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Partial Melting of the Segment around Pseudouridine in Yeast 5S RNA

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Pseudouridine in yeast 5S RNA was modified with 4-bromomethyl-7-methoxycoumarin(BMC). Temperature dependence of fluorescence intensity was measured at various concentrations of Mg^{2+} and K^{+} cations. Hyperchromicity was also measured. At 100mM KCl and 10mM Mg^{2+}, fluorescence intensity decreased with temperature as free BMC except a plateau at 45°C. Withdrawal of Mg^{2+} from the buffer resulted in a large quenching at 20°C and showed a gradual increase of fluorescence intensity with temperature, indicating a partial melting of the segment around pseudouridine. The temperature range agrees with the low melting temperature shown by hyperchromicity. In 10mM KCl solution, the effects are more exaggerated.

The function of 5S RNA in protein biosynthesis remains unclear, although it has been thought to take part in the binding of tRNA to ribosomes. The nucleotide sequences of 5S RNA from many organisms and the corresponding secondary structure have been summarized in literature(1-4). For yeast 5S RNA, several models were proposed by the studies of sequence homology(3-8), chemical modification(9), nuclease digestion(10-13), and optical measurements (14-16). Several studies indicate the presence of Mg^{2+} dependent tertiary structure(s) in 5S RNA(14-18). E.coli 5S RNA is known to exist in two different forms, native and stable denatured, depending on the concentration of Mg^{2+}(19-21). For yeast 5S RNA, no stable denatured conformation has been reported. Yeast 5S RNA molecule contains one pseudouridine(22). In this paper, we report the melting behavior of the segment of yeast 5S RNA modified with fluorescent reagent of 4-bromomethyl-7-methoxycoumarin in different buffers containing 10mM Mg^{2+} or no Mg^{2+} and 100mM or 10mM KCl, respectively.

MATERIALS AND METHOD

Yeast RNA was extracted by Holley's method(23). The RNA was separated into 5S RNA and tRNA by Sephadex A-50 chromatography. Finally, 5S RNA was purified by Sephadex G-100 chromatography as reported(24,25). The purified 5S RNA was examined by electrophoresis on 7.5% polyacrylamide gels under the condition described by Richard et al.(19), and was found to move as a single band.

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A fluorescent reagent, 4-bromomethyl-7-methoxycoumarin(BMC), was purchased from Wako pure chemical industries, Ltd.(Japan). Normal nucleotides in 5S RNA don't react with this reagent. Pseudouridine, located only at the 50 position in yeast 5S RNA, was modified with this reagent as reported by Yang and Söll (26).

The buffers added to 5S RNA contained: 100mM KCl, 10mM $MgCl_2$ and 10mM Tris-HCl(pH7.4) for buffer A, 100mM KCl, 1mM EDTA and 10mM Tris-HCl(pH7.4) for buffer B, 10mM KCl, 10mM $MgCl_2$ and 10mM Tris-HCl(pH7.4) for buffer C, and 10mM KCl, 1mM EDTA and 10mM Tris-HCl(pH7.4) for buffer D. In the preparation of the sample solution with no magnesium cation, 5S RNA was dissolved in buffer B or D, and then heated at 60°C for 10 min to completely catch magnesium cation. The solutions were cooled slowly to room temperature and then dialyzed against the buffer B or D at 4°C, respectively.

UV-absorbance and fluorescence spectra were measured with temperature controlled cell holders using a Hitachi M200-20 spectrophotometer and a Shimadzu RF-503 difference spectrophotometer, respectively. Samples were degassed before the measurement. The temperature was increased at a rate of 1°C/2 min, and equilibriated for 5 min before the absorbance was recorded. Absorbance was recorded at each temperature interval of 3°C. The samples heat treated for the hyperchromicity measurement were cooled to 20°C after the experiment, and found to recover their initial absorbance within an experimental error of 1%. No detectable effect of the heat treatment imposed on 5S RNA during the sample preparation remained in the melting behavior of the sample when the solutions were carefully annealed. The renatured sample was prepared by heat treatment after the addition of Mg^{2+}. The UV-thermal melting and mobility on polyacrylamide gel electrophoresis of BMC modified 5S RNA showed no detectable differences with those of intact 5S RNA.

RESULTS

UV-thermal melting curves of 5S RNA in solution A and B are shown in Fig.1. Curve 2, for solution in buffer B, shows clear two-step melting. This is also shown in curve 1, for solution in buffer A. The result is similar to that reported by Luoma et al.(16).

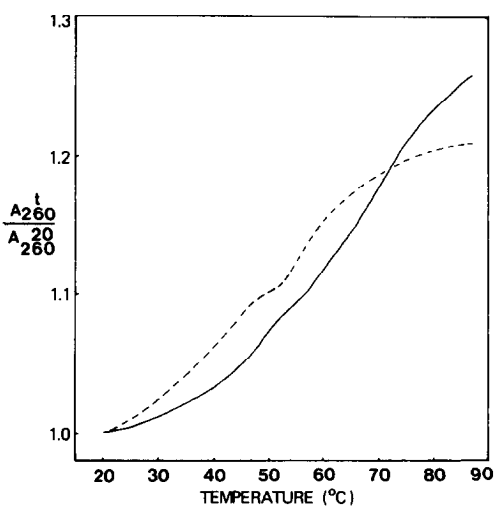

Fig.1 UV-thermal melting curves of 5S RNA are shown. (——); curve 1 for solution of buffer A and (---); curve 2 for solution of buffer B.

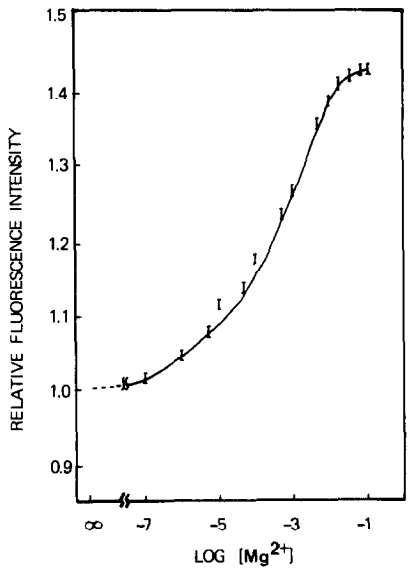

Fig.2 The Mg^{2+} dependent fluorescence intensity of BMC modified 5S RNA were measured in solution B without EDTA.

The fluorescence intensity of BMC modified 5S RNA was plotted against Mg^{2+} concentration and is shown in Fig.2. The sample was excited at the wavelength of 325nm, and fluorescence intensity at the maximum wavelength of 400nm was measured. Fluorescence intensity increased as Mg^{2+} concentration increased, showing a sigmoidal curve. Fig.3 shows the temperature dependence of fluores-

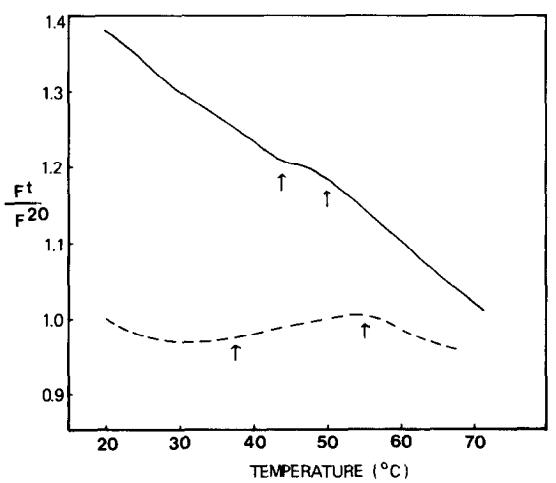

Fig.3 The temperature dependences of fluorescence intensity of BMC modified 5S RNA are shown. (——); curve 1 for solution A and (---); curve 2 for solution B.

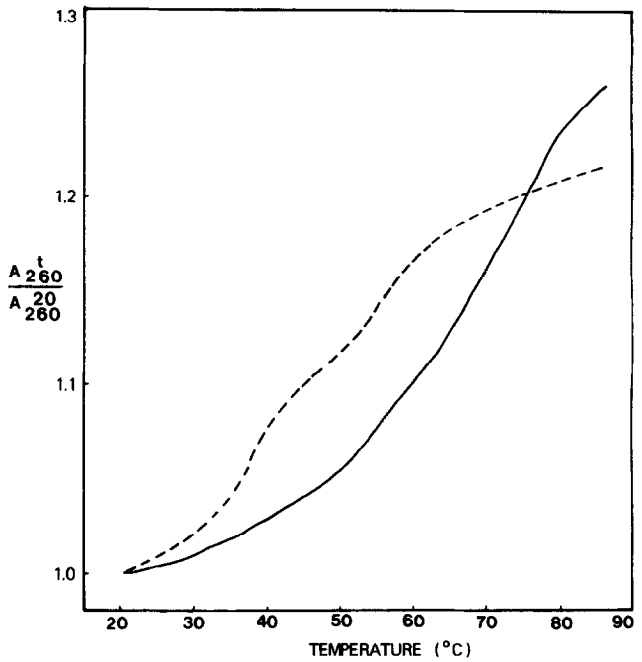

Fig.4 UV-thermal melting curves of 5S RNA are shown. (——); curve 1 for solution C and (---); curve 2 for solution D.

cence intensity of BMC modified 5S RNA in solution A and B, respectively. The values of ordinate, fluorescence intensities relative to those measured at 20°C, were plotted against temperature. At the temperature region of 45 to 50°C, curve 1 shows a small plateau. Curve 2 has a quite different shape compared to curve 1. First, the value of fluorescence intensity of curve 2 at 20°C is only 73% of that of curve 1, although the initial trend is the same as curve 1. Second, fluorescence intensity starts to increase at a low temperature of about 35°C and reaches a maximum at 55°C. The hypothetical melting points and the ends of the first melting in curves 1 and 2 in Fig.1 are shown by arrows in Fig.3.

UV-thermal melting curves in solution of buffer C and D, the low concentrations of KCl containing 5S RNA solution, are shown in Fig.4. The profiles resemble those in Fig.1. Melting, however, starts at a lower temperature, and curve 1 does not show clear two-step melting. The temperature dependence of fluorescence intensity in solution C and D are shown in Fig.5. Curve 1 shows a plateau at a temperature region around 35°C. The fluorescence inten-

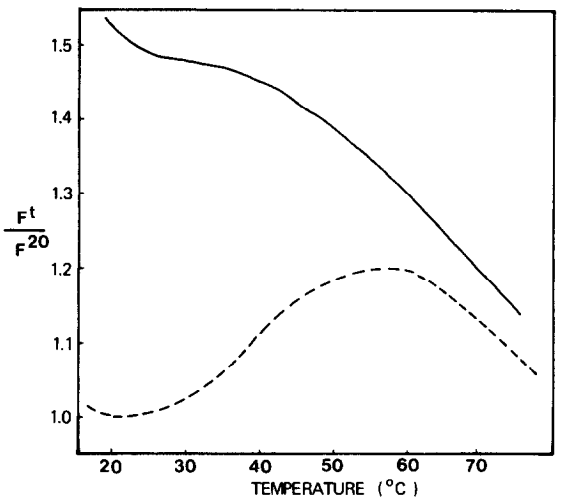

Fig.5 The temperature dependent fluorescence intensity of BMC modified 5S RNA are shown. (——); curve 1 for solution C and (---); curve 2 for solution D.

sity of curve 2 in solution D starts to increase at 20°C, and reaches a maximum at 55°C. These characteristic temperatures are close to the temperatures of the hyperchromicity curve in Fig.4.

DISCUSSION

UV-thermal melting curves of Figs. 1 and 4 show two-step melting. The first increase in hyperchromicity may reflect melting in the weak hydrogen bonded part(s) in the 5S RNA molecule. Concentrations of both Mg^{2+} and K^+ have an effect on the melting curves, although the effect of Mg^{2+} cation is more prominent than that of K^+ cation.

The temperature dependence of fluorescence intensity of BMC modified 5S RNAs shows, apparently, the melting behavior of the segment around pseudouridine. The fluorescence intensity of free BMC itself decreased monotonously as the temperature increased. The main part of curve 1 in Fig.3 agrees with this trend, and curve 2 has a quite different shape. A small plateau in curve 1 and a gradual increase in fluorescence intensity in curve 2 were observed with temperature increase. The increase in fluorescence intensity may be attributed to the increasing hydrophilic environment of the fluorescent probe as the melting of the segment around the probe proceed. The temperature region

exhibiting a plateau and a gradual increase of fluorescence intensity lies between the arrows. The low temperature melting region observed in UV-thermal melting curves can be attributed to the melting of the segment around pseudouridine. Secondary structures proposed for yeast 5S RNA have the same structure for the region extending from position 23 to position 54. This region contains the conserved sequence GAUC in the loop and pseudouridine in the stem at position 50. According to Tinoco's theory for estimating the most stable secondary structure, the double helical region containing pseudouridine is unstable(27). Theoretical results agree with the present experimental results.

The effect of magnesium and monovalent cations on the stabilization of the secondary and tertiary structure of tRNA is well known(28). When Mg^{2+} ions are absent, the tertiary structure of tRNA is greatly destroyed, and at lower ionic strength, the structure becomes more unstable. The experimental results of 5S RNA presented show analogous effects of the cations. The number of base pairs estimated by spectroscopic methods decreases slightly on removal of Mg^{2+}(14-18). These studies also suggest the presence of a Mg^{2+} dependent tertiary structure(13). Fluorescence intensity varied by experimental conditions shows the conformational change of the region around pseudouridine. The decrease in the relative fluorescence intensity at 20°C on removal of Mg^{2+} may be attributed to the disappearance of the Mg^{2+} dependent tertiary structure. Curve 2 in Fig.3 shows the initial decrease with temperature and the gradual increase of fluorescence intensity at about 35°C when Mg^{2+} ions are absent. The temperature where the increase occurs corresponds to that of first melting of curve 2 in Fig.1. It is considered that a kind of secondary structure starts to melt at this lower temperature. Wong et al. and Maruyama et al. reported a secondary structure containing fewer base pairs than those measured at high KCl and Mg^{2+} concentrations, and at room temperature(15,18). At high KCl and $MgCl_2$ concentrations, the region around pseudouridine remains intact at 45°C. On the other hand, at low KCl concentration and with no Mg^{2+}, the region starts to melt at 20°C.

Eukaryotic 5S RNA contains the conserved sequence from position 42 to position 44. It is a complementary sequence of GAUC in the T-loop of eukaryotic initiator tRNA. It is thought of as one of the recognition sites of 5S RNA which bind to ribosomes. The experimental results presented show that the pseudouridine containing the segment adjacent to the GAUC loop of yeast 5S RNA has adequate variability in conformation, which is required when the complementary base pair is formed between GAUC sequences of 5S RNA and initiator tRNA. Recently, however, Pace et al. reported that the complementary GAUC-T loop is not necessary for the process of protein biosynthesis(29).

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