THE EFFECTS OF SALTS ON THE OXYGEN AFFINITY OF HEMOGLOBIN

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1. Introduction

Since the description of the effect of specific binding of DPG and chloride on the oxygen affinity of hemoglobin [1], numerous studies dealing with anion binding to hemoglobin have been reported [2–4]. The competition between organic phosphates and inorganic anions, lead to the suggestion of a common binding site on the deoxyhemoglobin molecule [3]. More recently, an evidence pertaining to the location of the oxygen linked anion sites at the $\alpha_1\alpha_2$ interface, was presented [5,6].

In this communication we compare a number of inorganic anions such as ClO_4^-, NO_3^-, Br^- and Cl^- belonging to the Hofmeister series [7] with respect to their effect on the oxygen affinity of hemoglobin. In addition we also describe an effect of cesium and rubidium sulphates on the oxygenation of human hemoglobin.

2. Materials and methods

Hemoglobin was prepared by hemolysis of outdated blood and freed of organic phosphates by chromatography on a Sephadex G-25 (40 × 1.5 cm) column, at pH 7.5, 0.1 M NaCl [8]. Oxygen dissociation experiments were carried out in a tonometer at a 1.5×10^{-5} M concentration of the tetramer, 0.05 M bis–Tris buffer, pH 7.0, 25°C. The different salts were dissolved in the buffer prior to evacuation and the pH corrected whenever necessary. Spectra were recorded on a Cary 14 spectrophotometer.

Abbreviations: bis-Tris, *N,N*-bis (2-hydroxyethyl) iminotris (hydroxymethyl) methane; DPG, 2,3-diphosphoglycerate

3. Results and discussion

Oxygen affinity values of human hemoglobin in the presence of 0.05 M concentration of different salts are summarized in fig.1. Figure 2 describes the concentration dependence for p_{50} values of some of the salts. It is evident that oxygen affinity decreases in the order $Cl^- < NO_3^- < Br^- < ClO_4^-$, which represents the order of increasing chastropicity of the salts affecting proteins through solvent-mediated effects [7]. A similar order of effectiveness has been demonstrated for specific anion binding to proteins

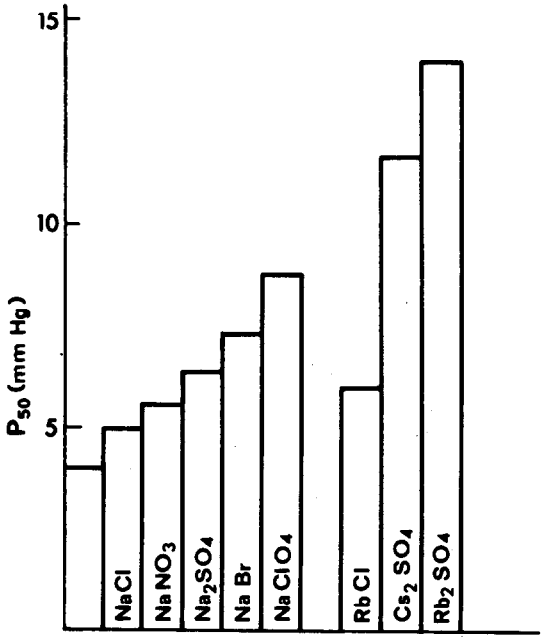

Fig.1. Oxygen affinity of the hemoglobin (1.5×10^{-5} M) in 0.05 M solution of salts, pH 7.0, 0.05 M bis–Tris buffer, 25°C.

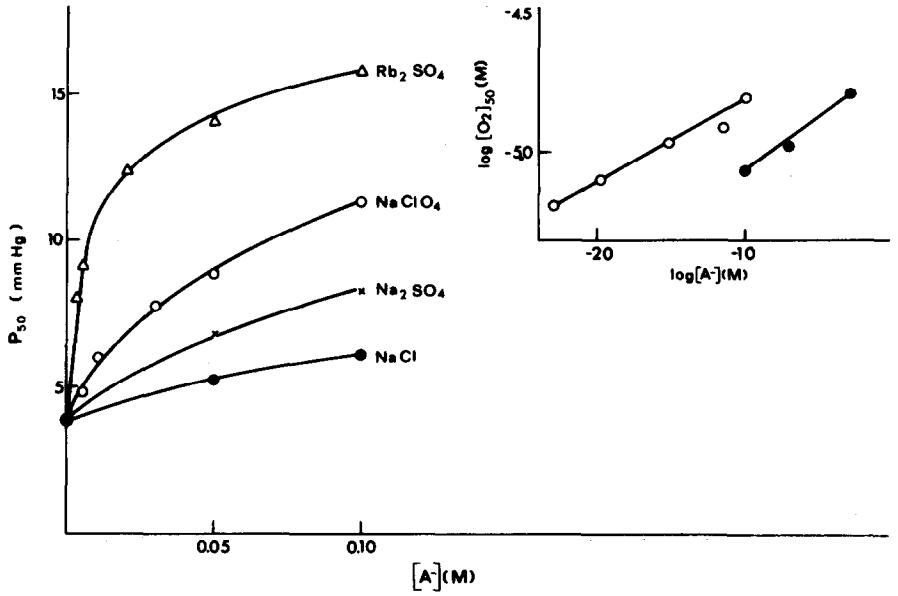

Fig.2. The dependence of p_{50} on the concentration of salt: (•) NaCl; (X) Na_2SO_4: (o) $NaClO_4$; (△) Rb_2SO_4. Inset: Oxygen concentration at half saturation as a function of the concentration of perchlorate (o) and chloride (•) 0.05 M bis–Tris buffer, pH 7.0, 25°C.

and enzymes [9]. In the case of hemoglobin, for which chloride binding has been established [3], the decreased affinity towards oxygen is to be attributed to the preferential anion binding capacity of the deoxygenated form. The results summarized in fig.1 imply that this capacity, as reflected in the reduction of the affinity towards oxygen, depends mainly on the nature of the anion.

The carbamylation rate of valine-1 (α) residue was enhanced by deoxygenation and inhibited by chloride [6]. These findings were attributed to the binding of monovalent anions, including cyanate, to positively charged groups in the vicinity of Val-1 (α). They demonstrated also that nitrate and iodide are competitive inhibitors of carbamylation at this residue. Following this suggestion, it may be inferred that anion binding close to the amino anion terminal residue and reflected in the decreased oxygen affinity and carbamylation rates, follows the order of the Hofmeister series [7].

The thermodynamics of the linkage between binding of chloride and oxygen to human hemoglobin have been studied [10]. By plotting the dependence of p_{50} as a function of chloride concentration, the apparent difference in the number of bound chloride ions per heme between deoxy and oxyhemoglobin was estimated to equal 0.4. In the insert in fig.2 a similar plot of the results obtained with sodium perchlorate is shown. For the calculation of oxygen concentration at half saturation we used the solubility coefficients reported [10] in the presence of low concentrations of chloride. The slope of 0.3 yielding an approximate value of 1.2 as the difference in the number of perchlorate ions bound to the tetramer, indicates that despite differences in affinity, the stoichiometry of the ion binding chloride and perchlorate is similar.

Substitution of Rb^+ or Cs^+ for Na^+ does not significantly affect the oxygen affinity of hemoglobin when chlorides of these cations are compared (fig.1,2). Sulfates the rubidium and cesium (Merck Com.) reduce significantly, however, the affinity for oxygen. This effect cannot be attributed to the sulfate anion since the extent of increase of p_{50} in Na_2SO_4 is much lower. The reasons for these findings are unclear and are probably related to some feature of the salt as a whole and not to its components.

References

[1] Benesh, R. E., Benesh, R. and Yu, CI (1969) Biochemistry 8, 2567–2571.
[2] Shimizu, K. and Bucci, E. (1973) Biochemistry 12, 809–814.
[3] Chiancone, E., Norne, Y. E., Forsen, S., Antonini, E. and Wyman, J. (1972) J. Mol. Biol. 70, 675–688.
[4] Desbois, A. and Banerjee, R. (1975) J. Mol. Biol. 32, 478–483.
[5] Nigen, A. M. and Manning, Y. M. (1975) J. Biol. Chem. 250, 8248–8250.
[6] Nigen, A. M., Bass, B. D. and Manning, Y. M. (1976) J. Biol. Chem. 251, 7638–7643.
[7] Von Hippel, P. H. and Schleick, T. (1968) in: Biological Macromolecules (Timashef, S. T. and Fasman, G. eds) vol. 2, pp. 471–573, Marcel Dekker, New York.
[8] Berman, M., Benesh, R. and Benesh, R. E. (1971) Arch. Biochem. Biophys. 145, 236–242.
[9] Warren, Y. C., Stowring, L. and Morales, M. F. (1966) J. Biol. Chem. 241, 309–316.
[10] Haire, R. N. and Hedlund, B. E. (1977) Proc. Natl. Acad. Sci. USA 74, 4135–4138.