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Nucleotide sequence and thermal property of 5S rRNA from the elder aphid, Acyrthosiphon magnoliae

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ABSTRACT

The nucleotide sequence of 5S rRNA from the elder aphid, Acyrthosiphon magnoliae was determined by using postlabeling sequencing techniques. The aphid 5S rRNA consists of 120 nucleotides and the sequence differs from those of Bombyx and Drosophila 5S rRNAs in 14 and 16 positions, respectively. A secondary structure model based on the sequence has two distinctive features : the helix I is shorter and the total free energy lower. Judging from the thermal profile, the aphid 5S rRNA likely assumes a conformation somewhat different from those of the other two insects'.

INTRODUCTION

A comparison of primary structures of 5S rRNAs from various organisms provides valuable information on phylogenetic relationships among those organisms. At the same time, the invariant nucleotide sequences among those molecules will be taken to represent those sequences important to function. In addition, secondary structure models can be deduced by comparative analysis of primary structures.

Of more than thirty sequences of eukaryotic 5S rRNAs so far reported (1), only the two are from Insecta, the class with the most numerous number of species, Drosophila melanogaster (2) and Bombyx mori (3). In the meantime, in terms of molecular properties of ribosomal RNA, the aphid is distinguished from the rest of insect species. The aphid 28S rRNA lacks the hidden break which those from the other protostomes generally share at or near the midpoint (4). Also, the 18S rRNA from the aphid is with molecular weight significantly larger than 0.7×10^6, the common value for most of the eukaryotic 18S rRNAs (4). All these circumstances prompted us to determine the nucleotide sequence of the aphid 5S rRNA. Also reported is its secondary structure model, which hints at the unique properties likely pertinent to this molecule.

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MATERIALS AND METHODS

The aphids, Acyrthosiphon magnoliae (Essig et Kuwana) were collected from sprouts of the elder trees on the Tokyo University campus. The whole tissues of insects were homogenized and the total RNA extracted by the cold phenol method. The total RNA, treated with DNase, was fractionated into 28S, 18S and low molecular weight RNAs by sucrose density gradient centrifugation (5). The low molecular weight RNAs were resolved on 10% polyacrylamide slab gel in 7 M urea and the 5S rRNA, located by UV-absorbance, eluted from the gel slices, precipitated in ethanol and taken to dryness (6).

Upon treating with alkaline phosphatase from calf intestine (Boehringer), the 5S rRNA was labeled at the 5'-terminus with [γ-^{32}P]ATP (3,000 Ci/mmol, Amersham) in use of polynucleotide kinase (BRL) (7). The 3'-labeled RNA was prepared with [5'-^{32}P]pCp (2,000-3,000 Ci/mmol, Amersham) and T4 RNA ligase (P-L Biochem.) (6). The end-labeled RNA was electrophoresed again in 10% polyacrylamide-7 M urea slab gel. Thus purified RNA was subjected to ladder analysis following partial chemical hydrolysis (6) or partial base-specific hydrolysis with ribonucleases T1 and U2 (Sankyo) (8). Mobility shift analysis was performed after partial hydrolysis with alkali (9, 10). Also employed was the modified method of Stanley and Vassilenko (11). The RNA was partially hydrolyzed with formamide and the resulted fragments labeled at the 5'-termini. On seperated by gel electrophoresis, each of the 5'-labeled fragments was digested completely with nuclease P1 (Yamasa) and subjected to two dimensional thin-layer chromatography on Avicel SF cellulose plate (7).

Thermal denaturation analysis was performed at 260 nm in a temperature-programmed recording spectrophotometer (Gilford). The RNA was dissolved in 150 mM NaCl and 15 mM Na-citrate (SSC) or in 3 x SSC at a final concentration of about 1 A_{260} unit. Temperature was raised at 0.5°C/min and the data points taken every one fifth of a degree.

Polyacrylamide gel electrophoreses were performed at constant voltage in TEB buffer (50 mM Tris, 1 mM EDTA and 50 mM borate, pH 8.3) (12).

RESULTS

Fig. 1 represents the nucleotide sequence of 5S rRNA from Acyrthosiphon magnoliae, aligned with those from Bombyx and Drosophila. The sequence of about 90 nucleotides from the 3'-terminus was determined, using the 3'-labeled RNA, mainly by the partial chemical digestion method of Peattie (6) and for confirmation by the partial enzymatic digestion method of Donis-Keller et al. (8). The sequences determined by these two methods were in

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                          10         20         30         40         50
Acyrthosiphon     GGCAACGACCAUACCACGUUGAAUACACCAGUUCUCGUCCGAUCACUGAA
Bombyx            .C.....U........U............G................C...
Drosophila        .C................C........U.G................C...

          60         70         80         90        100        110        120
GUUAAGCAACGUCGGGCGUAGUUAGUACUUGGAUGGGUGACCGCUUGGGAACACUACGUGCCGUUGGCAU
..C.......A........G..C.....................C.........C.....AU......U.
A.......G.........CG..........A......G................CG....UU......C.
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Fig. 1. Comparison of the nucleotide sequence of Acyrthosiphon magnoliae 5S rRNA with those of Bombyx mori and Drosophila melanogaster 5S molecules.

complete agreement except for positions around 110, where the bands were compressed in lower concentration gels. Employing 30% polyacrylamide gel overcame the difficulty and the nucleotide sequence at the position 107-112 was determined, without uncertainty, to be CGUGCC. The sequence down to position 50 from the 5'-terminus was determined, using the 5'-labeled RNA, mainly by a combination of the partial enzymatic digestion method and mobility shift analysis (9, 10). We encountered some difficulty in determinig the 5'-terminal sequence. The 5'-terminal pentanucleotide was GGPyAA by the former method whereas $-{}^{A}/_{C}\,{}^{A}/_{C}\,{}^{A}/_{C}\,{}^{A}/_{C}$ by the latter analysis (the labeled terminal nucleotide can not be determined by this analysis). These taken together, the terminal sequence was deduced to be GXCAA (X is nucleotide not identified). Like in the present results, due to unknown reasons, the 5'-terminal sequence determined by mobility shift analysis is sometimes inconsistent with that by partial enzymatic digestion method. By employing the modified method of Stanley and Vassilenko (11), the nucleotide of position 3 was confirmed as C. And yet, the same method could not identify nucleotide X at position 2 because it was only poorly labeled. Finally the nucleotide was regarded as G in considering the high specificity of RNase T1 since the position was sensitive to this enzyme but not to either RNase U2 or A.

It was noticed that electrophoretic mobility of the aphid 5S rRNA in 10% polyacrylamide gel was very slow under the conditions that the gel plate was slightly warmed up (Fig. 2). However, when electrophoresed at 4°C or with urea, the mobility was not different from those of Bombyx and Drosophila 5S rRNAs (data not shown). Therefore, these molecules are supposed to take a compact form in common at 4°C and unfolded forms with urea whereas under the moderate conditions likely the aphid 5S rRNA behaves differently. This possibility was tested by investigating hyperchromicity of these RNAs due to in-

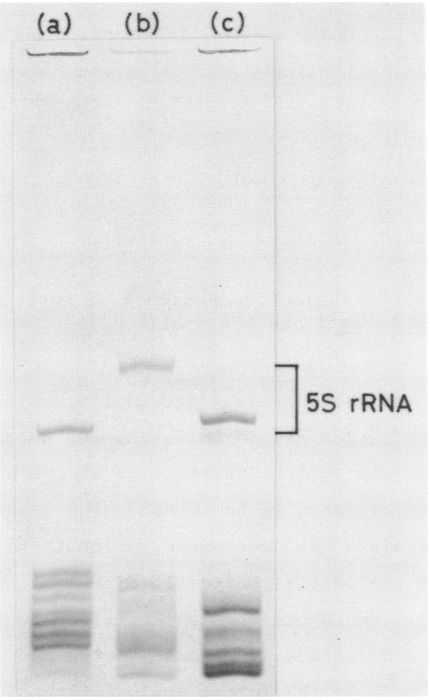

Fig. 2. Electrophoretic pattern of the low molecular weight RNAs from three insect species in 10% polyacrylamide gel. These RNAs were electrophoresed in TEB buffer under the partially-denaturing conditions. (a) Drosophila, (b) Acyrthosiphon, (c) Bombyx.

creased temperature. Shown in Fig. 3 were the thermal denaturation curves of aphid and Bombyx 5S rRNAs in 3 x SSC at 260 nm. A similar experiment in SSC resulted in the curves very similar, though shifted to lower temperatures, to the ones in Fig. 3 (data not shown). The Tm for denaturation of aphid 5S rRNA was lower than that of Bombyx molecule by 4 to 5°C.

DISCUSSION

Table 1 indicates the numbers of the different nucleotides between the corresponding positions in the 5S rRNA sequences of several animals. Though Bombyx and Drosophila are closer to each other than to the aphid from the morphological and physiological points of view, the homologies between 5S rRNA sequences of every two insects are almost equal. Table 1 also suggests that the differences between insect and crustacean RNAs are in the same range as those between arthropod and vertebrate molecules. It will be noteworthy

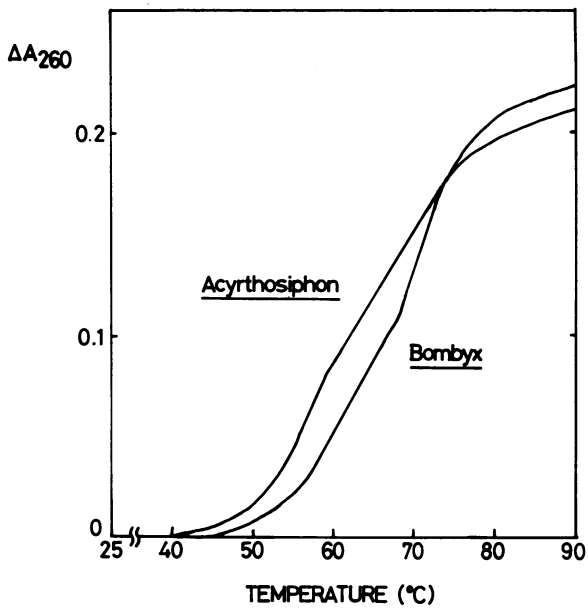

Fig. 3. Thermal denaturation profiles of the Acyrthosiphon and Bombyx 5S rRNA in 3 x SSC. A_{260} values are normalized to 1 at 25°C.

that the difference between the each two sequences of insects is 14 or more while, among the vertebrates, the mean divergence between two organisms belonging to different classes (fish, amphibia, reptiles, birds and mammals) is limited to 9 positions (Table 1 and ref. 1). Apparently, a rate of base substitution of the 5S rRNA has been considerably higher in arthropods than in vertebrates.

At 4°C or with urea, 5S rRNAs of aphid and the other two insects showed

Table 1. The numbers of nucleotide substitutions between 5S rRNAs from several animals.

		A.m.	B.m.	D.m.	A.s.	X.l.	HeLa
insects	Acyrthosiphon magnoliae	-	14	16	25	29	27
	Bombyx mori (3)		-	16	21	27	27
	Drosophila melanogaster (2)			-	27	29	26
crustacean	Artemia salina (13)				-	25	25
amphibian	Xenopus laevis (1)					-	8
mammalian	HeLa cell (1)						-

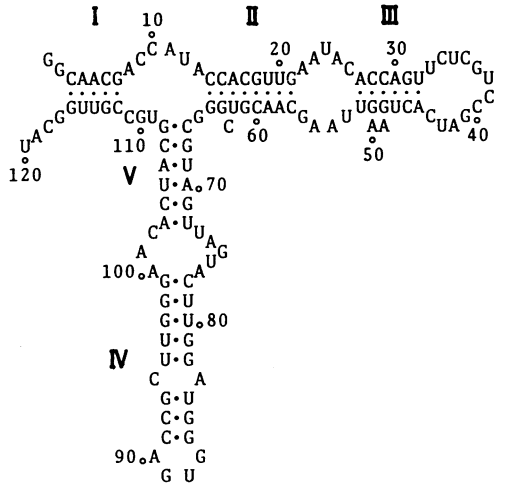

Fig. 4. Secondary structure model for the Acyrthosiphon 5S rRNA. I-V indicate helices so named.

an identical mobility in 10% polyacrylamide gels. When electrophoresed at room temperature under the conditions that the gel plate was slightly warmed, the aphid 5S rRNA moved very slowly (Fig. 2). These will suggest that the aphid 5S rRNA takes more expanded form than the other two under the latter conditions. This possibility was supported by the thermal denaturation profiles (Fig. 3), which indicated the aphid 5S rRNA is with a lower Tm than of the Bombyx molecule. In an effort to explain the unique property of aphid 5S rRNA found above, we constructed its secondary structure model (13) based on the nucleotide sequence (Fig. 4).

The model has two principal features that distinguish it from those of Bombyx and Drosophila 5S rRNAs (see Table 2).

First, the free energy (14), ΔG, of a double stranded structure of aphid 5S rRNA relative to its single stranded one is exceptionally low. The lower Tm observed with aphid 5S rRNA is probably due to its lower free energy.

Second, the helix I which consists of the 3'- and 5'-end of the molecule contains only 5 nucleotide pairs. It is because of loss of two base pairs, position 2-117 and 8-111, as a result of base substitutions. So far, several secondary structure models have been proposed for the 5S rRNA species (15, 16). In common to all the models including the prokaryotic ones, the helix I has been conserved and also cosidered to be fairly stable. For example, the helix I of 5S rRNA from Thermus thermophilus, which is known to be able to

Table 2. Helix I and total free energy of assumed secondary structures of arthropod 5S rRNAs

	base-paired nucleotides of helix I	total free energy (kcal)
Acyrthosiphon	5	-28.4
Bombyx (3)	9	-43
Drosophila (2)	9	-47.8
Artemia (13)	8	-48.8

Values were taken and calculated (14) on the basis of the secondary structure models (13).

grow at 85°C, is made up of 11 nucleotide pairs including 8 G•C pairs (17). The free energy of the helix I of aphid 5S rRNA is only -8.8 kcal whereas those of Bombyx and Drosophila ones are -17.8 kcal. The difference of 9 kcal at the helix I makes up two thirds of the total difference of free energy between the aphid and Bombyx 5S rRNA (Table 2). The actual difference of thermal denaturation profiles between the two RNAs may suggest that the aphid 5S rRNA is with such unstable helices in reality.

In addition to uniqueness in the 18S and 28S rRNA, the aphid seems to contain the 5S rRNA with a distinguished feature as above. Biological significances pertinent to this unique property are yet to be unveiled.

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