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Effect of Dangling Ends on the Double-Helix Melting of Ribooligonucleotides at Different Na^+ Concentrations

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We report quantitatively the effects of 3' and 5' dangling ends on the melting of the self-complementary double-helix of AUGCAU at various Na^+ concentrations. The dependences of the melting temperature on the salt concentration are large for dangling G and U, but are small for the dangling A and C at both 3' and 5' ends.

Unpaired terminal nucleotides (dangling ends) play very important roles in the structure and interaction of ribonucleic acids (RNAs). There are extensive studies about qualitative effect of dangling ends.[1-3] For example, it is well known that the dangling ends determine the stability of codon-anticodon associations[1,2] and are responsible for some codon context effects.[3] It was also reported that the dangling ends determine the important tertiary structure of transfer RNA (tRNA).[4] On the other hand, little is reported about the quantitative effect of the dangling ends on the stability of an RNA duplex except at a high salt concentration such as 1 mol dm^{-3} NaCl.[4,5] At the condition of high salt concentration, 3' dangling purines add more stability than 3' dangling pyrimidines and 5' dangling ends.[4,5] However, since a salt condition should affect the stability of the RNA duplex,[6,7] the conclusion at the high salt concentration can not be applied to the other salt concentration and the result makes it difficult to predict the effect of dangling ends at the low salt concentration which is much closer to the conditions of a biological system.

In this paper, we report quantitatively the effects of 3' and 5' dangling ends on the melting of the self-complementary double-helix of AUGCAU at various Na^+ concentrations (Eq. 1). The study can provide insight into the contribution of the salt to a base pairing and stacking in RNA double helix.

$$2\ \mathrm{AUGCAUX} \rightleftharpoons \underline{\mathrm{AUGCAUX}}\atop{\mathrm{XUACGUA}}, \qquad 2\ \mathrm{YAUGCAU} \rightleftharpoons \underline{\mathrm{YAUGCAU}}\atop{\mathrm{UACGUAY}} \qquad (1)$$

(X and Y; A, C, G, and U)

The oligonucleotides, AUGCAUX and YAUGCAU (X and Y; A, C, G, and U), were synthesized on solid support with a phosphoramidite method.[8] The oligonucleotides were finally purified and desalted with a C-18 Sep-Pak cartrige(Waters). Oligonucleotide concentrations(C_t), strand concentrations, were calculated from the high-temperature (50 or 60°C) absorbance at 260 nm. Single-strand extinction coefficients were calculated from extinction coefficients of dinucleotide monophosphates and nucleotide.[9] The buffer was 10^{-2} mol dm^{-3} Na_2HPO_4 and 10^{-3} mol dm^{-3} Na_2EDTA, pH 7.0, containing various concentration of NaCl. Prior to dilution of the oligonucleotides for experiments, buffers were degassed by heating at 90°C for 10 min. Absorbance vs. temperature curves (melting curves) were measured at 260 nm on a Hitachi U-3200 programable spectrophotometer. The heating rate was 0.5 or 1 °C/min regulated with a Hitachi SPR-7 temperature controller.

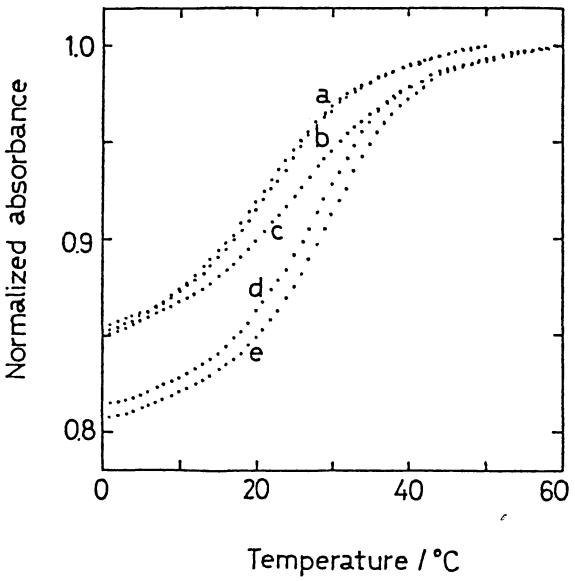

Fig. 1. Melting curves of about 30 μmol dm^{-3} (a)AUGCAU, (b)AUGCAUU, (c)AUGCAUC, (d)AUGCAUG, and (e)AUGCAUA in 100 mmol dm^{-3} NaCl buffer.

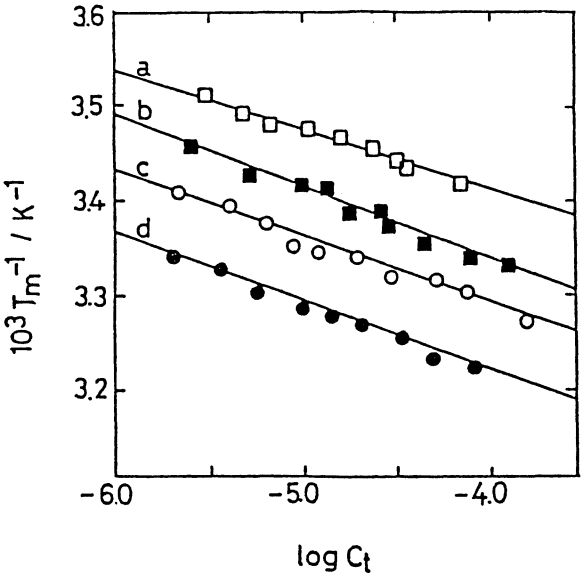

Fig. 2. Plots of T_m^{-1} vs. log(C_t) for AUGCAUG in (a) 0, (b) 30, (c) 100, and (d) 400 mmol dm^{-3} NaCl buffers.

Figure 1 shows the typical melting curves of the AUGCAU and AUGCAUX in 0.1 mol dm^{-3} NaCl buffer. In each salt concentration, about 10 melting curves were measured over a 50-fold range in strand concentration of each oligonucleotide. The melting temperature, T_m, where half of the strands was in the duplex, was determined as described previously.[10] Melting curves were analyzed with a model of two-state transition, that is, double-helix to single-strand transition in Eq. 1. For the two-state transition, the reciprocal melting temperature, T_m^{-1}, should depend linearly on the logarithm of C_t.[11] Figure 2 shows the typical plots of T_m^{-1} vs. log(C_t)

for the AUGCAUG duplex at various NaCl concentrations. The linearities of T_m^{-1} vs. $\log(C_t)$ were observed for all the oligonucleotides and the salt concentrations. The results suggest that the oligonucleotide duplexes having the dangling ends melt from the double helix to the single strands at any salt concentration. The suggestion is supported by the results of CD spectra of the oligonucleotides at low and high temperatures.[12]

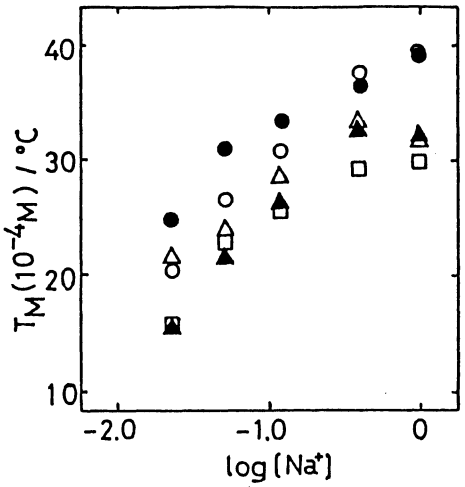

Fig. 3. Plots of T_m vs. log[Na+] for AUGCAU(□), AUGCAUA(●), AUGCAUG(○), AUGCAUU(▲), and AUGCAUC(△).

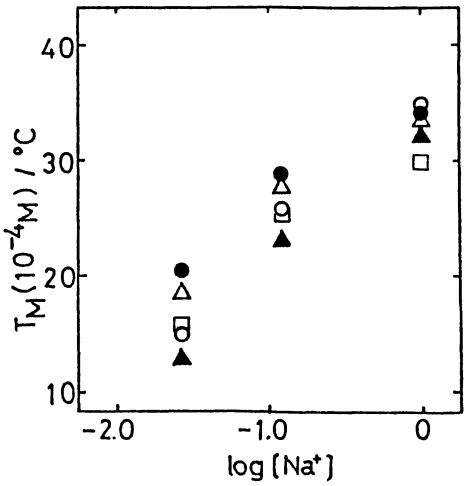

Fig. 4. Plots of T_m vs. log[Na+] for AUGCAU(□), AAUGCAU(●), GAUGCAU(○), UAUGCAU(▲), and CAUGCAU(△).

Figures 3 and 4 show the plots of T_m calculated for 0.1 mmol dm^{-3} strand concentration of the oligonucleotides having 3' and 5' dangling ends vs. log[Na+], respectively. The values of T_m at 1.02 mol dm^{-3} Na^+ in Figs. 3 and 4 were obtained from Ref. 4. The Na^+ concentration was calculated as the sum of the concentrations from NaCl, Na_2HPO_4, and Na_2EDTA. In Fig. 3, the all 3' dangling ends stabilize the AUGCAU duplex at 1.02 mol dm^{-3} Na^+. On the other hand, at low Na^+ concentrations 3'U dangling end does not stabilize the duplex. The 5'U and 5'G dangling ends have the clearer tendency; both dangling ends stabilize and instabilize the core duplex at high and low salt concentrations, respectively. The results show that the effect of the dangling ends on the stability of the core double-helix at a high salt concentration is not always the same at a low salt concentration.

Table 1. The values of $d(T_m)/d(\log[Na^+])$ for the oligonucleotides having 3' and 5' dangling ends

AUGCAUX	X:	A	C	G	U	-
$d(T_m)/d(\log[Na^+])$ /°C		7.9	6.3	11.6	10.5	9.0
YAUGCAU	Y:	A	C	G	U	
$d(T_m)/d(\log[Na^+])$ /°C		8.4	9.1	12.0	11.5	

The values of $d(T_m)/d(\log[Na^+])$ are listed in Table 1. The value is larger for 5' dangling than 3' dangling at each terminal nucleotide. It may suggest that the 3' dangling nucleotide has significant overlap with 5' terminal A on the opposite strand, while the 5' dangling nucleotide does not stack so tightly 3' terminal U; therefore, the 5' dangling ends can be affected slightly larger than the 3' ends. For different nucleotides, dependences of T_m on the salt concentration are large for the dangling G and U, but are small for the dangling A and C at both 3' and 5' ends. The results suggest that the dependence of the effect of the dangling ends on the Na^+ concentration is dangling nucleotide-specific. The results in this work should be useful for the prediction of structures of RNA at low salt concentrations which are similar to those of biological systems.

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