The N-terminal residues of five different human haemoglobins

Since the introduction of SANGER's dinitrophenyl (DNP) procedure for the identification of N-terminal residues in proteins[1] this technique has also been applied to some human haemoglobins. PORTER AND SANGER[2] for instance found 5 valyl groups in N-terminal position in the normal adult haemoglobin (Hb-A), while in the haemoglobin from cord blood only 2.6 N-terminal residues of the same amino acid were present. Later, HAVINGA[3] described the presence of 5 N-terminal valyl residues in sickle cell haemoglobin (Hb-B).

In the experiments described below the number of different human haemoglobins studied in this way is extended to Hb-C and Hb-E, while moreover the procedure is repeated for the purified foetal haemoglobin (Hb-F). The Hb-A was obtained from blood samples of laboratory workers; the Hb-B from patients with a sickle cell anaemia; the Hb-C from patients with homozygous Hb-C disease[4] and the pure Hb-F from some samples of cord blood, the adult component being removed by the alkaline denaturation technique described by CHERNOFF[5]. The Hb-E was derived from the blood of a patient with the heterozygous Hb-E disease[6]. As we had not yet succeeded in separating this abnormal haemoglobin from the adult component the estimation was carried out using a mixture of these two haemoglobins (55.5 % Hb-A and 44.5 % Hb-E, estimated by the Tiselius moving boundary method). The haemoglobins were prepared by the method previously described[7] and then converted to globin.

SANGER's DNP procedure was essentially used for the analysis of the N-terminal amino acids: the DNP-globin, obtained by treating the protein with FDNB[2], was hydrolyzed by refluxing with 6 *N* hydrochloric acid for 48 hours. The dinitrophenyl amino acids were extracted from the acid hydrolysates into ether and analysed in the dark by the paper chromatographic method recently developed by LEVY[8]. The identification was carried out by adding known DNP-amino acids. Quantitative results were obtained by cutting out the spots and extracting them with water. The optical densities at 360 mμ were read in the Beckman model DU spectrophotometer.

Only valine was present as N-terminal group in the five human haemoglobins studied. The quantitative results are given in Table I. The assumed mol. wt. of the proteins was 66,000. A breakdown correction factor of 1.55 was used as 65 % of DNP-valine remained during the acid hydrolysis.

TABLE I

N-TERMINAL RESIDUES OF FIVE HUMAN GLOBINS

Globin	*Number of valine residues*	*Mean*	*Assumed*
Hb-A	4.7; 4.2; 5.0	4.6	5
Hb-B	4.85; 4.8	4.8	5
Hb-C	4.8; 4.7	4.75	5
Hb-E	5.1		5
Hb-F	2.5; 1.8; 2.4; 2.0	2.2	2

From the data presented in Table I it will be clear that the haemoglobins A, B, C and E contain five molecules of valine as N-terminal amino acid, while in the purified foetal haemoglobin only 2 N-terminal valyl groups are present. The results of the analyses of Hb-A, Hb-B and Hb-F are in good agreement with the data mentioned above. It will be noted that both the number of N-terminal residues and the amino acid composition of the haemoglobins A, B, C and E[4,6] are nearly the same, while the Hb-F differs from all these haemoglobins in many respects[7]. The differences between the haemoglobins A, B, C and E may be caused by changes in the structure of the polypeptide chains of these proteins, the foetal component being a quite different protein.

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