SEPARATION OF YEAST AMINO ACID - ACCEPTOR RIBONUCLEIC ACIDS BY COUNTERCURRENT DISTRIBUTION IN MODIFIED KIRBY'S SYSTEM

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Separation of yeast amino acid-acceptor ribonucleic acids by countercurrent distribution procedures has been reported (Doctor et al, 1961; Holley and Merrill, 1959; Holley et al, 1959; Holley et al, 1960). In this report we wish to describe an application of a modified solvent system of Kirby (1960) for the separation of amino acid-acceptor "soluble fraction" RNA isolated from yeast.

Experimental: The s-RNA from the yeast and the amino acid activating enzymes from rat liver homogenates were prepared by modifications of procedures described by Holley et al (1960, 1961). The s-RNA fractions were assayed for amino acid incorporation activity by the procedure of Holley et al (1960).

The solvent system of Kirby was modified as follows in order to employ it for the separation of s-RNA isolated from yeast: thirty grams of crystalline ammonium sulfate were dissolved in 50 ml of distilled water and 1.0 ml of glacial acetic acid, and 1.0 ml of 0.1 M magnesium chloride was added. The solution was adjusted to pH 4.0 with concentrated ammonium hydroxide (0.3 ml) and the volume was adjusted to 100 ml. Four milliliters of forma-

mide and 40 ml of 2-ethoxy ethanol were added and the mixture was shaken vigorously in a glass-stoppered bottle. The solvent system was allowed to stand at room temperature for 3 to 4 hrs with occasional shaking. The procedure described by Doctor et al (1961) for performing the countercurrent distribution and the isolation of s-RNA fractions suitable for enzyme assays was employed.

Results and discussion: Figure 1 shows the 200-transfer distribution of yeast s-RNA in modified Kirby's system. Also shown are the locations of the alanine-, valine-, threonine-, serine- and tyrosine-acceptor activities. The recovery of the activity and the specific activities of various fractions are summarized in Table 1. The alteration of ammonium sulfate and 2-ethoxy ethanol concentration and the addition of formamide were found to be necessary to obtain the gross partition coefficient (K) of s-RNA close to one. A

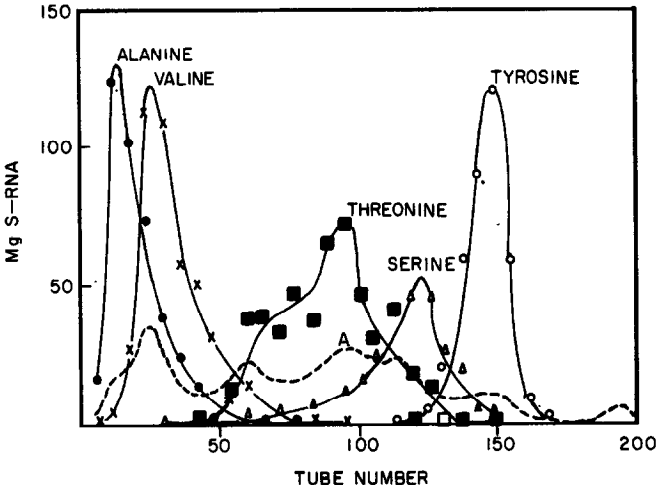

Fig. 1. 200 transfer countercurrent distribution of 200 mg of yeast s-RNA in modified Kirby's solvent system at pH 4.0. ---A---, mg s-RNA per tube after dialysis and evaporation (calculated from the absorbancy at 260 mu, 1 mg/ml = 19.0); ●——●, alanine acceptor activity; ×——×, valine acceptor activity; ■——■, threonine acceptor activity; Δ——Δ, serine acceptor activity; o——o, tyrosine acceptor activity. The activities are plotted on a scale such that the activity of the starting mixture would coincide with its weight, and the specific activities of the fractions relative to the starting material are given by the ratio of activity to weight.

TABLE 1

Recovery and Amino Acid-Acceptor Activity of Countercurrent Distribution Fractions of Yeast S-RNAs

Tube No.	Amino Acid	Recovery of Activity* %	CCD Fraction** cpm/uM	Starting Mixture** cpm/uM	Increase in Activity (multiples of originals)
14-15	Alanine	85	135,000	18,000	7.5
25-26	Valine	95	201,000	54,000	3.7
93-94	Threonine	95	114,000	42,000	2.7
121-122	Serine	55	189,000	63,000	3.0
149-150	Tyrosine	82	828,000	69,000	12.0

* Calculated on the basis of the s-RNA recovered after dialysis and evaporation.

** Assuming a molecular weight of 30,000.

The results presented here demonstrate once more the power of the Craig countercurrent distribution technique. Also it furnishes another solvent system that can be employed in the separation of amino acid-acceptor RNAs.

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greater number of ultraviolet absorbing material peaks was obtained in this solvent system compared to the pH 6 phosphate buffer system. Although the patterns of distribution do not appear to be different in the two solvent systems, better recovery of specific activity was obtained in this system. Another advantage of this solvent system is the absence of salts in the isolated s-RNA fractions which is quite desirable for the further studies of these fractions.

From these results it appears that with further modifications better separation of yeast s-RNA may be accomplished in this system. The broad peak

for threonine acceptor activity which was described previously (Doctor et al, 1961) is also observed in this system. However in the light of the recent findings of Berg et al (1961) and Benzer et al (1961), it will be interesting to test the threonine acceptor activity of these fractions with the amino acid activating enzymes isolated from the yeast.

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