Studies on Nucleic Acids of Living Fossils

III.* A Classification of Transfer Ribonucleic Acids by the Elution Profiles on Gel Filtration and Sedimentation Profiles on Sucrose Density Gradient

Nobuyoshi SHIMIZU
The Institute of Molecular Biology, Faculty of Science, Nagoya University, Chikusa, Nagoya, 464

Received for publication, September 24, 1970

Elution profiles on gel filtration (Sephadex G-100) and sedimentation profiles on sucrose density gradient were compared for transfer RNAs (tRNAs) from two “living fossils” and from eleven species of “present organisms.” These various species of tRNAs showed a similar coefficient of distribution, Kd value, in the denatured state, which reflects a similar molecular length on an average.

However, they were classified into two groups from their Kd values in the native state. Group I (Kd=0.17) includes five bacterial tRNAs (E. coli, S. typhimurium, B. subtilis, B. cereus and P. vulgaris) and yeast tRNA. Group II (Kd≥0.19) includes five species of tRNAs from fungus (A. oryzae), plant (spinach), insect (silkworm) and mammalian (rat liver and rabbit liver). tRNAs from living fossils (brachiopod Lingula and horseshoe crab Limulus) are also included in the group II, although their Kd values are somewhat larger. Sedimentation profiles on sucrose density gradient supported this classification.

During the study of nucleic acids from a so-called “living fossil,” brachiopod Lingula of which ancestor is found in the deposits before 4×10⁸ years (1, 2), we found that tRNA of this rare marine animal sedimented more slowly than tRNAs of microorganisms (E. coli and yeast) and was eluted later on Sephadex G-100 column (3, 4). In order to know whether these physico-chemical properties are characteristic of the living fossil’s tRNA, tRNA preparations from the following sources were compared: tRNA from another “living fossil,” horseshoe crab Limulus and tRNAs from 11 species of “present” organisms, that is, bacteria (Escherichia coli, Salmonella typhimurium, Bacillus subtilis, Bacillus cereus and Proteus vulgaris), yeast (Torulopsis utilis), fungus (Aspergillus oryzae), plant (spinach), insect (silkworm) and mammalian (rat liver and rabbit liver). In this paper a classification of tRNAs based on the distribution coefficient, Kd value, on gel filtration and the sedimentation profiles on sucrose density gradient is described.

**MATERIALS AND METHODS**

**tRNA Preparations**—Bacterial tRNAs were extracted from the cell pastes obtained in the logarithmic phase by the method of von Ehrenstein and Lipmann (5). Spinach and A. oryzae tRNAs were also prepared by the same method as that for bacterial tRNAs. Yeast tRNA, prepared from *Torulopsis utilis* by the phenol method (6), was kindly supplied to Dr. S. Takemura by Jujo Paper Co. (Akita, Japan). Rat liver and rabbit liver tRNAs were extracted by the method of Delihas and Staehelin (7). *Bombyx mori* post silk gland tRNA was kindly supplied by Prof. K. Shimura, Tohoku University. [3H] uracil-containing tRNA (specific activity, 10,300 cpm per A260 unit) prepared from *E. coli* B(H) was supplied by Dr. Y. Hayashi in our laboratory. Horseshoe crab *Limulus* (*Limulus longispinus*, (1, 8)) was supplied from the Marine Institute of the Hiroshima University. Brachiopod *Lingula* was supplied by Dr. M. Kuwano, Kyushu University. tRNAs from these living fossils were prepared as described previously (3). All of the tRNA preparations were purified by DEAE-cellulose column chromatography and deacylated as described previously (9).

Yeast tRNA methylated with 6H-labelled dimethyl sulfate was prepared as follows: the reaction mixture (5.7 mg of yeast tRNA and 300 μmoles of 6H-dimethyl sulfate (170 μCi/μmole, The Radiochemical Centre, Amsham) in 260 μl of 5 mM MgCl₂, 10 mM Tris-HCl, pH 7.5)) was incubated for 150 min at room temperature. Specific activity of the 'H-labelled yeast tRNA collected by ethanol precipitation is 8,050 cpm per A260 unit and its alanine accepting ability is almost retained. Since the tRNA appeared as a symmetrical peak of nearly constant specific activity on sucrose density gradient and Sephadex G-100 gel filtration, the tRNA is labelled homogeneously with tritium.

Formaldehyde-treated tRNA was prepared according to the method of Boedtker (10). Formaldehyde (37%, reagent grade) was diluted to 30% (11M HCHO) by adding one-fifth volume of 0.5 M Tris-HCl buffer containing 0.1% SDS (sodium dodecyl sulfate) and 10 mM EDTA, pH 7.5. One volume of the diluted solution was then added to 9 volumes of tRNA solution (1 mg/ml). The resultant solution was heated at 63°C for 15 min, cooled rapidly in ice-water and tRNA was precipitated with two volumes of cold ethanol.

**Assay of Amino Acid Acceptance of tRNA**—The aminoacyl-tRNA synthetase [EC 6.1.1 group] partially purified from baker's yeast was supplied by Mr. M. Kawata in our laboratory. Method of the assay for amino acid accepting activity was described previously (9).

**Gel Filtration on Sephadex**—Sephadex G-100 (Pharmacia Fine Chemicals, Inc., Uppsala) was suspended in the elution buffer (0.1 M NaCl and 10 mM Tris-HCl, pH 7.6) and equilibrated with the same elution buffer for 2 days at room temperature. Prior to packing, the gel suspension was transferred to a large suction flask and briefly deaerated under vacuum.

Each sample, containing 4 to 10 A₂₆₀ units of tRNA and 3,380 cpm of 'H-labelled yeast tRNA (0.42 A₁₉₀ unit) and 10 μl of 5% Blue-dextran 2000 (Pharmacia Fine Chemicals Inc., Uppsala) in 0.1 ml of elution buffer, was applied on the top of the column (1.0 cm X137 cm) and washed into the gel with addition of 0.5 ml of buffer. Chromatographic runs were made without overhead pressure at room temperature. The flow rate was 5.4 ml/hr. Twenty-drop fractions (0.62 ml) were collected. Optical density at 260 μm and the TCA-insoluble radioactivity of each fraction were measured as described previously (9). Each fraction was characterized with a coefficient of distribution, Kd value, defined by Flodin (11) as,

\[
Kd = \frac{V_e - V_0}{V_i}
\]

(Ve, elution volume; V₀, void volume; Vi, inner volume). Using a column mentioned above, V₀, determined by Blue-dextran 2000, was 30.3 ml and Vᵢ, determined by Bromphenol-blue, was 96.8 ml. Similar procedure was used in the presence of 7 M urea and under the conditions of other salt concentrations and pH's.

**Sucrose Density Gradient Centrifugation**—About 5 A₁₉₀ units of tRNA to be tested and
3,380 cpm of $^3$H-labelled yeast tRNA (0.42 $A_{260}$ unit) in 0.1 ml of the top solution for the gradient were layered on the top of a linear gradient of 20 to 5% (w/v) sucrose in 1 M NaCl, 1 mM EDTA and 10 mM Tris-HCl, pH 7.5. After centrifugation at 25,000 rpm for 16 hr in the SW 27 rotor of the Spinco model L ultracentrifuge at 4°C, 22-drop fractions (0.59 ml) were collected as described previously (12). Optical density at 260 m$\mu$ and the TCA insoluble radioactivity of each fraction were measured as described previously (9).

**Analyses of Terminal Residues and Total Phosphates**—One mg of tRNA in 0.2 ml of 10 mM ammonium acetate buffer (pH 4.5) was incubated with 2 units of RNase T$_s$ [EC 2.7.7.17, Sankyo Co.] at 37°C for 18 hr. The digest was chromatographed on a column (0.2 cm x 20 cm) of Dowex-1 (Cl$^-$) with a linear concentration gradient generated by 20 ml of 0.004 N HCl and 20 ml of 0.2 M NaCl in 0.01 N HCl. The separated components were identified by their chromatographic behaviors and ultraviolet absorption spectra (at pH's 2.0 and 12.0) and estimated quantitatively by their molar extinction coefficient at pH 2.0. Further details are described in Ref. (9).

Analysis of organic phosphorus was carried out according to Chen et al. (28).

**RESULTS**

(a) **Sedimentation Profiles on Sucrose Density Gradient**—In order to compare the sedimentation properties, each tRNA preparation of living fossils (Limulus and Lingula) was co-sedimented with $^3$H-labelled yeast tRNA in a sucrose density gradient at neutral pH in

![Fig. 1. Sedimentation profiles of tRNAs from various organisms on sucrose density gradient centrifugation. Various kinds of tRNA preparations were co-sedimented with yeast $^3$H-labelled tRNA. Centrifugation was done at 25,000 rpm for 16 hr in the SW 27 rotor at 4°C. The gradient was made in 5 to 20% sucrose in 1 M NaCl, 1 mM EDTA and 10 mM Tris-HCl, pH 7.5. Sample: 4 to 6 $A_{160}$ units of each tRNA and 3,380 cpm (0.42 $A_{260}$ unit) of $^3$H-labelled yeast tRNA. Each fraction was analyzed as described in "METHODS." Absorbance at 260 m$\mu$ (O), acid-insoluble $^3$H-radioactivity (●), specific activity (cpm per $A_{160}$ unit) (x).](https://academic.oup.com/jb/article-abstract/69/4/761/800128)
the preparative ultracentrifuge. As shown in Fig. 1-a, b, Lingula and Limulus tRNAs sedimented more slowly than yeast tRNA. Specific activity (expressed as counts/min per A_{260}) of each fraction indicated clearly the difference of sedimentation rate among them. When three species of bacterial tRNAs (E. coli, S. typhimurium and B. cereus) were co-sedimented with ^3H-labelled yeast tRNA, they sedimented at the same rate as that of yeast tRNA (Fig. 1-d–f). In these cases specific activity of each fraction was constant. The sedimentation behaviors on sucrose density gradient presented here is consistent with the previous observations that Lingula tRNA has a sedimentation coefficient of 3.7±0.1 S, while yeast and E. coli tRNA has 4.1 and 4.0±0.1 S, respectively (4). So, the sedimentation behaviors of tRNAs of living fossils seemed to be unique.

However, as shown in Fig. 1-c, rabbit liver tRNA showed slower sedimentation rate as well as living fossils' tRNAs. The order of the sedimentation rate is as follows: bacterial and yeast > rabbit, Lingula Limulus tRNAs.

Identical patterns were obtained even after these tRNAs were treated with 1% (w/v) sodium dodecyl sulfate, indicating that the sedimentation behaviors of these tRNAs were not influenced by interaction with contaminating protein components. Kaji and Tanaka reported that in the case of E. coli tRNA, some aminoacylated tRNAs sediment more slowly than the corresponding tRNAs and other aminoacylated tRNAs sediment relatively close to the corresponding ones on sucrose density gradient centrifugation (13). The difference observed here on total tRNAs from various

Fig. 2. Elution profiles of yeast and bacterial tRNAs on Sephadex G-100 column. Each tRNA preparation was run separately on the same column with yeast ^3H-labelled tRNA and eluted with 0.1 M NaCl in 10 mM Tris-HCl, pH 7.6, at room temperature. In (f), ^3H]uracil-containing tRNA of E. coli B(H) and non-labelled yeast tRNA were co-chromatographed. Column size, 1.0 cm × 137 cm; fraction volume, 0.62 ml; flow rate, 5.4 ml/hr; loaded sample, 4 to 10 A_{260} units of each tRNA sample and 3,380 cpm (0.42 A_{260} unit) of yeast ^3H-labelled tRNA. The arrow indicates the elution position of Blue-dextran 2000 (void volume of the column). Each fraction was analyzed as described in "METHODS." Absorbance at 260 m\(\mu\) (○), acid-insoluble ^3H-radioactivity (●). In all cases the recovery of total ultraviolet absorbance or radioactivity applied to the gel was greater than 90%.

J. Biochem.
CLASSIFICATION OF tRNAs

sources is due to deacylated tRNAs (see "MATERIALS"). Terminal deficient tRNAs would not affect the sedimentation profiles since these tRNAs have almost all of the terminal residues (see Table I).

From these sedimentation behaviors it is suggested that tRNAs from various organisms are classified into two categories: some tRNAs sediment more slowly than yeast tRNA and other tRNAs at the same rate as that of yeast tRNA. The former are animal tRNAs, such as rabbit, Lingula and Limulus tRNAs and the latter are bacterial tRNAs, such as E. coli, S. typhimurium and P. vulgaris tRNAs as well as yeast tRNA.

(b) Gel Filtration Analyses on Sephadex G-100 Column.

(i) Elution Profiles of Native tRNAs from Various Organisms—Here, gel filtration method was used to compare the physico-chemical properties of tRNA molecules. Various species of tRNAs were co-chromatographed with \(^{3}H\)-labelled yeast tRNA on Sephadex G-100 column under the condition of high ionic strength. As shown in Fig. 2-a-f, yeast and five species of bacterial tRNAs (B. cereus, P. vulgaris, B. subtilis, S. typhimurium and E. coli) were eluted at the same position as that of \(^{3}H\)-labelled marker tRNA (yeast). These elution patterns were identical even if a different radioactive indicator tRNA (\(^{3}H\) uracil-containing E. coli tRNA) was used (for example: Fig. 2-f).

On the other hand, two living fossils' tRNAs were eluted later than yeast tRNA from Sephadex G-100 column (Fig. 3-a, b). These elution profiles were extremely distinct from those of bacterial tRNAs, and again question arose as to whether living fossils' tRNAs have unusual properties or not. However, further detailed study revealed that five species of tRNAs from silkworm, rat liver, rabbit liver, spinach and A. oryzae were also eluted later than yeast tRNA. Since similar results were obtained at the same salt concentration but at pH 4.5 (10 mM ammonium acetate) and also in 0.6 M NaCl at the same pH 7.6, these elution profiles are not influenced by salt concentration and/or pH.

(ii) Kd Values of Native tRNAs and

![Fig. 3. Elution profiles of tRNAs of higher organisms on Sephadex G-100 column. Each tRNA preparation was run separately on the same column with yeast \(^{3}H\)-labelled tRNA. Other conditions were the same as in Fig. 2. Absorbance at 260 nm (O), acid-insoluble \(^{3}H\)-radioactivity (●).](https://academic.oup.com/jb/article-abstract/69/4/761/800128)
### Table I. \(K_d\) values of tRNAs from various organisms on Sephadex G-100 column as obtained under the conditions described in Figs. 2, 3 and 4. (mole \% of the terminal residues are described, too.)

<table>
<thead>
<tr>
<th>Group</th>
<th>tRNA Source</th>
<th>Native (K_d)</th>
<th>Denatured (K_d)</th>
<th>Adenosine mole%1)</th>
<th>pGp</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Yeast</td>
<td>0.173 ± 0.0052)</td>
<td>0.135 ± 0.0033)</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td><em>B. cereus</em></td>
<td>0.168</td>
<td>0.139</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td><em>P. vulgaris</em></td>
<td>0.174</td>
<td>—</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td><em>B. subtilis</em></td>
<td>0.168</td>
<td>0.138</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td><em>S. typhimurium</em></td>
<td>0.173</td>
<td>0.138</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>0.173</td>
<td>0.140</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>II.</td>
<td><em>A. oryzae</em></td>
<td>0.189</td>
<td>0.138</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Spinach</td>
<td>0.191</td>
<td>0.137</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td><em>Bombyx mori</em></td>
<td>0.186</td>
<td>0.138</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Rat liver</td>
<td>0.193</td>
<td>—</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Rabbit liver</td>
<td>0.193</td>
<td>0.139</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td><em>Lingula</em></td>
<td>0.196</td>
<td>0.140</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td><em>Limulus</em></td>
<td>0.202</td>
<td>—</td>
<td>0.9</td>
<td>1.0</td>
</tr>
</tbody>
</table>

1) Expressed as moles of terminal residues per 100 moles of organic phosphates (see "METHODS").
2) Average of 13 experiments.
3) Average of 10 experiments.

**Classification**—The distribution coefficients, \(K_d\) values, of tRNAs on a Sephadex G-100 column, as obtained under conditions described in Figs. 2 and 3, are listed in Table I. As shown in Table I, \(K_d\) value of yeast tRNA is 0.173 ± 0.005 and the accuracy of the method is limited by the resolution of the column to about ±3%. \(K_d\) values of five bacterial tRNAs are 0.17 as well as that of yeast tRNA. \(K_d\) values of five tRNA species of higher organisms (an insect, two mammalians, a fungus and a plant) are 0.19. \(K_d\) values of two living fossils’ tRNAs are 0.20. From these characteristic \(K_d\) values tRNAs can be classified mainly into two groups. Group I \((K_d=0.17)\) includes bacterial tRNAs (*E. coli*, *B. cereus*, *P. vulgaris*, *S. typhimurium*) and yeast tRNA (*Torulopsis utilis*). Group II \((K_d>0.19)\) includes tRNAs from higher organisms (*A. oryzae*, Spinach, *Bombyx mori*, rat liver and rabbit liver). tRNAs from living fossils (*Lingula* and *Limulus*) are also included in the latter group, but their \(K_d\) values are somewhat larger than others. It is noted that the group II-tRNAs indicated sharper elution peaks as compared with those of group I-tRNAs. No species of which tRNAs have smaller \(K_d\) value than 0.17 has been found yet. This classification agrees well with the results obtained from sedimentation profiles (cf. (a)).

**iii) Native and Denatured Conformations**—tRNAs in the denatured state occupy larger molecular volumes than their native counter-

---

Fig. 4. Elution profiles of formaldehyde-tRNA on Sephadex G-100 column. Formaldehyde-treated tRNA of yeast was co-chromatographed with \(^3\)H-labelled yeast tRNA. Other conditions were the same as in Fig. 2. Absorbance at 260 mµ (○), acid-insoluble \(^3\)H-radioactivity (●).

*J. Biochem.*
parts, so that they retarded less on gel filtration (14). This idea was supported by the following experiments using tRNAs denatured with formaldehyde. As shown in Fig. 4, it is clear that the formaldehyde treatment had a marked effect on the elution characteristics of yeast tRNA molecules, in as much as they were eluted substantially earlier than \( ^3 \)H-labelled yeast tRNA. Whereas the \( K_d \) value was 0.17 for native yeast tRNA, this value was altered to 0.14 after denaturation with formaldehyde. Such a behavior is likely to be due, at least in part, to tRNA molecules adopting more expanded conformation. Similar results were obtained as to group I-tRNAs (E. coli and B. subtilis) of which \( K_d \) values were altered to 0.14 from 0.17. Lingula and rat liver tRNAs (group II) were also affected with the formaldehyde treatment in such a way that their \( K_d \) values became close to 0.15. These results suggest that each tRNA preparation used in Figs. 2 and 3 did not contain any denatured tRNA molecules. Group II-tRNAs would hold more compact conformation than group I-tRNAs on an average, if they have a similar chain-length.

(iv) **Elution Profiles of Denatured tRNAs**—In order to compare the chain-length of tRNAs, after heat denaturation similar gel filtration was done in the presence of 7 M urea in the elution buffer. As shown in Fig. 5 (b–e), heat-denatured tRNAs (Lingula, rabbit, E. coli and B. cereus) were eluted somewhat later than \( ^3 \)H-labelled yeast tRNA, while Lingula tRNA was eluted at the same elution position, as that of \( ^8 \)H uracil-containing tRNA of B. cereus.

Fig. 5. Elution profiles of the heat-denatured tRNAs on Sephadex G-100 column in the presence of 7 M urea. Each tRNA preparation was heated with \( ^3 \)H-labelled yeast tRNA (or \( ^8 \)H uracil-containing E. coli tRNA) in distilled water at 100°C for 5 min, and then solid urea was immediately added to make 7 M, followed by rapid cooling in an ice-water bath. These heat-denatured tRNA samples were chromatographed on a Sephadex G-100 column in the presence of 7 M urea, 0.1 M NaCl in 10 mM Tris-HCl (pH 7.6). Other conditions were the same as in Fig. 2. Absorbance at 260 m\( \mu \) (○), acid-insoluble \( ^3 \)H-radioactivity (●).
coli (Fig. 5-f). \( K_d \) values of these tRNAs are \( \approx 0.14 \) except yeast tRNA (Table I). From these results it seems most reasonable to conclude that all native tRNAs, which belong to different groups, might have a similar molecular length on an average. Therefore these results eliminated the possibility that group II-tRNAs have a smaller molecular length than group I-tRNAs.

(v) Distribution of Amino Acid-Specific tRNAs—In order to know whether individual amino acid-specific tRNAs are eluted at the same position, a portion of each fraction eluted from the Sephadex G-100 column was

![Fig. 6. Distribution of the amino acid specific tRNA of yeast on Sephadex G-100 column. Four milligrams of yeast tRNA were chromatographed on Sephadex G-100 column (1.0 cm x 146 cm) under the conditions described in Fig. 2. After elution each fraction was measured for absorbance at 260 m\( \mu \) (top figure) and 0.1 ml of each fraction (0.63 ml) was assayed for amino acid accepting ability (O or •). For further details, see "METHODS."](image1)

![Fig. 7. Elution profiles of rat liver \(^{3}H\) seryl-tRNA and yeast \(^{14}C\) seryl-tRNA on Sephadex G-100 column. Rat liver tRNA and yeast tRNA were incubated with yeast seryl-tRNA synthetase to attach \(^{3}H\) serine and \(^{14}C\) serine, respectively, at 37°C for 20 min, and after immediate cooling in ice-water sodium pyrophosphate was added in these reaction mixtures at final concn. of 0.02 M to inhibit the aminoaacyl-AMP formation. These two reaction mixtures were combined and immediately loaded on the top of the column of Sephadex G-100 equilibrated with 0.02 M sodium pyrophosphate in 0.01 M ammonium acetate (pH 6.6, 5.5 and 4.5). After elution absorbance at 260 m\( \mu \) (-----) and radioactivity (\(^{3}H\), \(^{14}C\), •) of each fraction was measured.](image2)
assayed for the ability of amino acid acceptance. As shown in Fig. 6, it is noted that yeast tRNA specific for serine was eluted much earlier than tRNAs specific for leucine, valine and tyrosine, and tRNA specific for alanine was eluted still more lately. Isoleucine tRNA was eluted at the same position as that of valine tRNA. Thus, individual amino acid-specific tRNA occupies a definite position, for example $K_d$ for serine tRNA is 0.16, on the elution pattern from the Sephadex G-100 column. However, their elution order can not be explained by only their chain-length (15–19).

It has been known that baker's yeast aminoacyl-tRNA synthetase can sufficiently aminoacylate a heterologous rat liver tRNA (20), although total uncharged yeast and rat liver tRNAs showed markedly different elution profiles as described in (iv). As shown in Fig. 7, rat liver [3H] seryl-tRNA and yeast [14C] seryl-tRNA were eluted at the same position on a Sephadex G-100 column at three different pH conditions. The radioactivity recovered as seryl-tRNA decreased with the increase in pH value. At pH 7.6 (10 mM Tris-HCl and 0.1 M NaCl) almost all of the radioactive serine attached to the tRNA was released during chromatography. Similar experiments were performed for Lingula tRNA. As shown in Fig. 8, Lingula [3H] seryl-tRNA and yeast [14C] seryl-tRNA were eluted at the same position. Thus, we couldn't find out the difference among three species of seryl-tRNAs, although total uncharged tRNAs showed markedly different elution profiles. These results are consistent with the interpretation that aminoacylation changes the conformation of the tRNA (13). The primary structure of yeast tRNA$^{ser}$ extremely resembles that of rat liver tRNA$^{ser}$ only except that rat liver tRNA$^{ser}$ contains three more methyl groups than yeast tRNA$^{ser}$ (19, 21). Judging from the elution profiles on the gel filtration, the effect of methyl groups on molecular conformation may be negligible in the aminoacylated state.

**DISCUSSION**

Measurements of the sedimentation coefficient on sucrose density gradient and the elution position on Sephadex column (22, 23) are convenient procedures for determining the molecular weight of nucleic acids. However, it is generally difficult to obtain reliable molecular weight by these methods because it has been known that the conformation of a particular RNA depends not only on its overall chain-length but also on its specific secondary and/or tertiary structure (10, 24). The base composition and the molecular charge of the individual components as well as the ionic strength of the buffer must be taken into account (23). In the case of tRNA molecules, the chain-length is similar (80±5 nucleotides, (25)), and a clover leaf model is commonly proposed for the secondary structure of every amino acid-specific tRNA from various organisms (15, 25). The success of crystalization of unfractionated yeast tRNA also suggests that every amino acid-specific tRNA resembles each other in the tertiary structure (26).

However, it was found that there are differences in the sedimentation and gel filtration profiles among various species of tRNAs, and that tRNAs can thus be divided into two distinct groups as summarized in Table I. These observations were given under several experimental conditions, but were restricted to cases of the deacylated tRNAs. Further-
more, the data of the present study (Fig. 6) support the earlier report that the efficiency of gel filtration procedure varies with the molecular species of tRNAs, probably depending on the volume difference (14). Therefore, it can be concluded that the characteristic Kd values of bulk tRNAs shown in Table I reflect the mean molecular volume of the population of tRNA molecules. Bacterial tRNAs might have the largest molecular volumes and living fossils' tRNAs the smallest ones on an average.

Recently it has been suggested that a precursor tRNA from Krebs 2 ascites tumour cells might have a more open molecular conformation than that of mature tRNA on the basis of the observations that the precursor tRNA sediments at a position somewhat less than 4S marker (27). However, E. coli tRNA was used as a sedimentation marker. As shown in Fig. 1, animal tRNAs sediment more slowly than bacterial tRNAs. We must use a homologous tRNA or, if not so, a member of tRNAs in the same group (Table I) as a marker in such experiments.

In this paper emphasis has been placed on a classification, in which there was a clear difference between bacterial and higher organisms' tRNAs including living fossils' tRNAs. Further studies are necessary before the significance of such a phenomenon can be interpreted. The fractionation and purification of Lingula tRNA, of which Kd value (0.20) is one of the largest, is now in progress.

I am grateful to Dr. K. Miura and Dr. S. Takemura for their continuing interest. This work was partly supported by a grant from the Ministry of Education.

REFERENCES