A HAEMOGLOBIN CONTAINING ONLY δ-CHAINS

N. Dance and E.R. Huehns*

Department of Biochemistry, University College London, England

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The simultaneous in vivo occurrence of haemoglobins which do not contain α-chains, namely haemoglobin H (β_4^A) (Rigas et al., 1955; Gouttas et al., 1955; Jones et al., 1959) and haemoglobin "Bart's" (γ_4^F) (Ager and Lehmann, 1958; Hunt and Lehmann, 1959; Kekwick and Lehmann, 1960) has been reported in a number of individuals (Ramot et al., 1959; Huehns et al., 1960; Fessas, 1960; Silvestroni et al., 1960). Recently, a further abnormal haemoglobin besides haemoglobins H and "Bart's" has been detected in two individuals with haemoglobin H disease (Huehns, 1962), and this haemoglobin has now been isolated from the red cells of one of them (Case 1, Bingle et al., 1958) by column chromatography followed by starch block electrophoresis. Analysis of the peptides obtained by tryptic hydrolysis and recombination experiments have shown it to consist solely of δ^{A_2}-chains.

Method and Results

Haemolysates were prepared from the washed red cells by the addition of toluene and water. The haemoglobin solution was dialysed for 18 hours against a 0.01M sodium phosphate buffer pH 7.0 before application to a column of carboxymethyl cellulose which had already been equilibrated with the same buffer. Most of the haemoglobin A was retained on the column. The eluted haemoglobin solution was concentrated in vacuo with concurrent dialysis against the same buffer before

* Beit Memorial Fellow

being subjected to starch block electrophoresis in a barbiturate buffer pH 8.6 and I 0.05. Three haemoglobin zones were resolved by this analysis. The pigment of the slowest of these zones was eluted, concentrated and again subjected to starch block electrophoresis, this time using a 0.04M sodium phosphate buffer pH 7.0. Two haemoglobin zones were seen, one of which migrated with the same mobility as haemoglobin A. The haemoglobin of the other zone, which migrated nearer to the anode, was eluted and concentrated. This haemoglobin was used in the recombination and "fingerprint" experiments described.

Recombination of this haemoglobin with haemoglobin α^A (Huehns et al., 1961) at pH 7.0 and 25°C produced one new haemoglobin zone which on starch gel electrophoresis migrated with haemoglobin A_2. Comparison of the tryptic hydrolysate of this haemoglobin with that of haemoglobin A_2 by electrophoresis followed by chromatography as described by Baglioni (1961) showed that those peptides present in the peptide pattern of haemoglobin A_2 which arise solely from the α-chain were absent and the remaining peptides corresponded to those of the δ^{A_2}-chain. In particular, the two peptides found only in the hydrolysate of haemoglobin A_2, called peptide A_2T-26 and peptide A_2a by Ingram and Stretton (1961) were clearly visible.

Discussion

The small number of peptides found in the tryptic hydrolysate shows that this haemoglobin consists only of one type of polypeptide chain. The absence of the peptides derived from the α-chain and the presence of peptides A_2a and A_2T-26 indicate that this is the δ^{A_2}-chain. The formation of a new species with an electrophoretic mobility identical with that of haemoglobin A_2 in the recombination of the newly isolated haemoglobin with haemoglobin α^A confirms that this haemoglobin contains a polypeptide chain with the same net charge as the δ^{A_2}-chain of haemoglobin A_2. This haemoglobin is therefore called haemoglobin δ^{A_2}.

It is of particular interest that the recombination of haemoglobins δ^{A_2} and α^{A} occurred at 25°C and neutral pH. The only other haemoglobins which are known to react with haemoglobin α^{A} under these conditions are haemoglobin H (Huehns and Shooter, 1962) and haemoglobin "Bart's" (Huehns and Beaven, 1962) which also consist of a single type of polypeptide chain.

Starch gel electrophoresis in phosphate buffer pH 7.4 shows that haemoglobin δ^{A_2} migrates between haemoglobins "Bart's" and A, and examination of the haemolysates from the three other individuals with haemoglobin H disease available shows that each of these carries three abnormal haemoglobins migrating more rapidly than haemoglobin A, namely haemoglobins H, "Bart's" and δ^{A_2}. These four patients were found in three Greek Cypriot families living in London.

The isolation and positive identification of haemoglobin δ^{A_2} from the haemolysate of one individual with haemoglobin H disease and the probable presence of this haemoglobin in three others lend strong support to the hypothesis that this disease is caused by deficient α-chain synthesis and that the α-chains of haemoglobins A and A_2 arise from a common metabolic pool. As all normal adults carry haemoglobin A_2, it might be expected that all individuals with haemoglobin H disease would also carry haemoglobin δ^{A_2}. If it is assumed that in these cases the synthesis of δ^{A_2}-chains is proceeding at a normal rate, then the proportion of haemoglobin A_2 would be expected to be lower than normal if some δ-chains are used in the formation of haemoglobin δ^{A_2}, and this has been found to be so in all four cases examined here. Several other authors have also found a decreased proportion of haemoglobin A_2 in haemoglobin H disease and this suggests that haemoglobin δ^{A_2} might be present in many or all cases of this disease. Indeed, several reports (Fessas, 1960; Koler and Rigas, 1961) of a second abnormal haemoglobin in haemoglobin H disease which resembles haemoglobin δ^{A_2} rather than

haemoglobin γ_4^F have appeared and it is possible that these represent further examples of the haemoglobin described here.

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