

CHROM. 7960

Note

Computer programs for calculations in amino acid analysis

J. LAMAZIÈRE and R. MIGLIERINA

Laboratory of Immunochemistry, Research Institute on Blood Diseases, Hôpital Saint-Louis, 2 place du Dr. Fournier, 75475 Paris Cedex 10 (France)

(First received July 15th, 1974; revised manuscript received September 16th, 1974)

Nowadays, amino acid analyzers permit the running of four, six or more chromatograms daily, which are generally automatically "read" by using a peak integrator. A constituent is characterized by its retention time and peak area measured at one or two wavelengths. A detailed assessment of the data is necessary in order to obtain values such as residues per mole or per gram of protein.

This paper describes computer programs that give these results easily and quickly.

EQUIPMENT AND METHODS

The study was carried out with a JEOL JLC 5AH amino acid analyzer and the integration of peaks was effected with a Model DK integrator with a printer giving the area at 570 and 440 nm for each peak.

The integration data were treated with a Hewlett-Packard 9810 A desk-top computer equipped with an "α-printer ROM" with a capacity for 500 instructions and 49 memories. The α-printer gives a final result in which every amino acid is listed by one or several letters according to the international code. Only one magnetic card was necessary to store all the instructions. For computers without an α-printer, another program is given in which the amino acids are listed by numerals ("dumb" program). The concentration of one amino acid is obtained by comparing the areas of the peaks for the sample and for a standard mixture analyzed under the same conditions (the standard mixture is composed of equal and known amounts of amino acids).

The number of residues, R_i , of an amino acid i is given by the following equation:

$$R_i = \frac{[S_i/S_i^{st}] M}{\sum (S_i/S_i^{st}) m_i}$$

where

- i (or j, k, \dots) = number of the amino acid according to the code (Fig. 1);
- | | | |
|------------|---|--|
| S_i | = sample peak area | } data obtained from an integrator or using the height-width method; |
| S_i^{st} | = standard mixture peak area | |
| m_i | = molecular weight of anhydro amino acid; | |
| M | = molecular weight of analyzed protein. | |

1 = W	= TRY	12 = S	= SER
2 = K	= LYS	13 = E	= GLU
3 = H	= HIS	14 = P	= PRO
4 = R	= ARG	15 = G	= GLY
5 = GLUC	= GLUCOSAMINE	16 = A	= ALA
6 = GAL	= GALACTOSAMINE	17 = C	= 1/2 CYS
7 = CY	= CYSTEIC ACID	18 = V	= VAL
8 = CMC	= CARBOXYMETHYLCYSTEINE	19 = M	= MET
9 = D	= ASP	20 = I	= ILE
10 = MS	= METHIONINE SULPHONE	21 = L	= LEU
11 = T	= THR	22 = Y	= TYR
		23 = F	= PHE

Fig. 1. Numbering and codes of amino acids.

The program does not use the internal standard because usually absolute concentrations are not required and the data for only one standard are used for the calculation of residues. In any event, a complete program cannot be carried out on our computer without additive memories and program steps. A short program has been written, using a second card that lists each amino acid (A.A.) and its concentration in the analyzed sample, after the calculation of residues (Fig. 2).

The capacity of the computer and the needs of the laboratory led us to treat not more than 23 amino acids and their derivatives possibly present in a sample. Besides

A. A. QUANTITIES NM IN STANDARD

DATE	10.00*
NAME	
W	0.00
K	46.99
H	12.08
R	28.74
GLUC	0.00
GAL	0.00
CY	15.98
CMC	0.00
D	65.45
MS	3.99
T	63.43
S	98.17
E	100.97
P	45.27
G	48.01
A	55.21
C	0.00
V	50.92
M	0.00
I	27.71
L	58.47
Y	40.62
F	41.35

Fig. 2. Results obtained in nanomoles.

the calculation and printing instructions, the program memorizes molecular weights according to the order in Fig. 1. The programming plan is given in Fig. 3.

The programs are used in the same manner and they differ only in the printing of the results. The order in which the data are entered has no influence. The "SET FLAG" instruction after the last entry starts the final calculation. The machine is self-prepared for a new run after printing the results. It is not necessary to disconnect the computer and to reload it with the program card.

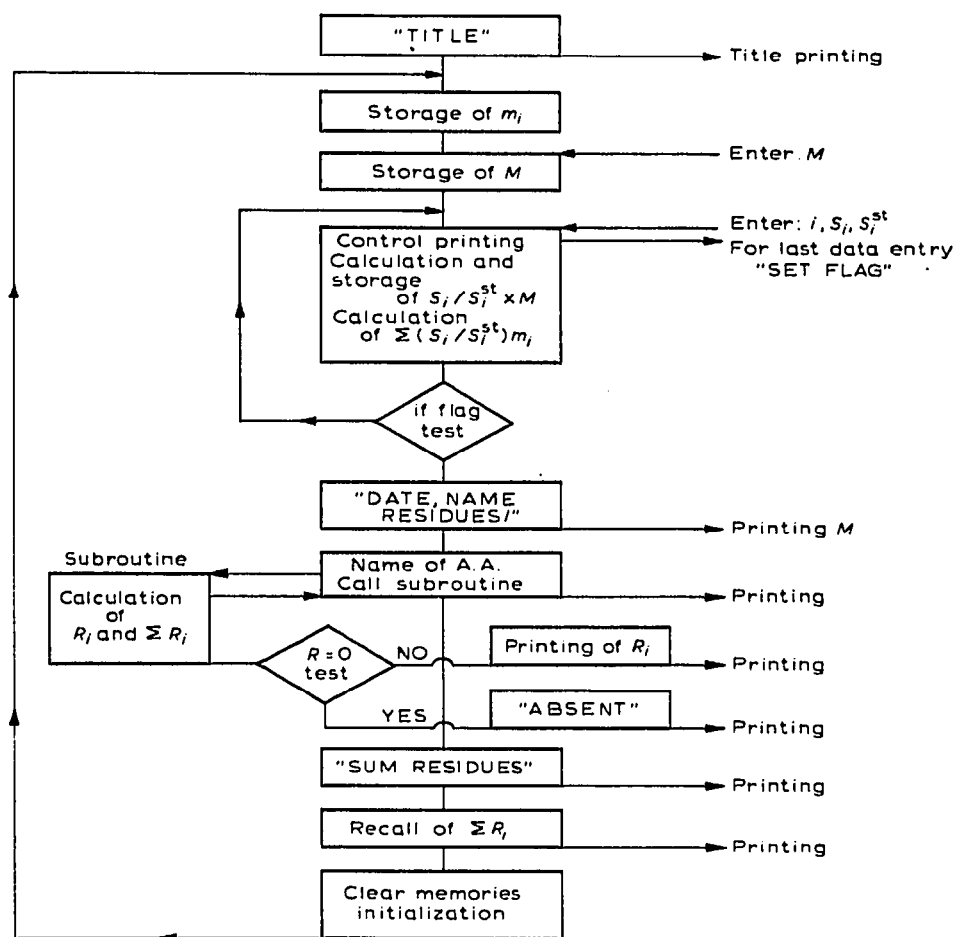


Fig. 3. Programming plan.

RESULTS AND DISCUSSION

The results obtained for protein analyses are given in Figs. 4 and 5 with and without the use of an α -printer. The user can exchange one or more amino acids written in the program by any other derivative.

This program considerably reduces the time required for the calculations. It

			22500.00
			1.00*
			0.00
			2.00*
			11.81
			3.00*
			0.04
			4.00*
			7.22
			5.00*
			0.00
			6.00*
			0.00
RESIDUES/22500.00			7.00*
W			4.02
	.ABSENT		
K			6.00*
H		11.81	0.00
R		3.04	
GLUC		7.22	9.00*
	.ABSENT		16.45
GAL			10.00*
	.ABSENT		1.00
CY			
CMC		4.02	11.00*
	.ABSENT		15.94
D			
MS		16.45	17.00*
T		1.00	0.00
S		15.94	
E		24.67	18.00*
P		25.38	12.00
G		11.38	
A		12.07	19.00*
C		13.87	0.00
	.ABSENT		20.00*
V			6.92
M		12.80	
	.ABSENT		21.00*
I			14.69
L		6.96	
Y		14.69	22.00*
F		10.21	10.21
		10.39	
			23.00*
			10.39
SUM RESIDUES			
		201.89	201.89

Fig. 4. Protein analysis with the program described.

Fig. 5. Protein analysis as in Fig. 3 but using the "dumb" program. (For convenience, the list has been cut between results 11 and 17.)

uses only one magnetic card, and can be developed in such a way as to obtain a "universal" program using internal and external standards and giving concentrations and residues using only one card. The performances are greater than those obtained with the procedures of Mondino¹ using 23 cards and Damoglou² using 6 cards. The only manual operation that remains is to enter the number and peak area. We are studying the storage of integration data on a punched tape that would be read directly by the computer, which would completely automate the method and avoid on-line connection of the computer and analyzer.

Copies of the programs, listings and user instructions on magnetic card are available from the authors on request.

REFERENCES

- 1 A. Mondino, *J. Chromatogr.*, 39 (1969) 262.
- 2 A. P. Damoglou, *J. Chromatogr.*, 47 (1970) 257.