AMINO-ACID COMPOSITION OF HUMAN CRYSTALLIZED MYOGLOBIN AND HAEMOGLOBIN*

by

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INTRODUCTION

In 1940 it was shown by one of us[1] that myoglobin (Mb) and haemoglobin (Hb) have a different chemical constitution. This result was later confirmed by other authors. The research on the chemistry of Mb has mainly been confined to the protein extracted from various types of animals (horse, cow *etc.*). With regard to the comparative chemistry of human Mb and Hb, only the N partition, iron and suphur analyses, and the content in few amino-acids[2] are reported in the literature. Recently the pattern of the amino-acids contained in the two proteins has been compared by filter paper chromatography[3]. In the present research we report the quantitative amino-acid composition of the two crystallized pigments as evaluated by the chromatographic method on ion-exchange resin developed by MOORE AND STEIN[4].

EXPERIMENTAL

The human Mb and Hb were purified, crystallized and hydrolized as previously described[3]. The quantity of protein present in each sample used for the chromatography was calculated on the base of the N content obtained in quadruplicate microkjeldahls. The values of 16.9% for crystalline Hb[5,6] and 16.7% for crystalline Mb[2] have been taken as the percentage nitrogen for the calculations. About 3 mg of hydrolysate were used for each determination. The technical procedure was essentially that proposed by the authors of the method[4], with the following slight variations. The elution and fractionation of the effluent were made in a closed space through which passed a flow of ammonia-free air. This was found necessary for minimizing the oscillation of the blanks[7]. The ninhydrin reagent was prepared as described by MOORE AND STEIN[8], with the difference that a half quantity of ninhydrin was used. Ninhydrin was a technical sample completely freed from ammonia by passing its solution in methylcellosolve through a column of Dowex 50 in the H-form. Citric acid, dissolved in water, was also passed through Dowex 50. The results were as good as with the original reagent with the advantage of a much lower cost. All the fractions (1 ml) were boiled with the ninhydrin reagent for 30 min. The optical density was read with a Coleman spectrophotometer. When density values greater than 1.0 were obtained, the samples were diluted to the appropriate amount in order to bring the reading below 1.0. Standard curves for any one of the amino-acids were made in the same conditions. We did not obtain as good a separation of proline from glutamic acid as that reported by other authors, especially when glutamic acid was present in large quantity. By taking a double reading at 570 and 440 mμ we obtained satisfactory quantitative data, even when proline was completely covered under the glutamic acid peak. The following formulae were utilized for the calculation:

$$O.D._{570} = \frac{L_{570} - L_{440} \times \alpha}{\gamma}; \quad O.D._{440} = \frac{L_{440} - L_{570} \times \beta}{\gamma}$$

where $O.D.$ represents the optical density at 570 mμ for glutamic acid and at 440 mμ for proline,

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used for the integration; L is the photometric reading at 570 and 440 mμ, subtracted from the respective blanks; α and β are constants representing the ratios of $O.D.$ at 570 and 440 mμ for proline and at 440 and 570 mμ for glutamic acid, calculated separately on pure samples; γ is the constant $1 - \alpha \times \beta$.

The quantitative value reported for cystine in the case of Hb is not definitive since the present method is not appropriate for the determination of this amino-acid[9]. The values for tryptophan and amide nitrogen are included here for completeness and are from data reported elsewhere[10].

RESULTS

In Figs. 1–4 are given the elution curves of typical experiments of 24 hours hydrolysate of Mb and Hb with long and short columns. Table I shows the percentage values of amino-acids obtained by taking the mean average of three different determinations. The percentages of the amino-acids obtained for Hb are in fairly good accord with the results reported by SCHROEDER, KAY AND WELLS[6], using starch chromatography. The differences found, namely in the case of phenylalanine, may be imputable to the different method used rather than to the different race of the Hb donors; SCHROEDER *et al.* analysed Hb of negro subjects. We found it impossible to determine

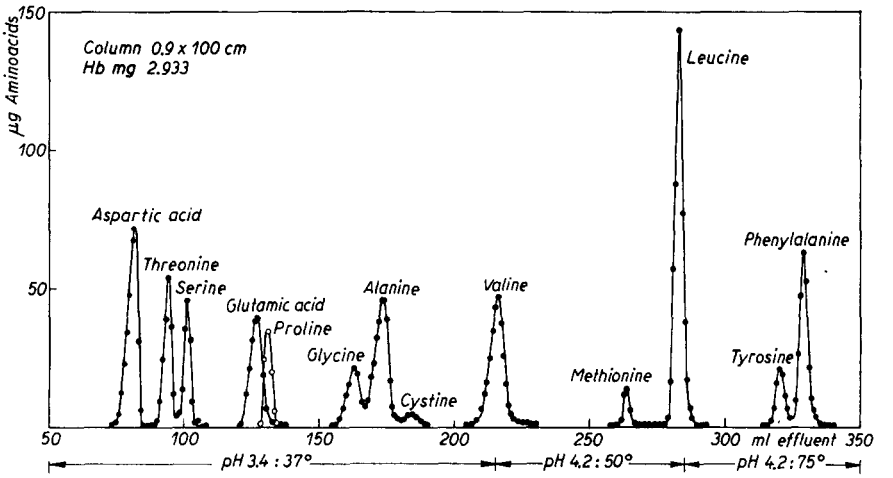

Fig. 1. Elution curve of hydrolysate of crystalline human Hb determined by the ninhydrin method on a Dowex 50 column 0.9 × 100 cm, for the acidic and neutral amino-acids. Ordinate: amount of amino-acid in µg; abscissa: ml of effluent fractionated in 1 ml amount.

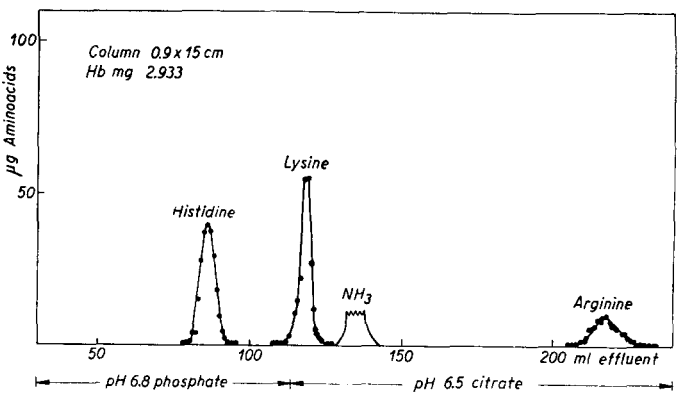

Fig. 2. Elution curve for the determination of the basic amino-acids on a 0.9 × 15 cm column of an hydrolysate of crystalline human Hb. Conditions as in Fig. 1.

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the *iso*leucine content in contrast with SCHROEDER *et al.* who report a result of 0.21% using starch chromatography.

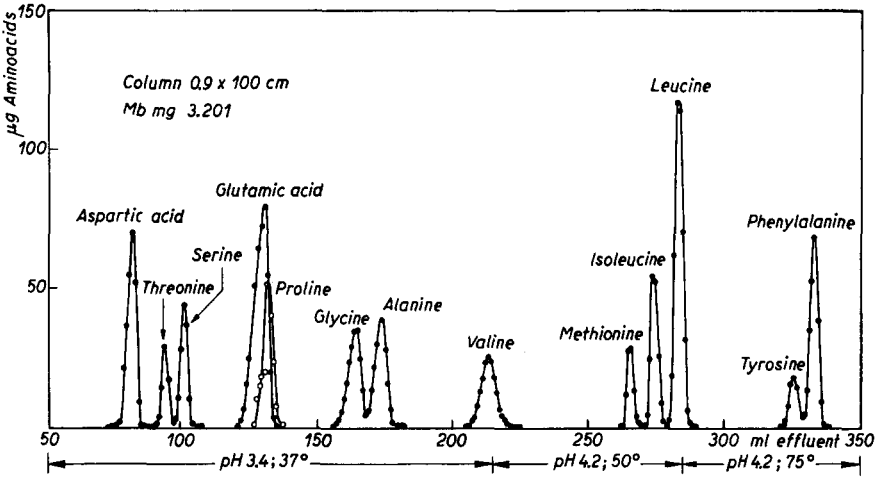

Fig. 3. The same as in Fig. 1 for an hydrolysate of crystalline human Mb.

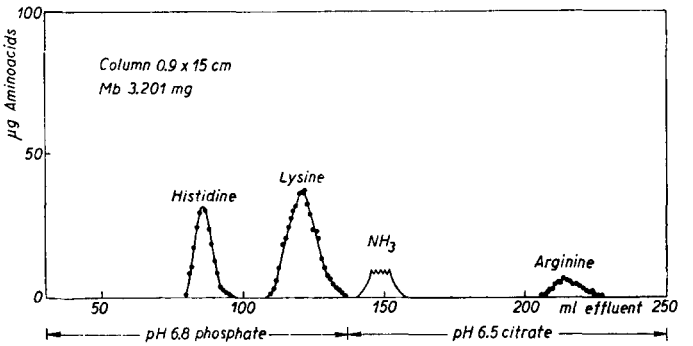

Fig. 4. The same as in Fig. 2 for an hydrolysate of crystalline human Mb.

The data in Table I show that the proteins are formed by a significantly different mixture of amino-acids. The human Mb is richer in glutamic acid, lysine, glycine and methionine; poorer in threonine, alanine, valine and arginine. We found again, as previously reported that *iso*leucine is present in Mb and absent in Hb, while cystine is absent in Mb but present in Hb.

From the above reported data it has been possible to evaluate the distribution of the polar and non-polar groups in the molecules of the two pigments. These values, according to BAILEY[11], are extremely useful for characterizing the chemical nature of the proteins. As the data in Table II show, the two proteins have a prevalently basic nature since they present in the molecule a larger number of cationic than anionic groups. The total number of the ionic groups (cationic plus anionic) is different in the two proteins. Mb has a higher number of ionic groups so that it is a stronger electrolyte than Hb. In this respect human Mb and Hb resemble the corresponding horse pigments except for some small quantitative differences.

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TABLE I

COMPOSITION OF HUMAN CRYSTALLINE Hb AND Mb (AVERAGE VALUES OF THREE DETERMINATIONS)

	Hb			*Mb*		
	Grams amino-acid per 100 g protein	*N as % of total N*	*Assumed no. of residues*	*Grams amino-acid per 100 g protein*	*N as % of total N*	*Assumed no. of residues*
Aspartic acid	9.99	6.21	51	8.27	5.30	10
Threonine	6.03	4.20	34	2.85	2.04	4
Serine	5.07	4.00	33	4.43	3.59	7
Glutamic acid	7.41	4.17	34	16.17	9.39	19
Proline	5.02	3.61	30	5.40	4.00	8
Glycine	4.28	4.73	39	6.08	6.89	13
Alanine	9.83	9.11	75	5.82	5.58	11
Cystine	0.89	0.59	5	0.00	—	—
Valine	11.00	7.78	64	4.64	3.38	6
Methionine	1.23	0.68	5.5	2.69	1.53	3
Isoleucine	0.00	—	—	5.27	3.41	6
Leucine	14.90	9.41	77	13.67	8.90	18
Tyrosine	2.90	1.32	11	2.19	1.03	2
Phenylalanine	9.62	4.83	39	8.22	4.25	8
Histidine	8.49	13.61	37	7.79	12.86	8
Lysine	10.64	12.07	49.5	19.09	22.31	22
Arginine	3.48	6.63	14	2.47	4.82	2
Tryptophan	2.00	1.60	11	3.40	2.84	3
Amide-N	(1.13)	5.86	48	(1.22)	6.57	13
Total	112.78	94.55	609	118.42	102.12	150

TABLE II

Derived data from Table I*

	Haemoglobin	*Myoglobin*
Mean residue weight	109.5	112.4
Total number residues	621	151
Groups % of total groups		
Cationic	16.2	21.2
Free anionic	5.9	10.6
Total ionic	22.1	31.8
Amide	7.7	8.6
Total polar	45	51
Lipophilic	53	48.3

* Following SPRINGALL[12].

SUMMARY

The amino-acid composition of human crystallized myoglobin and haemoglobin has been analysed by quantitative ion-exchange chromatography. Qualitative and quantitative differences in the amino-acid content indicate that the two proteins are substantially different in composition.

RÉSUMÉ

On a analysé à l'aide de la chromatographie quantitative par échangeur d'ions la composition en acides aminés de l'hémoglobine et de la myoglobine humaines crystallisées. Les différences quantitatives et qualitatives trouvées font prévoir pour les deux protéines une structure complètement différente.

ZUSAMMENFASSUNG

Mit Hilfe der quantitativen Ioneaustauschchromatographie wurde die Aminosäurezusammensetzung des menschlichen kristallisierten Myoglobins und Haemoglobins untersucht. Es wurden quantitative und qualitative Unterschiede gefunden, die für eine gänzlich verschiedene Struktur der beiden Proteine sprechen.

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