FRACTIONATION OF SOLUBLE RNA BY CHROMATOGRAPHY ON DEAE ION EXCHANGERS

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One of the current methods for purification of soluble RNA (sRNA) specific to a single kind of amino acid involves charging a sRNA preparation with that amino acid and eliminating those sRNA molecules without attached amino acid by periodate oxidation followed by suitable techniques (Zamecnik et al., 1960; von Portatius et al., 1961; Zachau et al., 1961). In order to obtain specific sRNA in amounts useful for structural studies, it is highly desirable to have some preliminary fractionation methods, such as column chromatography or countercurrent distribution, before performing periodate oxidation, to reduce the amount of sRNA sacrificed through the above procedure. We have found that the columns of DEAE ion exchangers and of methylated albumin serve this purpose well for various amino acid acceptor RNA's. The results obtained with DEAE ion exchangers are presented here.

Nishiyama et al. (1961) could concentrate leucyl RNA considerably by chromatography on DEAE cellulose. More recently, Stephenson and Zamecnik (1962) obtained highly purified valyl RNA by chromatographing on DEAE-Sephadex the sRNA which had been charged with valine, treated with periodate and coupled to a tetrazo-compound. In our experiments, amino acid-free sRNA was used throughout.

sRNA was prepared from brewer's yeast by a modified method of Monier et al. (1960) and treated with dilute alkali to remove attached amino acids. It was adsorbed to a DEAE cellulose (Brown Co., 0.85 meq./g) or DEAE-Sephadex

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(Pharmacia, A-25, medium, 3.8 meq./g) column and eluted in stepwise fashion with sodium chloride solutions of increasing molarity, buffered to pH 5 (0.02 M sodium acetate). The eluates were pooled into several fractions and, after being concentrated, they were tested for their amino acid accepting activity under the standardized conditions. The results on five amino acids are shown in Fig. 1 and 2. The distribution of various sRNA's was qualitatively very similar with the two ion exchangers, but DEAE-Sephadex (Fig. 2) seemed to be somewhat more effective (note that the fractionation scheme of Fig. 2 is simpler than that of Fig. 1). The valine acceptor RNA was eluted

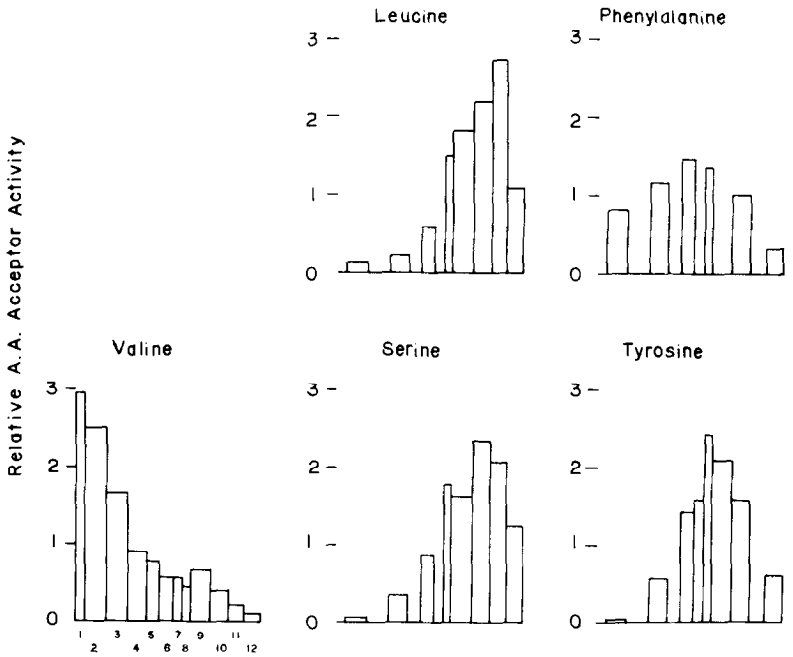

Fig. 1. Fractionation by DEAE-cellulose. Yeast sRNA (500 mg) was eluted from a 5 cm x 18 cm column successively by 0.46 M (Fr. 1 - 8), 0.48 M (Fr. 9 and 10), 0.50 M (Fr. 11) and 1.0 M NaCl (Fr. 12) at pH 5. The abscissa corresponds to the amount of RNA in the fraction (total recovery, 97 %) and the ordinate to the amino acid accepting activity relative to the average value. The area of the rectangle is, therefore, proportional to the amount of the acceptor RNA in the fraction. The activity of the peak fraction (mμM amino acid incorporated/mg RNA) was: valine 4.9; leucine 5.2; phenylalanine 2.6; serine 3.4; and tyrosine 1.8. The recovery of the activities was 80 % or higher.

very early, resulting in high degrees of purification, in agreement with Stephenson and Zamecnik (1961) who used sRNA with attached valine. This and other experiments using C^{14}-amino acyl RNA suggested that the presence of the amino acid residue is not likely to be the major factor that determines the elution characteristics of a particular species of sRNA. sRNA other than valine RNA could also be concentrated severalfold, though not as efficiently. sRNA's for glycine, phenylalanine and proline had broad distributions; this might be an indication of their heterogeneity within themselves. Combining other techniques, further purification of some acceptor RNA's is now in progress.

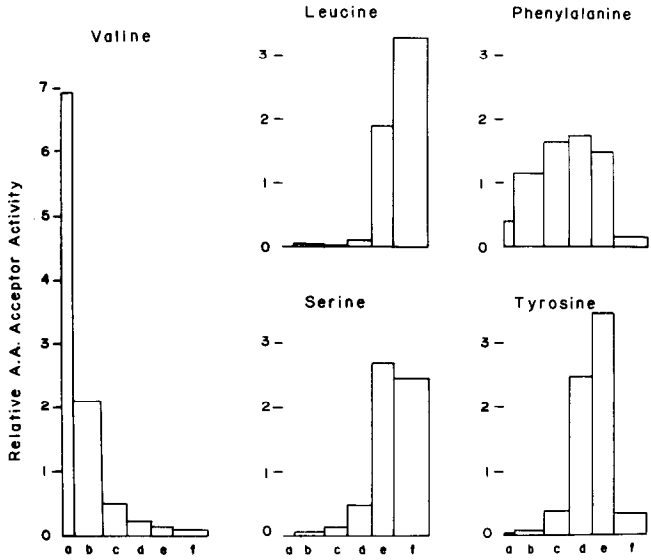

Fig. 2. Fractionation by DEAE-Sephadex. Yeast sRNA (40 mg) was eluted from a 1.2 cm x 16 cm column successively by 0.56 M (Fr. a and b), 0.58 M (Fr. c, d and e) and 1.0 M NaCl (Fr. f) at pH 5. The recovery of RNA was 92%. The peak activity was (mμM amino acid/mg RNA): valine 13.7; leucine 5.1; phenylalanine 4.2; serine 4.2; and tyrosine 3.3. The recovery of the activities was 80 % or higher.

sRNA forms a complex with mercuric chloride (see the accompanying paper) which can be similarly adsorbed to DEAE-Sephadex and eluted with sodium perchlorate solutions. Surprisingly, no significant fractionation could be achieved in this case (valine and leucine tested). In a control experiment

in which sRNA without mercuric chloride was eluted with sodium perchlorate instead of sodium chloride, the separation was poorer than that of Fig. 2. Since the secondary structure of sRNA is profoundly affected by complexation with mercuric ion and also high concentrations of perchlorate have a strong denaturing power on DNA (Hamaguchi and Geiduschek, 1962), a hypothesis may be proposed that the secondary structure of sRNA molecules plays the major role in the column fractionation.

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