Table 2 Rate and equilibrium constants for O_2, CO and methyl isocyanide binding to native (His-E7) and mutant (Gly-E7) sperm whale myoglobin and human α- and β-chains in R-state haemoglobin at 20 °C

Protein	O_2			CO			Methyl isocyanide		
	k' ($\times 10^{-6}$ M^{-1} s^{-1})	k (s^{-1})	K ($\times 10^{-6}$ M^{-1})	k' ($\times 10^{-6}$ M^{-1} s^{-1})	k (s^{-1})	K ($\times 10^{-6}$ M^{-1})	k' ($\times 10^{-6}$ M^{-1} s^{-1})	k (s^{-1})	K ($\times 10^{-6}$ M^{-1})
Mb(E7 His)	14±3	12±2	1.2±0.3	0.51±0.06	0.019±0.005	27±8	0.12±0.02	4.3±0.3	0.028±0.005
Mb(E7 Gly)	140	1,600	0.087	5.8	0.038	150	10	6.3	1.6
α(E7 His)	29±5	10.1±4.6	2.9±1.4	3.2±0.4	0.0047±0.005	680±110	0.14±0.06	1.8±0.1	0.078±0.033
α(E7 Gly)	220	620	0.4	19	0.0067	2,900	0.21	0.28	0.75
β(E7 His)	100±24	21±6	5.0±2.0	9.8±1.9	0.0086±0.0030	1,100±500	0.57±0.13	6.4±0.8	0.089±0.023
β(E7 Gly)	100	37	3.0	5.0	0.0130	390	0.67	4.2	0.16

All reactions were carried out in 0.1 M K phosphate, pH 7.0 for myoglobin (Mb) or 0.1 M Bis-Tris, 0.1 M KCl, pH 7.0 for haemoglobin (α- and β-mutants). The exact protein concentration varied: 2–10 μM haem concentrations were used in stopped-flow, rapid mixing and conventional photolysis experiments, whereas 20–50 μM haem concentrations were used in laser photolysis experiments. Under these conditions liganded or R-state haemoglobin does dissociate partially into dimers; however, all previous work indicates that the functional properties of the α- and β-chains in dimers are essentially identical to those in R-state, high-affinity tetramers[14–16]. The dissociation rate constants (k) were measured by stopped-flow techniques as described in Table 1. Association rate constants (k') for ligand binding to R-state haemoglobin were determined from partial photolysis experiments. Only time courses showing <10% photodissociation were considered to ensure that $Hb_4X_3 + X \rightarrow Hb_4X_4$ was the only tetrameric reaction being measured. The methyl isocyanide and CO complexes were photolysed by a square-wave, 0.5-ms light pulse whereas the O_2 derivatives were photolysed by a 500-ns dye laser pulse. The parameters for the His-E7 proteins were obtained from multiple experiments (>4), and the errors represent standard deviations from the mean. The constants for the *E. coli* wild-type proteins were averaged with those for the native proteins as systematic differences were not observed. Fewer experiments were done with the individual Gly-E7 derivatives, but the errors for the His-E7 parameters are assumed to apply to the mutant rate and equilibrium constants. The equilibrium constants (K) were calculated from the ratio of the rate constants (k'/k).

oxygen affinity due to a ~100-fold increase in the O_2 dissociation rate constant. This mutation also resulted in a five-fold increase in CO affinity for these two proteins, due mainly to an increase in the association rate constant. There were also marked increases (10–60-fold) in methyl isocyanide affinity for the Gly-E7 derivatives of myoglobin and α-chains, with different kinetic effects in the two proteins. In myoglobin the His-E7 to Gly mutation caused a ~100-fold increase in the association rate constant for isonitrile binding with little effect on the dissociation rate constant, whereas the same mutation in α-chains had no effect on the isonitrile association rate but decreased the dissociation rate about 10-fold. In contrast, the His-E7 to Gly substitution produced little or no effect on any of the ligand-binding properties of β-chains in R-state haemoglobin (Table 2, last two rows).

Neutron diffraction studies have demonstrated the presence of a hydrogen bond between His-E7 and the oxygen ligand in oxy-myoglobin[17]; no such hydrogen bond is found in CO-myoglobin[18]. The high resolution X-ray crystallographic structure of oxy-haemoglobin suggests that there is a similar hydrogen bond in the α-subunits but not in the β-subunits[5]. Shaanan has pointed out in detail the similarities of the distal pockets in oxygen-liganded myoglobin and R-state α-subunits. He also concluded that β-subunits behave more like model haem compounds than either of the other proteins[5]. Our experiments complement this structural work and clearly demonstrate different functional contributions of the distal histidine to ligand binding in these globins. In both myoglobin and α-subunits, His-E7 stabilizes bound O_2 by about -1.4 kcal mol^{-1}, presumably by hydrogen bonding, and sterically hinders CO binding by about 1.0 kcal mol^{-1}. The differences between the geometry of O_2 and CO bound to haemoglobin and myoglobin have been discussed elsewhere[18,19]. In the case of methyl isocyanide binding, the steric hindrance by His-E7 in myoglobin is much larger (~2.0 kcal mol^{-1}) than it is in α-chains (~1.0 kcal mol^{-1}), in agreement with Shaanan's observation of less hindered distal pockets in both subunits of oxy-haemoglobin. The insensitivity of oxygen affinity to the His-E7 to Gly substitution in R-state β-chains is also consistent with earlier calculations indicating unlimited freedom of rotation of bound O_2 and the apparent lack of a hydrogen bond with the distal histidine in the β-subunit of oxy-haemoglobin[5].

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Corrigenda

Evidence from cathodoluminescence for non-volcanic origin of shocked quartz at the Cretaceous/Tertiary boundary

Michael R. Owen & Mark H. Anders
Nature **334,** 145–147 (1988).
WE regret that we failed to acknowledge that sample material was provided by Glen A. Izett, US Geological Survey.

Structure of antibody hypervariable loops reproduced by a conformational search algorithm

Robert E. Bruccoleri, Edgar Haber & Jiří Novotný
Nature **335,** 564–568 (1988).
THE reference to the crystallographic structure of the monoclonal antibody Fab fragment HyHEL-5 was inadvertedly omitted. The reference reads as follows: S. Sheriff *et al., Proc. natn. Acad. Sci. U.S.A.* **84,** 8075–8079; 1987. Also, in the legend to Fig. 3, the amino-acid residue H 100 was incorrectly specified as aspartate. This residue is, in fact, an asparagine.