

Arctic adaptation in reindeer

The energy saving of a hemoglobin

Bruno Giardina, Ole Brix⁺, Matti Nuutinen[†], Said El Sherbini*, Astrid Bardgard⁺,
Giuseppe Lazzarino and Saverio G. Condò

*Department of Experimental Medicine and Biochemical Sciences, II University of Rome, Tor Vergata, Via Orazio Raimondo, 00173 Rome, Italy, ⁺Zoological Laboratory, University of Bergen, Allegaten 41, N-5007 Bergen, Norway, [†]Department of Pediatrics, University of Oulu, SF-90220 Oulu 22, Finland and *Department of Biochemistry, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt*

Received 20 February 1989

Previous results [(1988) *Arct. Med. Res.* 47, 83–88] have shown that hemoglobin from reindeer is characterized by a low overall heat of oxygenation. This particular aspect has been investigated further in a series of precise oxygen equilibrium experiments. The results obtained show a peculiar dependence of the temperature effect on the fractional saturation of hemoglobin with oxygen, which could be regarded as a very interesting case of molecular adaptation to extreme environmental conditions.

Cold adaptation; Hemoglobin; Oxygen binding; (Reindeer)

1. INTRODUCTION

Our expanding knowledge of biochemistry and physiology of hemoglobin is derived to a great extent from the careful investigation of human mutant hemoglobins and the systematic observation of animal hemoglobins, whereby biochemical and physiological differences can be related to evolutionary development. In fact the allosteric properties of hemoglobins, especially their responses to ligands other than oxygen, vary widely in different classes of vertebrates living in different environments. Hence the oxygen affinity of the hemoglobin of most vertebrates is influenced by various chemical factors, known as heterotropic ligands, and by temperature. Arguing along these lines, and aiming at a better understanding of the control mechanisms which underlie molecular adaptation to different environments, we have

studied in detail the functional properties of hemoglobin from the reindeer with special regard to the effects of temperature since it is known that this animal encounters a great range of temperature changes (-40°C to $+20^{\circ}\text{C}$).

2. MATERIALS AND METHODS

The hemoglobin, collected as described previously [1], was stripped by passing the hemolysate on a mixed-bed ion-exchange column (Dowex AG 501 \times 8). The oxygen equilibria were measured with a diffusion chamber technique [2] except that the stepwise increases in oxygen tension were generated by cascaded gas mixing pumps (Wosthoff) while absorbance changes between zero and full saturation were monitored on recorders with high sensitivity units (Eppendorf, Radiometer). The application of small amounts of hemoglobin solution (layer thickness about $10\ \mu\text{m}$) minimized equilibration time and methemoglobin formation, which was always less than 1%.

Alternatively oxygen dissociation curves were obtained spectrophotometrically by the tonometric method [3] at a protein concentration of 3–5 mg/ml.

Concentrated stock solutions of 2,3-diphosphoglyceric acid (DPG) were prepared by dissolving the sodium salt of 2,3-DPG (Sigma) in water or in buffer.

Correspondence address: B. Giardina, Department of Experimental Medicine and Biochemical Sciences, II University of Rome, Tor Vergata, Via Orazio Raimondo, 00173 Rome, Italy

3. RESULTS AND DISCUSSION

Reindeer Hb is characterized by a very low oxygen affinity, even in the absence of organic phosphates e.g. 2,3-DPG [1]. Under these conditions at pH 7.0 and 20°C (in 0.1 M Bis-Tris + 0.1 M NaCl), P_{50} is 15.8 mmHg, a value slightly lower than that of human hemoglobin A fully saturated with 2,3-DPG [4]. Hence, hemoglobin from reindeer, like that of other ruminants [5,6], does not need to be modulated in vivo by the presence of organic cofactors. This observation is supported further by the low level of intraerythrocytic 2,3-DPG which is more than ten times lower than that in human erythrocytes (0.4 mM vs 5 mM).

Fig.1 shows the Bohr effects of red blood cells from both reindeer and man. As can be seen, at each pH value (and despite the low level of intraerythrocytic 2,3-DPG) reindeer erythrocytes display an oxygen affinity about 35% lower than that of human red cells.

The oxygen affinity of reindeer Hb is very much affected by changes in Cl^- concentration (see inset to fig.1). Moreover, the data clearly show that when 2,3-DPG is added to reindeer Hb in 0.1 M Cl^- (near physiological concentration), the oxygen affinity remains unchanged, confirming previous results obtained with bovine Hb [7,8]. The fact that reindeer Hb is particularly sensitive to increasing concentrations of Cl^- is reminiscent of the behavior of Hb from *Lemur fulvus fulvus* [9] and strongly indicates that in some species small anions may substitute for 2,3-DPG in modulating the oxygen affinity of Hb in vivo.

However, the most surprising characteristics of reindeer Hb concerns its response to changes in temperature. The overall heat of oxygenation, obtained indirectly from the van 't Hoff equation, was found to be two to three times lower than that of human Hb under the same experimental conditions (overall $\Delta H = -4.5$ kcal/mol at pH 7.4) (see inset to fig.2). This finding could be of particular significance from an evolutionary point of view since it should be considered in connection with the great range of temperature changes (-40°C to $+20^\circ\text{C}$) that reindeer encounter in the wild [10].

For this reason, a set of oxygen binding experiments have been carried out in the presence of chloride and carbon dioxide (4%) at two

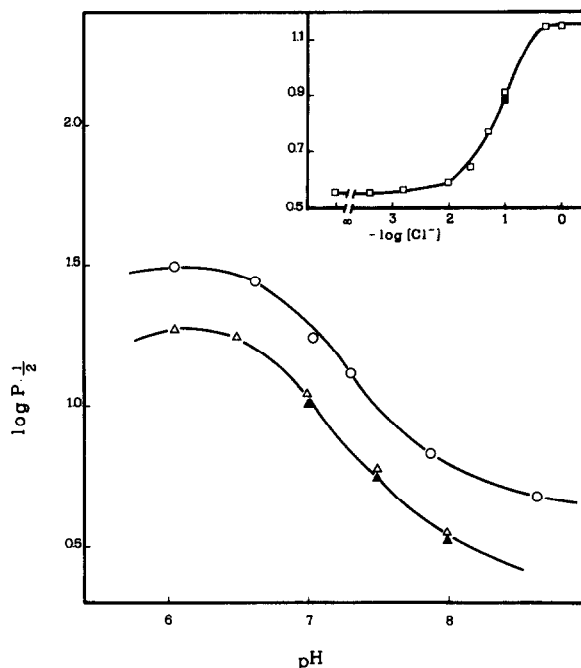


Fig.1. Oxygen Bohr effect of human (triangles) and reindeer (circles) erythrocytes at 20°C (\circ , Δ) 0.05 M Bis-Tris or Tris-HCl buffer plus 0.1 M NaCl; (\blacktriangle) 80 mM phosphate buffer. The inset shows the effect of increasing concentration of chloride ions (open squares), as observed in 0.1 M Hepes at pH 7.4 and 20°C, on the oxygen affinity (indicated as $\log P_{1/2}$) of stripped reindeer hemoglobin; closed square represents the value obtained by adding 3 mM 2,3-DPG.

temperatures (10°C and 20°C). The data, presented in the form of a Hill plot, extend over a saturation range broad enough to permit an evaluation of a number of thermodynamic parameters. These are summarized in table 1. One feature, which shows up very clearly in fig.2, is the strong temperature dependence of the shape of the binding curve. Thus, an increase in temperature brings about a great decrease in the association constant for the binding of the first oxygen molecule without significantly affecting that for the binding of the last molecule. These constants, which are referred to as K_T and K_R , respectively, by analogy with the T and R states of human hemoglobin A, are estimated by lines drawn asymptotically to the experimental curves at extremely high and low O_2 saturation.

The differential effect of temperature on K_T and K_R raises the free energy of heme-heme interactions ΔG_i (which equals $RT \ln(K_T/K_R)$, where R is

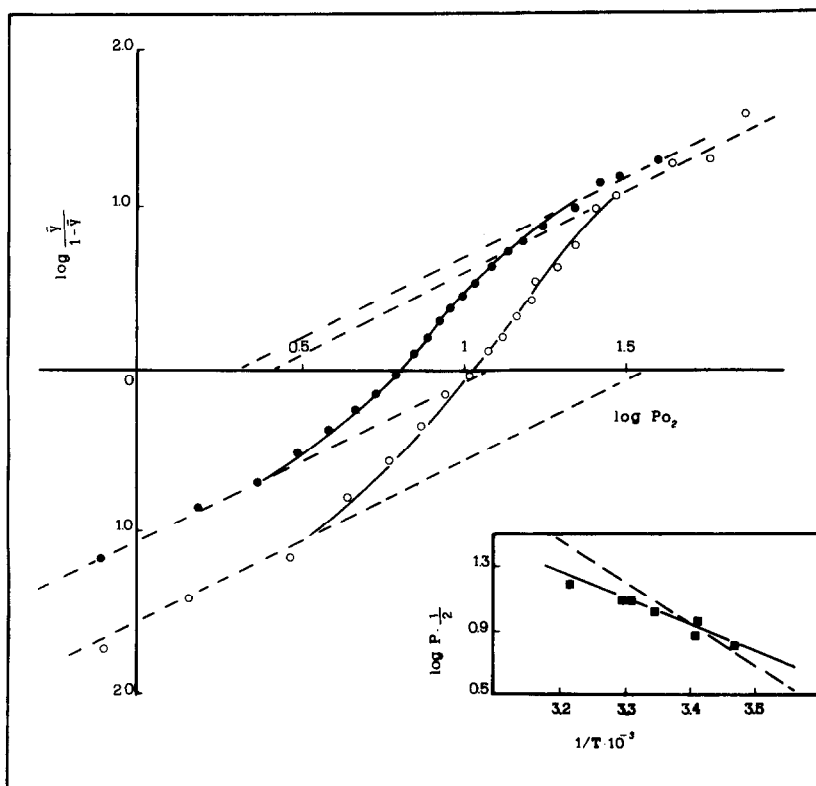


Fig.2. Effect of temperature on oxygen equilibria of reindeer Hb measured in 0.05 M Tris-HCl buffer in the presence of 4% of carbon dioxide. Closed and open circles refer to 10 and 20°C, respectively. The inset shows the van 't Hoff equation plot obtained in the case of human hemoglobin A (dashed line) and reindeer hemoglobin (closed square) in 0.1 M Hepes plus 0.1 M NaCl, pH 7.4 and $T = 20^{\circ}\text{C}$, at 50% saturation. Data on HbA are obtained in the presence of 3 mM 2,3-DPG.

the gas constant and T the absolute temperature). Under the experimental conditions reported in fig.2 an increase of temperature of 10°C results in about a 30% increase in the free energy of interaction from 1.0 kcal/mol at 10°C to 1.5 kcal/mol at 20°C . This is mainly due to the difference in the overall heat of oxygenation relative to the T and R states of the molecule. Thus, while ΔH of oxygen binding to the T state is strongly exothermic, that of the R state is very close to zero or even positive (after correction for the heat of the oxygen solution) (see table 1). It should be recalled that this finding, although different from data obtained for human hemoglobin A [11], shows a trend which is similar, though not as extensive as that of sheep Hb [12].

A very unusual and striking feature which emerges from the analysis of reindeer Hb is the fact that the position of the upper asymptote is almost temperature independent; to our

knowledge this is the first time that the R state of a hemoglobin has been found to display such a small ΔH for ligand binding.

Moreover, it should be emphasized that the dramatic difference in the thermodynamics of the two conformational states of reindeer Hb results in

Table 1
Thermodynamic parameters for the binding of oxygen to reindeer Hb

	T state	R state
ΔG	-6.5	-8.0
ΔH	-15.9	-0.79
$T\Delta S$	-9.4	+7.2
P_{50}	37.1 (mmHg)	2.5 (mmHg)

The parameters refer to the reaction of a molecule of ligand with a single site in a T or R quaternary state. Enthalpy, entropy and free-energy changes are expressed as kcal/mol of oxygen in solution. Conditions as in fig.2

a particular dependence of the temperature effect on the degree of oxygen saturation (\bar{Y}) of the protein. This behavior is outlined in fig.3. For values of \bar{Y} higher than 0.6, i.e. within the range of O_2 saturation in which the protein is working in vivo, the overall heat of oxygenation is of the order of -3.8 kcal/mol O_2 , approaching zero as \bar{Y} tends to 1.0.

As far as proton concentration is concerned it should be noted that H^+ acts mainly (at least in the absence of carbon dioxide) on K_T by shifting the deoxy-asymptote to lower affinities ($K_T = 12.6$ mmHg at pH 7.46; $K_T = 42.6$ mmHg at pH 7.0) affecting slightly the upper asymptote ($K_R = 0.6$ mmHg at pH 7.46; $K_R = 0.9$ mmHg at pH 7.0). This follows, at least within this pH range, the behavior of human hemoglobin where lower pH reduces K_T slightly altering K_R [13]. The present data allow the estimation of the Bohr effect for binding of the first and the last oxygen molecules (i.e. $\Delta \log K_T / \Delta pH$ and $\Delta \log K_R / \Delta pH$, respectively) as -1.15 and -0.37 , thus showing that most protons are released on binding of the first oxygen molecule.

The results reported here show that the functional behavior of reindeer Hb displays some peculiar features which make this protein an example of molecular adaptation to extreme environment. In fact, present day reindeer graze like their wild ancestors, beyond latitude 65° , showing a high degree of physical fitness [10] to the environmental conditions. In our opinion a great part of this fitness could be attributed to the unusual thermodynamic properties of its hemoglobin which, being characterized by a small negative ΔH in the upper part of the binding curve, does not require much energy during its oxygenation-deoxygenation cycle. In other words, at the level of the peripheral tissues, i.e. legs, skin, etc., where temperature is $\sim 10^\circ C$ lower than that of the lungs ($37^\circ C$), oxygen delivery is not drastically impaired, thus requiring 1/3 or less of the heat necessary in other mammals [14].

The energy saving displayed by reindeer Hb is certainly one of the most elegant examples of the different strategies which have been adopted during evolution to solve the problem of oxygen

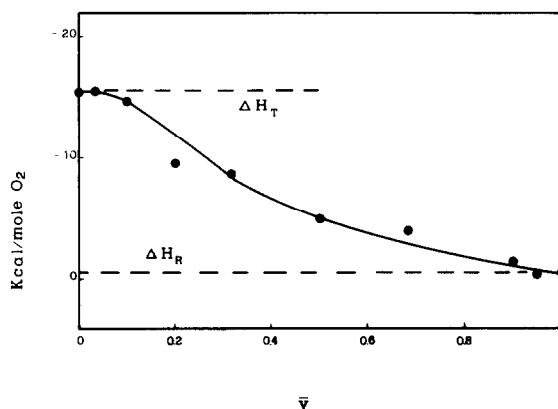


Fig.3. The overall ΔH of oxygen binding, calculated from the data reported in fig.2, reported as a function of fractional saturation (\bar{Y}). Data were obtained from the experimental points and refer to O_2 in solution.

transport to respiring tissues. These data call for a structural investigation to elucidate the molecular mechanism which is at the basis of the unusual thermodynamics of this arctic hemoglobin.

REFERENCES

- [1] Condò, S.G., El-Sherbini, S., Shehata, Y.M., Serpe, E., Nuutinen, M., Lazzarino, G. and Giardina, B. (1988) *Arct. Med. Res.* 47, 83–88.
- [2] Lykkeboe, G., Johansen, K. and Maloiy, G.M.O. (1975) *J. Comp. Physiol.* 104, 1–11.
- [3] Giardina, B. and Amiconi, G. (1981) *Methods Enzymol.* 76, 417–427.
- [4] Antonini, E., Condò, S.G., Giardina, B., Joppolo, C. and Bertollini, E. (1982) *Eur. J. Biochem.* 121, 325–328.
- [5] Bunn, H.F. (1971) *Science* 172, 1049–1050.
- [6] Perutz, M.F. (1984) *Adv. Protein Chem.* 36, 213–243.
- [7] Fronticelli, C., Bucci, E. and Orth, C. (1984) *J. Biol. Chem.* 259, 10841–10844.
- [8] Perutz, M.F. and Imai, K. (1980) *J. Mol. Biol.* 136, 183–191.
- [9] Bonaventura, C., Sullivan, B. and Bonaventura, J. (1974) *J. Biol. Chem.* 249, 3768–3775.
- [10] Soppela, P., Nieminen, M. and Timisjarvi, J. (1986) *Rangifer* 1, 273–278.
- [11] Imai, K. and Tyuma, I. (1973) *Biochem. Biophys. Res. Commun.* 51, 52–58.
- [12] Roughton, F.J.W., Otis, A.B. and Lyster, R.L.J. (1955) *Proc. R. Soc. B* 144, 29–54.
- [13] Imai, K. and Yonetani, T. (1975) *J. Biol. Chem.* 250, 7093–7098.
- [14] Bunn, H.F. (1981) *Blood* 58, 189–197.