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Note

pH-dependent elution volume of some pyrroles chromatographed on Sephadex G-15

HANSJÖRG A. W. SCHNEIDER

Botanisches Institut der Universität zu Köln, 5 Gyrhofstrasse 15, Köln 41 (G.F.R.)

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Gel chromatography is a technique frequently used for separating solute substances according to their molecular size and for determining molecular weights. The behaviour of small molecules, however, does not always fit the common concept of molecular sieving, to which separation on highly crosslinked polymers is attributed. Side-effects have been reported that are thought to be caused by ion exchange, ion exclusion and absorption[1]. The present paper describes the unusual connection between hydrogen ion concentration and the chromatographic behaviour of some pyrroles.

EXPERIMENTAL AND RESULTS

Chromatography was performed with a 270 × 15 mm column of Sephadex G-15. A 2-ml volume of a $2 \cdot 10^{-2}$% solution of the substances shown in Fig. 1 was placed on top of the column, and elution was carried out with 0.01 *M* buffers (sodium acetate, potassium phosphate and tris–HCl) and 0.01 *N* HCl or ammonia solution, as these were also used for equilibration of the column. Porphobilinogen (PBG) was taken from an enzymatic preparation[2], and ALA-pyrrole and AA-pyrrole were synthesized by condensation of ALA and AA with acetylacetone[3].

During purification procedures on Sephadex columns, it was observed that

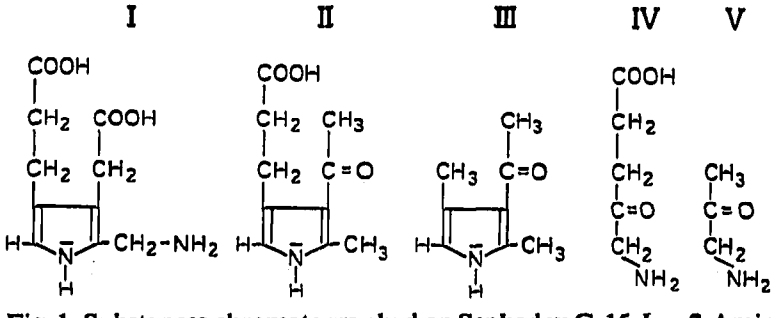

Fig. 1. Substances chromatographed on Sephadex G-15. I = 2-Aminomethyl-3-acetic acid-4-propionic acid-pyrrole, porphobilinogen (PBG); II = 2-methyl-3-acetyl-4-propionic acid-pyrrole (ALA-pyrrole); III = 2,4-dimethyl-3-acetylpyrrole (AA-pyrrole); IV = δ-aminolevulinic acid (ALA); V = aminoacetone (AA).

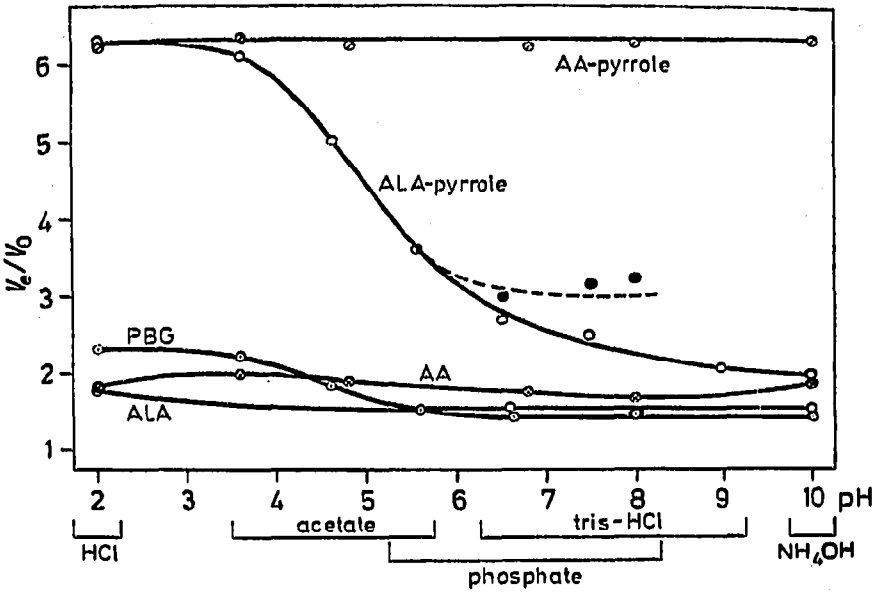

Fig. 2. PBG, ALA-pyrrole, AA-pyrrole, ALA and AA chromatographed on a column of Sephadex G-15, showing the ratio V_e/V_0 (elution volume/void volume) *versus* pH.

the elution volume of PBG did not remain constant when buffers of different hydrogen ion concentration were used. Under defined conditions, it could then be shown that the elution volume decreased with increasing pH (Fig. 2). The shape of the line correlating elution volume with pH resembles a titration curve. In a similar manner, the elution volume of ALA-pyrrole is dependent on the hydrogen ion concentration, but the difference between the highest and the lowest V_e/V_0 ratio (6.3 and 2.0, respectively) is much greater than that between the corresponding ratios for PBG (2.3 and 1.4, respectively). The pH dependence of the elution behaviour of ALA-pyrrole is in complete agreement with absorption, ion-exclusion and counter-ion effects described in a survey[4] of amino acid behaviour on Sephadex G-10. Concerning ALA-pyrrole and its elution volume in the region of higher pH values, the type of buffer is also of importance. The elution volume in phosphate is higher than in tris–HCl buffer of the same pH. Only the values obtained with tris–HCl, however, are situated on a continuous line, which, on extrapolation, gives the elution volume at pH 10 in ammonia solution. Apart from this observation, all of the elution volumes are remarkably independent of the type of buffer and the ionic strength (Table I).

TABLE I

RELATIONSHIP BETWEEN THE ELUTION VOLUME AND THE TYPE OF BUFFER AND IONIC STRENGTH

Buffer	*Molarity*	*pH*	V_e/V_0
Sodium acetate	0.01	5.6	3.38
	0.05		3.66
	0.10		3.53
Potassium phosphate	0.01	5.6	3.45
	0.04		3.66

The difference in the elution volume of ALA-pyrrole in tris–HCl and potassium phosphate buffer at pH values where the pyrrole has an anionic charge may be due to the greater size of the tris salt compared with the potassium salt of the pyrrole. However, there must also be an influence of the anion because of the small elution volume in ammonia solution.

The elution volume of AA-pyrrole is as high as the elution volume of ALA-pyrrole at low pH values, but in contrast to the behaviour of ALA-pyrrole the elution volume of this substance remains constant over the whole range of hydrogen ion concentrations studied. Small amounts of AA-pyrrole (5%), however, are eluted much faster with increasing pH in another peak. Similar to this observation, trace amounts of ALA-pyrrole are found with higher elution volumes.

The elution of ALA and AA themselves is hardly influenced by the pH. These substances leave the column before the larger molecules of ALA-pyrrole and AA-pyrrole.

In columns equilibrated and run with twice-distilled water, AA is strongly retained and hardly eluted. This behaviour can be ascribed to the influence of the few carboxyl groups in the gel matrix[5] on the amino group of AA. The retention is completely eliminated in 0.01 *M* buffer. This effect is not observed with the other substances. The interactions of their amino groups with the gel are obviously masked by the influence of other groups.

DISCUSSION

On account of the aromatic structure of the pyrroles, it can be assumed that their high affinity for the gel has a similar origin as the retention of aromatic substances[5,6]. However, great differences occur in the pH-dependent behaviour of some pyrroles. The structures of ALA-pyrrole and AA-pyrrole differ only in the side-chain at position 4, which is elongated in ALA-pyrrole by an acetic acid group. This group must therefore be responsible for the different behaviour of the two substances under the influence of hydrogen ions. The ionization of the acidic group is shown by the reduced elution volume at high pH values. An enlargement of the molecule cannot account for the observed large decrease in the elution volume. This effect must be due to a change in the interactions of gel and substance. Independent of pH, the affinity for the gel is also weakened by the additional groups in PBG, which do not increase the net charge of the molecule. The range of the influence of hydrogen ions is limited by the resulting general decrease in elution volumes.

Although only a few experimental results are available, the observations indicate that the affinity of the tested pyrroles for the gel is decreased by side-chains and modified by the propionic acid group.

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