>
<th>$\Delta T_m$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U:U</td>
<td>84.6</td>
<td>91.0</td>
<td>6.4</td>
</tr>
<tr>
<td>U:A</td>
<td>91.8</td>
<td>93.0</td>
<td>1.2</td>
</tr>
<tr>
<td>A:U</td>
<td>89.5</td>
<td>90.7</td>
<td>1.2</td>
</tr>
<tr>
<td>A:A</td>
<td>84.6</td>
<td>85.7</td>
<td>1.1</td>
</tr>
</tbody>
</table>

$^{*}$10 mM sodium cacodylate-cacodylic acid (pH 6.8) and 100 mM sodium perchlorate. $^{*}$$\Delta T_m = T_m (+Hg) - T_m$ (Hg).

Table 2: Melting temperatures of the duplex RNA, APM-F25Y-RNA:APM-R25Z-RNA (Y:Z=C:C, C:G, G:C, and G:G), at pH 6.8 with or without silver (I) nitrate, obtained from UV melting.

<table>
<thead>
<tr>
<th>Y:Z</th>
<th>$T_m$ (-Ag) (°C)</th>
<th>$T_m$ (+Ag) (°C)</th>
<th>$\Delta T_m$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C:C</td>
<td>67.0</td>
<td>71.0</td>
<td>4.0</td>
</tr>
<tr>
<td>C:G</td>
<td>76.1</td>
<td>76.8</td>
<td>0.7</td>
</tr>
<tr>
<td>G:C</td>
<td>75.4</td>
<td>76.0</td>
<td>0.6</td>
</tr>
<tr>
<td>G:G</td>
<td>68.5</td>
<td>68.7</td>
<td>0.2</td>
</tr>
</tbody>
</table>

$^{*}$10 mM sodium cacodylate-cacodylic acid (pH 6.8) and 100 mM sodium nitrate. $^{*}$$\Delta T_m = T_m (+Ag) - T_m (-Ag)$

U:U, U:A, A:U and A:A) in the absence or presence of mercury (II) cation. In the absence of mercury (II) cation, the melting temperatures of the homoduplex containing the perfectly matched base pairs (W:X=U:A and A:U) were significantly higher than that of the heteroduplex containing the mismatch base pair (W:X=U:U). Addition of mercury (II) cation increased the melting temperature of the heteroduplex containing the U:U mismatch base pair by about 6 °C. On the other hand, the melting temperatures of the homoduplex containing the U:A and A:U perfectly matched base pairs were not significantly changed by the addition of mercury (II) cation. These results indicate that mercury (II) cation specifically increased the thermal stability of the heteroduplex containing the U:U mismatch base pair.

Table 2 summarizes melting temperatures of a series of duplex RNA, APM-F25Y-RNA:APM-R25Z-RNA (Y:Z=C:C, C:G, G:C and G:G) in the absence or presence of silver (I) cation. In the absence of silver (I) cation, the melting temperatures of the homoduplex containing the perfectly matched base pairs (Y:Z=C:G and G:C) were significantly higher than that of the heteroduplex containing the mismatch base pair (Y:Z=C:C). Addition of silver (I) cation increased the melting temperature of the heteroduplex containing the C:C mismatch base pair by about 4 °C. On the other hand, the melting temperatures of the homoduplex containing the C:G and G:C perfectly matched base pairs were not significantly changed by the addition of silver (I) cation. These results indicate that silver (I) cation specifically increased the thermal stability of the heteroduplex containing the C:C mismatch base pair.

The present results suggest that the mercury (II) cation-U:U mismatch base pair interaction and the silver (I) cation-C:C mismatch base pair interaction can be specifically formed in duplex RNA. Replacement by hydroxyl group at the 2' position of sugar moiety, and removal of methyl group at the 5 position of thymine base may not perturb the specific interaction between metal cation and mismatch base pair.

CONCLUSION

We conclude that the specific interaction between metal cation and mismatch base pair can be formed in duplex RNA as in the case of duplex DNA.

REFERENCES


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