EFFECT OF CO_2 BINDING TO HAEMOGLOBIN ON THE REACTIVITY OF THE SH GROUPS AT POSITION $ß_{93}$

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SUMMARY

In deoxygenated human haemoglobin A_{II} and sheep haemoglobin B, in the presence of CO_2 the rate of reaction of the SH groups at position $ß_{93}$ decreased significantly, but did not change in deoxygenated haemoglobin A_{Ic}, where the N-terminal α amino groups of the ß chains are blocked. In the absence of CO_2 the SH reaction rates were identical for all three haemoglobins in deoxy form, but differed for the respective oxyhaemoglobins. In the presence of CO_2 the individual rate constants for oxyhaemoglobin were not altered. It is concluded that binding of CO_2 to haemoglobin leads primarily to a stabilisation of the tertiary deoxy structure of the individual subunits, rather than to a stabilisation of the deoxy quaternary structure of the tetramer.

INTRODUCTION

Carbon dioxide is an important allosteric effector of haemoglobin, but unlike as in the case of organophates a plausible explanation for the change in haemoglobin function upon CO_2 binding has not yet been advanced. It is generally accepted that CO_2 is bound at the N-terminal amino groups of the α and ß chains (1) and that all four groups are involved in oxygen-linked CO_2 binding though predominantly the ß chains (2, 12).

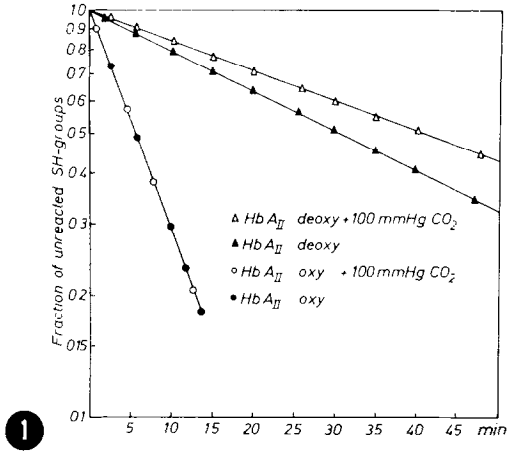
We have investigated the effect of CO_2 binding to haemoglobin on the reactivity of sulfhydryl groups in position $ß_{93}$, these groups being sensitive indicators for both tertiary and quaternary conformational changes. Three different haemoglobins namely human haemoglobin A_{II}, A_{Ic} and sheep haemoglobin B

(here the SH group is at position β_{92} due to deletion of residue $NA\beta_2$) were examined. While in human haemoglobin A_{II} and sheep haemoglobin B all four N-terminal amino groups are free to react with CO_2, the β N-terminal amino groups of haemoglobin A_{Ic} are blocked by an unidentified hexose (3).

The experiments were conducted in order to obtain information if binding of CO_2 leads to structural changes that reflect itself in the reactivity of the β_{93} SH groups and specifically if this is the case for haemoglobin A_{Ic} where CO_2 binding is possible only at the α chain N-termini.

EXPERIMENTAL

Sheep haemoglobin B was obtained from a sheep homozygous for haemoglobin B. Haemoglobins A_{II} and A_{Ic} were separated on Biorex 70 with Developer No 4 (4). The purity of the separated fractions was assessed by isoelectric focussing on polyacrylamide gel. Haemoglobin was deoxygenated by evacuation in 250 ml tonometer cuvettes and was considered free of ligand when the ratio OD_{555}/OD_{540} was 1.24 or higher (5). The ratio was also checked after each experiment, to make sure that the sample had remained deoxygenated during the course of the reaction. An amount of CO_2 equivalent to 100 mm Hg was injected into the tonometer cuvette via a rubber stopped sidearm and the sample equilibrated for 20 min at 20° C. Buffers were either 0.05 MTris 0.16 MCl^-, pH 7.3, or $NaHCO_3/NaCl$ buffer of I =0.16 M, pH 7.3. The reactive haemoglobin SH groups were determined as described by Ampulski et al. (6), with minor modifications using 4,4' dithiopyridine (4-PDS) as reagent. The change in absorbance at 324 nm was followed in a Hitachi model 124 spectrophotometer connected to a recorder. Methaemoglobin formation did not exceed 5% at the end of each experiment.



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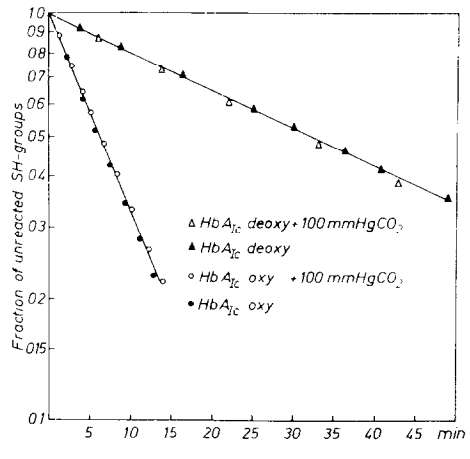

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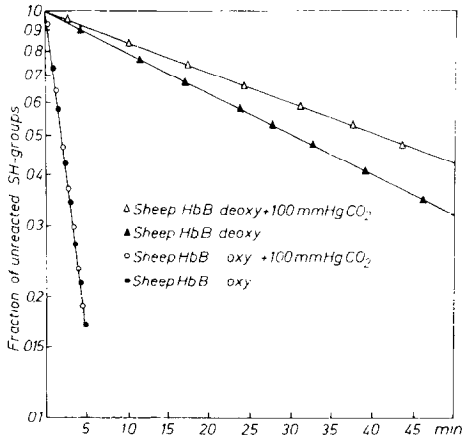

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Pseudo-first order plots for the reaction of 4-PDS with SH groups at β_{93} in human oxy and deoxyhaemoglobins A_{II} (fig. 1) and A_{Ic} (fig. 2) and SH groups at β_{92} of sheep haemoglobin B (fig. 3) respectively in the absence and presence of 100 mm Hg CO_2. pH 7.3, T = 20°, I = 0.16, Hb_4 = 1 x 10^{-5}M, 4-PDS = 30^2 x 10^{-5}M for experiments with deoxyhaemoglobin and 10x10^{-5}M for experiments with oxyhaemoglobin.

RESULTS

The kinetics of the reaction of the SH groups with 4-PDS were pseudo-first order for both the various oxy and deoxyhaemoglobins under our experimental conditions (Fig. 1, 2 and 3). k' the apparent first order rate constant was calculated from the slope of the pseudo-first order plots and the numerical values are presented in Table 1. These values are derived from

Table 1

	Hb A_{II} K' sec^{-1} x 10^4	Hb A_{Ic} K' sec^{-1} x 10^4	Sheep Hb B K' sec^{-1} x 10^4
oxy	21	18.0	64
oxy + 100 mm Hg CO_2	21	17.5	64.5
deoxy	3.7	3.6	3.8
deoxy + 100 mm Hg CO_2	2.8	3.7	2.8

Pseudo-first order rate constants for the reaction of the reactive SH groups of human haemoglobins A_{Ic} and A_{II} and sheep haemoglobin B. Experimental conditions as in legend to fig. 1, 2, 3.

4-10 experiments in each case.

In the presence of CO_2 the rate constants for deoxyhaemoglobin A_{II} and deoxyhaemoglobin B of sheep are moderately but significantly ($2p < 0.01$) decreased, while in the case of haemoglobin A_{Ic} no change was observed.

In the absence of CO_2 the reaction rates for all three deoxyhaemoglobins are almost identical. For oxyhaemoglobins the reaction rate remained independent of CO_2, here, however, a pronounced difference of rate constants exists between sheep haemoglobin B, and human Hb A_{II} and A_{Ic} respectively.

DISCUSSION

The reactivity of the SH groups at position $ß_{93}$ is influenced by the state of ligation of the ß chains (7), and by haemoglobin quaternary structure (8). According to Perutz (9), the lower reactivity of these groups in deoxyhaemoglobin is due to the formation of a salt-bridge between the C-terminal Histidine $ß_{146}$ and aspartate $ß_{94}$ of the same chain, which restricts access of reagent. As these residues and presumably the salt-bridges are invariant in the haemoglobins studied aside from human Hb A_{II} in this investigation, this hypothesis offers a plausible explanation for the invariance of the deoxy reaction rates in the absence of CO_2, in haemoglobins which differ widely both in primary structure and function. Likewise McDonald et al. (10) reported nearly identical reaction rates for the reactive SH groups of human deoxyhaemoglobin F as compared to human HbA, whereas pronounced differences existed for the respective rates in oxyhaemoglobin.

The different reaction rates for oxyhaemoglobin presumably reflect localized changes in the tertiary structure of the

ß chains which may be due to the different primary structures. As no difference is observed for the SH reaction rates in the absence or presence of CO_2 in the case of oxyhaemoglobin, the results of this study give no indication that binding of CO_2 to oxyhaemoglobin alters the tertiary and/or quaternary conformation in a substantial way. This conforms with Salhany's (11) observation, that the kinetics of CO release from fully ligated human haemoglobin are unaffected by CO_2 binding to haemoglobin.

The binding of CO_2 to human haemoglobin A_{II} and sheep haemoglobin B in the deoxy state results in a moderate but significant reduction of the SH reactivity while no reduction can be observed in the case of haemoglobin A_{Ic}. In view of the fact that there is a definite oxygen-linked binding of CO_2 to haemoglobin A_{Ic}, reducing both the Bohr effect and oxygen affinity (12), one has to conclude that the conformational changes responsible for the alteration of functional behaviour are in this case confined to the α subunits and not transmitted to the ß subunits. If CO_2, like organophosphates, stabilised the deoxy quaternary conformation (8), one would expect that the reactivity of the SH groups changes upon CO_2 binding to the α chains in deoxy haemoglobin A_{Ic}. The results of this study seem to indicate that the main effect of CO_2 binding to haemoglobin is due to a stabilisation of the deoxy tertiary structure of the individual chain to which CO_2 is bound, rather than to a stabilisation of the deoxy quaternary structure of the tetramer.

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