Observations on the use of cellulose ion exchangers for the chromatographic separation of nucleotides

Ion-exchange chromatography of nucleotides on Dowex 1 is a standard laboratory procedure of proven utility. A major limitation, however, is the necessity for large volumes of solutions of high molarity or low pH for elution of the firmly bound nucleotides. Two of the more convenient procedures, for example, employ a gradient-elution system with formic acid–ammonium formate buffers[1]. In one, the final concentrations of the reagents are 4 *M* formic acid and 0.8 *M* ammonium formate (pH 2.6), and in the other, 1.75 *M* ammonium formate and 0.5 *M* formic acid (pH 4.15). Despite the fact that formic acid is volatile and ammonium formate may be removed by sublimation, it is not easy to prepare nucleotides, thus eluted, free of salt, particularly since they are usually obtained in at least several hundred ml of buffer. In many instances one must resort to adsorption of the nucleotides onto charcoal even though their subsequent elution is far from quantitative. The low pH of these systems further complicates the isolation of acid-labile compounds such as adenosine-3′-phosphate-5′-phosphosulfate[2] which are unstable under such conditions. An ideal ion exchanger would be one from which the nucleotides could be eluted in a relatively small volume of buffer of low molarity and with a pH not far from neutrality.

Cellulose anion exchangers were originally designed for chromatography of macromolecules but their low pK's, the relative ease of elution of adsorbed compounds, and the high degree of resolution usually attained suggested their application to the separation of nucleotides. "ECTEOLA" was prepared by reacting cellulose powder with epichlorohydrin and triethanolamine in NaOH according to the procedure of PETERSON AND SOBER[3]. It was then converted to the formate form and washed well with water. In a preliminary experiment, it was found that a known mixture of adenylic acid, adenosine diphosphate and adenosine triphosphate was separable by a gradient elution from 0.0 to 0.35 *M* ammonium formate, pH 4.5.

This system was then employed in a search for nucleotides linked to sulfated glucosamine and glucuronic acid derivatives which might be possible intermediates in the biosynthesis of heparin[4]. A boiled, aqueous extract of 5 g of mouse mast-cell tumor[5] was applied to an ECTEOLA formate column, 2.5 × 20 cm. The column was washed well with water overnight until the absorbance of the eluate was less than 0.1 at 260 mμ. A gradient-elution system was then established with a mixing-chamber volume of 250 ml and a flow rate of 10–12 ml/h. Samples of 4 ml were collected. Initially, the mixing chamber contained distilled water and the reservoir contained 0.1 *M* ammonium formate, pH 4.2. When 250 ml buffer had passed through the column, the solution in the reservoir was changed to 0.5 *M* ammonium formate, pH 4.2. The molarity of the eluate was calculated to be 0.058 at tube 62, and 0.35 at tube 120.

By this simple procedure, 13 compounds were partially or completely separated from each other (Fig. 1). No fraction exceeded a volume of 25 ml. The ratios of absorbance at 250 mμ/260 mμ and at 280 mμ/260 mμ are given in Table I. Although these ratios are somewhat distorted owing to the presence of a contaminant in the ammonium formate that absorbs in the u.v., it is apparent that derivatives of adenine, guanine, cytosine and uracil are represented in the fractions obtained[1]. Unfortunately for the original purpose of this experiment, neither glucosamine nor glucuronic acid

was found in any of the samples. None of the compounds was further characterized.

TABLE I

Tube number	Ratios of absorbance	
	250 mμ/260 mμ	280 mμ/260 mμ
36	0.8	1.1
41	0.77	1.1
45	0.75	1.4
49	0.77	1.1
56	0.75	0.64
63	0.66	0.30
66	0.92	0.21
78	0.97	0.37
83	0.87	0.51
85	0.96	0.57
91	0.89	0.36
101	0.93	0.6
108	0.96	0.72

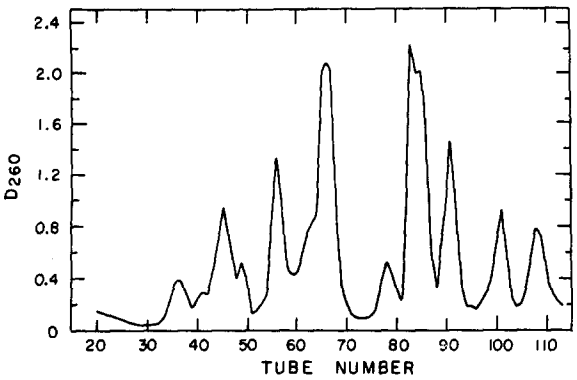

Fig. 1. Chromatography of a boiled, aqueous extract of mouse mast-cell tumor on ECTEOLA formate.

The procedure employed in this experiment was chosen arbitrarily, and no attempt has been made to modify it. It is anticipated, however, that good chromatographic results would be obtained with buffers of even lower molarities at pH's approaching neutrality. Since ECTEOLA has a pK of approximately 7 (ref. [3]) even the most firmly bound compounds should be readily eluted, without recourse to solutions of high molarity, merely by increasing the pH of the eluting buffer. In any event, the above observations are sufficient to indicate that chromatography on ECTEOLA formate is a useful procedure for the separation of complex mixtures of nucleotides.

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[1] R. B. HURLBERT, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. III, Academic Press Inc., New York, 1957, p. 85.
[2] P. W. ROBBINS AND F. LIPMANN, *J. Biol. Chem.*, 229 (1957) 837.
[3] E. A. PETERSON AND H. A. SOBER, *J. Am. Chem. Soc.*, 78 (1956) 751.
[4] E. D. KORN, *J. Am. Chem. Soc.*, 80 (1958) 1520.
[5] T. B. DUNN AND M. POTTER, *J. Natl. Cancer Inst.*, 18 (1957) 587.

Received September 12th, 1958

Scattering of light by tobacco-mosaic virus and X-protein from infected tobacco plants

In a study of the inactivation of tobacco-mosaic virus (TMV) by u.v. light the fraction of absorbed light which is not scattered was estimated from the absorption by ribonucleic acid (RNA) from TMV and TMV-protein[1]. The scattering of light by TMV arises from the enormous size of the elementary particle or molecule[2]. A more direct way of estimating the scattering would be to compare the absorbance of two solutions, one of which would consist of a low-molecular-weight protein and the other would contain