Note

Use of a programmable pocket calculator in processing amino acid analysis data

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Modern methods of automatic amino acid analysis have reduced the time required for the analysis of protein hydrolysates to less than 2 h, so that about 10 hydrolysates can be analysed in 24 h.

In order that calculations of amino acid composition can keep pace with the data output of modern amino acid analysers, it is necessary to use electronic computation (for review see ref. 1). Computer programs can be written to make full use of the analysis data, providing in addition to a print-out of the amount of each amino acid, a print-out of the composition of the protein hydrolysate in a number of alternative forms, *e.g.* amino acid residues/1000 residues, μg nitrogen, % nitrogen, % amino acid, or amino acid composition/molecular weight. The use of computers for dealing with analysis data is most efficient, but computer facilities are not necessarily available to everyone carrying out amino acid analyses, and it may also be necessary to have some knowledge of one of the computer languages if specialist help is not available. Damoglou[2] has described the use of a desk-top computer for the rapid calculation of amino acid analysis data.

There are available small electronic calculators capable of a limited number of program steps, and these calculators can be easily programmed to carry out simple, repetitive routine calculations. I would like to outline the use of one of these small programmable electronic calculators in calculating amino acid analysis data. The program described in this paper and included in the Appendix has been written for the Hewlett-Packard HP25, which is a programmable calculator with the capability of carrying out 49 separate program steps.

METHODS

The calculation of the amino acid composition on which the program is based uses the ratio of the area of each amino acid in the hydrolysate to the area of an internal amino acid standard (A_x/A_{Nle}). The following expression defines the calculation executed by the program:

$$\text{nmoles of amino acid } x = \frac{A_x}{A_{Nle}} \cdot \text{CF} \cdot 40$$

where A_x is the area of the amino acid, A_{Nle} the area of the norleucine standard and CF is the colour factor defined as the ratio A_{Nle}/A_x; this ratio is obtained from a

standard chromatogram. The amount of each amino acid used to obtain the standard chromatogram is 40 nmoles, hence 40 appears in the above expression.

Fig. 1 is a block diagram of the program scheme. Two programs are shown in this scheme, and both programs have the first 28 steps in common. Program A calculates the color factor for each amino acid, and program B calculates the amount of each amino acid present in the sample hydrolysate. In program A, steps 01–32 are used to calculate the CF for each amino acid. This calculation is carried out using the data from a calibration analysis of a standard amino acid mixture containing norleucine (Nle) as an internal standard. It can be seen that in this scheme some parts of the calculation routine are repeated in each program, *i.e.* the group of operations contained in steps 01–15 are similar to those in steps 16–27. Both these groups calculate amino acid peak areas. Steps 01–15 are used to calculate the Nle peak area and store it in register 2 (R_2) for use later in the calculation. Steps 16–27 calculate peak areas of the other amino acids in the analysis. When the calibration mixture is being analysed program A is used, and the amino acid CF is found by dividing the Nle peak area by the amino acid peak area (step 30). When doing the amino acid
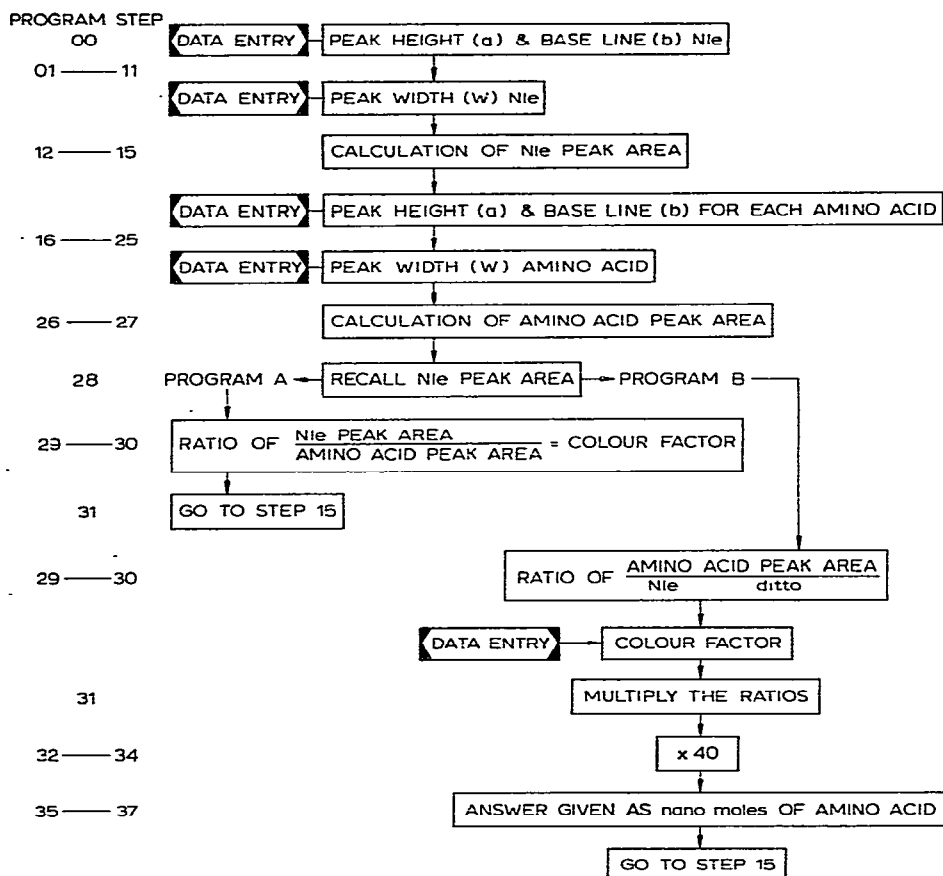

Fig. 1. Schematic representation of the two programs A and B. The sequence of operations is indicated by arrowed interconnecting lines and data entry points are shown.

analysis of a hydrolysate, program B is used. The program modification to convert program A to program B starts at step 29. Step 29 divides the area of the amino acid peak by the area of the Nle peak, *i.e.* the reciprocal of the ratio calculated by program A. Care should therefore be taken when using a program that the correct one has been entered in the calculator. The use of these programs is discussed in more detail in the Appendix.

Measuring amino acid peak areas

Normally each amino acid is displayed on the amino acid analyser recorder chart as a gaussian distribution of optical density *vs.* time, and the area under the peak represents the amount of amino acid present in the protein hydrolysate. The program uses the height–width method of calculating the peak area. To determine the peak area, it is necessary to measure the height of the peak, H, and the width of the peak, W, at half height. The width (W) is obtained by counting the number of dots on the top half of the peak, so that multiplying $H \times W$ gives the area of the amino acid peak. This method of calculating the peak area assumes that the peak is symmetrical. The accuracy of the amino acid analysis depends on the precision with which the measurement of H and W are made.

Calculating the Nle peak area (steps 01–15)

From Fig. 1 it can be seen that in the first part of the program the Nle peak area is calculated. To calculate this area steps 01–15 of the program are used. Step 14 stores the value for the Nle peak area in R_2 for later use.

Calculating the amino acid composition of the protein hydrolysate (steps 16–37 of program B)

The peak area of each amino acid ($H \times W$) in the hydrolysate is calculated using steps 16–27 of the program, and the areas converted to nmoles of amino acid using the above expression. The area of the amino acid peak, A_x, is already in the calculator (step 27), so step 28 recalls the area of Nle peak (A_{Nle}) from R_2 and step 29 gives the ratio amino acid peak area/Nle peak area (A_x/A_{Nle}). The color factor CF is now entered (data entry point) before step 31 and steps 31–34 calculate the nmoles of amino acid. This value is then stored in register R_5, *i.e.* step 35. The calculator is then ready to operate on the data for the next amino acid peak, *i.e.* step 36 sends the program back to the start of the calculation at step 16. When all the data from one analysis has been processed, the total amount of amino acid recovered is obtained by recalling the total from register R_5, *i.e.* press RCL 5.

Data entry points

There are five data entry points in this program. These entries are made before steps 01, 12, 16, 26 and 31, the last being an entry point in program B. Table I lists the data entry points and what data is entered in the calculator at these points. It is important to note that in every case the data for the Nle peak should be processed first.

RESULTS AND DISCUSSION

The complete program appears in the Appendix, along with an explanation of each step of the program. It takes about 1 min to enter the program and about 40 sec

TABLE I

SEQUENCE OF DATA ENTRIES AND THEIR POSITION IN THE PROGRAM

| Data entry	Comment
Before step 01: Nle peak height a followed by Nle baseline b.	Enter the two values a followed by b and run the program. The first calculator display is H (cf. Fig. 2). The next display, step 11, is the half height, i.e. $H/2 + b$ (cf. Fig. 2). The half height, given to 3 decimal places, is drawn on the Nle peak. The dot counting method is used to determine the width W of the Nle peak.
Before step 12: Enter number of dots representing the width W of the Nle peak.	The program calculates the area of the Nle peak (ANle) and stores the value in R_2.
Before step 16: Amino acid peak height a followed by baseline b.	These two values are entered as above for the Nle peak height and baseline values and the width of the amino acid peak determined.
Before step 26: Enter the width W of the amino acid peak.	This part of the program calculates the area of the amino acid peak (A_x).
Before step 31: Enter the colour factor CF for the appropriate amino acid.	This entry is only made when the program B is used.
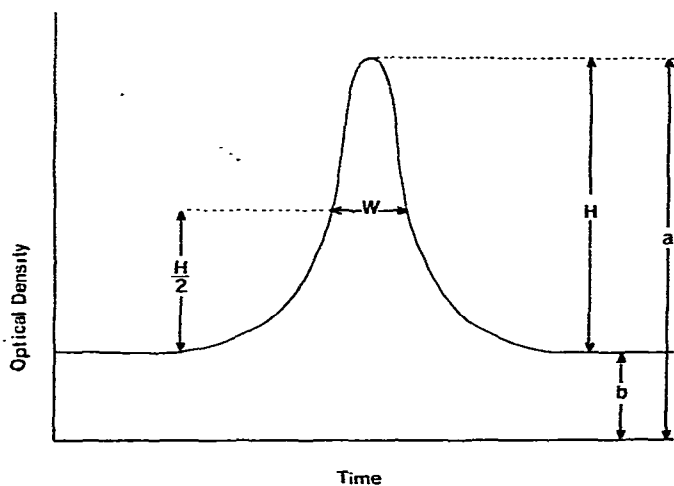
Fig. 2. A representation of an amino acid peak obtained from an analyser chart. a = Total peak height, b = baseline, H = net height, W = width of peak at the half height.

to check that it has been correctly entered. The program check is carried out using a calculation known to be correct. The average total calculation time for each peak is about 1.5–2.0 min, although this time could be reduced by an adept operator.

Certain intermediate steps in the calculation are displayed, i.e. steps 03 and 19, the net peak heights. It is not necessary to display these values, but some analysis records may require them to be noted, so they are included in this program.

The program illustrates the use of this type of calculator applied to amino acid analysis calculations and assumes that peak areas have to be calculated. If a peak area integrator is available, a considerable saving in time results and the program is much simpler than the one described. The advantages of using this type of calculator are that it is much cheaper than a computer and hence more readily available, no knowledge of programming language is necessary and therefore modifications to

the program can be made to suit the needs of either the individual or the format of the data. One disadvantage is that errors can occur in keying in the data or entering the program. A check calculation can be used to eliminate the latter error; however, errors in data entry can only be found by careful checks.

APPENDIX

Program A is used to calculate amino acid colour factors (CF). The data for this calculation is obtained by analysing a mixture of amino acids plus norleucine of known concentration. For amino acid analyses, steps 01–28 are combined with steps 29–37 to form program B. The data entry points are indicated in the program, and entries of peak heights, base line values and colour factors should be made only at these points. Instructions:

(1) To key in the program switch PRGM/RUN to PRGM mode and then press fPRGM. Now key in the program steps shown.

(2) To run the program switch PRGM/RUN to RUN press fPRGM and make first data entry.

Program A

Step	Key	Data entry	Comments	Data output
00		*a* then *b* Nle	Peak height and baseline for Nle	
01	Sto 0		Base line *b* stored in R_0	
02	—		$a - b$	
03	R/S			Net height *H*
04	Sto 1		Net height *H* stored in R_1	
05	.			
06	5			
07	X		Net half height $H/2$	
08	RCL 0		Baseline *b* recalled from R_0	
09	+		$H/2 + b$	
10	f FIX 3			Half height to 3 decimal places
11	R/S			
		W for Nle	*W* number of dots on top half of peak	
12	RCL 1		Recall net height *H*	
13	X		$H \times W$	
14	Sto 2		Area $H \times W$ stored in R_2	
15	R/S			Area of Nle peak (A_{Nle})
		a then *b*	Peak height and baseline for next amino acid	
16	Sto 3		Baseline *b* stored in R_3	
17	—		$a - b = H$	
18	Sto 4		*H* stored in R_4	
19	R/S			Net height (*H*)
20	.			
21	5			
22	X		Net half height $H/2$	
23	RCL 3		Baseline *b* recalled from R_3	
24	+		$H/2 + b$	
25	R/S			Net half height
		W for amino acid	*W* number of dots on top half of peak	

Program A (continued)

Step	Key	Data entry	Comments	Data output
26	RCL 4		H recalled from R_4	
27	X		$H \times W$ = area of amino acid peak	
28	RCL 2		Area of Nle peak recalled from R_2 Program A and B branch at this point	
29	X $\rightleftarrows$ Y		Exchange the contents of the X and Y registers	
30	÷		Nle peak area/amino acid peak area	
31	GTO 15		Program ready to process next data entry at step 16	
32	R/S			Colour factor for amino acid (CF)

Since steps 01–28 are common to programs A and B, B is entered in the program memory of the calculator as follows. If program A is in the calculator, then with the PRGM/RUN switch in the RUN mode press GTO 28 and switch to PRGM. Now key in the following program steps

Program B

Step	Key	Data entry	Comments	Data output
29	÷		Area of amino acid peak/area of Nle peak	
30	R/S			Ratio of peak areas (A_x/A_{Nle})
		CF for amino acid		
31	X			
32	4		Concentration of amino acid	
33	0		(40 nmoles in the standard solution)	
34	X			
35	Sto + 5		nmole of each amino acid stored additively in R_5	
36	GTO 15		Program ready to process next data entry at step 16	
37	R/S			nmoles of amino acid

When all the amino acid data has been processed the total amount of amino acid analysed (nmoles) is obtained by pressing RCL 5. This total can then be used in additional calculations to express the analysis as residues/1000 or other alternative forms.

REFERENCES

1 B. Sheldrick, *Qart. Rev., Chem. Soc.*, 24 (1970) 454.
2 A. P. Damoglou, *J. Chromatogr.*, 47 (1970) 257.