The Use of Ammonium Acetate in the Precipitation of Ribonucleic Acid

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Ammonium acetate (0.24M) in combination with cold 66% (v/v) ethanol quantitatively precipitated RNA from very dilute solutions ($\geq 5 \mu\text{g/ml}$) after centrifugation for $2.5 \times 10^6 \text{ g}\cdot\text{min}$. There was also less co-precipitation of detergents with ammonium acetate than NaCl.

Precipitation of RNA from aqueous solutions is often done by cold ethanol and a salt, e.g. NaCl (Magasanik, 1955), or sodium acetate or potassium acetate (Zubay, 1962; Kirby, 1965; Davidson *et al.*, 1973). However, it is a common experience that, if the initial RNA solution is dilute (less than $50 \mu\text{g/ml}$), complete precipitation of RNA with ethanol and these salts is not always predictable without prolonged ultracentrifugation. Moreover, detergents used to isolate RNA may co-precipitate with the RNA in the presence of these salts.

This communication shows that the precipitation of RNA with ammonium acetate and ethanol minimized the problems described above.

Materials and methods

RNA from whole mouse liver and rat brain was prepared by a procedure based on the methods of Kirby (1965), Brawerman *et al.* (1972), Crippa *et al.* (1967) and Davidson *et al.* (1966). The tissue was first extracted at 20°C with phenol equilibrated with 0.1M-sodium acetate, pH5.0; the phenol/aqueous interphase was then extracted with phenol equilibrated with 0.1M-Tris-HCl, pH9.3. Both buffers contained 0.5% sodium naphthalenedisulphonate and 0.5% tripropylnaphthalenedisulphonate. The combined aqueous phases at this stage are designated 'crude extracts', from which RNA-containing material was precipitated with ethanol in the presence of ammonium acetate or NaCl. Final purification involved treatment with deoxyribonuclease (Worthington Biochemical Corp., Freehold, N.J., U.S.A.) and Pronase (Calbiochem, San Diego, Calif., U.S.A.) and further deproteinization with phenol-chloroform mixtures.

Pulse-labelled nuclear RNA was prepared from crude liver nuclei from mice injected with $700 \mu\text{Ci}$ of [^3H]orotic acid (New England Nuclear Corp., Boston, Mass., U.S.A.) 30 min before death. The liver was homogenized in 0.32M-sucrose–3mM-MgCl₂ and centrifuged at 3000 g for 10 min at 2°C . The RNA was then extracted from the crude nuclear

pellet by the above procedure. RNA preparations were analysed on 5–20% (w/v) sucrose density gradients in 0.1M-sodium acetate–0.1mM-EDTA, pH5.0, and centrifuged at 260000 g for 2 h at 4°C in a Beckman–Spinco SW 50.1 rotor. This ^3H -labelled nuclear RNA preparation contained substantial amounts of unlabelled rRNA.

tRNA was the gift of Dr. Paul Denny, Department of Dental Biochemistry, University of Southern California.

J. T. Baker reagent-grade ammonium acetate and NaCl were used without further purification.

To precipitate RNA, an ammonium acetate solution was added to the aqueous RNA solution to a concentration of 0.24M. Then 2 vol. of pre-chilled ethanol (-20°C) was added and the solution was chilled for at least 2 h at -20°C . The precipitated RNA was separated by centrifugation for 30 min at $82500 \text{ g}_{\text{av}}$ at 0°C , in a Beckman SW-27 rotor.

Absorbance was measured with a Beckman Acta CIII spectrophotometer, and radioactivity was measured in a Beckman LS-230 liquid-scintillation counter with Aquasol scintillant (New England Nuclear Corp.).

Results and discussion

Efficient precipitation of pure tRNA (Table 1) or purified mixtures of rRNA and ^3H -labelled nuclear RNA (Table 2) was obtained by adding 2 vol. of chilled ethanol to solutions of the RNA in 0.24M-ammonium acetate. This method thus precipitates RNA over a molecular-weight range of 4S to $>50\text{S}$.

Ammonium acetate is also useful in the purification of RNA by precipitation from 'crude extracts' (see under 'Materials and methods'). RNA was precipitated with ammonium acetate or NaCl from the crude extracts of phenol-extracted tissue which contained substantial quantities of detergents. The sucrose-density-gradient profile of the NaCl-precipitated RNA preparation region contained 18% of the material in a broad u.v.-absorbing region at 4S and below; this 'peak' had E_{260}/E_{280} of 2.3–2.6,

Table 1. Recovery of tRNA precipitated from 0.24M-ammonium acetate solutions

Concn. of initial RNA solution ($\mu\text{g/ml}$)	Mean recovery (%)	
1.0	51	(47, 48, 59)*
2.6	77	(70, 78, 81)*
5.1	$94 \pm 2^\dagger$	
8.4	101*	
10.3	99	(96, 102)*
21.2	102	(102, 102)*

* Individual measurements.

 † Mean $\pm$ s.e.m., five measurements.

which indicates the presence of detergents. RNA preparations at this stage which have been passed over a Sephadex G-25 column to remove detergents have E_{260}/E_{280} of 1.9–2.0. However, the density-gradient profile of the ammonium acetate-precipitated RNA preparation showed a distinct peak (approx. 4S), about 13% of the total material, with E_{260}/E_{280} of 1.90–1.98; there was no u.v.-absorbing material of less than 4S. RNA recovery with ammonium acetate-ethanol precipitations was highly reproducible: we obtained 9.2, 8.8 and 8.7 mg of highly purified RNA/10 g of rat brain, with E_{260}/E_{280} of 2.0–2.2, from successive preparations.

Finally, any ammonium acetate which may be precipitated by cold ethanol is easily removed from the RNA by evaporation from an aqueous solution *in vacuo* or by freeze-drying (Rouser *et al.*, 1963).

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Table 2. Recovery of rRNA and pulse- ^3H -labelled nuclear RNA precipitated from 0.24M-ammonium acetate solutions

In the initial RNA solution, more than 80% of the 260 nm-absorbing material is rRNA. The specific radioactivity of the ^3H -labelled nuclear RNA was 38400 c.p.m./mg. Virtually all of the ^3H -labelled RNA material was larger than 4S on sucrose density gradients.

Initial RNA solution			Recovery			
			rRNA		³ H-labelled nuclear RNA	
(μg/ml)	(μg)	(c.p.m.)	(μg)	(%)	(c.p.m.)	(%)
11.1	134	5140	127	95	4850	95
5.5	67	2570	61	91	2540	99

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