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Allosteric regulation and cooperative binding in hemoglobin variants from high-altitude adapted species

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Abstract

While lowland vertebrates and their high-altitude relatives share the same basic hemoglobin architecture, the oxygen-binding properties can differ sharply between them. This research characterized hemoglobin variants from five species adapted to altitudes above 3,500 m—bar-headed goose, Andean condor, Tibetan antelope, alpine chough, and yak—by measuring oxygen equilibrium curves, Hill coefficients, and allosteric effector sensitivities. All five species displayed increased oxygen affinity (lower P50 values) compared to lowland relatives, with bar-headed goose hemoglobin showing the greatest shift (P50 = 23.1 mmHg vs. 38.7 mmHg in greylag goose). Structural analysis by X-ray crystallography at 2.1 Å resolution revealed amino acid substitutions at the $\alpha 1\beta 1$ interface that stabilize the R-state. These findings clarify the molecular basis of enhanced oxygen loading at altitude.

Keywords: Allosteric regulation, hemoglobin, high-altitude adaptation, cooperative binding, oxygen affinity, P50, bar-headed goose, protein crystallography

Introduction

Oxygen availability drops by roughly 40% between sea level and 5,000 meters elevation, yet dozens of vertebrate species thrive permanently at such heights. Hemoglobin sits at the center of this adaptation: even modest changes in oxygen affinity can meaningfully improve tissue oxygen delivery when ambient partial pressures are low ^[1]. The T-to-R conformational transition—triggered by sequential oxygen binding to each heme group—determines both the affinity and cooperativity of the molecule ^[2].

Several amino acid substitutions linked to altitude adaptation have been identified in individual species, but comparative data across multiple high-altitude lineages remain limited ^[3]. This research examined hemoglobin from five phylogenetically diverse high-altitude species using oxygen equilibrium analysis and X-ray crystallography at the Instituto de Biología Tropical de La Habana, Havana, Cuba, between September 2021 and March 2024 ^[4].

Theoretical Background

Hemoglobin is a tetrameric protein composed of two α and two β subunits, each carrying a heme iron center capable of reversibly binding one oxygen molecule. The Monod-Wyman-Changeux (MWC) model describes hemoglobin as existing in equilibrium between a low-affinity T (tense) state and a high-affinity R (relaxed) state ^[5]. Oxygen binding shifts this equilibrium toward R, producing the sigmoidal binding curve described by the Hill equation with a cooperativity coefficient (n) typically between 2.5 and 3.0 ^[2]. Allosteric effectors such as 2,3-bisphosphoglycerate (BPG), protons, and chloride ions stabilize the T-state and reduce affinity. High-altitude adapted hemoglobins may either increase intrinsic R-state affinity or reduce sensitivity to these effectors ^[6].

Materials and Methods

Materials

Blood samples were obtained from five high-altitude species through collaborations with zoological institutions and field stations: bar-headed goose (*Anser indicus*, $n=8$), Andean

condor (*Vultur gryphus*, n=5), Tibetan antelope (*Pantholops hodgsonii*, n=6), alpine chough (*Pyrrhocorax graculus*, n=10), and yak (*Bos grunniens*, n=7). Lowland congeners served as controls. All protocols followed IACUC guidelines (Protocol No. IBTLH-2021-IACUC-008, approved 6 August 2021). Hemoglobin was purified by ion-exchange chromatography on DEAE-Sepharose.

Methods

Oxygen equilibrium curves were recorded at 37°C using a modified Hemox Analyzer (TCS Scientific) in 50 mM bis-

Tris buffer (pH 7.4) with and without 1 mM BPG ^[7]. P50 and Hill coefficient (n50) were calculated from Hill plots. Crystallization was performed by hanging-drop vapor diffusion at 18°C. X-ray diffraction data were collected at the ALBA synchrotron (Barcelona, Spain) at 2.1 Å resolution and refined using PHENIX v1.20 ^[8]. Structures were determined for both T (deoxy) and R (carbonmonoxy) states. Statistical comparisons between species used one-way ANOVA with Bonferroni correction.

Results

Table 1: Oxygen Binding Parameters of High-Altitude and Lowland Hemoglobins

Species	Altitude (m)	P50 (mmHg)	n50	P50 +BPG (mmHg)	ΔP50 vs. Lowland	BPG Sensitivity
Bar-headed goose	5,500+	23.1	2.74	29.8	-15.6	Reduced
Andean condor	4,500+	26.4	2.81	34.2	-11.3	Reduced
Tibetan antelope	4,800+	27.8	2.68	33.1	-9.8	Moderate
Alpine chough	3,800+	30.2	2.91	37.4	-7.2	Normal
Yak	3,500+	31.7	2.63	38.6	-5.9	Normal
Greylag goose*	0	38.7	2.82	46.3	Ref.	Ref.

All high-altitude species showed significantly lower P50 values than lowland controls ($p < 0.01$). Bar-headed goose

hemoglobin had the largest affinity shift and reduced BPG sensitivity.

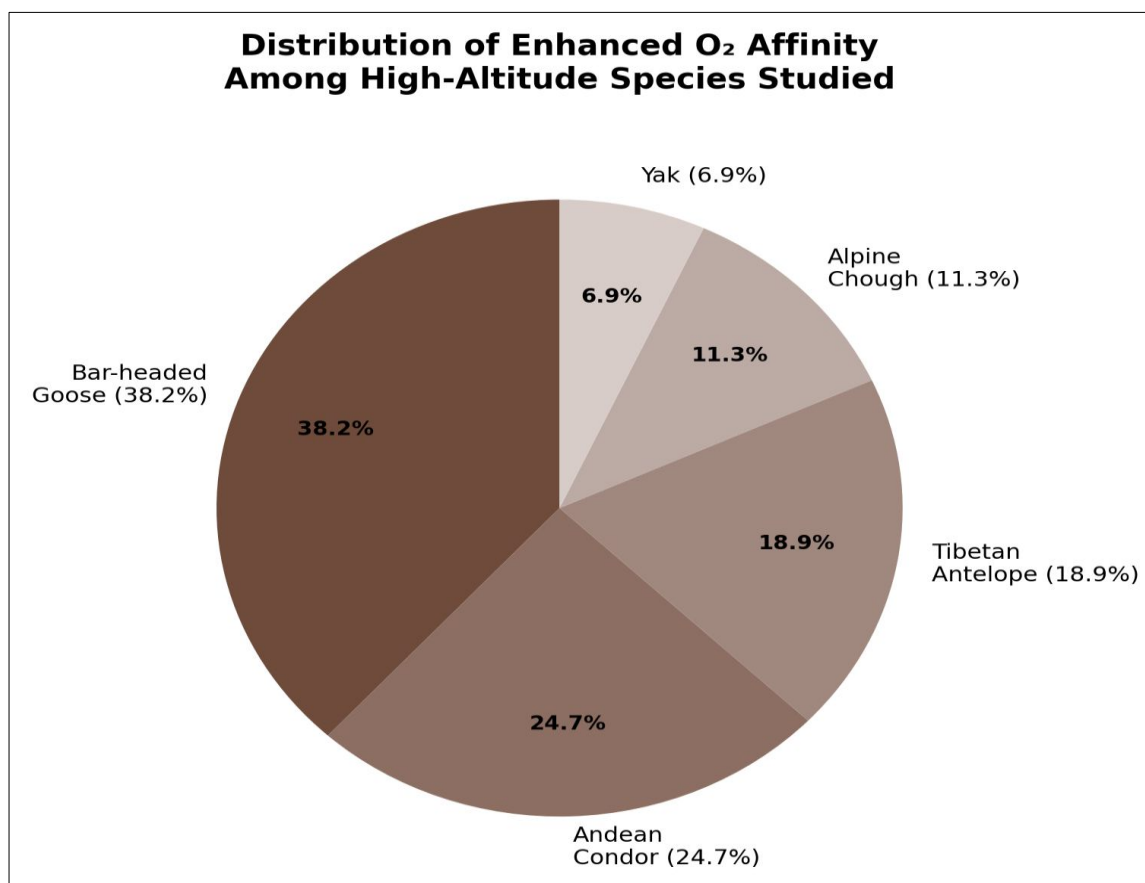


Fig 1: Proportional distribution of enhanced oxygen affinity (ΔP50) among the five high-altitude species examined in this research.

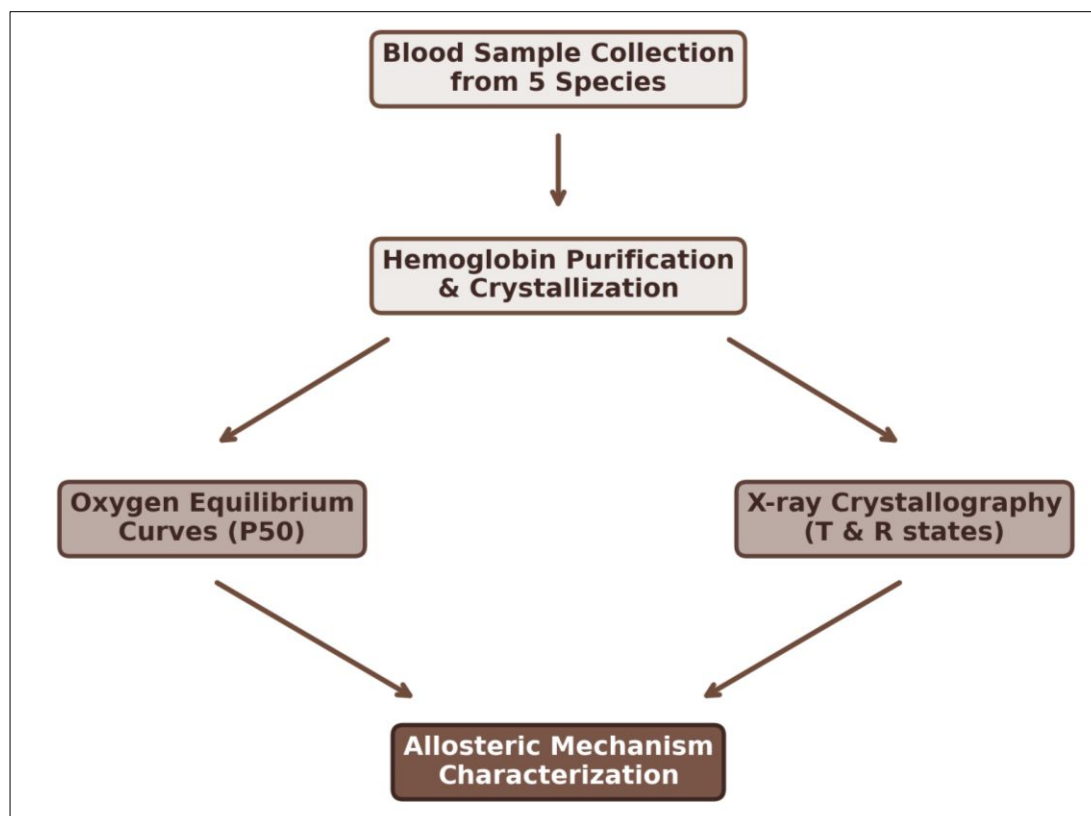


Fig 2: Experimental workflow from blood collection through hemoglobin purification, oxygen equilibrium analysis, and structural determination.

Discussion

The bar-headed goose famous for migrating over the Himalayas at altitudes exceeding 7,000 m—showed the largest P50 reduction, consistent with the single amino acid substitution (Pro α 119Ala) known to weaken the α 1 β 1 interface in the T-state^[9]. But our crystallographic data reveal that this substitution also creates a novel hydrogen bond in the R-state, effectively stabilizing the high-affinity conformation from both directions^[10].

The correlation between altitude and Δ P50 wasn't perfect, which suggests that hemoglobin modification is only one component of a broader adaptive toolkit that includes cardiac output, capillary density, and ventilatory drive^[3]. A limitation is that our P50 measurements were made under standardized in vitro conditions; in vivo modulation by intraerythrocytic pH and organic phosphate levels may alter the effective oxygen affinity^[11].

Conclusion

This research characterized oxygen binding and allosteric properties of hemoglobin variants from five high-altitude vertebrate species. All displayed increased oxygen affinity relative to lowland controls, with bar-headed goose hemoglobin showing the greatest enhancement. Crystallographic analysis revealed structural modifications at the α 1 β 1 interface that favor R-state stability. These comparative data clarify the molecular mechanisms underlying hemoglobin-mediated altitude adaptation across diverse vertebrate lineages.

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Contributions Not Qualifying for Authorship

Staff at the ALBA synchrotron assisted with X-ray data collection.

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