FRACTIONATION OF SOLUBLE RNA BY A METHYLATED ALBUMIN COLUMN OF INCREASED CAPACITY

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The usefulness of methylated albumin columns for fractionation of nucleic acids has been well demonstrated. The column discriminates between various molecular species of DNA by their size, base composition and hydrogen bond content (Mandell and Hershey, 1960; Sueoka and Cheng, 1962). It also separates different classes of RNA molecules such as soluble RNA (sRNA), two kinds of ribosomal RNA, messenger RNA and virus RNA (Mandell and Hershey, 1960; Sueoka and Cheng, 1962; Otaka, Mitsui and Osawa, 1962; Kubinski and Koch, 1962; Fukada and Kawade, 1963). Furthermore, Sueoka and Yamane (1962, 1963) showed that the column can fractionate sRNA and reveal the heterogeneity of various amino acid acceptor RNA's. However, the column now usually employed, consisting of kieselguhr coated with methylated albumin, has relatively low capacities for nucleic acids and, therefore, is not very suitable for preparative purposes. We found that silicic acid can adsorb much more methylated albumin than kieselguhr and the column thus made has a large capacity for sRNA, which makes it a promising preparative tool for purification of specific acceptor RNA's.

The sRNA mainly used in this study was obtained from brewer's yeast by a modification of Monier, Stephenson and Zamecnik (1960), and freed of amino acids by mild alkali treatment. Silicic acid (Merck, Darmstadt) was suspended in water and decanted 10-20 times to remove fine particles, washed with 1 N HCl

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followed by water and dried. Methylated albumin, prepared from bovine serum albumin (Armour, Fraction V) (Mandell and Hershey, 1960) was dissolved in water and added to a suspension of the silicic acid in 0.2 M NaCl-0.02 M Na acetate buffer, pH 5. The amount of methylated albumin adsorbed was found to be from 17 to 45 mg per gram dry silicic acid depending on the batch. Under comparable conditions, one gram of kieselguhr adsorbed 1.3 mg methylated albumin. Columns of silicic acid-methylated albumin thus prepared could adsorb from 3 to 8 mg sRNA per ml of packed volume.

For fractionation, sRNA in amounts considerably smaller than the maximum capacity was charged to the column and eluted by sodium chloride solutions of stepwise increasing concentrations buffered at pH 5. A typical elution profile of yeast sRNA is shown in Fig. 1. The eluates were divided into 14 fractions as indicated, and the RNA in each fraction was recovered by adsorption to and elution from a DEAE cellulose column and ethanol precipitation. It was then assayed for amino acid acceptor activities. The results on lysine, phenylala-

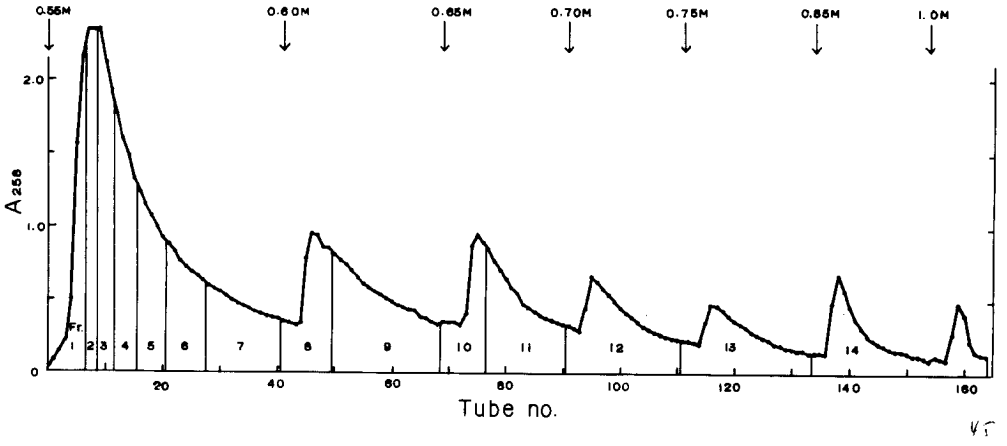

Fig. 1. Chromatography of yeast sRNA on a methylated albumin-silicic acid column. A suspension of 60 g silicic acid in 0.02 M Na acetate buffer at pH 5.4 containing 3 g methylated albumin was packed into a column (4 cm x 20 cm) which was then washed with the same buffer. 206 mg sRNA was adsorbed to the column and eluted by a series of NaCl solutions (containing the above buffer) of stepwise increasing concentrations as indicated. 90% of sRNA was recovered. The experiment was done at room temperature (about 25° C) with a flow rate of 10 ml/min and a fraction size of 50 ml.

nine, proline, serine, tyrosine and valine acceptor RNA's are presented in Fig. 2. Considerable separation and enrichment of various acceptor RNA's is apparent. Most promising results were obtained with glycine acceptor RNA of brewer's yeast as well as torula, as shown in Fig. 3. The highest activity, 11.0 mμM glycine attached/mg RNA, corresponds to 25% purity, if the molecular weight of sRNA is taken as 23,000. Attempts are being made to obtain highly purified glycyl RNA by applying the polyacrylic acid hydrazide procedure (von Portatius, Doty and Stephenson, 1961) to such fractions.

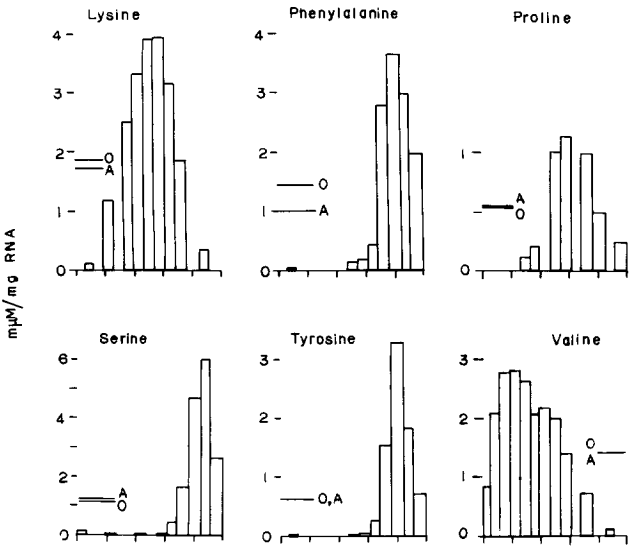

Fig. 2. Amino acid acceptor activities of the 14 fractions obtained in the run shown in Fig. 1. The ordinate indicates the activity in mμM amino acid attached/mg RNA, and the abscissa is proportional to the amount of RNA in the fraction. The height of the bars marked with O and A indicate the amino acid acceptor activity of the original unfractionated RNA and the weighted average of all the recovered fractions, respectively; these are mainly to show the recovery of the acceptor activities.

The positions of the acceptor RNA's examined here are in general agreement with those found by Sueoka and Yamane (1963) with C^{14}-amino acid-labeled sRNA of yeast using kieselguhr-methylated albumin columns. Comparison of these results with those obtained by DEAE ion exchangers (Kawade, Okamoto and Yamamoto, 1963) indicates marked differences in the distribution of some acceptor RNA's. Thus, phenylalanine acceptor RNA, which showed a broad distribution

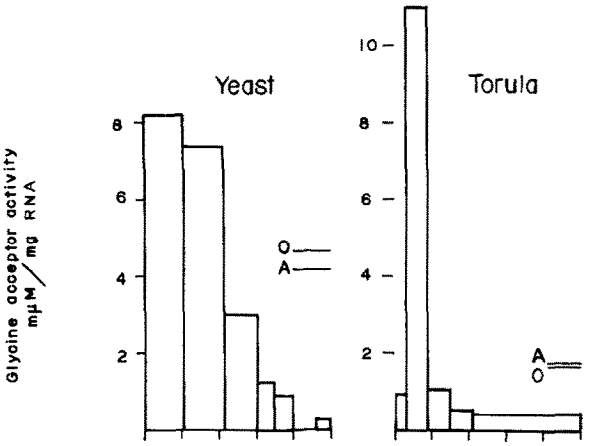

Fig. 3. Distribution of glycine acceptor RNA of brewer's yeast and torula. Presented in the same way as in Fig. 2.

on DEAE ion exchangers, was concentrated in the later fractions from the methylated albumin column. On the other hand, valine acceptor RNA, which was highly concentrated in the earliest eluates from the DEAE ion exchangers, was rather broadly distributed in this chromatography. These results suggest that a combined use of these two different columns may be useful for purification of certain specific acceptor RNA's and also for detecting their heterogeneity.

It is to be noticed, however, that the recovery of the acceptor activity was not always satisfactory. For example, in the experiment represented by Figs. 1 and 2, about one-third of phenylalanine acceptor activity was lost, while the other five activities were essentially recovered. Experiments are in progress to determine whether such an inactivation can be prevented.

Preliminary experiments on ribosomal RNA indicated that the capacity of the silicic acid-methylated albumin column is about one-third of that for sRNA, but still is much larger than that of the kieselguhr column. The elution profile of E. coli ribosomal RNA was very similar to that from the kieselguhr column. Our column will therefore be useful also for the separation of relatively large amounts of high molecular weight RNA's.

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References

Fukada, T. and Kawade, Y., Virology 19, 409 (1963).

Kawade, Y., Okamoto, T. and Y. Yamamoto, Biochem. Biophys. Res. Commun. 10, 200 (1963).

Kubinski, H. and Koch, G., Virology 17, 219 (1962).

Mandell, J. D. and Hershey, A. D., Anal. Biochem. 1, 66 (1960).

Monier, R., Stephenson, M. L. and Zamecnik, P. C., Biochim. Biophys. Acta 43, 1 (1960).

Otaka, E., Mitsui, H. and Osawa, S., Proc. Natl. Acad. Sci. U. S. 48, 425 (1962).

Sueoka, N. and Cheng, T. Y., J. Mol. Biol. 4, 161 (1962).

Sueoka, N. and Yamane, T., Proc. Natl. Acad. Sci. U. S. 48, 1454 (1962).

Sueoka, N. and Yamane, T., "Informational Macromolecules", (Vogel, H. J., Bryson, V. and Lampen, J. O., ed.) p. 205. Academic Press, 1963.

von Portatius, H., Doty, P. and Stephenson, M. L., J. Am. Chem. Soc. 83, 3351 (1961).