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The influence of eluent salt composition on the elutions of riboflavin and adenine in poly-N-vinyl pyrrolidone column chromatography

Evidence from various laboratories has indicated that hydrogen bonding is an important factor governing the selectivity of poly-N-vinyl pyrrolidone (PVP) toward various naturally occurring phenolic compounds[1-3]. For example, GUSTAVSON[1,2] first showed that urea could disrupt PVP–vegetable tannin complexes, and ANDERSEN AND SOWERS[3] presented spectral data which established that phenols attached to the polymer by hydrogen bonds. The tenacity of PVP binding has been shown to be a function of the number of phenolic hydroxyl groups, and it is diminished under alkaline conditions[3]. LERNER *et al.*[4] have speculated that hydrogen bonding occurs between protons on ring nitrogens in nucleotide derivatives and the pyrrolidone carbonyl groups during purine and pyrimidine separations on PVP columns. The present study is an examination of the mode of interaction of riboflavin and adenine with PVP in column chromatography.

Details of preparation of PVP for column chromatography have been reported previously[4]. Columns were equilibrated with eluent before each experiment. Solutes were dissolved in distilled water and applied to the columns in saturated solutions

TABLE I

EFFECT OF ELUENT COMPOSITION ON RETENTION VOLUMES OF RIBOFLAVIN AND ADENINE

Compound	*Eluent*	*Ionic strength of eluent*	*Elution volume*[a,b] *(ml)*	*Band width*[c] *(ml)*
Elution volumes on a 0.9 × 31.8 cm column				
Riboflavin	Distilled water (pH 6)	—	23.8, 23.1, 21.5	
Riboflavin	0.1 m*M* NaCl	0.0001	25.2, 25.7, 25.2	
Riboflavin	1.0 m*M* NaCl	0.001	22.7, 23.0	
Riboflavin	10 m*M* NaCl	0.01	23.6, 23.7	
Riboflavin	100 m*M* NaCl	0.1	23.2, 23.1	
Riboflavin	1.0 m*M* K_2SO_4	0.003	25.0, 25.7	
Riboflavin	10 m*M* K_2SO_4	0.03	23.7, 24.6	
Elution volumes on a 0.9 × 12.5 cm column				
Adenine	Distilled water	—	37.3, 35.3	8 (approx.)
Adenine	1.0 m*M* K_2SO_4	0.003	33.3, 32.8, 30.3	5.4
Adenine	10 m*M* K_2SO_4	0.03	26.3, 26.8	5.1, 5.3
Adenine	50 m*M* K_2SO_4	0.15	19.8, 20.3[d]	2.5, 3.0
Adenine	100 m*M* K_2SO_4	0.3	21.8, 20.3	3.2, 3.3
Adenine	1.0 m*M* NaCl	0.001	31.8, 36.3, 34.3	
Adenine	10 m*M* NaCl	0.01	25.3, 28.8, 26.3, 27.3	
Adenine	100 m*M* NaCl	0.1	23.8, 22.8	
Riboflavin	100 m*M* K_2SO_4	0.3	9.8, 9.8	
2,6-diaminopurine	50 m*M* K_2SO_4	0.15	29.8[d]	

[a] Volume of eluate to maximum concentration.
[b] Each figure is the value obtained in a single experiment.
[c] Peak width at half-maximum concentration.
[d] Column length = 12.0 cm.

having volumes of either 0.3 ml (long column) or 0.8 ml (short columns). Eluents were delivered at 25° and at atmospheric pressure. Flow rates averaged 0.12 ml/min for the long column and 0.5 ml/min for the short columns. Eluate was collected in 0.3 or 0.5 ml fractions and monitored spectrophotometrically at 260 mμ for adenine, 280 mμ for 2,6-diaminopurine, and 440 mμ for riboflavin.

Riboflavin was previously found to be retarded in its passage through PVP columns relative to vitamin B_{12} (which may be excluded because of size) during elution with distilled water[4]. Presumably, three factors can contribute to its behavior on PVP, namely, nonpolar attraction of the isoalloxazine ring to hydrocarbon portions of the polymer backbone, electrostatic bonding of both the sugar–alcohol moiety D-ribitol and the secondary ring nitrogen to the polarized pyrrolidone groups, and gel permeation. Of these factors, electrostatic interactions are readily distinguished from adsorption (or filtration) effects in that they can be normalized by the presence of salt in the eluent[5]. The elution of riboflavin with distilled water was compared with its elution under various conditions of eluent salt composition. The data shown in Table I indicate that the retention of this compound is not influenced by the salts; thus electrostatic effects can be ruled out. Furthermore, the absence of ribitol–hydroxyl group binding can be contrasted to the behavior of phenolic hydroxyls[1–3]. On the other hand, salt markedly affects the elution of adenine (Table I). The tenacity of adenine binding decreases as the eluent ionic strength increases, thus implicating polarity in the retention process. Elution profiles also are sharper and more symmetrical under salt conditions than with distilled water. The free amino group is of major importance in retarding adenine, as evidenced by the still greater affinity of 2,6-diaminopurine. The observation that salt concentrations above a certain ionic strength do not cause a further reduction in elution volume of adenine means that other interactions are responsible for retarding this compound relative to riboflavin when charge effects have been normalized.

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