Supplementary material

Figure S1. Alignment of α-type globin sequences from bar-headed goose and greylag goose. The α^A- and α^D-globins comprise the α-type subunits of the major HbA and the minor HbD isoforms, respectively.

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                    1         10        20        30        40        50        60        70
Anser indicus (αA)  VLSAADKTNVKAVFSKISGHAEEYGAETLERMFTAYPQTKTYFPHFDLQHGSAQIKAHGKKVVAALVEAV
Anser anser (αA)    ..........G.....G.....................................................A.......
Anser indicus (αD)  M...D..KIIAQLWE.VA..QD.F.N.A.Q...VT.............VHP..E.VRS.....A...GN..
Anser anser (αD)    M.T.D..KIIAQLWE.VA..QD.F.N.A.Q...VT.............HP..E.VRS.....A...GN..

                    80        90        100       110       120       130       141
Anser indicus (αA)  NHIDDIAGALSKLSDLHAQKLRVDPVNFKFLGHCFLVVVAIHHPSALTAEVHASLDKFLCAVGTVLTAKYR
Anser anser (αA)    ...............................................P.....................
Anser indicus (αD)  KSL.N.SQ...E..N...YN.....A...L.SQ..Q..L.V.LGKDY.P.M..AF....S..AA..AE...
Anser anser (αD)    KSL.N.SQ...E..N...YN.....A...L.SQ..Q..L.V.LGKDY.P.M..AF....S..AA..AE...
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Figure S2. Purification of HbA and HbD by anion exchange chromatography of bar-headed goose (A, left panel) and graylag goose (B, left panel) hemolysates, and corresponding analysis of peaks by isoelectric-focusing (right panels). In the chromatograms (absorbance, continuous lines), peak 1 passes unbound through the column and corresponds to the major HbA, peak 3 corresponds to the minor HbD, while peak 2 contains HbA, presumably due to protein washout at the onset of the salt gradient (dashed line).

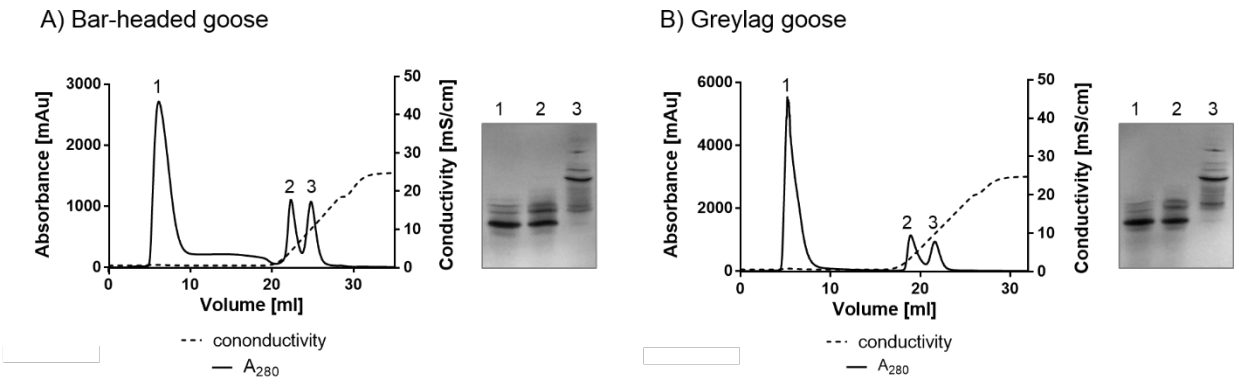

Table S1. O_2 affinity (P_{50}, torr), Hill cooperativity coefficient (n_{50}) and Bohr effect (expressed as Bohr factor Φ = ΔlogP$_{50}$/ΔlogpH) of HbA and HbD from bar-headed goose and graylag goose. Experiments were performed in the absence (stripped) and presence of 0.1 M KCl and 0.15 mM IHP, added separately and in combination. Experiments were done at a heme concentration of 0.3 mM, 37 °C, in 0.1 M Hepes buffer, pH 7.4. Data are means of duplicate experiments (variation was < 10%), except for the Bohr effect, where Φ is derived from linear regression (linear plots of Fig. 2E,F). n.d., not determined.

Species	Conditions	HbA				HbD			
		P_{50} (torr)	logP_{50}	n_{50}	Φ	P_{50} (torr)	logP_{50}	n_{50}	Φ
Bar-headed goose	stripped	3.72	0.57	2.35	-0.36	2.79	0.45	2.15	-0.35
	KCl	4.38	0.64	2.58	n.d	3.20	0.51	2.13	n.d.
	IHP	46.22	1.66	3.21	-0.55	29.84	1.47	2.94	-0.45
	IHP+KCl	34.71	1.54	3.67	n.d.	24.29	1.39	2.95	n.d.
Greylag goose	stripped	4.24	0.63	2.29	-0.35	4.60	0.66	1.93	-0.39
	KCl	6.05	0.78	3.00	n.d.	6.47	0.81	1.51	n.d.
	IHP	60.22	1.78	2.94	-0.45	45.28	1.66	3.18	-0.43
	IHP+KCl	49.37	1.69	2.78	n.d.	35.24	1.55	3.50	n.d.

Table S2. Thermodynamic MWC allosteric parameters for O_2 binding of HbA and HbD from bar-headed goose and graylag goose in the absence (stripped) and presence of 0.15 mM IHP, fitted within 95% confidence limits. Experiments (shown in Fig. 4) were performed at 37 °C (heme concentration 0.3 mM, 0.1 M Hepes buffer, pH 7.4). MWC parameters of recombinant adult human Hb wild type (wt) and α119Pro→Ala mutant obtained under stripped conditions at 25 °C (heme concentration 0.146 mM, 0.1 M Hepes, pH ~7.25) (Weber et al., 1993) are shown for comparison. P_{50}, O_2 tension at half-saturation; P_m, median O_2 tension; n_{50} and n_{max}, half-saturation and maximal Hill's cooperativity coefficients; K_T and K_R (torr^{-1}), O_2 association equilibrium constant of T- and R-states, respectively; $c = K_T/K_R$; ΔG (kcal mol^{-1}), free energy of cooperativity (ΔG = -RT lnK_T/K_R, where R is the gas constant and T is the absolute temperature). Fitting correlation coefficient r^2 is indicated.

Parameter	Bar-headed goose				Greylag goose				Human Hb at 25°C (Weber et al. 1993)	
	HbA		HbD		HbA		HbD		Hb wt	Hb α119Pro→Ala
	stripped	IHP	stripped	IHP	stripped	IHP	stripped	IHP	stripped	stripped
P_{50} (torr)	3.37	44.68	2.74	29.39	4.10	57.30	4.05	47.21	2.00	1.12
P_m (torr)	3.64	43.82	2.98	29.27	4.31	54.46	4.39	47.04	1.84	1.05
n_{50}	2.67	3.44	1.97	3.05	2.57	3.09	2.17	3.01		
n_{max}	2.74	3.45	1.93	3.05	2.60	3.13	2.13	3.01		
K_T (torr^{-1})	0.012	0.002	0.040	0.003	0.022	0.003	0.023	0.002	0.170	0.448
K_R (torr^{-1})	1.20	0.76	0.86	0.38	1.07	0.61	0.68	0.22	6.22	6.22
log$L$	2.57	6.08	1.62	4.18	2.65	6.10	1.90	4.08	4.24	3.28
c	0.010	0.002	0.046	0.009	0.021	0.004	0.033	0.010		
ΔG (kcal mol^{-1})	2.69	3.77	1.65	2.89	2.33	3.30	1.90	2.83	2.13	1.56
r^2	0.998	0.998	0.998	0.999	0.998	0.998	0.998	0.998		

References

Weber, R. E., Jessen, T. H., Malte, H. and Tame, J. (1993). Mutant hemoglobins (alpha 119-Ala and beta 55-Ser): functions related to high-altitude respiration in geese. *J. Appl. Physiol.* **75**, 2646-2655.