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Note

High-performance liquid chromatography of peptides

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Usually, mixtures of peptides are separated by thin-layer chromatography[1] or ion-exchange column chromatography[2], although high-performance liquid chromatography (HPLC) has been used for the isolation of single peptides[3,4,5]. In this communication, a reversed-phase method is described for the separation of small peptides of different polarities and chain lengths. The advantages of octadecylsilane (ODS, reversed-phase) columns in comparison with ion-exchange columns are: (1) they are pressure-stable; (2) re-equilibration of the columns to their initial conditions after use of a gradient is more rapid; (3) bleeding seldom occurs; (4) ODS is resistant to most organic solutes at neutral and acid pH; (5) retention on ODS is less dependent on temperature; and (6) ODS can be re-used many times without deterioration of its resolving properties.

MATERIALS AND METHODS

Peptides (Serva, Heidelberg, G.F.R.), phosphoric acid and potassium phosphate (p.a. quality; Merck, Darmstadt, G.F.R.) were used. Methanol (p.a.; Merck) and water were distilled at least three times before use; the latter with addition of alkaline potassium permanganate during the second distillation to oxidise trace impurities that might give spurious peaks on the chromatogram. The chromatograph assembly (Waters Assoc., Königstein, G.F.R.) consisted of two pumps (model 6000), a programmer (model 660), an injector (model U6K) and a detector (Perkin-Elmer, model LC 55). The column (200 × 4 mm I.D.) was packed by the slurry method at 6000 p.s.i. with 5-μm ODS particles (Nuleosil 5 C-18; Macherey and Nagel, Düren, G.F.R.) and its temperature was maintained at 31° during chromatography. A linear gradient was applied, the initial eluent being 0.05 *M* potassium dihydrogen phosphate (adjusted to pH 2 with phosphoric acid) and the final eluent being methanol; the gradient was terminated after 60 min. The sample was 5 μl of peptide solution (1 mg/ml), the flow-rate was 3 ml/min, and the detection wavelength was 230 nm.

RESULTS AND DISCUSSION

Preliminary experiments showed that acid pH and the addition of potassium phosphate resulted in narrower peaks and better resolution; probably this is because

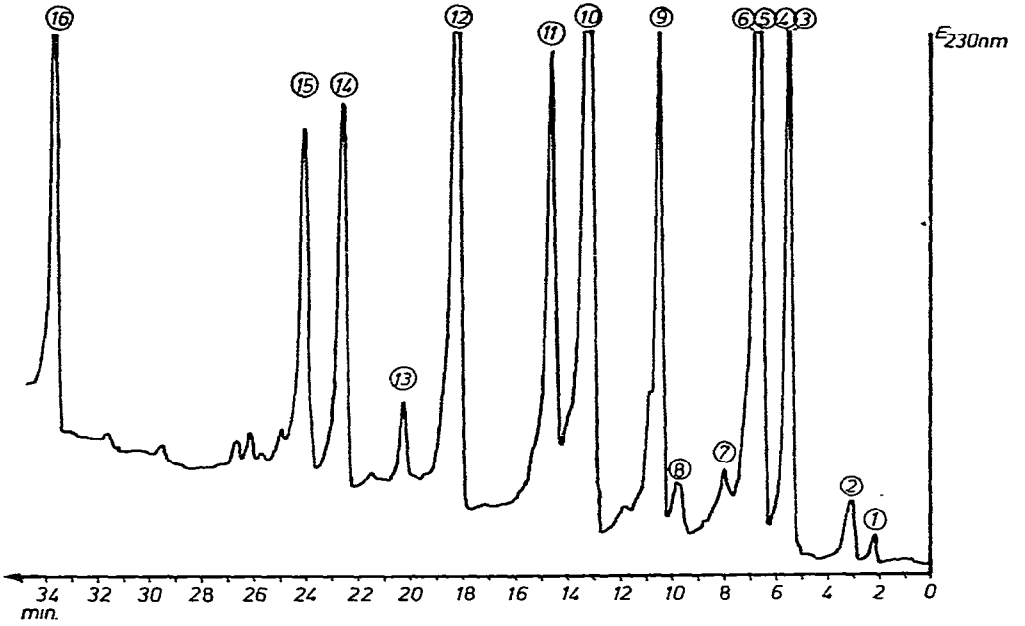

Fig. 1. Chromatographic profile showing separation of a peptide mixture. For conditions of separation see "Materials and Methods", and for identities of numbered peaks see Table I.

TABLE I

SEPARATION OF PEPTIDES BY HPLC

For conditions of separation see "Materials and Methods".

Peak No.	*Peptide*	*Retention time, min*
1	Val-Gly	2.1
2	Gly-Val	3.0
3	Tyr-Gly	5.5
4	Leu-Gly	5.5
5	Gly-Tyr	6.8
6	Gly-Leu	6.8
7	Phe-Gly	8.0
8	Gly-Phe	9.8
9	Ala-Ala-Tyr-Ala-Ala	10.5
10	Gly-Trp	13.2
11	Phe-Tyr	14.6
12	Leu-Trp-Met-Arg	18.3
13	Unknown*	20.2
14	Met-Glu-His-Phe-Arg-Trp-Gly	22.5
15	Leu-Leu-Val-Tyr	24.0
16	Renin inhibitor (an octapeptide)	33.6

* Probably an impurity.

peptides are more strongly bound to ODS at acid pH, and thus retention times are increased.

The results are shown in Fig. 1 and Table I, from which it can be seen that only two pairs of the peptides tested were not separated. From the values presented, it can be deduced that the retention time of a peptide in this system is determined by its polarity. The C-terminal amino acid of a dipeptide seems to be of particular importance in this respect, *e.g.*, the retention times of Gly-Tyr and Tyr-Gly are different, the retention time being longer when the less polar Tyr is the C-terminal amino acid; the same is true for other dipeptides. The polarities of some of the amino acids used, as indicated by their solubility in water, is shown in Table II (see also ref. 6). With oligopeptides, the pentapeptide Ala-Ala-Tyr-Ala-Ala, in which the polar Ala predominates, is retained to a much lesser extent than the tetrapeptide Leu-Leu-Val-Tyr, which is composed of non-polar amino acids.

TABLE II

SOLUBILITIES OF SOME AMINO ACIDS IN WATER AT 25°

Amino acid	*Solubility, g/100 g of water**
Glycine	24.99
L-Alanine	16.51
L-Valine	8.85
L-Phenylalanine	2.96
L-Leucine	2.19
L-Tryptophan	1.13
L-Tyrosine	0.45

* Values taken from ref. 6.

The chain length of a peptide also influences its retention on the column, oligopeptides usually having longer retention times than dipeptides. However, non-polar dipeptides (*e.g.*, Gly-Phe) are eluted at about the same time as oligopeptides predominantly composed of polar amino acids (such as Ala-Ala-Tyr-Ala-Ala).

The elution profiles so obtained suggest that mixtures of small peptides can be separated by using reversed-phase HPLC as described here.

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