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Flight and heat dissipation in birds

A possible molecular mechanism

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Birds during normal sustained flight must be able to dissipate more than 8 times as much heat as during rest in order not to be overheated. The experiments reported in this note on the hemoglobin systems from two different birds indicate the existence of a molecular mechanism by which hemoglobin is used simultaneously for oxygen transport and heat dissipation.

Heat; Bird; Hemoglobin; Temperature; Flight

1. INTRODUCTION

The unique allosteric properties of hemoglobin are such that protons, carbon dioxide and increased temperature, features which pertain in the tissues, all promote the release of oxygen contributing to meet the metabolic requirements of the given species. However it should be outlined that, due to the exothermic character of the oxygenation process of hemoglobin, a significant increase of body temperature may result in a substantial decrease of oxygen loading at the level of the lungs. This, in turn, could also imply a decrease of the amount of oxygen released by hemoglobin at the level of the tissues whose metabolic requirements may not be fully met. This situation may well be that of birds during flight. In fact, flight is a very energy-requiring way of locomotion so that the metabolic rates of flying birds increase to more than 8 times the resting rate [1,2]. This observation implies that birds during normal sustained flight must be able to dissipate more than 8 times as much heat as during rest in order not to be overheated [3,4]. Although some controversy does exist, it has been suggested that regulation of heat loss during flight takes place by an active process whereby the bird is able to greatly increase the heat loss through the feathers substantially reducing their insulation. If this was not the case it would not be possible to account for the removal of the extra heat transferred by the circulation of blood to the skin surface.

Moreover, recent studies [5,9] on hemoglobins from arctic mammals have clearly pointed out that much interesting information can be overlooked when experiments are carried out at a single temperature not taking into account that the thermodynamic parameters of oxygen binding are of extreme importance also from a physiological point of view.

For all the reasons reported above we have investigated the effect of temperature on the functional properties of hemoglobins from two different species of birds which are characterized by different life-behaviour. Hence *Gallinula chloropus* is a species seasonally subjected to prolonged flights while *Columba livia* although flying at a quite high velocity (~50 km/h) is not able to maintain the corresponding muscular activity for more than 10 min.

The results obtained suggest the possibility that in some species special molecular mechanisms may be at work to increase heat dissipation and to stabilize body temperature at a reasonable level.

2. MATERIALS AND METHODS

The two species of birds were captured near Cagliari (Sardinia, Italy). Blood samples from pigeon (*Columba livia*) and water-hen (*Gallinula chloropus*) were collected into an isotonic NaCl solution containing 2 mM EDTA. The cells were washed 3 times by centrifugation at 3000 rpm in isotonic NaCl solution and the packed cells lysed by adding 2 vols of cold distilled water. The stroma were removed by centrifugation at 10 000 rpm for 30 min. Electrophoretic analysis of hemoglobin components was performed by alkaline polyacrylamide gel electrophoresis. Stripped hemoglobin was obtained by passing the hemolysate first through a Sephadex G-25 column, equilibrated with 0.01 M Tris buffer, pH 8.0, containing 0.1 M NaCl, and afterwards through a column of mixed-bed ion-exchange resin (Bio-Rad AG 501X8).

Concentrated stock solutions of P_6-inositol (0.1 M) were prepared by dissolving the sodium salt of phytic acid (Sigma) in water and adjusting the pH to the desired value with concentrated phosphoric acid.

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Oxygen dissociation curves were determined spectrophotometrically [10] at a protein concentration of 3-5 mg/ml. Spectrophotometric measurements were carried out with a Kontron 860 Uvikon spectrophotometer. The change in enthalpy, ΔH, accompanying oxygenation was calculated from the integrated van 't Hoff equation [8].

3. RESULTS

Electrophoretic analysis of the hemolysate from pigeon shows only one hemoglobin component. In contrast the hemolysate from gallinula is characterized by the presence of 3 hemoglobin components, one of which (Hb1) represents about 78% of total pigment and the other two account for the remaining 22% (Hb2 = 7%, Hb3 = 15%).

The oxygen binding properties of the two avian hemoglobin systems were investigated, as a function of temperature, in the pH range 6.0-8.5.

Fig. 1 (panel A) shows the effect of pH on the oxygen affinity of pigeon Hb at two different temperatures (15 and 20°C) and in the presence of 3 mM P_6-inositol. We have focused our attention on this effector even if the physiological co-factor in avian red cells is, generally, P_5-inositol because most of the available data on human HbA and on hemoglobin from other species concern the interaction with P_6-inositol; in this respect it was shown that P_5-inositol is only slightly less effective than P_6-inositol [11,12]. Furthermore, previous investigations [13] on the functional properties of bird erythrocytes and hemoglobin solution containing 3 mM P_6-inositol have pointed out only slight differences clearly indicating that considerations based on the effect of P_6-inositol, at this concentration, on the functional properties of bird hemoglobins are certainly valid and physiologically interpretable.

In the same figure, for comparison, the oxygen Bohr effect of human HbA is reported at 20°C in the presence of its physiological effector namely 2,3-DPG (3 mM). As may be observed the oxygen affinity of pigeon hemoglobin is lower than that of HbA in the presence of 2,3-DPG at the same temperature. As far as the Bohr effect is concerned, its amplitude appears to be only slightly affected by temperature within the range explored and smaller than that of human HbA being the value of its Bohr coefficient ($\Delta \log P_{50}/\Delta pH$) -0.36 at 20°C versus -0.51 for HbA.

Panel B of Fig. 1 reports the effect of pH on the oxygen affinity of gallinula Hb at different temperatures and in the presence of 3 mM P_6-inositol. A peculiar feature is represented by the temperature dependence of the amplitude of the Bohr effect which increases as temperature increases ($\Delta \log P_{50}/\Delta pH = -0.39$ at 15°C and -0.53 at 20°C). It should be pointed out that this behaviour is just the opposite of what has been previously seen on human HbA [14]. Hence, in the case of gallinula Hb the enhancement of the Bohr effect brought about by the increase in temperature results in an increase of the overall ΔH of oxygen binding as the pH is lowered. This is further evidenced by Fig. 2 in which both the temperature dependence, at different pH values, of oxygen affinity (panel A) and the pH dependence of the overall ΔH of oxygen binding (panel B) are reported for gallinula Hb, pigeon Hb and human HbA. As evident the overall ΔH of oxygen binding for pigeon Hb is almost pH independent representing an intermediate case with respect to human HbA and gallinula Hb.

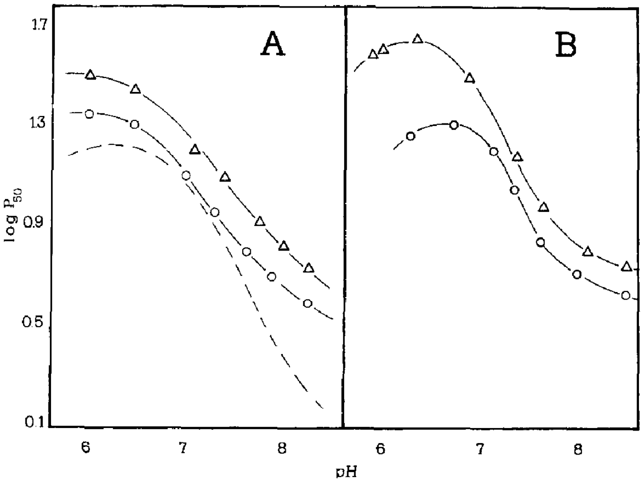

Fig. 1. Oxygen Bohr effect of *Columba livia* (panel A) and *Gallinula chloropus* (panel B) hemoglobin in 0.1 M Bis-tris or Tris buffer plus 0.1 M NaCl plus 3 mM P_6-inositol at (○) 15°C and (△) 20°C. Dashed line refers to human HbA in the same experimental conditions at 20°C and in the presence of 3 mM 2,3-DPG.

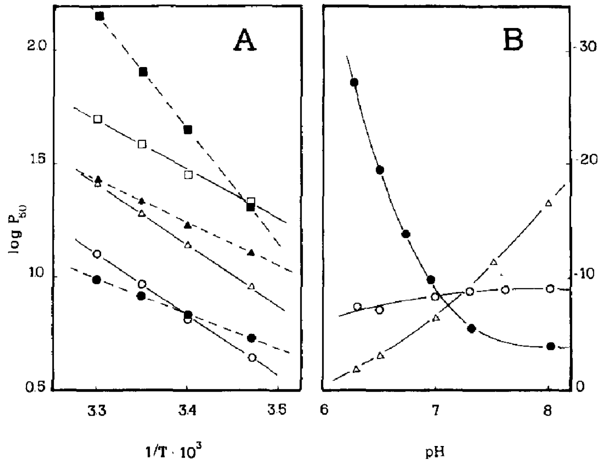

Fig. 2. Effect of temperature on the oxygen affinity of avian hemoglobins and, for comparison, of human HbA at different pH values. Panel A: van 't Hoff isochore (log P_{50} versus 1/T degrees K°) of *Columba livia* (open symbols) and *Gallinula chloropus* (closed symbols) hemoglobins at different pH values. (circles) pH 8.0, (triangles) pH 7.3 and (squares) pH 6.5. Conditions: 0.1 M Bis-tris or Tris buffer plus 0.1 M NaCl plus 3 mM P_6-inositol. Panel B: apparent heat of oxygenation for (●) *Gallinula chloropus* Hb, (○) *Columba livia* Hb and (△) human HbA as a function of pH calculated from the integrated van 't Hoff equation by using the data obtained from oxygen equilibria experiments. The values are corrected for the heat contribution of oxygen in solution (−3 kcal/mol). Conditions: 0.1 M Bis-tris or Tris buffer plus 0.1 M NaCl plus 3 mM P_6-inositol (for avian Hbs) or 3 mM 2,3-DPG (for HbA).

4. DISCUSSION

The results reported in this paper show that the reactions of hemoglobin from both pigeon and gallinula with oxygen and protons are functionally linked and qualitatively similar to those found for other hemoglobins. However the effect of P_6-inositol deserves some special comment. Thus, the total change in log P_{50} induced by this organic polyanion is somewhat smaller (by about 60% and 25% for gallinula and pigeon respectively at pH 7.0 and 20°C) as compared to that observed in the case of human HbA saturated with its physiological cofactor (2,3-DPG). Within the framework of the two states allosteric model [15] this implies that the difference in the organic phosphate binding constant between T and R states of the molecule is lower than that measured for human HbA leading to a decrease of the differential interaction. This finding could be tentatively attributed to the existence in bird hemoglobins of a higher number of positively charged residues at the phosphates' binding site which may lead to a high affinity for organic phosphates even in the oxygenated state [16,17]. Moreover, from a physiological point of view, it could be interesting to outline that the physiological phosphate P_5-inositol appears to be particularly inert from a metabolic point of view [18-20]. This introduces a different perspective when looking at the role of organic phosphates in the different species, since the modulation related to the effect of 2,3-DPG in mammals and its correlations to the metabolic properties of the cell are not parallelled in bird blood. This fact may suggest the existence of other adaptation mechanisms which may possibly involve the interplay between P_5-inositol and carbon dioxide. This hypothesis is worth exploring in some detail.

Another peculiar feature is represented by the unusual effect of temperature on the amplitude of the Bohr effect of gallinula Hb as it is outlined in panel B of Fig. 2 in which values of the apparent ΔH for oxygen binding were reported as a function of pH. It could be very informative and appropriate to try and correlate the observations above and the various contributions which, at a molecular level, are included in the overall thermal effects measured upon oxygen binding. These may be summarized as follows: (i) intrinsic heat of oxygenation; (ii) heat of ionization of oxygen-linked acid groups (endothermic); (iii) heat of oxygen solubilization (exothermic); (iv) heat involved in the $T \rightarrow R$ allosteric transition (always endothermic in the cases examined so far) [14,21] and (v) heat of binding of other ions.

It should be recalled that in the case of human HbA the more exothermic value is observed at very alkaline pH values where the Bohr effect is over and the contribution of the Bohr protons (endothermic) is abolished [14]. In the case of HbA going towards acid pH values the apparent ΔH of oxygen binding becomes less and less exothermic due to the increasing contribution of the Bohr protons that cancels some of the heat released upon oxygen binding.

However in the case of gallinula Hb we have a completely different situation since the apparent heat of oxygenation is at its minimum (in absolute value; -4.0 kcal/mol of oxygen) at alkaline pH values and tends to more exothermic values (up to -27 kcal/mol of oxygen at pH 6.3) going towards lower pHs in spite of the increasing endothermic contribution of the Bohr protons. Therefore, leaving constant the (intrinsic) ΔH values of oxygen binding and solubilization, the only contribution which may be exothermic and thereby explaining the observed change of ΔH as a function of pH, are the ones mentioned in points (iv) and (v). If we assume that the $T \rightarrow R$ conformational transition is endothermic as in most of the oxygen carriers tested so far, this implies an exothermic oxygen-linked binding of other ions whose relative weight becomes more and more important as the pH is decreased.

In any case these thermodynamic properties seem to have a great physiological significance in relation to the problem of heat dissipation that birds have to solve when flying for a long period of time. In this respect, it seems of particular significance that also in the case of pigeon we observe an unusual effect of pH on the overall ΔH of oxygenation that is slightly affected (from -7 to -9 kcal/mol) by the variations of proton concentration showing at alkaline pHs a value remarkably lower (-9 versus -17 kcal/mol at pH 8.0) and at acid pHs remarkably higher (-7 versus -3 kcal/mol at pH 6.5) with respect to human HbA. This unusual behaviour could be of great significance from a physiological point of view since it could well be related to the necessity of the animal to optimize the heat exchange at the level of the lungs. Moreover, the heat required at the level of the tissues for the deoxygenation of the hemoglobin molecule is about double in respect to other vertebrates thereby contributing to remove the heat metabolically released at the same level and to cool the whole organism.

In this respect the hemoglobin from gallinula is certainly a very elegant example of adaptation to specific physiological requirements. Thus its functional properties and their dependence on both pH and temperature provide a molecular mechanism by which the problems of an adequate oxygen supply to muscles, of buffering the increased amount of protons produced at muscular level and of dissipating as much heat as possible at the same level are all contemporaneously solved.

Thus, during the activity linked to the prolonged flight, at the muscular level we observe an increased demand of oxygen, a great production of heat due to the increased rate of the metabolic reactions and a contemporaneous decrease of pH brought about either by lactic acid production and/or by the increase in temperature. Hemoglobin reaching the muscular tissue, finds a more acid pH which lowers its oxygen affinity and increases both its ΔH of deoxygenation and its Bohr effect to maintain the body temperature at a reasonable level. In fact, assuming a pH value of approx. 6.6 at the level of the muscles, the hemoglobin from gallinula would require, during the deoxygenation process, at least 3 times more heat than human HbA thereby contributing to lower the amount of heat that has to be dissipated by other ways such as evaporation of water [3] and convection [22].

Unfortunately our data on gallinula Hb cannot be compared with those reported recently [23] on the temperature dependence of the oxygen binding properties of the migratory bird, swift (*Apus apus*). In fact the measurement of the oxygen affinity of the swift hemoglobin at different pH values and temperatures has been performed only under 'stripped conditions' (no P_6-inositol).

Finally it should be mentioned that in the absence of information about the effect of carbon dioxide we are not able to discuss in detail the Bohr shift during oxygen unloading. However it should be recalled that, during prolonged flight, birds utilize mainly fat as energy supply and that under these conditions the Bohr shift would be reduced due to the Haldane effect. However, even if protons would be buffered at the level of muscles, the temperature increase would significantly decrease the pH bringing the 'mechanism' outlined above into operation.

Although further studies are needed in order to understand how general is in birds the behaviour described and to elucidate its structural basis, it is very suggestive that these unusual thermodynamic properties seem to fit very well with the known physiological requirements of the species under investigation. It is also significative that *Columba livia*, that represents an intermediate case, is not able, like other pigeons reported in literature, to fly for more than 10 min [4].

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