A HAEMOGLOBIN CONTAINING ONLY α-CHAINS

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Although the in vivo occurrence of haemoglobins which do not contain α-chains, namely haemoglobin H (β_4^A) (Gouttas et al., 1955; Rigas et al., 1956; Jones et al., 1959) and haemoglobin "Bart's" (γ_4^F) (Ager and Lehmann, 1958; Hunt and Lehmann, 1959; Kekwick and Lehmann, 1960) is now generally accepted, the analogous haemoglobin α_4^A has not yet been found. In this report, the in vitro preparation of a haemoglobin consisting solely of α^A-chains is described and the significance of this finding briefly discussed.

Method

Haemolysates from washed normal human red cells were prepared either by the addition of water and toluene (Singer et al., 1951) or by breaking the packed cells with ultrasonic vibrations. The non-haem proteins in the haemolysate were removed by chromatography on carboxymethyl cellulose (CMC) using 0.01M phosphate buffer of pH 6.5 as eluting buffer. The haemoglobin was then eluted from the column with 0.04M phosphate buffer of pH 8.0. The eluted haemoglobin solution was dialysed against 0.08M acetate buffer of pH 4.7 and then applied to a column packed with CMC which previously had been equilibrated with 0.3M acetate buffer of pH 4.7. When all the haemoglobin had been applied, the column was developed with the 0.3M acetate buffer. The haemoglobin was eluted from the column in one broad peak. The eluate

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was bulked into consecutive 100 ml. fractions and concentrated with concurrent dialysis against water (Huehns and Shooter, 1961). The concentrated haemoglobin solutions were dialysed against phosphate buffer of pH 6.8 and I 0.02, the haemoglobin was converted to the carbonmonoxy form and then subjected to starch block electrophoresis in 0.04M sodium phosphate buffer of pH 7.0. Under these conditions all the concentrated fractions showed three haemoglobin zones, a major zone corresponding to haemoglobin A and two minor zones clearly separated from haemoglobin A, one nearer the anode and the other migrating more rapidly than haemoglobin A towards the cathode.

Results

The isolated haemoglobin from the zone nearest to the cathode had an absorption spectrum of a typical carbonmonoxy haemoglobin with the δ-band at 342.5 mμ and a ratio of the optical densities at 418 and 540 mμ of 10.3. The latter ratio for carbonmonoxy haemoglobin A is 10.5. This haemoglobin was also examined by the recombination technique (Itano and Singer, 1958; Gammack et al., 1960). When it was dissociated and recombined with haemoglobin G/C, $\alpha_2^G\beta_2^C$, (Raper et al., 1960) the only new haemoglobin species detected on subsequent starch gel analysis was haemoglobin C. It has previously been shown that when haemoglobin G/C is recombined with haemoglobin A, both haemoglobin C and haemoglobin G form according to the equation:

$$\underset{\text{Hb-G/C}}{\alpha_2^G\beta_2^C} + \underset{\text{Hb-A}}{\alpha_2^A\beta_2^A} \longrightarrow \underset{\text{Hb-G/C}}{\alpha_2^G\beta_2^C} + \underset{\text{Hb-C}}{\alpha_2^A\beta_2^C} + \underset{\text{Hb-G}}{\alpha_2^G\beta_2^A} + \underset{\text{Hb-A}}{\alpha_2^A\beta_2^A}$$

Thus, the appearance of haemoglobin C without the concomitant appearance of haemoglobin G suggests that the minor haemoglobin isolated from the starch block contains only α_2^A subunits. The possibility that it also contains an abnormal β_2 subunit which, when combined with α_2^G

subunits would have the mobility of haemoglobin C, is ruled out because the starting material consisted of purified haemoglobin A.

Dissociation and recombination of the minor haemoglobin fraction with canine haemoglobin produced one new haemoglobin species corresponding to the haemoglobin $\alpha_2^A\beta^{Can}$ which is formed in the recombination of normal adult and canine haemoglobins (Huehns et al., 1961). The haemoglobin $\alpha_2^{Can}\beta_2^A$, which is the only new species in the recombination of canine haemoglobin and haemoglobin H, was not found. The recombination experiment with canine haemoglobin confirms that only α_2^A subunits are present in the minor haemoglobin. Analysis of the tryptic peptides (Baglioni, 1961) from this haemoglobin by combined electrophoresis at pH 6.4 and chromatography in the solvent pyridine : isoamyl alcohol : water (35 : 35 : 30) revealed only about half the number of peptides obtained from haemoglobin A. Using the numbering system of Ingram, peptides 4, 5, 6, 12, 14, 15β, 17β, 19, 24, 25, 26 were absent, whereas peptides 3, 9, 10, 11, 13, 15α, 16, 17α, 18, 20, 21, 22, 23 were clearly visible. One other peptide was visible in the "fingerprints", between peptides 9 and 15 but as this is often present in the digest prepared from purified haemoglobin A, no special significance could be attached to it. This analysis corresponds to that obtained with the separated α-chains of globin prepared from haemoglobin A.

The electrophoretic behaviour of this haemoglobin is distinctive. On starch gel electrophoresis in sodium phosphate buffer of pH 7.4 (Gammack et al., 1960) it migrates towards the cathode faster than haemoglobin G/C. This behaviour is the opposite of that of haemoglobin H.

Thus, both the recombination experiments and the tryptic peptide analysis of this new <u>in vitro</u> formed haemoglobin show that it consists solely of α^A-chains. Its behaviour on recombination suggests that

these are in the form of α_2^A subunits and that it could have the molecular composition α_4^A. Alternatively, if this haemoglobin consists of single α-chains, then these chains are only able to take part in the formation of tetramer haemoglobin molecules in the presence of β-chains.

The other new minor haemoglobin which is formed has a mobility similar to that of haemoglobin H (β_4^A). If the preparation is carried out with normal neonatal haemolysate, this new minor haemoglobin has the mobility characteristic of haemoglobin "Bart's" (γ_4^F).

The electrophoretic mobility of the α^A-chain haemoglobin is such that it could easily be detected if it exists in vivo. It can be argued that a haemoglobin of this type is most likely to occur in individuals with thalassaemia major (or β-thalassaemia). Six individuals with this disease have now been examined and this haemoglobin has not been detected. Whereas the in vivo appearance of haemoglobins H and "Bart's" shows that the β^A or γ^F-chains are free to polymerise, the failure to observe the α^A-chain haemoglobin in vivo, even though it can be made in vitro, suggests that this is not so for the α^A-chains. Since it has been previously suggested (Raper et al., 1960) that the final stage in haemoglobin synthesis is the combination of pairs of independently synthesized α- and β-chains, then one possible explanation is that free β_2 subunits must be present for the release of the α-chains and the formation of normal haemoglobin.

References

Ager, J.A.M. and Lehmann, H., Brit.med.J., 1, 929 (1958)
Baglioni, C., Biochim.Biophys.Acta, 48, 392 (1961)
Gammack, D.B., Huehns, E.R., Shooter, E.M. and Gerald, P.S., J.Mol.Biol., 2, 372 (1960)
Gouttas, A., Fessas, P., Tsevremis, H. and Xefteri, E., Sang, 26, 911, (1955)
Huehns, E.R. and Shooter, E.M., J.Mol.Biol., in press.
Huehns, E.R., Shooter, E.M., Beaven, G.H. and Gratzer, W.B., unpublished
Hunt, J.A. and Lehmann, H., Nature (London), 184, 872 (1959)

Itano, H.A. and Singer, S.J., Proc.Natl.Acad.Sci., U.S., 44, 522 (1958)
Jones, R.T., Schroeder, W.A. and Vinograd, J.R., J.Am.Chem.Soc., 81, 4749 (1959)
Kekwick, R.A. and Lehmann, H., Nature (London), 187, 158 (1960)
Raper, A.B., Gammack, D.B., Huehns, E.R. and Shooter, E.M., Brit.med.J., ii, 1257 (1960)
Rigas, D.A., Koler, R.D. and Osgood, E.E., J.Lab.Clin.Med., 47, 51 (1956)
Singer, K., Chernoff, A.I. and Singer, L., Blood, 6, 413 (1951).