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Note

Effect of pH on the elution of cytokinins from polyvinylpyrrolidone columns

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Cytokinins may be separated by column chromatography using insoluble poly-N-vinylpyrrolidone (PVP) as the solid phase and phosphate buffer pH 6.4 as the eluant[1–3]. PVP may also be used as a highly efficient purifying agent during the extraction of plant hormones[2,4] because it strongly binds phenols[5] which may otherwise interfere with bioassays. As the pH is lowered, phenols become more strongly bound to PVP, with maximum adsorption occurring at pH 3.5 (ref. 6). However, both adenine[7] and the cytokinin zeatin[4] bind less strongly to PVP as the pH declines and it is assumed that other adenine-derived cytokinins exhibit the same binding characteristics. Purification of cytokinins in celery seed extracts can be achieved by elution from a PVP column at pH 3.5 (ref. 8) although the elution profiles of individual cytokinins at pH 3.5 have not been reported. The phenolic nature of the N^6 side-chain of the naturally occurring cytokinin N^6-(*o*-hydroxybenzyl)aminopurine riboside (hyd-BA riboside)[9] suggests that this and other related compounds may bind very strongly to PVP, which could result in loss of these cytokinins from plant extracts purified with PVP. However, it may be possible to prevent this loss by lowering the pH and thus weakening the cytokinin–PVP bond. Changes in the elution profile of different cytokinins in response to adjustment of the eluant pH may also aid in the separation and characterisation of individual cytokinins. This report describes the influence of pH on the elution of eleven cytokinins, including hyd-BA riboside and its free base (hyd-BA) from PVP columns.

EXPERIMENTAL

Preparation of columns

Polyclar AT (PVP) purchased from GAF (G.B.), Manchester, Great Britain, was mixed with distilled water and the finer particles were repeatedly decanted off prior to column packing so that a good flow-rate could be achieved. The PVP slurry was poured into glass columns and allowed to settle by gravity flow. The columns were washed with distilled water followed by the eluant buffer which was either 0.013 *M* KH_2PO_4 acidified to pH 3.5 with 1 *N* HCl, 0.013 *M* KH_2PO_4/Na_2HPO_4 at pH 6.4, or 0.013 *M* Na_2HPO_4 adjusted to pH 9.5 with 1 *N* NaOH.

Cytokinins

Most of the cytokinins were purchased commercially and 2×10^{-4} *M* solu-

tions were prepared with the appropriate eluant buffer. Hyd-BA and hyd-BA riboside were kindly donated by Dr. R. Horgan, University College of Wales, Aberystwyth, and because of the limited quantities available were prepared as 10^{-4} *M* and 5×10^{-5} *M* solutions, respectively.

Column chromatography

It was assumed that hyd-BA and hyd-BA riboside would be strongly bound to PVP, so a small column (10 × 1.0 cm) was used for their separation. The other cytokinins were applied to either a 25 × 1.7 cm column or to a 60 × 2.2 cm column. Cytokinins were applied separately to the columns in 4.0 ml of buffer and eluted with each of the three buffers.

Detection of cytokinins

The elution profiles of all except two of the cytokinins were obtained by monitoring the transmittance of UV light at 254 nm through the eluate using an LKB Uvicord II absorptiometer. The exceptions were hyd-BA and hyd-BA riboside, which, because of their low concentrations in the eluate, were collected in 5-ml fractions from the column, absorbances being measured at 270 nm.

RESULTS AND DISCUSSION

The results in Table I show that at pH 6.4 cytokinin bases may be separated as previously described[1,2]. All the ribosides eluted before their respective bases. At pH 3.5 elution was more rapid although the effect of low pH on elution volume was much greater with free bases than with ribosides, with the result that at pH 3.5 each riboside eluted slightly later than its respective base. The elution volume of 2iPA riboside overlapped with that of kinetin riboside at pH 6.4 but, because it responded more to the pH reduction, a discrete separation of these compounds was achieved at pH 3.5.

The use of buffer at pH 3.5 or 6.4 ensured most of the cytokinins tested could be separated from each other on the 25 × 1.7 cm column with very little overlap. The exceptions were zeatin and zeatin riboside from dihydrozeatin and zeatin riboside from 2iPA riboside. The use of pH 9.5 buffer did not improve the separation of these cytokinins except zeatin riboside from dihydrozeatin because although the elution peaks were more widely separated, the elution bands were much broader. They were separated, however, at pH 6.4 using a larger column (60 × 2.2 cm). Buffer at pH 9.5 was not used with cytokinins which could be separated successfully at pH 6.4 or 3.5.

Hyd-BA and hyd-BA riboside had slower and wider elution bands than the other cytokinins. This could be due to the phenolic nature of these compounds although the decrease in affinity for PVP at pH 3.5 is contrary to the expected behaviour of phenols[6]. The estimated peak elution volumes of the two compounds on the 25 × 1.7 cm column are given in Table I. It is assumed that these are accurate since an error of only 4% was evident when an estimate was made of the BA peak elution volume from this column based on its elution from the 10 × 1.0 cm column. Its elution band width, however, was over-estimated by 100% and the true elution spread of hyd-BA and its riboside on the 25 × 1.7 cm column would probably be much smaller than was estimated.

TABLE I

ELUTION VOLUMES OF CYTOKININS ON PVP COLUMNS (25 × 1.7 cm, EXCEPT WHERE OTHERWISE STATED) USING A 0.013 *M* PHOSPHATE BUFFER AT DIFFERENT pH VALUES

Cytokinin	*Peak elution volume (ml)*			*Elution range (ml)*		
	pH 3.5	*pH 6.4*	*pH 9.5*	*pH 3.5*	*pH 6.4*	*pH 9.5*
Dihydrozeatin	65	150 590*	195	50–75	120–190 540–640*	145–240
Zeatin	68	170 700*	225	55–85	140–215 630–770*	175–280
Zeatin riboside	87	110 440*	120	70–100	80–150 380–510*	90–150
N^6-(Δ^2-Isopentenyl)-aminopurine (2iPA)	75	255	—	60–90	210–300	—
2iPA riboside	105	155 590*	160	80–130	120–190 530–660*	120–195
N^6-Furfurylaminopurine (kinetin)	125	360	—	100–150	285–475	—
Kinetin riboside	160	185	—	125–200	145–230	—
N^6-Benzylaminopurine (BA)	150	620	—	120–190	500–710	—
BA riboside	210	310	—	150–280	230–400	—
N^6-(*o*-Hydroxybenzyl)-aminopurine (hyd-BA)	735** 105***	2800** 400***	—	420–1260** 60–180***	1680–3990** 240–570***	—
Hyd-BA riboside	875** 125***	1225** 185***	—	420–1540** 60–220***	630–1890** 90–270***	—

* Elution from a 60 × 2.2 cm column.
** Estimated for a 25 × 1.7 cm column based on the results obtained with a 10 × 1.0 cm column.
*** Elution from a 10 × 1.0 cm column.

Cytokinins may thus be readily purified from plant extracts on PVP columns using a buffer at pH 3.5. A comparison of elution at pH 6.4 and 3.5 may also help to decide if a cytokinin is a free base or a riboside.

With suitable variation of column size and eluant pH, PVP column chromatography offers a cheap and reproducible means of achieving efficient separation of different cytokinins.

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