Effect of Carbon Dioxide on Human Hemoglobin

KINETIC BASIS FOR THE REDUCED OXYGEN AFFINITY*

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SUMMARY

The kinetic basis for the reduction in the oxygen affinity of hemoglobin due to carbon dioxide at constant pH, is studied by measuring the deoxygenation rates of oxyhemoglobin with dithionite, as well as the rate of binding of carbon monoxide to deoxyhemoglobin. These studies were performed in buffer containing 0.05 M potassium phosphate, as well as various mixtures of potassium chloride and potassium bicarbonate all at a constant pH of 7.5 and 23°C. The results indicate that the reduction in the ligand affinity of hemoglobin by carbon dioxide is the result of a reduced rate of ligand binding with no significant change in the rate of release. It is pointed out that this result is in contrast to salts which reduce the ligand affinity of hemoglobin by increasing the rate of ligand dissociation as well as reducing the rate of ligand binding.

The three major nonheme ligands of physiological importance in the reaction of hemoglobin with oxygen are protons, CO₂ and 2,3-DPG. The effect of 2,3-DPG on the hemoglobin-oxygen interaction has recently been the subject of intensive study employing a number of techniques (1-7). Carbon dioxide, on the other hand, has been known to influence the oxygen affinity of blood since the studies of Bohr, Hasselbalch, and Krogh in 1904 (8). However, it has been only recently that significant insight has been gained concerning the mechanism by which CO₂ exerts this influence on the ligand-reacting properties of hemoglobin (9-15). The studies of Kilmarin and Rossi-Bernardi (13-15) have shown that the terminal amino groups of hemoglobin are responsible for the oxygen-linked binding of CO₂. They demonstrated that when all four terminal amino groups are specifically reacted with cyanate no reduction in oxygen affinity due to CO₂ is detected, nor was there any observable difference in CO₂ binding between oxy- and deoxyhemoglobin. The purpose for undertaking this study was to ascertain, with kinetic techniques, whether the reduction in ligand affinity due to CO₂ results from an increase in the rate of ligand dissociation, or a decrease in the rate of ligand binding, or both.

EXPERIMENTAL PROCEDURE

Preparation of Hemoglobin—Plasma and membrane-free hemolysates of adult venous blood were prepared as described previously (5). Isolated hemoglobin was prepared by subjecting the above hemolysates to ion exchange chromatography on DEAE-Sephadex according to a modification of the methods of Huisman and Dossy (16) in 0.05 M Tris-HCl. The isolated hemoglobin was then dialyzed against buffer consisting of 0.2 M KC1 and 0.05 M potassium phosphate (for the condition where no CO₂ was present); 0.025 M KHCO₃, 0.175 M KC1, and 0.05 M potassium phosphate (for the 19 mm Hg pCO₂ condition, used for equilibrium studies); 0.05 M KHCO₃, 0.15 M KC1, and 0.05 M potassium phosphate (for the 38 mm Hg pCO₂ condition) and 0.1 M KHCO₃, 0.1 M KC1, and 0.05 M potassium phosphate (for the 76 mm Hg pCO₂ condition). All studies of the effect of CO₂ were performed at pH 7.5 and 23°C.

Oxygen Equilibrium—The oxygen equilibrium studies were performed with the spectrophotometric methods of Keys, Mizukami, and Lumry (17), employing a Cary 14 recording spectrophotometer, and scanning from 650 to 520 nm. The hemoglobin solutions, prepared as described above, were placed in the Keys-Mizukami-Lumry (KML) tonometer and deoxygenated with a vacuum. Then one atmosphere of analyzed gas (Matheson Co.), containing the appropriate partial pressures of CO₂ balance N₂ was added to the hemoglobin solutions. When no CO₂ was present, one atmosphere of pure N₂ was added. The hemoglobin in the N₂- CO₂ (or N₂) environment was allowed to equilibrate in the deoxy form for 20 min. Then, with the lower chamber of the KML tonometer closed, the upper chamber was degassed, following which analyzed gas, containing the same partial pressure of CO₂ balance a mixture of N₂ and O₂ was added at a total pressure of one atmosphere and the equilibrium curve was constructed. Calculations of p50 (half oxygen saturation point) were performed as described elsewhere (17).

Deoxygenation Kinetics—These kinetics were performed with sodium dithionite (1 g per liter) in a Durrum-Gibson stopped flow apparatus on the above described hemoglobin preparations and a pseudo-first order deoxygenation rate constant (k in sec⁻¹) was calculated as previously described (5). For the CO₂ experiments, the hemoglobin solutions were flushed with gas containing the appropriate partial pressures of CO₂ balance O₂ (150 mm Hg) and N₂. The dithionite was in 0.05 M potassium.
phosphate, 0.2 M KCl buffer at pH 7.5, which was flushed with pure nitrogen before the solid dithionite was added. The hemoglobin dithionite reactions were all followed at 577 nm and 23°C.

Carbon Monoxide-binding Kinetics—The CO-binding experiments were performed by flushing the hemoglobin solutions with the given partial pressures of CO₂ balance N₂. The buffer solution (0.05 M potassium phosphate and 0.2 M KCl) was flushed with analyzed gas containing CO so as to yield a solution containing 2.4 × 10⁻³ M CO (after mixing in the stopped flow). The CO reactions were followed at 555 nm and 23°C. The calculation of the CO-binding constant (k', in M⁻¹ sec⁻¹) was performed exactly as described by Gibson and Roughton (18). In all kinetic studies at least three to five experiments were performed per experimental point reported. The values reported are the mean of the experiments. The standard deviations never exceeded ±5% of the reported means.

RESULTS

The results of the hemoglobin-oxygen equilibrium studies in the presence of increasing pCO₂ at constant pH are presented in Fig. 1. At pH 7.5, the pNO increases (affinity decreases) with a rise in pCO₂. These results appear to be in good agreement with those of Kilmartin and Rossi-Bernardi (15) who observed a 2.7 mm Hg increase in pNO with the addition of 46 mm Hg pCO₂ at pH 7.4 and 25°C in the same buffer system. The results in Fig. 1 show a 3 mm Hg increase in pNO at pH 7.5, at the 46 mm Hg pCO₂ point.

The effect of equilibrating oxyhemoglobin with CO₂ on the rate of deoxygenation over the same range in partial pressure is shown in Fig. 2. The value of k was entirely invariant with a rise in the partial pressure of CO₂. However, when the rate of CO binding to deoxyhemoglobin was measured under identical conditions, the value of k became significantly smaller with a rise in the partial pressure of CO₂ (Fig. 3).

DISCUSSION

The results of this paper seem to define clearly the kinetic basis for the reduced oxygen affinity observed due to CO₂ at constant pH. It is of interest to discuss further the possible mechanism by which these kinetic effects occur, especially as compared qualitatively with the effects of salts.

As mentioned in the introduction, CO₂ reacts covalently with the terminal amino groups of hemoglobin in a manner which is oxygen-linked (13–15). This oxygen linkage is shown by the greater binding of CO₂ to deoxyhemoglobin as well as the reduction in oxygen affinity at constant pH. However, as shown herein, the reduction in oxygen affinity due to CO₂ is not associated with an increase in the rate of deoxygenation. Since CO₂ is also bound to oxyhemoglobin, the above kinetic results suggest that carbamino formation on the oxy conformation has no effect on the functional properties of that conformation. The lack of an effect of CO₂ on k is different from the known effect of salts, particularly inorganic and organic phosphates, on the rate of deoxygenation (5, 6, 19, 20). These phosphates reduce the oxygen affinity of hemoglobin not only by increasing the deoxygenation rate, but also by reducing the rate of ligation (6, 19, 20), whereas CO₂ only has the latter effect on hemoglobin at constant pH.

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REFERENCES

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