Poly-N-vinyl pyrrolidone chromatography

Effect of pH on elution of bases and a proposed mechanism for the adsorption of purines

Recently the separation of purine and pyrimidine bases by poly-N-vinyl pyrrolidone (PVP) chromatography was reported[1]. The principal mechanism of adsorption was attributed to hydrogen and hydrophobic bonding. Electrolytes tend to strengthen hydrophobic bonds whereas they weaken salt linkages[2]. Adenine in distilled water elutes at approximately 80 ml, while adenine in 0.3 ml saturated $(NH_4)_2SO_4$ elutes at 56 ml (1 × 39.5 cm column). The elution volume of guanine is also decreased in salt[3]. These observations seem to indicate that hydrophobic bonds are not of major importance in the adsorption of adenine or guanine to PVP.

Compared with the pyrimidines, adenine and guanine bind tenaciously to PVP. Our present studies, reported below, indicate that hydrogen bonding increases the electrophilic character of the carbonyl carbon of PVP and hence the center of high electron density in adenine and guanine adjacent to the point of hydrogen bonding may also be involved.

Materials and methods

Adenine, guanine, cytosine and thymine were purchased from Nutritional Biochemical Company* and 1-methylguanine from Sigma Chemical Company. Insoluble PVP (sold under the trade name of Polyclar AT Powder) was obtained from GAF Corporation, New York.

The Polyclar AT was mixed with distilled water, and the fines were discarded by repeated decantation. The suspension was poured into a column and allowed to pack with gravity flow.

Columns were equilibrated with the eluting solution before standard samples were added. A 0.5 ml solution containing 0.08 mg each of thymine, cytosine, guanine and adenine (adjusted to the pH of the eluting medium) was applied to a 1.0 × 39.5 cm column. Elution was carried out at room temperature and atmospheric pressure with solutions containing 0.02 *M* NH_4Cl + 0.2 *M* NH_4OH at pH 10.3 and pH 7 or 0.005 *N* HCl adjusted to pH 3.5 with dilute NH_4OH. 1-Methylguanine (0.1 mg in 0.5 ml) was eluted at pH 7.

The effluent was collected in 1.25 ml fractions and monitored at 260 mμ in a Gilford 220 spectrophotometer. Identification of the bases was made from the ultraviolet absorbance spectra.

Results and discussion

The dissociations of adenine and guanine of pK' values 4.2 and 3.3, respectively, may be attributed to the amino groups and the guanine dissociation of pK' 9.6 to the $-N_1H-C_6O-$ group. The dissociation of adenine of pK' 9.8 and the dissociation of guanine of pK' 12.3 are almost certainly the dissociation of –NH– in the imidazole ring[4].

ELUTION OF BASES WITH VARIATION IN pH

Dissociation constants (pK') are taken from JORDAN[4].

	pK'			Ve (ml)[a]		
	$-NH_3^+$	$-N_1H-C_6O-$	*Imidazole* $-NH-$	*pH 3.5*	*pH 7*	*pH 10.3*
Adenine	4.2		9.8	43.8	73.8	70.0
Guanine	3.3	9.6	12.3	50.0	67.5	37.5
Cytosine	4.6			31.2 }		32.5
Thymine		9.8		36.2 }	34	28.8
1-Methylguanine					52.5	

[a] Ve = elution volume.

Table I shows the variation in elution volume (Ve) of adenine, guanine, cytosine and thymine under different elution conditions. There appears to be little contribution from the imidazole –NH– of adenine, since there is only a slight difference between the elution volumes at pH 7 and pH 10.3 (74 *vs.* 70 ml). The only large change in elution volume of adenine occurred at pH 3.5 (44 ml). Hence the amino group of adenine (pK' 4.2) must play a significant role in binding to PVP. Hydrogen bonding alone could not account for the greater attraction of adenine to PVP compared with that of the pyrimidines, which have the same number of possible hydrogen bonding sites. Because of the electrophilic nature of the pyrrolidone carbonyl group, it is reasonable to implicate the high electron density at $N_{(1)}$ of adenine as a site of adsorption. With hydrogen bonding of the amino group, the electrophilic character of the carbonyl carbon is increased[5], and the center of high electron density at $N_{(1)}$ of adenine is attracted to the carbonyl carbon of PVP, making ring formation possible as indicated in Fig. 1. When the hydrogen bond involving the adenine amino group is ruptured at pH 3.5 the ring is broken and adenine elutes much sooner.

Similarly, guanine could bind to PVP through formation of a six-membered ring. Once the $-N_1H-$ of guanine is hydrogen bonded, the guanine keto oxygen may be attracted to the electrophilic center of the PVP molecule (Fig. 1). At pH 10.3 guanine has a much smaller elution volume (37.5 ml) than at pH 7 (67.5 ml). This same 30 ml change (as indicated above with adenine) can be attributed to the

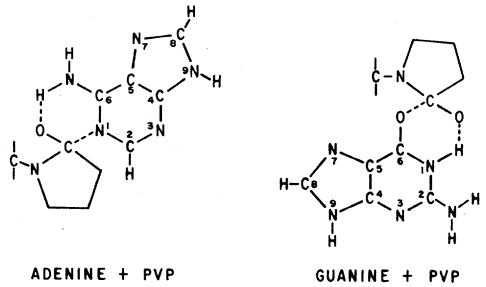

Fig. 1. Postulated adsorption of adenine and guanine to PVP.

ionization of $-N_1H-$ (pK' 9.6) disrupting the hydrogen bonding and hence the ring.

The *Ve* of 1-methylguanine (pH 7) is less than that of guanine. Substitution of the hydrogen atom at $N_{(1)}$ of guanine with a methyl group leads to a decrease in adsorption, presumably due to lack of ring formation.

Hence, although hydrogen bonding has been considered the principal mechanism by which substances adsorb to PVP, the hydrogen bonding may be concerted with a nucleophilic attack at the pyrrolidone carbonyl carbon, resulting in ring formation and increased adsorption. The feasibility of the stereo-structural relationships shown in Fig. 1 has been confirmed by use of molecular models.

As a practical application of altering the elution pH, cytosine and thymine can be separated by PVP chromatography at pH 3.5, since the $-N_1H-$ of thymine is hydrogen bonded to PVP at this pH and elutes at 36 ml. The two bases may also be resolved at pH 10.3 where the $-N_1H-$ of thymine is ionized (pK' 9.8), disrupting the hydrogen bonding and allowing thymine to be eluted first.

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1 J. LERNER, T. M. DOUGHERTY AND A. I. SCHEPARTZ, *J. Chromatog.*, 37 (1968) 453.
2 W. KAUZMANN, *Advan. Protein Chem.*, 14 (1959) 49.
3 T. M. DOUGHERTY AND A. I. SCHEPARTZ, *J. Chromatog.*, 40 (1969) 299.
4 D. O. JORDAN, *The Chemistry of Nucleic Acids*, Butterworths, Washington, 1960, p. 134.
5 E. H. CORDES AND W. P. JENCKS, *J. Am. Chem. Soc.*, 84 (1962) 4319.