LOCATION OF THE REACTIVE SULPHYDRYL GROUP IN HUMAN GLOBIN

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Human globin in a neutral medium was allowed to react with labelled *p*-chloromercuribenzoic acid (^{203}Hg) and resolved into the α and β chains by electrophoresis in a starch gel containing 8M urea. The reacted sulphydryl group has been detected in the β chain. This observation is regarded as one more proof that the α1β1 contacts of the globin molecule remain preserved.

The reactivity of sulphydryl groups of the six cysteine residues in a molecule of human haemoglobin has been the subject of a number of papers[1–4]. In neutral media only two of these groups were accessible to SH-reactants. The remaining four became reactive on exposing the protein to conditions producing dissociation of the tetrameric structure to dimeric or monomeric subunits. With the aid of N-ethylmaleimide it has been demonstrated[3] that the reactive SH groups in an intact molecule belong to the cysteine residues of the β-chains at the position βF 9.

A part of our study on the conformation of globin in solutions was the investigation of reactivity of its cysteine residues. We observed[5] that in neutral media the αβ units of globin behaved like the αβ units of haemoglobin: in reactions with N-ethylmaleimide, 5,5'-dithiobis-(2-nitrobenzoic acid) and *p*-chloromercuribenzoic acid (PCMB) one SH group reacts fast, whereas those of the remaining two cysteine residues react much slower. The behaviour of carbonylhaemoglobin reconstituted from globin with one blocked sulphydryl group, and the behaviour of bovine globin in the reaction with PCMB suggest that the readily reacting SH group in human globin, as in haemoglobin, belongs[5] to the cysteinyl βF 9.

The objective of this paper was to substantiate the hitherto indirect evidence of the equal reactivities of the cysteine residues in haemoglobin and globin. Since we previously compared[5] the kinetics of the reactions of these two proteins with PCMB we used this reagent again. Direct location of the modified cysteine residue on a peptide map (the fingerprinting method) is difficult because all the cysteine residues of globin reside in the trypsin-resistant "core", insoluble in neutral media. Chymotrypsin cleaves only the α-core[6]. In the use of pepsin[7] or in the trypsin digestion of aminoethylated[8] globin the mercuribenzoate group might split off. The same risk would have to be considered in the use of 2-mercaptoethanol, necessary for an efficient separation of the α and the β chains of globin by chromatography

on CM-cellulose[8]. For these reasons the present study is restricted to specifying the polypeptide chain in which the reactive cysteine residue occurs. The two polypeptide chains were separated electrophoretically and the substituent was detected by measuring the γ-radiation of ^{203}Hg from PCMB.

EXPERIMENTAL

Globin was prepared from its hydrochloride by the method of repeated precipitation in a neutral medium[9]. The substitution on the readily reacting sulphydryl group was effected with PCMB labelled with ^{203}Hg (Amersham, England). The agent was dissolved in a minimum of 1M-NaOH and added in an equimolar ratio to a 1% solution of globin in a 0·05M phosphate buffer, pH 7·0. After 2 h at 2°C the reaction mixture was dialysed against the same buffer. In order to achieve a better electrophoretic separation of the α and β chains, the remaining two sulphydryl groups were blocked in a sample of the preparation by reaction with non-labelled PCMB. To render these groups reactive urea was added to a dialysed sample of the modified globin until the solution was 8M in respect to it, then an amount of unlabelled PCMB was added to attain a final molar ratio of the SH-reagent to the protein equal three. The α and the β chains of the modified globins were separated by electrophoresis in a starch gel at pH 8·6 in the presence of 8M urea (Chernoff and Petit[10]). The starch gel was prepared from a hydrolysed starch (Koch-Light, England). The urea employed had been twice crystallized in order to minimize the formation of artifact zones, produced by carbamylation of the protein[11]. To obtain sharper zones of non-modified globin, as a reference sample, 2-mercaptoethanol was added to its solution to a concentration of 0·1M. After electrophoresis the layer of the starch gel was cut horizontally in the middle with a thin wire. The upper part was turned downside up and stained with amidoblack 10 B. The developed bands were cut off and removed. Knowledge of their location made it possible to obtain the same zones from the non-stained part of the gel. The radioactivity of the samples was measured with a well-type scintillation counter, Tesla NRQ 612 I.

RESULTS AND DISCUSSION

The electrophoresis in starch gel at pH 8·6 in the presence of 8M urea separates the polypeptide chains of globin very efficiently; the β chain migrates to the anode, the α chain to the cathode (Fig. 1). The bands are sharper in the use of buffers containing 0·1M 2-mercaptoethanol, which prevents the sulphydryl groups of the two chains

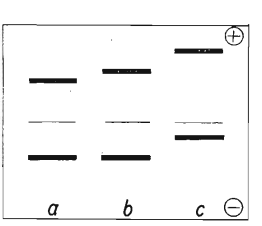

FIG. 1

Starch Gel Electrophoresis of Human Globin in a Veronal Buffer, pH 8·6, 8M in Urea

The symbol *a* designates non-modified globin, *b* and *c* globins with 1 and 3 SH groups blocked with PCMB.

from their possible interactions. The avoid the risk of splitting-off of the mercuribenzoate group, 2-mercaptoethanol was not used with the modified globin and interactions of the remaining SH groups were prevented by blocking them with non-radioactive PCMB. However, the separation of the chains proved good even when the remaining sulphydryl groups were not blocked. Measurement of the activity of γ-radiation of the electrophoretic zones cut off from the starch gel showed that the β chain (zone) contained 98·5% of the total radioactivity if the most reactive sulphydryl was the only substituted group, and 92% if all the SH groups had reacted. The same results were obtained with the zones developed with amidoblack 10 B. The behaviour of the modified globins in electrophoresis was in agreement with the finding that the most reactive SH group is in the β chain. Substitution of a mercuribenzoate group for the hydrogen atom of a sulphydryl group adds one elementary negative charge to the chain, with the consequential shift (at pH 8·6) of the zone to the anode. The substitution on the most reactive cysteine residue brings about a marked shift of the β chain zone. The α chain zone is shifted after reaction with more PCMB, in which case the other SH group of the β chain also reacts, with the corresponding further shift of its zone toward the anode (Fig. 1).

The fact that the accessible sulphydryl group of renatured human globin is located in the β chain strongly suggests that, as with haemoglobin, the residue βF 9 is involved. The same conclusion was drawn previously from the properties of human globin reacted with one molecule of PCMB and from the reactivity of the one cysteine residue of bovine globin[5]. The low reactivity of SH groups of the residues α 104 and β 112, occurring in the tetrameric form of haemoglobin in the contact region α 1 β 1, accords with the view that these contacts are preserved even in globin, as was deduced from other properties of its molecule[12].

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REFERENCES

1. Riggs A.: J. Biol. Chem. *236*, 1948 (1961).
2. Benesch R., Benesch R. E.: J. Biol. Chem. *236*, 405 (1961).
3. Guidotti G., Konigsberg W.: J. Biol. Chem. *239*, 1474 (1964).
4. Antonini E., Brunori M.: J. Biol. Chem. *244*, 3909 (1969).
5. Hrkal Z., Vodrážka Z.: Biochim. Biophys. Acta *257*, 324 (1972).
6. Baglioni C.: Biochim. Biophys. Acta *65*, 389 (1962).
7. Konigsberg W., Hill R. J.: J. Biol. Chem. *237*, 3157 (1962).
8. Clegg J. B., Naughton M. A., Weatherall D. J.: J. Mol. Biol. *19*, 91 (1966).
9. Vodrážka Z., Holeyšovská H., Šípalová H.: This Journal *29*, 1287 (1964).
10. Chernoff A. I., Pettit N. M.: Blood *24*, 750 (1964).
11. Čejka J., Vodrážka Z., Salák J.: Biochim. Biophys. Acta *154*, 589 (1968).
12. Vodrážka Z., Hrkal Z.: Unpublished results.

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