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RNA partitioning accompanied by adsorption: high-molecular-mass RNA adsorbed at the interface like a particle

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Abstract

In a potassium phosphate–poly(ethylene glycol) (PEG) system, RNA partitioning was accompanied by adsorption at the interface, depending on the molecular mass. Low-molecular-mass RNA showed the typical partition behavior of a soluble substance. Conversely, high-molecular-mass RNA was significantly adsorbed at the interface in the potassium phosphate–PEG1500 system. The adsorbed amount was proportional to the added amount, regardless of the phase volume ratio. The partitioning of high-molecular-mass RNA showed an amount–ratio-dependent distribution like that of a particle.

Keywords: Partitioning; RNA; Nucleic acids

1. Introduction

In the partitioning of particles in an aqueous two-phase system, adsorption at the interface is a common phenomenon [1]. However, there are only a few papers containing quantitative data for the adsorption accompanying partition [2–4]. From the observation of particle partitioning, Albertsson [5] revealed that the characteristic behavior of particle partition accompanied by adsorption was different from the partition of a soluble substance and distinct from the usual adsorption on a solid material.

Partitioning of nucleic acids showed the typical behavior of a soluble macromolecule in polymer–polymer two-phase systems [6,7]. In some systems containing salts in high concentration, the occurrence of adsorption of nucleic acids at the interface was described but there was no analytical study of it [8–11]. In a previous paper [12], we quantitatively demonstrated RNA partition accompanied by adsorption as a function of the poly(ethylene glycol) (PEG) molecular mass in the salt–PEG two-phase systems. In the system of PEG1000–PEG2000, remarkable adsorption of high-molecular-mass RNA at the interface was observed, with more than 90% of high-molecular-mass RNA being concentrated at the interface.

In the present paper, we analyzed the RNA partitioning in detail in the potassium phosphate–PEG system. Low-molecular-mass RNA showed the typical partition behavior of a soluble substance. On the contrary, high-molecular-mass RNA was partitioned depending on the amount–ratio. RNA is certainly a soluble macromolecule, but the characteristic behavior of high-molecular-mass RNA is shown to act just like a particle would. This interesting phenomenon is discussed.

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2. Experimental

2.1. Materials

PEG 600, PEG1500 and PEG3000, expressed as the average molecular mass in this study, were PEG 600, 1540 and 4000, respectively. All chemicals were derived from Wako Pure Chemical Industries (Osaka, Japan). Potassium phosphate was a mixture of K_2HPO_4 and KH_2PO_4, with a molar ratio of 10 to 7 [1].

The RNA used in this study was a "total RNA" and was prepared from Baker's yeast by phenol–chloroform extraction and ethanol precipitation with diethylpyrocarbonate [13]. The RNA preparation was suspended in sterile distilled water and stored at −80°C. The RNA preparation contained less than 2% DNA, as measured by Burton's method [14], and had an A_{260}/A_{280} value of 2.15. The preparation showed three bands on agarose gel electrophoresis, corresponding to 26S, 17S and a mixture of less than 5.8S.

2.2. Partition experiment

The two-phase systems used were potassium phosphate–PEG systems with 5 m*M* Na_2HPO_4 and 5 m*M* NaH_2PO_4. The two-phase system, prepared by weighing, was mixed with the diluted RNA preparation in a graduated test tube at a total weight of 5–10 g and was mixed by inversion. After sampling of the mixed system to determine the initial RNA concentration, the system was partitioned at 4°C following centrifugation (at 200 *g* for 2 min or 600 *g* for 10 min) to separate the two phases. After the measurement of the phase volumes, the top and bottom phases were withdrawn separately, to determine the RNA concentrations. All test tubes, tips and the solutions, with the exception of the RNA preparation, were autoclaved before use.

In order to examine the effect of the phase volume ratio, a series of systems with various phase volume ratios on the same tie-line had to be accurately prepared. The series of systems was prepared by mixing the solutions of the top phase and bottom phase which were separately withdrawn after partitioning without RNA.

The RNA concentration was determined using a GPC-mode HPLC system [13]. A high-speed gel permeance was performed with a Shimadzu HPLC system (SCL-6A and LC-6A, Shimadzu, Kyoto, Japan), equipped with an ultraviolet absorbance detector and an integrator (SPD-6A and C-R4A, Shimadzu). A sample solution of the phase system solution diluted with sterile distilled water was eluted on an Asahipak GS620S column (50 × 7.6 mm I.D., Asahi Chemical Industry, Kawasaki, Japan) with a 50 m*M* Tris–HCl buffer solution (pH 9.0), containing 0.1 *M* NaCl, at a rate of 0.5 ml/min at 30°C. The RNA concentration was estimated from the chromatogram after correction using the appropriate baseline chromatogram. The baseline chromatogram of the phase solution, without RNA, was subtracted from the sample chromatogram, as the absorbance of the phase solution resulting from potassium phosphate and PEG could not be neglected. For the presentation of data in this study, RNA was demonstrated to be isolated as two groups: "high-molecular-mass RNA" comprised of 26S and 17S ribosomal RNA and "low-molecular-mass RNA" comprised of 5.8S, 5S and lower. The composition of the RNA preparation was 67–71% of high-molecular-mass RNA and 29–33% of low-molecular-mass RNA.

2.3. Definition of partition behavior

The following relationships were used for the estimation and expression of the partition behavior of RNA in this report:

$$I = C_m \cdot V_m - C_t \cdot V_t - C_b \cdot V_b \quad (1)$$

$$K = C_t / C_b \quad (2)$$

$$G = (C_t \cdot V_t)/(C_b \cdot V_b) \quad (3)$$

$$G_i = (C_t \cdot V_t)/I \quad (4)$$

$$G_i' = (C_b \cdot V_b)/I \quad (5)$$

where I is the amount of RNA adsorbed at the interface, K is the partition coefficient, G is the partition ratio, G_i and G_i' are the special partition ratio, C_m is the concentration of RNA added to the system, C_t is the concentration in the top phase, C_b is the concentration in the bottom phase, V_m is the total volume, V_t is the volume of the top phase and V_b is the volume of the bottom phase. The RNA amount

adsorbed at the interface (I) was estimated by subtracting the amounts partitioned between the phases from the amount initially added, as shown in Eq. 1.

3. Results and discussion

In a previous study [12], we demonstrated that the molecular mass of PEG had a significant effect on RNA partitioning in a salt–PEG two-phase system. In the system with some PEG molecular mass ranges, RNA was significantly adsorbed at the interface. For example, in the potassium phosphate–PEG1500 system, more than 95% of the high-molecular-mass RNA was adsorbed at the interface.

In the potassium phosphate–PEG1500 system, RNA partitioning, with various concentrations of RNA added to the system, is shown in Fig. 1 (divided into high and low-molecular-mass RNA). The partition coefficient of the high-molecular-mass RNA had a lower value than that for the low-molecular-mass RNA. However, this value could not be estimated often because the partition was so one-sided that there was no detectable high-molecular-mass RNA in the top phase. About 95% of the high-molecular-mass RNA was adsorbed at the interface, in spite of the added concentration. The

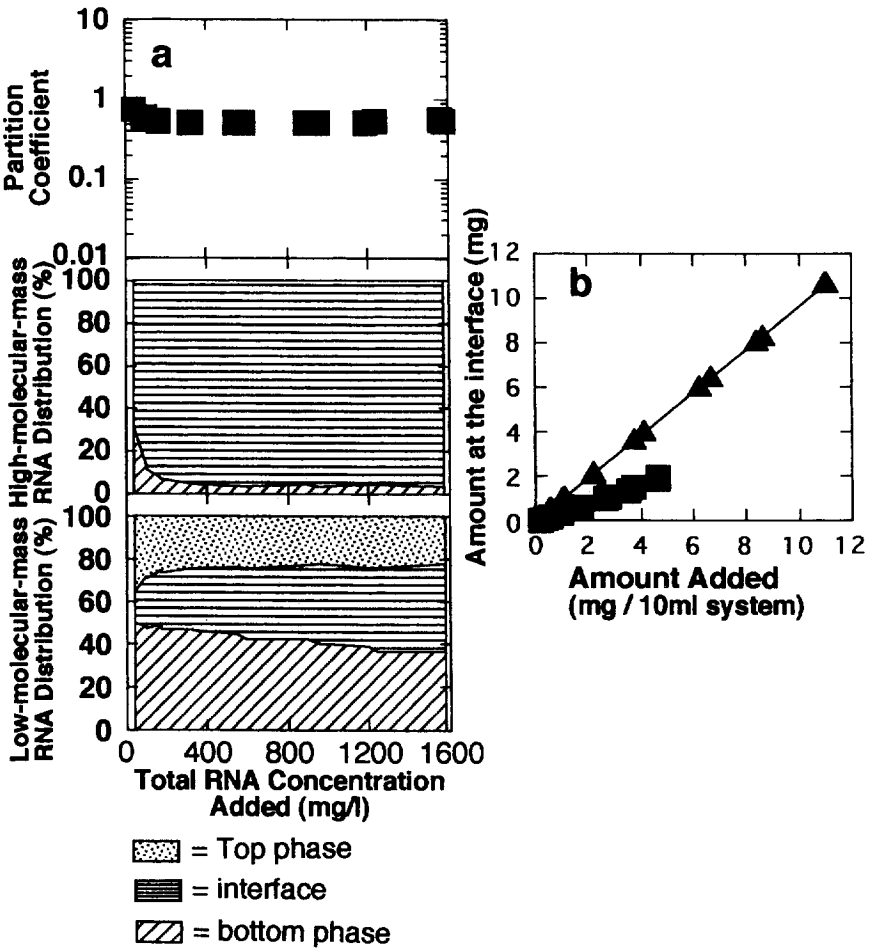

Fig. 1. (a) Effect of RNA concentration on RNA partitioning in a 14% (w/w) potassium phosphate–12% (w/w) PEG1500 system. (b) Relationship between RNA amounts added and adsorbed. (▲) High-molecular-mass RNA (the partition coefficient of the high-molecular-mass RNA could not be estimated as mentioned in Section 3); (■) low-molecular-mass RNA.

low-molecular-mass RNA showed a constant partition coefficient with around 40% being adsorbed at the interface. On the whole, low-molecular-mass RNA was partitioned between the two phases and the interface.

In Fig. 1b, the relationship between the RNA amounts added to the system and those adsorbed at the interface is shown individually for the high and low-molecular-mass RNA, based on the data given in Fig. 1a. The RNA amount adsorbed was proportional to the added amount in both cases up to the highest concentration. It seems that there is no saturated concentration for adsorption accompanying partition.

Low-molecular-mass RNA usually was not adsorbed at the interface when it was partitioned alone without high-molecular-mass RNA (unpublished data). When the mixture with high-molecular-mass RNA was partitioned, low-molecular-mass RNA was adsorbed in proportion to the amount of high-molecular-mass RNA adsorbed. Thus, it is probable that adsorption of low-molecular-mass RNA occurs by "co-adsorption" with high-molecular-mass RNA and/or by aggregation together with high-molecular-mass RNA.

Fig. 2 shows the effect of the phase volume ratio on RNA partitioning accompanied by adsorption on the same tie-line as the 14% (w/w) potassium phosphate–12% (w/w) PEG1500 system. If RNA is partitioned in the usual manner of a soluble sub-

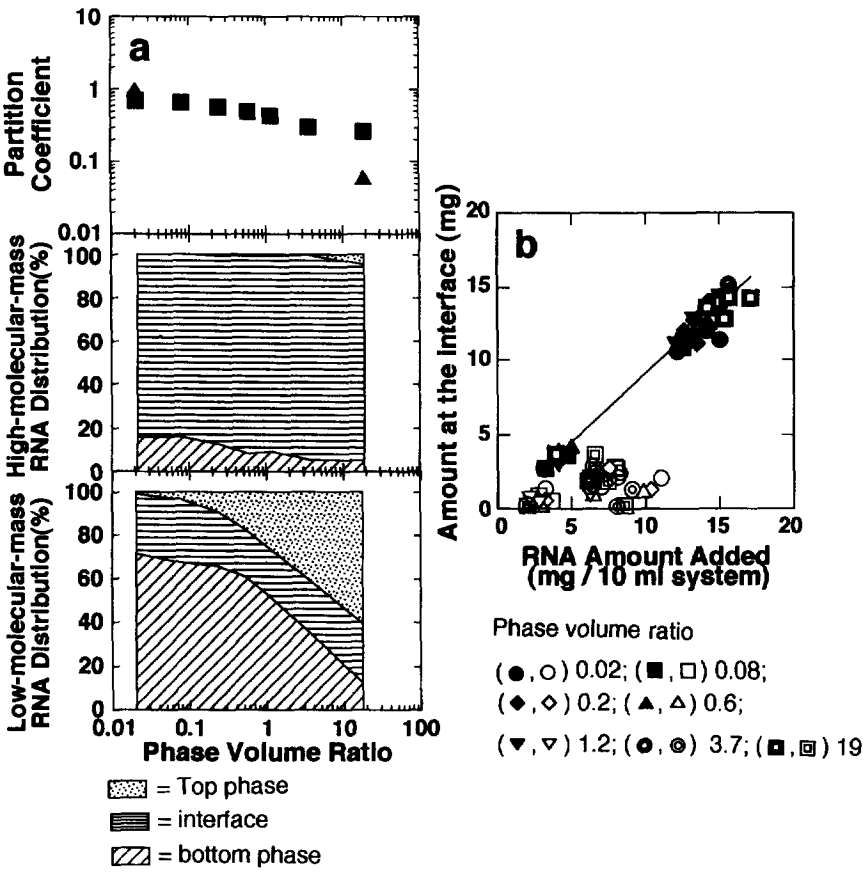

Fig. 2. (a) Effect of phase volume ratio on RNA partitioning in the potassium phosphate–PEG1500 system. Each system was prepared to be on the tie-line as the 14% (w/w) potassium phosphate–12% (w/w) PEG system. RNA was added to the system at a total RNA concentration of 50 mg/l and 200 mg/l, respectively. (▲) High-molecular-mass RNA (see the legend for Fig. 1); (■) low-molecular-mass RNA. (b) Relationship between RNA amounts added and adsorbed. Black symbols are shown for high-molecular-mass RNA and open ones for low-molecular-mass RNA.

stance such as a protein, it would show a constant partition coefficient regardless of the phase volume ratio. However, RNA partitioned differently depending on the molecular mass, as shown in Fig. 2a. The partition coefficient of high-molecular-mass RNA was significantly altered by the phase volume ratio while that of low-molecular-mass RNA slightly decreased with an increase in the phase volume ratio. The distribution of low-molecular-mass RNA shifted from the bottom phase to the top phase with increasing phase volume ratio. On the contrary, most of the high-molecular-mass RNA was constantly adsorbed at the interface regardless of the phase volume ratio.

The relation between the RNA amounts adsorbed and added for each phase volume ratio is shown in Fig. 2b. The amount of high-molecular-mass RNA adsorbed at the interface obviously had a linear relationship with the added amount, irrespective of the phase volume ratio; based on the slope shown in Fig. 2b, on average, 92% of the high-molecular-mass RNA was adsorbed at the interface. In the case of low-molecular-mass RNA, no relationship between the amount added and adsorbed could be found.

Comparison of the effect of phase volume ratio among the various molecular mass PEG systems is shown in Fig. 3. These systems with various PEGs

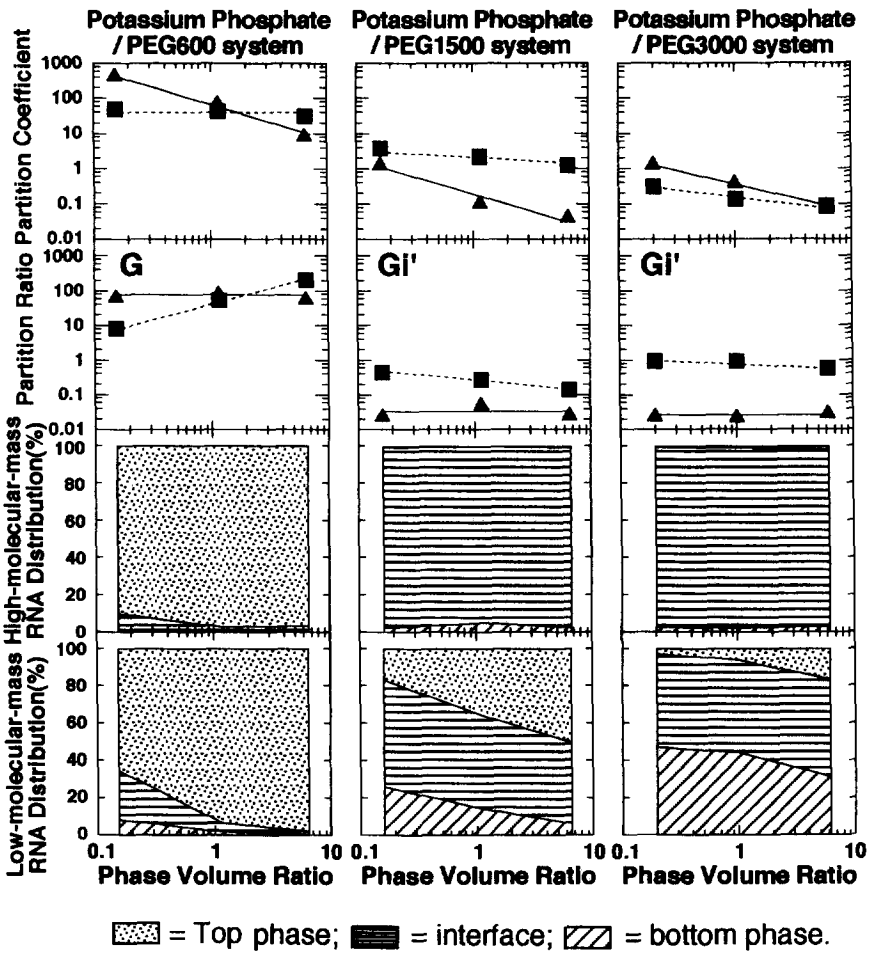

Fig. 3. Comparison of the effect of phase volume ratio on RNA partitioning among various molecular mass PEG systems. Each system was prepared to be on the tie-line as the 15% (w/w) potassium phosphate–16% (w/w) PEG system. RNA was added to the system at a total RNA concentration of 200 mg/l. (▲) High-molecular-mass RNA; (■) low-molecular-mass RNA.

had identical total compositions. Consequently, the composition of the phase system was removed from the binodial with the rise in PEG molecular mass.

In the case of high-molecular-mass RNA, with an increase in the phase volume ratio, the partition coefficient shifted drastically but the partition ratio remained almost constant. This tendency was almost the same for the three PEGs. In the systems with PEG1500 and PEG3000, in which severe adsorption occurred, the proportion of the adsorbed amount was almost constant in spite of the phase volume ratio, as shown by the stable value of G_i' (Eq. 5). In the system with PEG600, RNA adsorption at the interface was not significant, however, high-molecular-mass RNA was partitioned without a constant partition coefficient, but with a constant partition ratio, G (Eq. 3). In these potassium phosphate–PEG systems, partitioning of high-molecular-mass RNA seems to be amount–ratio-dependent whether the adsorption at the interface occurs or not. This phenomenon is unusual for the partitioning of a soluble substance.

As mentioned in the introduction, Albertsson et al. [2–4] found that in the partition of particles, such as starch, chloroplasts and bacterial cells, the number of particles adsorbed at the interface is proportional to the total number of particles, regardless of the particle concentration and the phase volume ratio. Albertsson [5] then indicated that adsorption of particles accompanying partition was defined in terms of the constancy of the G_i value (Eq. 4), i.e., it depended on the amount–ratio. That is, the adsorption of particles was distinct from the usual partitioning of a soluble substance, and was quite different from the usual adsorption which should follow an adsorption isotherm. In our study, partitioning accompanied by adsorption of high-molecular-mass RNA was shown to be identical to that of particles, as shown by Albertsson [5], in the independence of the concentration and the phase volume ratio. This may suggest that partition accompanied by adsorption is based on the amount–ratio-dependent distribution whether a substance is an insoluble particle or a soluble macromolecule.

In the case of low-molecular-mass RNA, the partition coefficient did not drastically change with the phase volume ratio. It was partitioned as a normal soluble substance, retaining the constant partition coefficient between the two phases and consequently changing the partition ratio with the phase volume ratio.

From these results, we can distinguish RNA partition behavior depending on its molecular mass. High-molecular-mass RNA was partitioned accompanied by adsorption at the interface, following the amount–ratio-dependent distribution. In contrast, low-molecular-mass RNA was partitioned in the typical manner of a soluble substance. It can be noted that RNA partition behavior shifts from the typical one of a soluble substance to an unique one, such as a particle, depending on its molecular mass.

RNA is a rather simple molecule judging from its primary structure, constructed using only four types of nucleotides. The high-molecular-mass RNAs used in this study have molecular masses of 600 000 (17S rRNA) and 1 200 000 (26S rRNA); the low-molecular-mass RNA is of 55 000 (5.8S rRNA), 42 000 (5S rRNA) and lower (tRNA and others). About a factor of ten difference in RNA molecular mass seems to cause a drastic shift in its partitioning. Up to some proportion of its molecular mass, RNA behaves as a soluble substance in the partitioning. Above a certain level, it acts like a particle. Whether the variation in partition behavior is successive, or whether there is a turning point with an increase in molecular mass, is not known at present.

We have not confirmed the conformation of RNAs in solution in this system. However, it can be imagined that a ribosomal RNA molecule without a ribosome-constructing protein would exist as a twisted and branched cord that has the twisted part of a double strand and the loop part of a single strand, based on observations made using electron microscopy in a water solution [15]. It is thought that ribosomal high-molecular-mass RNA in water has a much wider surface area than the RNA in ribosomes that have a folded compact structure together with ribosomal proteins. The fact that proteins with a molecular mass up to approximately 1 000 000 are generally partitioned between two phases, showing the typical partitioning of a soluble substance, is well-known [16]. In our case, high-molecular-mass RNA, that had molecular masses of 600 000 and 1 200 000, respectively, behaved like a particle. This may result from the rather wider surface area of RNA compared to proteins, which usually have a folded conformation even when their molecular

masses are similar. It might be noted that neither solubility nor molecular mass, but rather surface area, seems to determine whether the partitioning behavior is that of a soluble substance or that of a particle.

We know that many particle-like substances are apt to be adsorbed at the interface. By analysis of this type of partition behavior, a new, widely applicable principle for macromolecule partitioning is expected to be found. With this principle, we would apply this unique phenomenon to the efficient separation or concentration of particle-like useful biologicals.

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