Amino acid composition of human haemoglobin A_2

Haemoglobin is not a homogeneus protein[1–3]; by means of starch-block electrophoresis KUNKEL *et al.*[4,5] separated a component (Hb A_2), comprising about 2.5 % of normal uncrystallized adult haemoglobin, which moved more slowly than the main component in electrophoresis. A faster-moving component was also described. By means of ion-exchange chromatography it was shown by ALLEN *et al.*[6] and by CLEGG *et al.*[7] that various components are present in haemoglobin, even after crystallization; as yet no direct correspondence has been established between the chromatographic and electrophoretic fractions.

In a previous paper[8] we reported the amino acid composition of crystallized adult-human haemoglobin; in order to obtain a better picture of the chemical composition of human haemoglobin we have thought it of particular interest to isolate haemoglobin A_2 and to determine its amino acid content.

Human-adult haemoglobin from normal individuals was crystallized according to DRABKIN[9]. After repeated washing of the crystals in phosphate and dialysis against water, the solution was subjected to starch-block electrophoresis, using a 45 cm long, 30 cm wide, 8 mm thick preparative plate, in glycine–NaOH buffer, pH 8.8, I 0.12 as described by SILVESTRONI *et al.*[10,16], applying a potential of 300 V for 24–36 h in the cold room. About 700 mg haemoglobin were fractionated in each electrophoretic run. Under these conditions the slow component moves towards the cathode owing to the prevalence of electroosmosis, and a good separation from the main fraction is obtained. The starch zones containing haemoglobin A_2 were cut off from several such electrophoretic plates, and the pigment was eluted with water on a sintered-glass funnel. In order to obtain a better purification, the pooled A_2 fractions were lyophilized, and again submitted to electrophoresis on starch block and eluted. Haemoglobin A_2 thus obtained behaves like an electrophoretically homogeneous protein,

TABLE I

AMINO ACID COMPOSITION OF HUMAN HAEMOGLOBIN A_2

	g amino acid/100 g protein				
	I	*II*	*III*	*IV*	*average*
Aspartic acid	10.725	11.118	10.399	11.300	10.885
Threonine	4.241	4.300	4.200	4.161	4.225
Serine	5.899	6.188	6.060	5.960	6.026
Glutamic acid	7.304	7.667	7.642	7.848	7.615
Proline	4.090	3.747	4.529	3.832	4.049
Glycine	4.186	4.343	4.090	4.095	4.178
Alanine	7.504	7.783	7.343	7.709	7.584
Valine	7.449	7.354	7.544	7.149	7.374
Methionine	1.346	1.429	1.204	1.556	1.383
Isoleucine	1.019	1.145	1.095	1.199	1.114
Leucine	11.730	12.198	11.784	11.235	11.736
Tyrosine	2.744	2.969	2.656	2.904	2.818
Phenylalanine	6.574	6.417	7.016	6.379	6.596
Histidine	5.850	5.661	5.773	—	5.761
Lysine	8.650	8.470	8.667	—	8.595
Arginine	3.283	3.341	3.874	—	3.499

I, II, III and IV refer to four different analysis carried out on various amount of protein (ranging from 1.4–3 mg) from the same hydrolysate.

and migrates like the A_2 component of fresh crystalline haemoglobin analyzed on the same starch block or on paper[11].

After dialysis against water the pigment was denaturated in a boiling-water bath for 15 min; the precipitate was repeatedly washed to remove traces of glycine that could have escaped the dialysis, and traces of soluble starch possibly present; it was then hydrolized for 24 h in 6 *N* HCl under reflux. Amino acid analysis were performed in quadruplicate by chromatography on Dowex 50-X 8, according to MOORE AND STEIN[12] with the modifications previously reported[8]. The quantity of protein present in each sample used for chromatography was calculated on the basis of the N content determined by micro-Kjeldahl determinations in quadruplicate, assuming that the haemoglobin contained 16.9 % N. Values for serine and threonine were not corrected for decomposition on hydrolysis[13]. Cystine was detected as cysteic acid by paper chromatography. The results given in Table I show that haemoglobin A_2 is different both from adult and fetal human haemoglobin[8,14].

The presence of isoleucine is the most striking difference between haemoglobin A_2 and the main fraction, which lacks this amino acid[15]. It is interesting to note that ALLEN *et al.*[6] have observed the presence of isoleucine in the minor components they separated from human haemoglobin by chromatography on Amberlite IRC-50.

Haemoglobin A_2 is the chief minor component separated from crystallized haemoglobin by starch electrophoresis[4], and it is generally assumed not to exceed 2.5 % of the total pigment[4,5,16,17]. Therefore the isoleucine content of haemoglobin A_2 (1.1 %) agrees well with the finding that isoleucine is not detectable by column chromatography[12] in crystallized preparations of adult-human haemoglobin[8,18].

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