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Note

A simple and rapid procedure for desalting nucleotide-containing solutions

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During the course of our efforts to study spin-labelled tRNAs, we had to perform a series of enzymatic degradations of the tRNA molecules in order to prove the exact location of spin labelling[1,2]. Therefore, many chromatographic fractions containing mono-, di- and trinucleotides had to be desalted following salt gradient chromatography of each enzymatic digest. We would like to report in this paper a simple and rapid procedure that we have developed to obtain salt-free chromatographic fractions. The procedure is similar to the commonly used batch separation on Sephadex ion exchangers but does not necessitate any swelling of the resin because Dowex anion exchangers are used. The experimental set-up shown in Fig. 1 has been employed. The results obtained with two different spin-labelled $tRNA^{Glu}$ are reported and discussed.

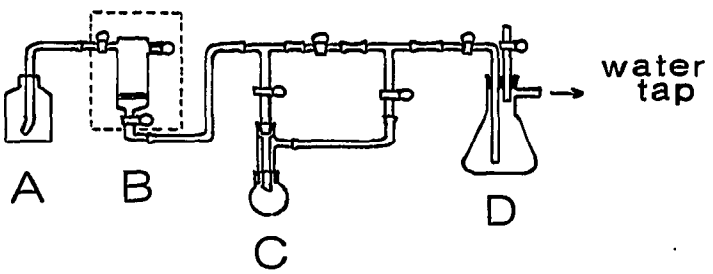

Fig. 1. Experimental set-up for desalting. (A) 125-ml bottle for washing and eluent solutions; (B) 200-ml Merrifield reaction vessel clamped to a Chipco shaker (rectangular box); (C) 50-ml round-bottom flask to collect the desalted material; (D) safety trap connected to a water tap aspirator. Connections are made with Tygon tubing.

EXPERIMENTAL

Materials

$tRNA^{Glu}$ (78% activity) was kindly provided by Dr. A. Kelmers of the Oak Ridge National Laboratory. DEAE-Sephadex A-25 was obtained from Pharmacia (Montreal, Canada) and Dowex 1-X2 (Cl^-) resin was a product from Bio-Rad Labs. (Richmond, Calif., U.S.A.). Buffer solutions were made by diluting the appropriate chemical of the purest form available in doubly distilled deionized water. Absorbances were recorded on a Cary 16 spectrophotometer.

Method

About 10 $A_{260\ nm}$ units of spin-labelled $tRNA^{Glu}$ were digested[2] with pancreatic ribonuclease A according to Ohashi *et al.*[3] and the resulting oligonucleotide mixture was separated by chromatography on DEAE-Sephadex A-25 in 7 *M* urea as described by Rushizki *et al.*[4]. Elution profiles essentially identical to those reported were obtained.

Desalting procedure. The fractions contained under each peak were combined (maximum volume of 25 ml), diluted once with doubly distilled deionized water and introduced directly into the Merrifield reaction vessel (part B of Fig. 1) containing about 10 mg of Dowex 1-X2 (Cl^-) resin that had been previously washed with water until the effluent showed no absorption at 260 nm. The resulting suspension was mechanically shaken for 30 min during which time the nucleotides adsorbed to the resin. The resin was then filtered by suction using part D of the system. The insoluble material was washed (3 × 80 ml) with the water contained in part A to remove any salt from the resin. The anion exchanger was converted to the formate form by shaking and rinsing the resin twice with 75 ml of 1 0.*M* ammonium formate for 20 min each time to reach equilibrium. The resin, now in the formate form, was washed again with water (3 × 80 ml) and finally shaken for 30 min with 25 ml of 10 *M* formic acid in order to liberate the nucleotides from the resin. The solution was collected by suction in flask C and the salt-free nucleotides were obtained after lyophylization.

RESULTS AND DISCUSSION

The procedure consists of adding to a small amount of Dowex resin, contained into a reaction vessel identical to the one used in solid phase peptide synthesis (Merrifield reaction vessel[5]), the combined chromatographic fractions containing nucleotides of a given peak in the elution profile of a nuclease digest of tRNA. Nucleotides are known to bind efficiently to anion exchangers[6] and consequently the salts used during the chromatography can be washed-off from the insoluble material by vacuum filtration using the device shown in Fig. 1. The nucleotides are then desorbed from the resin by a formic acid treatment (volatile eluent) and are recovered after lyophylization. Table I shows the recovery efficiency. By this procedure, 92–98% of the starting material was recovered and completely desalted.

TABLE I

RECOVERY EFFICIENCY FROM THE FIRST FOUR FRACTIONS OF TWO DIFFERENT SPIN-LABELLED $tRNA^{Glu}$

BSL and CSL represent a bromo and a carbodiimide spin label[2], respectively.

BSL-$tRNA^{Glu}$				*CSL-$tRNA^{Glu}$*			
Fraction number	*$A_{260\ nm}$ units before desalting*	*$A_{260\ nm}$ units after desalting*	*Recovery (%)*	*Fraction number*	*$A_{260\ nm}$ units before desalting*	*$A_{260\ nm}$ units after desalting*	*Recovery (%)*
1	1.638	1.620	98	1	1.387	1.332	96
2	1.346	1.290	96	2	1.194	1.122	94
3	1.268	1.191	94	3	1.063	0.978	92
4	0.564	0.546	97	4	0.490	0.475	97

The simple experimental device shown in Fig. 1 is judged to possess several advantages. It is quickly and easily assembled from inexpensive and readily available laboratory materials. The desalting of a peak from an elution profile is completed in less than 2 h and at no time during the procedure is the resin exposed. In comparison to other methods where additional chromatographies of the fractions obtained after the digestion are necessary, this method results in a considerable saving of time and of labour. It is also applicable to large numbers of samples. The technique may also be applied to the desalting of solutions of amino acids and small peptides by a proper choice of ion exchangers and buffers and is therefore of general application.

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