Preparation of ribonucleic acid in cold concentrated saline and some physico-chemical properties

It has been already reported that the microsomal ribonucleoprotein particles isolated by the method of LITTLEFIELD *et al.*[1] are dissociated to RNA and protein moieties when treated with concentrated saline in the cold, the latter being selectively precipitated under such conditions[2]. This property has been applied to the preparation of RNA[3]. This communication is concerned with the preparation of RNA by this method and some physical chemical properties of the isolated RNA.

A fresh calf liver was cut into strips about 1 cm thickness and frozen in dry ice. For each preparation of RNA, 200 g of the frozen liver was allowed to thaw at 5°, chopped very finely with a meat cleaver and then homogenized in 360 ml 0.25 *M* sucrose solution. The supernatant separated from the homogenate by centrifugation (15 min at 14,000 × *g* in the cold) was the starting material for the preparation.

To this supernatant, solid NaCl was added to a final concentration of 2 *M* under constant stirring, and kept overnight in the cold. After centrifugation for 15 min at 4,000 × *g*, the supernatant was carefully removed. The loosely packed sediment was collected and washed several times with 2 *M* NaCl in the cold. The gelatinous pellet obtained was suspended in 0.02 *M* KH_2PO_4–K_2HPO_4 buffer (pH 7.0) with the aid of a glass homogenizer and then the solution was centrifuged for 15 min at 14,000 × *g*. If the dissolution was incomplete, such a procedure was further repeated. The clear supernatant thus obtained was further submitted twice to the treatment with strong saline and dissolution in the 0.02 *M* phosphate buffer as described above. The pellet obtained after such purification procedures was suspended in the 0.02 *M* phosphate buffer and dialysed against the same buffer overnight in the cold. During dialysis, the solution became clear, the small amount of insoluble material being removed by centrifugation for 30 min at 20,000 × *g*.

About half of the RNA in the original mitochondrial supernatant was recovered in the final RNA solution, in which none or only a trace of DNA was detected by the diphenylamine test, and 1–3% protein contaminant was found by LOWRY's method[4]. The maximum and minimum of its absorbancy was found at 258 and 230 mμ respectively, average $A_{230\ m\mu}/A_{258\ m\mu}$ being 0.475.

The RNA prepared by this procedure is electrophoretically homogeneous, its ascending and descending mobilities in phosphate–saline buffer (pH 7.0, I 0.1) at 0° being -14.5 and $-13.6 \cdot 10^{-5}$ $cm^2 \cdot V^{-1} \cdot sec^{-1}$ respectively. Ultracentrifugation showed three components, the main one being the slowest (Fig. 1a). The sedimentation coefficient ($S^\circ_{20,w}$), were 11 S, 16 S and 20 S respectively. Viscosity measurements were carried out at 25° in an Ostwald viscosimeter having flow time of approximately 120 sec for water. The intrinsic viscosity $[\eta]$ of the RNA in 0.02 *M* potassium phosphate buffer (pH 7.0) was found to be 0.30 by extrapolation to zero concentration, which was in good agreement with the value calculated from HUGGINS' equation[5]. Assuming that the various components are homologous with regard to frictional properties, the intrinsic viscosity of 0.30 for the mixture was applied to SCHERAGA-MANDELKERN equation[6] to estimate the sedimentation-viscosity molecular weight of the 11 S component. The value calculated was $28 \cdot 10^4$.

Abbreviation: RNA, ribonucleic acid.

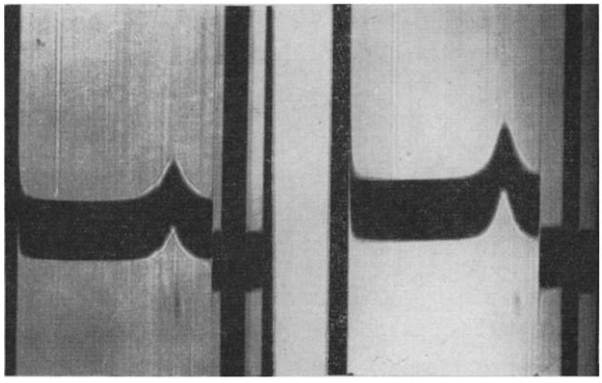

Fig. 1. Sedimentation photographs of (a) the RNA prepared by cold 2 *M* NaCl and (b) the heat-treated RNA (85°, 15 min). (a) Taken after run for 45 min at 47,300 rev./min. (b) Taken after run for 30 min at 60,000 rev./min. Concentration, 0.67 %; solvent, 0.02 *M* KH_2PO_4–K_2HPO_4 buffer (pH 7.0); bar angle 35° and 40°, respectively.

The absorbancy at 258 mμ in 0.02 *M* potassium phosphate buffer (pH 7.0) of the RNA rose by 18 % when the temperature was raised from 10° to 85° and more than 90 % recovery in the absorbancy was observed after cooling it again to 10°. The sedimentation patterns were, however, irreversibly altered; only a slower but remarkably homogeneous peak (8 S) was obtained (Fig. 1b). Its calculated molecular weight was about 10^5, suggesting that this component probably corresponds to the sub-unit RNA described by HALL AND DOTY[7].

As the molecular weight of the RNA prepared by aqueous phenol has been reported to be in the order of a million[8–11], the RNA isolated by the present method seems to have suffered considerable degradation during the isolation procedures.

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