ISOLATION OF VALYL-RNA OF A HIGH DEGREE OF PURITY

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We have recently (Stephenson and Zamecnik, 1961) reported the development of a technique for isolating valyl-RNA from the family of soluble RNA (S-RNA) molecules in a degree of purity of the order of 65-80%. Other investigators have also been engaged in purification procedures (cf. Stephenson and Zamecnik, 1961), and recently Zachau et al. (Zachau et al., 1961) have described a method of considerable promise. In our previously described technique, the possibility of further enhancement of purification was mentioned, and has now been realized, with a present calculated purification of over 90% for valyl-RNA. In addition, the yield of the enriched RNA has been improved. The method combines two techniques, a dye addition procedure (Zamecnik et al., 1960) followed by chromatographic separation using diethylamino ethyl-dextran (DEAE-dextran) (Stephenson and Zamecnik, 1961). Valyl-RNA emerges from the colum prior to any other species of S-RNA while the dye-RNA remains bound to the DEAE-dextran under these conditions.

The enhancement of purification over that previously reported has been obtained by three slight modifications of the parent procedure. These are described in Table I and include longer and narrower columns, a slight decrease in the sodium chloride gradient and a slower flow rate. The DEAE-dextran coarse grade (DEAE-Sephadex-A-25, coarse grade, Pharmacia, Uppsala, Sweden) has been more satisfactory in general than the medium and fine grades of the same material, permitting a satisfactory flow rate without application of undue pressure, and with operation of columns in a cold room at 4° C.

In Table I are summarized the results of seven of our recent experiments in which appreciable fractions of the RNA samples placed on the column

TABLE I

	Experiment Number						
	1	2	3	4	5	6	7
Mg RNA on column	49	25	25	25	25	29	25
Mg RNA eluted in valine peak	1.75	0.98	1.07	1.20	0.99	1.43	1.79
% of added RNA in valine peak	3.6	3.9	4.3	4.8	4.0	4.9	7.2
Mg valyl-RNA over 90% pure*	0.85	0.38	0.39	0.60	0.36	0.44	0.27
% of total valyl-RNA over 90% pure	49	39	36	50	36	31	15
% of total RNA 90% pure valyl-RNA	1.7	1.5	1.6	2.4	1.4	1.5	1.1

The S-RNA was prepared, labeled with C^{14}-valine and carried through the dye addition procedure as previously described (Stephenson and Zamecnik, 1961; Zamecnik et al., 1960). The DEAE-dextran chromatography was carried out as described (Stephenson and Zamecnik, 1961) with the following modifications: 1) the dimensions of the columns have been changed from 1.2 cm x 55 cm to 0.6 cm x 100 cm; 2) the linear gradient of NaCl in 0.1 M tris buffer at pH 6.0 has been reduced from 0.35-0.40 M NaCl to 0.33-0.38 M NaCl in a total volume of 2,000 ml; and 3) the rate of flow of the elution fluid has been decreased from 100 ml to 30 ml per hour.

*This includes all samples over 62,000 cpm/mg RNA. The C^{14}-valine contained 1.75×10^6 cpm/μmole; therefore there are 35 mμmoles of valine per mg RNA in these enriched samples. Many samples of RNA from the very early part of the valyl-RNA peak gave higher values. The amount of RNA in these samples was however too small for accurate determination of specific activity. The calculated 90% purity is based on an assumed molecular weight of 25,500 for valyl-RNA (Tissieres, 1959).

The original "mixed" RNA samples put on the columns contained about 1.5 mμmole of valine/mg RNA. About 75% of the radioactivity and 7% of the RNA were eluted from the columns under these conditions.

were obtained with purifications of valyl-RNA of over 90%. In the vicinity of 1.6% of the total RNA was collected with this degree of purification. This "purified" material represents about 42% of the valyl-RNA peak.

It is thus possible to isolate valyl-RNA in sufficient quantity to make it feasible to carry out degradative procedures (Yu and Zamecnik, 1960; Khym and Cohn, 1960; Naoi-Tada et al., 1959, McCully and Cantoni, 1961) in order to investigate the properties of this highly specific S-RNA. In prin-

ciple it should be possible to prepare certain other aminoacyl-RNA's of such a degree of purity by means of the same procedures.

Although valyl-RNA is eluted from the column prior to the bulk of the RNA the mechanism for this prior emergence is not known. Differences in base composition and variations in secondary structure of specific S-RNA molecules undoubtedly play a large role in the elution pattern. It is also interesting to speculate that the presence of valine on a free 2' or 3' ribosyl hydroxyl group of the terminal adenosyl portion of S-RNA may prevent the formation of hydrogen bonds by this hydroxyl group with the DEAE-dextran thus allowing this aminoacyl RNA to emerge very slightly ahead of the remaining RNA's more firmly bound to the DEAE-dextran. Since S-RNA is internally hydrogen-bonded (Brown and Zubay, 1960) the exposed hydroxyl groups of the terminal ribose may play a more important role in hydrogen bonding with the DEAE-dextran than would be initially anticipated. This situation would be somewhat analogous to the fact that methylated oligosaccharides travel more rapidly through cellulose columns and paper than do unsubstituted oligosaccharides (Isherwood and Jermyn, 1951; Lederer and Lederer, 1957).

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REFERENCES

Brown, G. L. and Zubay, G., J. Mol. Biol. 2, 287 (1960).

Isherwood, F. A. and Jermyn, M. A., Biochem. J. 48, 515 (1951)

Khym, J. X. and Cohn, W. E., J. Biol. Chem. 236, PC9 (1961)

Lederer, E. and Lederer, M., in: Chromatography: A Review of Principles and Applications. 2nd ed., Elsevier Press, New York, 1957, p. 246

McCully, K. and Cantoni, G., Biochim. Biophys. Acta 51, 190 (1961).

Naoi-Tada, M., Sato-Asano, K. and Egami, F., J. Biochem. 46, 757 (1959).

Stephenson, M. L. and Zamecnik, P. C., Proc. Natl. Acad. Sci. 47, 1627 (1961).

Tissières, A., J. Mol. Biol. 1, 365 (1959).

Yu, C.-T. and Zamecnik, P. C., Biochim. Biophys. Acta 45, 148 (1960).

Zachau, H. G., Tada, M., Lawson, W. B. and Schweiger, M., Biochim. Biophys. Acta 53, 221 (1961).

Zamecnik, P. C., Stephenson, M. L. and Scott, J. F., Proc. Natl. Acad. Sci. 46, 811 (1960)