Column chromatography of some methionine peptides

Column chromatography is useful for the separation of peptides, but little systematic information is available to help predict elution time. The present note is a step in this direction; it describes the elution behavior and color yield of 22 methionine peptides.

Experimental

Compounds. Samples of chromatographically homogeneous L-peptides, analyzed for C, H, N and S and with specific rotation data, were obtained from Mann Research Laboratories, Inc., New York City.

Chromatography. Chromatography was done with a commercial semiautomatic analyzer system (Technicon Corp., Ardsley, N.Y.) by the regular procedure recommended by the manufacturer, salient details of which are given elsewhere[1]. Compounds were run repeatedly, in amounts varying between 0.01 and 0.80 μmole and the peaks independently measured on two instruments, both by dot-count and automatically with a CRS-10A digital integrator (Infotronics Corp., Houston, Texas). Cysteic acid was used as the standard[2]. It emerges with the sample front, at one void volume, and elution times were measured from this peak. Molar color yields are in terms of the ratio of peak area for peptide to peak area for cysteic acid.

TABLE I

ELUTION TIME AND COLOR YIELD OF 22 METHIONINE PEPTIDES

Peptide	*Elution time (min after cysteic acid)*	*Molar color yield peptide/ cysteic acid*	*Emerges near or between*
MetAsp	494	1.22	Met-Ileu
MetSer	540	1.12	Ileu-Leu
MetGlu	581	1.11	Leu-Tyr
MetGlyGly	614	1.08	
MetGly	614	0.91	Tyr-Phe
GlyMet	619	0.61	
AlaMet	623	1.34	
MetAla	636	1.08	Tyr-βAIB
MetMetAla	664	0.91	βAIB-Eth-NH_2
MetGlyMetMet	683	0.64	
ValMet	684	0.24	
MetGlyMet	690	0.82	Eth-NH_2
MetLeuGly	706	1.0*	
MetHis	715	0.8*	NH_3
MetVal	718	1.2*	
MetMet	723	1.08	OH–Lys
MetMetMet	751	0.93	
MetLeu	771	0.97	
MetTyr	832	0.95	Orn
MetPhe	870	0.90	
MetPheGly	870	0.88	Lys
PheMet	887	0.92	Try

* Approximate; ammonia interference.

Results and discussion

Table I shows the results obtained. The nearest amino acids are indicated, for comparison (methionine emerges at 470 min). A number of elution data for other peptides are available in the literature[3,4], the methods used being sufficiently similar to, but not exactly the same, as that used here.

Inspection of the assembled information suggests some tentative empirical generalizations concerning peptide chromatography on cation exchange resins. (*a*) Neutral dipeptides having the same N-terminal substituent appear in the same sequence as the C-terminal substituent would if it were free, but later (MetHis is an apparent exception). With HAMILTON's[3] system the time difference is regularly about 210 min; with the Technicon system the difference decreases with increasing elution time and ranges from 350 to 220 min. If the N-terminal substituent is glutamyl, the order is generally the same, but the dipeptide may appear before either residue would if it were free or, with increasing elution time for the carboxyl substituent, between the positions the two would occupy as free amino acids. Glu-β-amino acid peptides emerge earlier than their sequence as free amino acids would suggest. (*b*) Neutral dipeptides with the same C-terminal residue appear in the sequence the N-terminal residue would if it were free, but 120–300 min later. (*c*) Reversal of substituents of neutral dipeptides (for example, AlaMet and MetAla) does not greatly affect elution time. (*d*) Neutral di- and tripeptides of similar structure (for example, MetAla and MetMetAla) emerge closely. (*e*) Molar color yield decreases with increasing chain length. (*f*) Effect of increasing chain length on elution time is not empirically predictable.

It will be useful to see how well future results with other peptides bear out these suggestions.

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