Short Communication

A Suggested Simple Chromatographic Method for Confirming the Amino Acid Analyses of Small Peptides

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Pardee (1951) derived the following theoretical relationship between the number of amino acid residues (n) in the peptides and their paper-chromatographic mobilities expressed as R_M values:

$$R_{M(\text{peptide})} = \frac{(n-1)A+B}{RT} + \sum R_{M(\text{amino acid})}$$

where A and B are constants. This equation was verified by using the chromatographic results reported in the literature for a number of peptides (Knight, 1951; Cook & Levy, 1950), and Pardee (1951) suggested that his equation could be used to check the molecular weight of a peptide of known amino acid composition and to confirm the experimental $R_F(\text{peptide})$ value. Moore & Baker (1958) demonstrated that this equation could account for the paper-chromatographic mobilities of a further 35 small peptides in nine solvent systems. These authors also stated that 'comparison of the R_F value for a peptide with those for the constituent amino acids showed no regular pattern'.

Notwithstanding the existence of these two papers (Pardee, 1951; Moore & Baker, 1958) relating the chromatographic properties of peptides to those of their constituent amino acids, no reports have yet been published whereby the results of amino acid analyses of the small peptides found in protein hydrolysates or in physiological fluids have been confirmed by the interpretation of the R_F value of the intact peptide. In view of the importance of the determination of the structure of peptides we decided to reinvestigate the relationship between the structure and chromatographic mobility of small peptides. We studied t.l.c. systems because of their speed and sensitivity and chose to use cellulose as the support, since such systems correspond well with paper chromatography (Schorn, 1964).

The R_F values of 26 commercially available synthetic di- and tri-peptides and their component amino acids were measured on thin layers of cellulose powder (Macherey Nagel MN 300) in a variety of neutral, acidic and basic solvent systems including (I) propan-2-ol-butanone-1M-hydrochloric acid

(12:3:5, by vol.) and (II) 2-methylbutan-2-ol-butanone-propanone-methanol-water-aq. NH_3 (sp.gr. 0.88) (10:4:2:1:3:1, by vol.) by the procedures described by Heathcote & Haworth (1969).

An empirical analysis of the chromatographic results given in Table 1 suggests that the following relationship exists between $R_F(\text{peptide})$ and the R_F values of the n component amino acids:

$$R_F(\text{peptide}) = (n-1)S_1 + (n-2)S_2 + \sum R_F(\text{amino acid})$$

where S_1 and S_2 are constants for the system. For the solvent system (I) these constants have the values -0.45 and $+0.03$, and for the solvent system (II) the values are -0.17 and 0.00 respectively. Published paper-chromatographic results (Moore & Baker, 1958) were then used to test the applicability of this simple equation to higher peptides for the solvents (III) propan-1-ol-water-aq. NH_3 (sp.gr. 0.88) (6:1:3, by vol.) and (IV) butan-1-ol-acetic acid-water (4:1:5, by vol.) (upper phase). The corresponding solvent constants S_1 and S_2 were found to be -0.40 and 0.00 , and -0.62 and -0.16 , respectively.

To discuss the validity of this equation it is necessary to determine quantitatively the range of reproducibility of the R_F results and to compare this with the differences shown in the column in Table 1. The t.l.c. R_F results given in this table are the mean values obtained from at least three determinations on separate plates and generally lay within the range ± 0.02 ; thus a δ value greater than 4 in Table 1 would imply that the equation was incorrect. Only eight of the 54 calculated R_F values differed by more than 0.04 from the experimental values. For the paper-chromatographic results Pardee (1951) adopted the value of $R_F \pm 0.04$ for the allowed deviation of duplicate determinations; thus a δ value greater than 8 in Table 1 would imply that the equation was incorrect. Only four of the 53 calculated R_F values differed by more than 0.08 from the experimental values. By using the same range of reproducibility, the application of Pardee's (1951) equation to the peptides studied leads to incorrect calculated R_F values in seven cases.

Table 1. Comparison of observed and calculated R_F values for small synthetic peptides on paper and thin layers of cellulose

Chromatography ...	$100 \times R_F$											
	T.l.c. (MN 300)			T.l.c. (MN 300)			Paper (Whatman no. 4)			Paper (Whatman no. 4)		
	(I)			(II)			(III)			(IV)		
	Obs.	Calc.	δ	Obs.	Calc.	δ	Obs.	Calc.	δ	Obs.	Calc.	δ
Peptide												
Ala-Ala	65	69	+4	26	19	-7	57	60	+3	39	40	+1
Ala-Asp	56	60	+4	1	3	+2	—	—	—	—	—	—
Ala-Glu	64	68	+4	5	5	0	—	—	—	—	—	—
Ala-Gly	50	49	-1	17	15	-2	50	48	-2	23	30	+7
Ala-Phe	94	94	0	52	56	+4	76	83	+7	62	64	+2
Ala-Ser	52	51	-1	17	24	+7	—	—	—	—	—	—
Gly-Ala	52	49	-3	18	15	-3	49	48	-1	27	30	+3
Gly-Asp	43	40	-3	0	-1	-1	33	17	-16	12	9	-3
Gly-Glu	—	—	—	—	—	—	24	23	-1	31	21	-10
Gly-Gly	34	29	-5	13	11	-2	41	36	-5	22	20	-2
Gly-His	7	3	-4	16	18	+2	—	—	—	—	—	—
Gly-Ile	81	82	+1	49	48	-1	70	72	+2	61	61	0
Gly-Leu	82	82	0	51	52	+1	71	71	0	61	57	-4
Gly-Lys	14	8	-6	11	10	-1	—	—	—	—	—	—
Gly-Met	—	—	—	—	—	—	64	63	-1	41	48	+7
Gly-Phe	75	74	-1	50	52	+2	—	—	—	—	—	—
Gly-Pro	47	50	+3	17	21	+4	—	—	—	—	—	—
Gly-Ser	32	31	-1	13	20	+7	41	37	-4	14	16	+2
Gly-Thr	—	—	—	—	—	—	48	45	-3	24	23	-1
Gly-Trp	—	—	—	—	—	—	64	62	-2	50	38	-12
Gly-Tyr	68	64	-4	28	24	-4	52	54	+2	41	38	-3
Gly-Val	72	71	-1	36	32	-4	58	65	+7	43	52	+9
Leu-Ala	97	>100	—	56	56	0	—	—	—	—	—	—
Leu-Gly	82	82	0	52	52	0	74	71	-3	54	57	+3
Leu-Val	100	>100	—	77	73	-4	—	—	—	—	—	—
Val-Gly	67	71	+4	38	32	-6	—	—	—	—	—	—
Val-Leu	100	>100	—	80	73	-7	—	—	—	—	—	—
Ala-Gly-Gly	47	47	0	13	12	-1	45	46	+1	20	25	+5
Gly-Ala-Gly	—	—	—	—	—	—	48	46	-2	24	25	+1
Gly-Gly-Ala	—	—	—	—	—	—	47	46	-1	23	25	+2
Gly-Gly-Gly	32	27	-5	8	8	0	34	34	0	15	15	0
Gly-Gly-Leu	—	—	—	—	—	—	68	69	+1	54	52	-2
Gly-Gly-Phe	—	—	—	—	—	—	64	69	+5	49	50	+1
Gly-Leu-Gly	—	—	—	—	—	—	73	69	-4	58	52	-6
Gly-Phe-Gly	—	—	—	—	—	—	66	69	+3	47	50	+3
Leu-Gly-Gly	—	—	—	—	—	—	—	—	—	50	52	+2
Val-Gly-Gly	65	69	+4	28	29	+1	—	—	—	—	—	—
Gly-Gly-Gly-Gly	—	—	—	—	—	—	32	32	0	12	8	-4
Gly-Ser-Asp-Gly	34	32	-2	5	2	-3	—	—	—	—	—	—
Gly-(Gly) ₃ -Gly	—	—	—	—	—	—	30	30	0	8	5	-3

A further check on the essential validity of our equation is provided by the observation that the isomeric pairs Gly-Ala, Ala-Gly; Ala-Leu, Leu-Ala; Gly-Val, Val-Gly; Gly-Leu, Leu-Gly; and Gly-Ala-Gly, Gly-Gly-Ala; have identical chromatographic mobilities in the systems studied.

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