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*GEL FILTRATION PROPERTIES OF CCD-PREPARED E. COLI B sRNA**

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Aggregation of polynucleotides is not an unknown occurrence. Crestfield *et al.*[1] observed a time-dependent association of yeast ribonucleic acid preparations maintained in dilute salt solutions at 0°. Examples of association of guanine-rich oligonucleotides have recently been described by Ralph *et al.*,[2] Ishikura,[3] and Lipsett.[4] Association of soluble ribonucleic acid (sRNA) polynucleotide chains with little loss of amino acid acceptor activity has been observed by Brown and

Zubay[5] following heating and rapid cooling in concentrated sRNA solution (*ca.* 10 mg/ml) of low ionic strength and neutral pH.

Recently, the results of an extensive fractionation of *E. coli* B sRNA by countercurrent distribution (CCD) were reported from this laboratory.[6] The present paper deals with an apparent aggregation-dissociation phenomenon which was first observed during the course of physicochemical measurements made upon CCD fractions of sRNA.

Materials and Methods.—Crude sRNA was prepared from fully grown *E. coli* B as described by Holley *et al.*[7] for yeast.

Deoxyribonucleic acid (DNA) contamination of crude sRNA was checked by utilizing the diphenylamine procedure as outlined by Dische.[8] Using the standard curve, constructed from thymus DNA solutions, and the weight of crude sRNA samples employed (*ca.* 1.7 mg), it was determined that less than 0.6% DNA contamination would have escaped detection.

Amino acid activating enzymes were prepared in the same manner as before.[6]

The assay procedure for the incorporation of radioactive amino acids into sRNA[6] was performed with the following modifications: GSH was omitted and replaced with 5 μmoles β-mercaptoethanol, and in some cases, 0.05–0.20 ml sRNA aliquots in 0.01 *M* pH 7.0 phosphate buffer or 1 *M* sodium chloride were used.

Countercurrent distribution (CCD): Studies were made on material obtained from the 970 transfer distribution (1.7 *M* phosphate system) previously described.[6] A second distribution of 1.8 gm of sRNA was performed in the same manner for 1889 transfers, except that after 1020 transfers the emerging upper phase from the train was collected in a sequential manner (single withdrawal procedure[9]). The material remaining within the train was removed by pooling the contents of every five successive tubes. Subsequent isolation procedures were performed as before.[6]

Gel filtration: These studies were carried out by using 0.9 × 150-cm columns of Sephadex G-100 (bead form) equilibrated with 1 *M* sodium chloride solution. The Sephadex was prepared by repeated washing and decantation with 1 *M* sodium chloride. Swelling of the gel was allowed to proceed for a minimum of 24 hr prior to the pouring of a column. Generally, flow rates averaging 30 ml/hr/cm^2 were obtained with the buffer reservoir 50 cm below the top of the column bed; such a column was used repeatedly until the flow rate dropped appreciably. Repourings yielded columns of excellent reproducibility. Fraction sizes ranging from 1.8 to 2 ml were collected. However, in the elution profiles presented, fraction sizes have been normalized to a volume of 2 ml.

sRNA fractions obtained from the CCD experiments were taken down to dryness from aqueous solution,[6] dissolved in 0.5 ml 1 *M* sodium chloride, and applied to the top of the Sephadex G-100 column bed. Washing in of the sample was performed with another 0.5 ml of 1 *M* sodium chloride.

In experiments involving the behavior of crude sRNA in the 1.7 *M* phosphate CCD system, approximately 5 mg of *E. coli* sRNA was dissolved in 2 ml of lower phase, then an equal volume of upper phase was added and the system equilibrated. After settling, the phases were separated and applied individually to the Sephadex G-100 column. This was followed by a sodium chloride wash.

Change of solvents in sRNA solutions was accomplished by dialysis using 23/32 Visking casing, or more conveniently by the use of 0.9 × 23-cm Sephadex G-25 columns equilibrated with the desired solvent.

Concentration procedures: The aqueous salt sRNA solutions were concentrated to about $^1/_{16}$ of their original volume at 30° under reduced pressure (Rotary Evapo-Mix, Buchler Instruments). Any salt deposition occurring upon concentration was discarded and only the supernatant employed. Concentration of formamide containing salt solutions was performed in the same manner, except that the remaining formamide was then removed by passage over a 0.9 × 23-cm Sephadex G-25 column and replaced with 0.01 *M* pH 7.0 phosphate buffer.

Dissociation of aggregates: Peaks 1 and 2 (contaminated with some of peak 3) obtained from the Sephadex G-100 chromatography of low partition coefficient (K) CCD-fractionated *E. coli* sRNA were pooled (*ca.* 1.7 mg), concentrated, and transferred from the sodium chloride solution to one containing 0.01 *M* phosphate buffer pH 7.0. One half of this solution was retained as a control. The remaining half was taken to dryness under reduced pressure, dissolved in a small volume of 4 *M* urea giving a final sRNA concentration of 0.83 mg/ml, heated in a 70° waterbath

for 15 minutes, removed, and allowed to cool to room temperature. The urea was then removed and substituted with phosphate buffer. Both the control and urea-treated samples were rechromatographed and tested for alanine acceptor activity.

Ultracentrifugation: The ultracentrifugation experiments were performed using the instrument and short column sedimentation equilibrium technique as described by Yphantis.[10] Three concentration levels, 0.01%, 0.03%, and 0.1% of sRNA in 1 *M* sodium chloride, were employed.

Results.—Several distinct molecular size species appear to be present in both unfractionated and fractionated sRNA when chromatographed in 1 *M* sodium chloride on Sephadex G-100 as is shown in Figure 1. The relative amounts of peaks 1, 2, and 3 vary considerably among the different 970 transfer sRNA fractions (Fig. 1*B*, *C*, *D*) and peak 4 only occurs in the high distribution coefficient material. Peak *B* (Fig. 1*A*) represents degraded low-molecular-weight material and is never present in the isolated (after dialysis) CCD fractions.

Peak *A* (Fig. 1*A*) could represent ribosomal RNA contamination (no contamination of crude sRNA by DNA was found) or alternatively a very high-molecular-weight unstable aggregate since it disappears when crude sRNA is subjected to a "one tube" distribution in the 1.7 *M* CCD system (Fig. 2). The elution pattern of the sRNA from the lower phase (Fig. 2, *left*) shows the presence of well-defined peaks 1 and 2. These peaks, in contrast, are essentially absent in the sRNA obtained from the upper phase (Fig. 2, *right*).

Low *K* sRNA obtained from a 1889 transfer CCD when chromatographed on Sephadex G-100 showed the same elution pattern as its counterpart obtained from

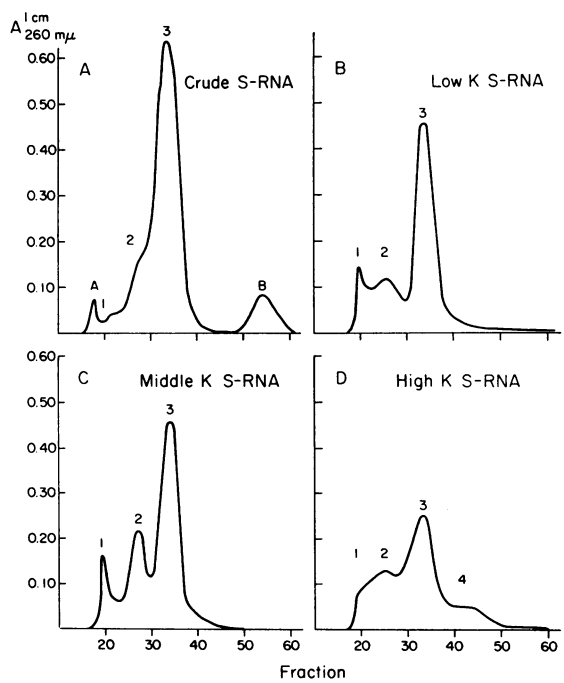

FIG. 1.—Gel filtration elution patterns (Sephadex G-100) for crude sRNA of representative partition regions from a 970 transfer CCD. Low *K* material is highly specific for alanine and proline; middle *K* material for glycine, proline, and valine; and high *K* material for tyrosine and leucine acceptance.

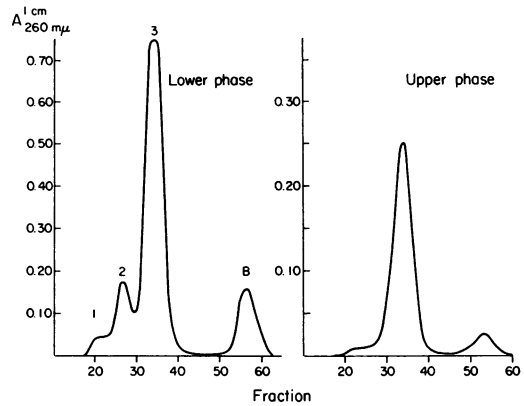

FIG. 2.—Elution patterns (Sephadex G-100) of crude sRNA which has sustained a "one tube" distribution in the 1.7 *M* CCD system. *Left*, profile for sRNA found in the lower phase; *right*, profile for sRNA found in the upper phase. The most retarded peak in both panels represents low-molecular-weight nucleotides plus the formamide of the CCD system.

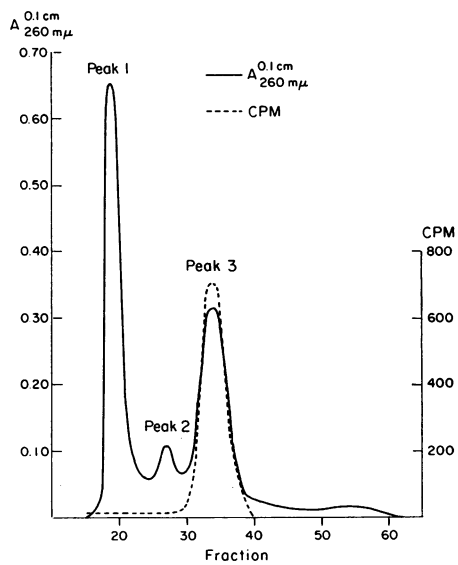

FIG. 3.—Elution profile (Sephadex G-100) of low partition coefficient sRNA highly specific for alanine and proline acceptance obtained from a 1889 transfer CCD. Counts per minute (cpm) is the expression of alanine acceptor activity per aliquot of 0.15 ml column effluent. Proline acceptor activity (not shown) tracks its alanine counterpart.

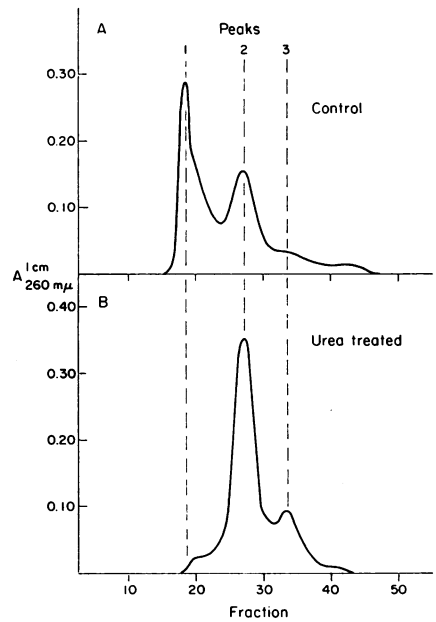

FIG. 4.—Elution patterns (Sephadex G-100) of an sRNA aggregate preparation (pooled peaks 1 and 2), (*A*) before and (*B*) after urea treatment. The area of each elution profile has been normalized to an sRNA concentration of 0.21 mg.

the 970 transfer experiment. Amino acid acceptor activity for both alanine and proline was found to be present only in peak 3 (Fig. 3). Peak 4, which is only found in high *K* sRNA, possesses negligible leucine acceptor activity (Fig. 1*D*).

In another experiment peaks 1 and 2 were separated from peak 3, pooled, isolated, and divided into two parts. One part served as a control, the other was transferred to solution in 4 *M* urea, heated at 70° for 15 min, followed by a change

TABLE 1

ALANINE ACCEPTOR SPECIFIC ACTIVITIES FOR AN sRNA AGGREGATE PREPARATION BEFORE AND AFTER UREA TREATMENT

Sample (pooled peaks 1 and 2)	Cpm per 100 γ *	% Peak 3†
Control	344	9
Urea-treated	635	22

* 50 γ/ml was taken to give an A_{260} 1 cm of 1.00 in 0.01 *M* pH 7.0 phosphate buffer for both control and urea-treated sRNA preparations (less than 3% change in extinction coefficient at 260 mμ for peak 1 to 2 transition). Cpm presented are the average of four determinations for alanine incorporation.
† Estimated from Fig. 4*A* and *B*.

of solution to dilute phosphate buffer. Sephadex elution profiles for the control and urea-treated samples are presented in Figure 4*A* and *B*, and Table 1 lists the respective alanine acceptor activities. The use of urea at elevated temperatures generates a twofold increase in alanine acceptor specific activity concomitant with a $2^1/_2$-fold increase of peak 3. The results of similar experiments performed with only peak 1, not in 4 *M* urea but in dilute phosphate buffer, reveal that the peak 1 to 2 transition can be effected without any generation of acceptor activity. It should also be noted that the two different aggregates are of considerable difference in stability.

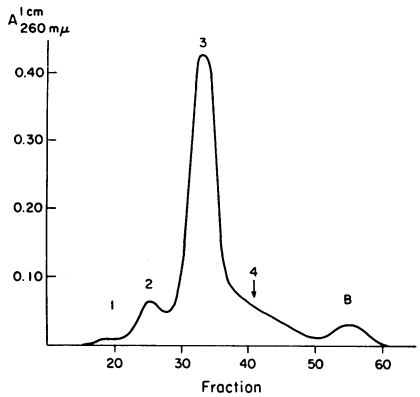

FIG. 5.—Elution profile (Sephadex G-100) for peak 3 sRNA concentrated 16-fold from a solvent system consisting of 1 *M* sodium chloride and 20% formamide.

Peak 3 itself can be concentrated to a small volume from 1 *M* sodium chloride with almost no formation of aggregates. However, if this same material is concentrated from a 20 per cent formamide–1 *M* sodium chloride solution, a significant quantity of aggregated and dissociated material is generated (Fig. 5).

Preliminary equilibrium sedimentation experiments are consistent with the notion of sRNA aggregation.

Discussion.—It is not presently known whether the various molecular sRNA species revealed in these experiments are the result of unique *in vitro* conditions or whether they are also present *in vivo*. Recently, Ames and Hartman[11] and Stent[12] have argued that modulation of sRNA, i.e., genetic adjustment of the availability of various active sRNA species, exists in the control of messenger RNA expression. A possible scheme of the way sRNA modulation could occur is suggested by our finding of acceptor activity only with peak 3 material. This would involve some sort of genetic control over the state of sRNA aggregation, possibly through the use of regulator enzymes. That this scheme could be feasible is suggested from both the work of Nishimura and Novelli[13] who found that certain chromatographic fractions of *B. subtilis* ribonuclease-treated sRNA showed a greater specific acceptor activity than the same fractions of untreated sRNA, and our urea treatment experiment where an increase of specific acceptor activity was found to accompany an increase in the amount of peak 3.

It is also interesting to note from the work of Goldstein and Holley[14] and Hele[15] that various sRNA preparations are able to modify the rate of amino acid-dependent

enzyme catalyzed adenosine triphosphate-pyrophosphate exchange; likewise that of Hele and Barth[16] which suggests the presence of an RNA "allosteric effector"[17] in "low activity" sRNA preparations which they believe is manifested by atypical rate changes of the ATP-P $\sim$ P exchange. Whether aggregation plays a role in this phenomenon is not yet known.

Summary.—Gel filtration of both crude and countercurrent distribution fractions of *E. coli* B sRNA employing Sephadex G-100 equilibrated with 1 *M* NaCl revealed the presence of several molecular species. Only one of these, peak 3, was found to have amino acid acceptor activity. The inactive species could be converted to active material through urea treatment. These results are discussed in terms of a possible modulation mechanism of sRNA.

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