CONCENTRATION AND DESALTING OF RIBONUCLEASES

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Kickhoefen and Buerger (1962) reported that bovine pancreatic ribonuclease (RNase) does not lose activity after exposure to phenol. Since dialysis of this RNase entails losses due to the low molecular weight of the enzyme, extraction with phenol was examined for the desalting of this and similar enzymes.

Crude B. subtilis RNase (Nishimura, 1960; Rushizky et al., 1963) in 25 ml of Nishimura's medium, (containing a total salt concentration of about 2M) was stirred for 15 minutes at room temperature with 5 ml. of water-saturated phenol (at 23°). The phases were separated by centrifugation, and the aqueous phase re-extracted with 2.5 ml of phenol. After centrifugation, the combined phenol phases were lyophilized and the residue dissolved in 2.5 ml of water. A small amount of insoluble material was spun off, and the clear solution examined for recovery of enzymatic activity and salt removal. As shown in Table 1, the same procedure yielded analogous results with purified RNase from B. subtilis, crude RNase T_1 (Takahashi, 1961; Rushizky and Sober, 1962), micrococcal nuclease (Cunningham et al., 1956; Rushizky et al., 1962) and purified pancreatic RNase

(Worthington). In each case, the amount of enzyme in the combined phenol phases was determined after a 1:200 (or higher) dilution, using appropriate blanks. After phenol treatment, lyophilization, and extraction of the aqueous enzyme solution with ether (5 times with an equal volume to remove traces of phenol), specific activity was determined. While the specific activity increased in the case of crude B. subtilis RNase and RNase T_1, those of the purified enzymes did not change.

B. subtilis RNase and RNase T_1 were also freed of phenol by the addition of 3 volumes of ice-cold acetone to the combined phenol phases (see above); after standing for 10 minutes at 0°, the precipitated enzymes were centrifuged out, quantitatively recovered, and found to be free of salt. Pancreatic RNase was only partially precipitated under these conditions, perhaps because of the low concentration used (1 mg/25 ml).

The phenol extraction procedure may be useful for the purification of low-molecular weight enzymes since losses due to desalting by dialysis are avoided and the volume is reduced. The method is faster than exclusion chromatography and may effect purification by selective denaturation of contaminating proteins. In the case of enzymes (micrococcal nuclease) derived from pathogenic bacteria, the phenol treatment offers a convenient procedure for biological inactivation of the starting material.

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Table 1. Phenol extraction of ribonucleases

Enzyme	Buffer	Enzyme Activity (total units)	Salts, measured as relative conductivity (in mmho)	Volume (ml)	Specific Activity*
Crude *B. subtilis* RNase	Nishimura's medium	125,000	700	25	39,000
Combined phenol phases before lyophilization		129,000		8.5	
Aqueous phase before lyophilization		460		23.6	
Aqueous solution of enzyme after lyophilization		128,000	24	2.5	78,000
Purified *B. subtilis* RNase	0.3 M sodium chloride 0.2 M sodium phosphate, pH 7.0	119,000	450	25	680,000
After phenol treatment and lyophilization		120,000	18	2.5	690,000
Crude RNase T_1	0.1 M sodium acetate, pH 7.0 0.2 M sodium chloride	400	150	25	41
After phenol treatment and lyophilization		395	8	2.5	57
Purified pancreatic RNase	0.3 M sodium chloride 0.2 M sodium phosphate pH 7.0	16,200	450	25	16.2×10^7
After phenol treatment and lyophilization		15,300	14	2.5	16.1×10^7
Micrococcal nuclease	0.3 M sodium chloride 0.2 M sodium phosphate, pH 7.0	3,550	450	25	2,550
After phenol treatment and lyophilization		3,570	13	2.5	2,600

* units of enzyme per $D_{280} = 1$, which is used as a measure of protein content.

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