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Fractionation of transfer ribonucleic acid

The formation of amino acid-ribonucleic acid compounds recently has been studied by several groups[1]. A specific amino acid-activating enzyme is needed for the incorporation into ribonucleic acid of a specific amino acid[2,3]. It has been suggested that a specific RNA might also be required for each amino acid[2,4]. In the present studies, that fraction of the soluble RNA active in the formation of amino acid-RNA (transfer RNA[5]) has been separated into fractions with increased specificity for tyrosine and leucine.

For fractionation, 3.5 mg of transfer RNA prepared from guinea-pig liver by phenol extraction[5], in 0.02 *M* Tris, pH 7.5, was adsorbed on an 0.8 × 40 cm column of large mesh (1/32 in.) Cato-8, a cationic starch exchanger[6]. The column was eluted successively with 0.125 *M*, 0.15 *M*, 0.20 *M* and 1.0 *M* NaCl. In each case, the elution was continued until the 260 mμ absorbance of the effluent fell to a small value. The pooled fractions contained 23, 34, 22 and 8 %, respectively, of the original RNA. The RNA in each fraction was precipitated with 2 vol. ethanol and then assayed for incorporation employing various ^{14}C-labeled amino acids. These assays were performed using the appropriate RNA-free activating-enzyme fractions[2,7] under conditions where the incorporation represents saturation of the RNA.

The results in Fig. 1 show that relative enrichment of tyrosine-specific RNA

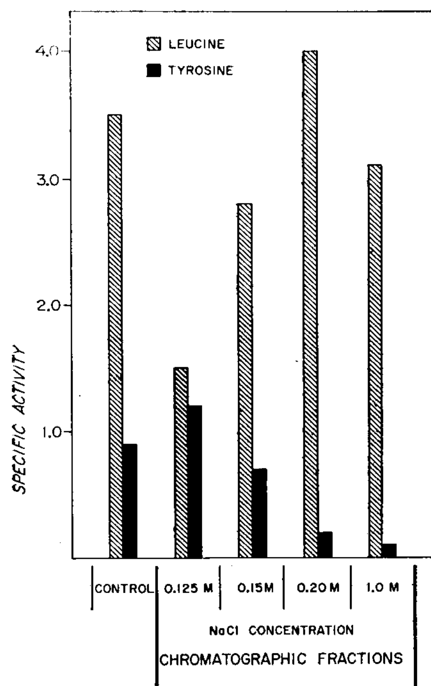

Fig. 1. Incorporation of ^{14}C-labeled tyrosine and leucine into RNA fractions prepared by column chromatography. The values are counts/min/mg RNA·10^{-3}. The amino acids used had a specific activity of 2,800 counts/min/mμmole.

Abbreviations: RNA, ribonucleic acid; Tris, tris(hydroxymethyl)aminomethane.

occurred in the 0.125 M NaCl peak, while leucine-specific RNA had the highest activity in the 0.2 M peak. The ratio of specific activities of leucine-RNA to tyrosine-RNA in the unfractionated material was 3.8:1. This ratio was changed to 1:1 in the first peak and was nearly 30:1 in the last peak. The RNA recovered (81% of that put on the column) contained 67% and 63% of the original leucine and tyrosine activity, respectively. Although the percentage of RNA which was eluted at each salt concentration varied to some extent for different preparations of RNA, enrichment of tyrosine-specific RNA always occurred in the first peak, and of leucine-specific RNA in the later fractions. Assays for the incorporation of lysine, isoleucine and threonine showed that some fractionation of RNA specific for these amino acids had also been achieved. Partial separation of transfer RNA and microsome RNA was obtained on these columns, since 85–90% of the former was eluted with neutral 1.0 M NaCl, while about 65% of the microsome-RNA remained adsorbed under these conditions and was eluted with alkaline salt solutions.

Ammonium sulfate fractionation has also given evidence for separation of amino acid-specific RNA. Soluble RNA in 0.1 M Tris, pH 7.5, was precipitated successively by the addition of solid $(NH_4)_2SO_4$ to 65, 80, 90 and 100% satn. at 4°. The fraction which precipitated at 65% satn. was inactive and probably contained microsome RNA (which in other experiments was precipitated at this salt concentration). Leucine incorporation was enriched over the original in the 65–80% fraction, while the 80–90% fraction contained RNA of the highest threonine and isoleucine specific activity.

These results provide evidence for the existence of RNA fractions specific for the attachment of individual amino acids. It is likely that this specificity is directed toward the enzyme-bound, activated amino acid[1], and resides in the particular sequence of bases in the RNA.

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