The sulfhydryl groups of four different human haemoglobins

In some previous papers[1,2] we reported the results of the analyses of the amino acid composition of four different human haemoglobins (A, B, C, and F), using the column chromatographic method of STEIN AND MOORE. With regard to the sulfhydryl content it was found that all these proteins contain 6 half-cystine residues. As the estimation of cystine by this method is not very accurate, and moreover some other investigators[3,4] found 8 sulfhydryl groups in normal adult haemoglobin, it seemed desirable to repeat these estimations with other methods. Besides a specific chromatographic method for the estimation of the total amount of sulfhydryl compounds an amperometric technique was also used in order to estimate the number of free –SH groups present in the four human haemoglobins studied.

The normal adult haemoglobin was obtained from blood samples of normal adults (laboratory workers); the sickle cell haemoglobin (Hb-B) from patients with sickle cell anaemia and the Hb-C from patients with the homozygous Hb-C disease. These patients are described elsewhere[5,6]. Haemoglobin solutions were prepared in the way described in a previous paper[1]. Foetal haemoglobin was purified according to the method of CHERNOFF[7]. The haemoglobins were pure in electrophoretic and chromatographic experiments[8].

The determination of the total cysteine/cystine content of these proteins was mainly carried out following the method of SCHRAM, MOORE AND BIGWOOD[9]. The globins derived from the different haemoglobins were oxidized by performic acid and hydrolyzed in 6*N* HCl by boiling under reflux for 20 hours. The cysteic acid formed during the oxidation was isolated by chromatography on a 0.9 × 25 cm column of Dowex-50 (× 8), using a 0.2 *M* citrate buffer at pH 3.42 as eluting solvent[10]. Upon oxidation of cystine the yield of cysteic acid was 94.5, 90.3 and 90.1% (mean 91.5%) of the theoretical amount. Hydrolysis of oxidized crystalline insulin* gave a recovery of 90.0; 84.7; 88.7; 86.7 and 91.8% (mean 88.4%) of the theoretical amount. In applying the method to the globins the quantities of cysteic acid measured are therefore divided by 0.88 to give the final number of cystine/2 residues present in these proteins.

The amperometric titration procedure was used for the determination of the free sulfhydryl groups in the four haemoglobins studied. The apparatus and technique were nearly similar to those described by INGRAM[4]. The diffusion current to a rotating platinum electrode (900 rotations per minute) was measured with a Kipp galvanometer (A 70). In all experiments 0.05 *M* NH_4NO_3 + 0.1 *M* NH_4OH was used as supporting electrolyte. The volume of the solution in the titration vessel was 50 ml and contained 0.4 to $0.7 \cdot 10^{-5}$ gram mole monocarboxyhaemoglobin, estimated spectrophotometrically at 4180° A. The titrations were carried out with a $AgNO_3$ solution (20 m*M*) and with a $HgCl_2$ solution (10 m*M*). Before the titration with $AgNO_3$ the platinum electrode was treated with an ammoniacal $HgCl_2$ solution (30–45 minutes at —0.2 volt S.C.E.), as it was found that the equivalent point of the $AgNO_3$ titration curve is hardly decreased without this pretreatment. In some denaturation experiments 1.0 ml of different haemoglobins solutions (0.4 to $0.7 \cdot 10^{-5}$ gram mole) was combined with 1.0 ml 1/12 *N* NaOH for different times (2 to 60 minutes). The reaction was stopped by adding slowly equimolar amounts of hydrochloric acid. After this procedure the number of free sulfhydryl groups was estimated by titration with $HgCl_2$. Finally it will be mentioned that the amperometric titration of glutathione and of cysteine (both obtained from Hoffman La Roche Ltd., Suisse) resulted in a number of 0.92 and 1.09 sulfhydryl groups respectively.

The results are given in Table I. The data obtained with the amperometric titration method are mean values of 4 to 8 individual titrations (mean errors from 0.06 to 0.26 for the titrations with $AgNO_3$ and from 0.16 to 0.47 for the titrations with $HgCl_2$).

From the data in Table I the following may be concluded:

1. The total half-cystine content of the haemoglobins A, B and C is 8 residues per mole. The foetal pigment differs in this respect from the other haemoglobins: it contains only 6 half-cystine residues per mole.

2. In a study of the haemoglobins A, B and C, the titrations with $AgNO_3$ show the presence of 8 moles of –SH per mole. All 8 sulfhydryl groups present in these haemoglobins seem to be fully reactive. So it may be concluded that the haemoglobins A, B and C do not contain any disulfide bonds. Foetal haemoglobin, however contains 4 moles of –SH groups per mole suggesting that two half-cystine residues are present in a disulfide linkage in this proteins. Since, moreover, the Hb-F contains two valyl groups in N-terminal position[11] and one histidine and one tyrosine molecule as C-terminal residues[12] it may be possible that this protein is built up of two polypeptide chains linked at least to each other by one disulfide bridge.

3. Titrations of the untreated haemoglobins A, B and C with $HgCl_2$ resulted in less titratable –SH groups. After denaturation of the adult haemoglobin with alkali for varying periods the

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TABLE I

THE SULFHYDRYL CONTENT OF DIFFERENT HUMAN HAEMOGLOBINS

(The values are given as half-cystine residues per mole haemoglobin, mol. wt. = 68,000)

	Chemical method			*Amperometric titration method*					
					with $HgCl_2$ after different denaturation times				
	*n**	*Found*	*Assumed*	*with $AgNO_3$*	*0'*	*2'*	*6'*	*10'*	*60'*
Hb-A	15	8.28 ± 0.17**	8	7.6	5.4	6.2	6.7	7.6	7.6
Hb-B	8	8.30 ± 0.18	8	8.2	7.0			6.8	7.2
Hb-C	15	8.38 ± 0.17	8	7.7	6.0	6.5	6.1		6.2
Hb-F	15	5.67 ± 0.15	6	4.0	4.8			3.6	2.8
Hb from cord blood 91% Hb-F				4.8					

* n = number of analyses.
** Standard deviations of the means.

amount was slowly increased and reached the same value found with the $AgNO_3$ titration. Studying the haemoglobins B and C different numbers of titratable –SH groups were established, which were not influenced by the alkali denaturation procedure. The results with foetal haemoglobin agree quite well with those obtained with the $AgNO_3$ titration. After longer periods of alkali denaturation, however, the amount of titratable –SH groups decreased. The differences between the results obtained with the $HgCl_2$ and $AgNO_3$ titrations may be explained by a steric handicap of the mutual –SH groups. This hindrance may be decreased (Hb-A), not altered (Hb-B and Hb-C), or increased (Hb-F) during the denaturation with alkali.

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Effects of monovalent cations on the incorporation of amino acids into protein*

It has been demonstrated rather clearly that magnesium ions promote the incorporation of amino acids into protein in a variety of experimental systems[1–5], while other divalent cations are generally inhibitory[5,6]. In contrast, little information is available on the effects of monovalent cations. Several observations, however, support the possibility that monovalent cations influence protein synthesis. Thus, STEWARD AND PRESTON[7] showed that potassium ions increase the rate of protein synthesis by potato slices. CANNON *et al.*[8] noted that utilization of amino acid mixtures for protein synthesis by starved rats depends on the simultaneous administration of potassium

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