CHANGES IN HUMAN HAEMOGLOBIN AFTER ITS REACTION WITH GLUTARALDEHYDE

Václava FRIČOVÁ, Tomáš I. PŘISTOUPIL, Marie KRAMLOVÁ, Stanislav ULRYCH and Tomáš MAŘÍK

Institute of Haematology and Blood Transfusion, 128 20 Prague

Received July 7th, 1979

The heterogeneity and molecular weight of human haemoglobin derivatives after their reaction with glutaraldehyde was examined. When subjected to polyacrylamide gel electrophoresis the intact and modified haemoglobin markedly differ: the former shows two fractions in the molecular weight range of 20 000 daltons whereas a series of fractions of molecular weight of 35 000—100 000 daltons are observed after the reaction with glutaraldehyde. Simultaneously the dissociation curves of oxyhaemoglobin vary as a function of glutaraldehyde concentration. The samples of modified haemoglobin show a higher affinity for oxygen than intact haemoglobin.

Glutaraldehyde is used in protein studies in cases requiring that the rigidity of protein molecules and their molecular weight be increased or that various compounds be covalently bonded to their molecules[1-4].

This study has been devoted to changes in human haemoglobin following its reaction with glutaraldehyde as regards the formation of inter- and intramolecular covalent bonds, the oxygenation equilibria, and the conversion of the haemo form into the hemi form. We examined therefore the changes in electrophoretic behavior in polyacrylamide gel, in the dissociation curve of oxyhaemoglobin, in the absorption of haemoglobin, and in methaemoglobin concentration.

EXPERIMENTAL

Material and Methods

The haemolysate of human erythrocytes[5,6] contained 90—95% of oxyhaemoglobin, and 5—10% of methaemoglobin. Methaemoglobin was determined by the method of Kaplan[7]. An 1—5% haemoglobin solution in 0·05M-PBS (phosphate-balanced solution containing 0·8 g of NaCl, 0·02 g of KCl, 0·282 g of $Na_2HPO_4.12\,H_2O$, and 0·02 g of KH_2HPO_4 in 300 ml of water) at pH 7·2 was treated with glutaraldehyde (Merck, 1·5—20 mg per 1 g of protein) and the mixture was stirred 30—60 min at 4—8°C at a rate of 450—500 rev/min. The unreacted glutaraldehyde was inactivated by the addition of lysine. Preparations which did not yield a gel were studied. The samples for polyacrylamide gel electrophoresis were filtered through a PM 30 Amicon (Lexington) membrane to remove protein components of a molecular weight lower than 30 000 daltons.

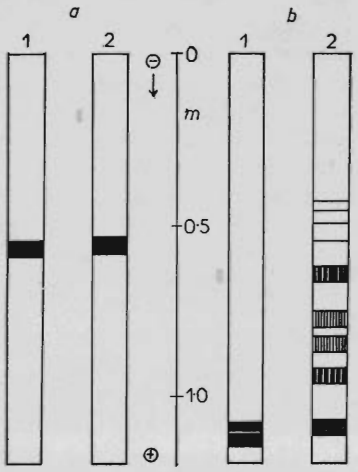

FIG. 1

Schematic representation of electrophoretic resolution of haemoglobin 1 and glutaraldehyde-treated haemoglobin 2. *a* Electrophoresis in phosphate buffer, pH 7·2; *b* electrophoresis of a sample incubated in 1% SDS and 1% mercaptoethanol in phosphate buffer, pH 7·2, containing 0·1% SDS; *m*, mobility

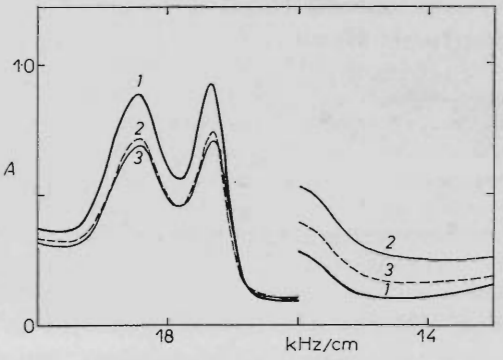

FIG. 2

Absorption spectra of haemoglobin and glutaraldehyde-treated haemoglobin. *1* haemoglobin; *2* haemoglobin treated with 15 mg of glutaraldehyde per 1 g of protein; *3* treated with 20 mg of glutaraldehyde per 1 g of protein. *A* Absorbance of samples; KHz/cm, wave number range in which the absorbance of the samples was measured

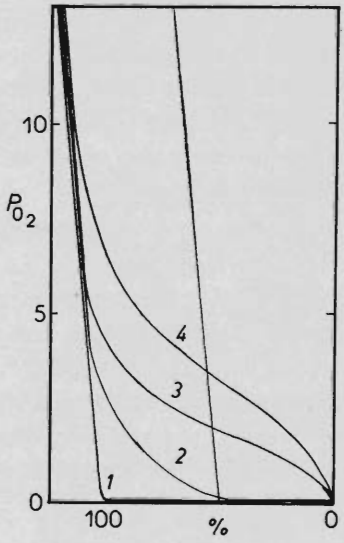

FIG. 3

Oxygenation curves obtained by the biotonometric method. *1* Phosphate buffer, pH 7·40; *2* haemoglobin treated with 15 mg of glutaraldehyde per 1 g protein; *3* haemoglobin; *4* fresh human blood. The measurements were carried out at 37°C. P_{O_2} in kPa; % haemoglobin saturation by oxygen (S_{O_2})

Polyacrylamide gel electrophoresis was carried out on 10 cm columns of 5% gel, 6 mm in diameter, in 0·1M phosphate buffer at pH 7·2, containig 0·1% sodium dodecyl sulfate (SDS, ref.[8]). The optimum experimental conditions for preelectrophoresis were 8 mA per column for 15 min. The dissociation of the sample was effected in a mixture of SDS and mercaptoethanol[8]. For molecular weight calibrations bovine serum albumin (Sevak, Prague), egg albumin (a preparation of the Institute for Haematology and Blood Transfusion, Prague), horse myoglobin (SERVA, Heidelberg), and a standard mixture of erythrocyte membrane proteins[9] were used. The molecular weights were calculated from the mobilities (m) of the individual samples using the formula $m = d_1 \cdot a/d_2 \cdot b$, where d_1 stands for the column length before the destaining, d_2 for the column length after the destaining, b for the distance of the standard substance (bromophenol blue) migration, and a for the distance of the protein migration[8]. The oxyhaemoglobin dissociation curves were automatically recorded and analyzed as described elsewhere[10,11]. The absorption spectra were measured in a Specord UV VIS (Zeiss, Jena) spectrophotometer.

RESULTS AND DISCUSSION

When buffers without the dissociating agents were used there was no essential difference between the polyacrylamide gel electropherograms of samples of native and glutaraldehyde-treated haemoglobin except for the slightly lower mobility of modified haemoglobin (Fig. 1*a*). The protein molecules were degraded to additional fragments after haemoglobin and glutaraldehyde-treated haemoglobin had been incubated in a mixture[8] of SDS and mercaptoethanol (Fig. 1*b*). Two main fractions in the 20 000 dalton range were observed with haemoglobin. Glutaraldehyde-treated haemoglobin yielded many other fractions of higher molecular weight, from 35 000 to 100 000 daltons. These results are indicative of the formation of covalent inter- and intramolecular bonds as a result of the glutaraldehyde treatment.

The methaemoglobin concentration increased from the original 5 – 10% to 10 – 15% during the reaction of haemoglobin with glutaraldehyde. No essential differences were observed in the absorption spectra of intact and modified haemoglobin (Fig. 2); this indicates that, except for the formation of methaemoglobin, the chromophore groups of the haemoglobin molecule, absorbing in the spectrum range studied, did not undergo any more profound changes.

The dissociation curve of oxyhaemoglobin changes its shape after the glutaraldehyde treatment from sigmoidal to a hyperbola-resembling shape (Fig. 3). The change of the shape of the dissociation curve of oxyhaemoglobin was dependent on the increasing glutaraldehyde concentration. The modified samples retain their oxygen-binding ability yet a marked drop of the P_{50}-values, as compared with intact haemoglobin, indicates an increased affinity of the modified haemoglobin for oxygen. These observations are obviously a result of a disturbance in the cooperation of the subunits of the haemoglobin tetramer molecule[12] after its reaction with glutaraldehyde.

REFERENCES

1. Wold F.: J. Biol. Chem. *236*, 106 (1961).
2. Dutton A., Adams M., Singer S. L.: Biochem. Biophys. Res. Commun *23*, 730 (1966).
3. Hartman F. C., Wold F.: Biochemistry *6*, 2439 (1967).
4. Hunter M. J., Ludwig W. L.: J. Amer. Chem. Soc. *84*, 3491 (1962).
5. Přistoupil T. I., Štěrba O., Ulrych S., Kramlová M., Paluska E., Jozová O., Špunda J.: Čas. Lék. Česk. *113*, 527 (1974).
6. Kramlová M., Přistoupil Z. I., Ulrych S., Hrkal Z.: Haematologia *10*, 365 (1976).
7. Kaplan J. G.: Rev. Franc. Étud. Clin. Biol. *10*, 856 (1965).
8. Weber K., Osborn M.: J. Biol. Chem. *244*, 4406 (1969).
9. Steck T. L.: J. Cell. Biol. *62*, 1 (1974).
10. Přistoupil T. I., Kramlová M.: Chem. Listy *74*, 965 (1980).
11. Neville J. R.: J. Appl. Physiol. *37*, 967 (1974).
12. Lehman H., Huntsman R. G. in the book: *Man's Haemoglobins*. North Holland, p. 67. Elsevier, Amsterdam 1974.

Translated by V. Kostka.