Expression, purification, and characterization of human hemoglobins Gower-1 ($\zeta_2\epsilon_2$), Gower-2 ($\alpha_2\epsilon_2$), and Portland-2 ($\zeta_2\beta_2$) assembled in complex transgenic–knockout mice

Zhenning He and J. Eric Russell

Embryonic $\zeta$- and $\epsilon$-globin subunits assemble with each other and with adult $\alpha$- and $\beta$-globin subunits into hemoglobin heterotetramers in both primitive and definitive erythrocytes. The properties of these hemoglobins—Hbs Gower-1 ($\zeta_2\epsilon_2$), Gower-2 ($\alpha_2\epsilon_2$), and Portland-2 ($\zeta_2\beta_2$)—have been incompletely described as they are difficult to obtain in quantity from either primary human tissue or conventional expression systems. The generation of complex transgenic–knockout mice that express these hemoglobins at levels between 24% and 70% is described, as efficient methods for their purification from mouse hemolysates. Key physiological characteristics—including $P_{50}$, Hill coefficient, Bohr effect, and affinity for 2,3-BPG—were established for each of the 3 human hemoglobins. The stability of each hemoglobin in the face of mechanical, thermal, and chemical stresses was also determined. Analyses indicate that the $\zeta$-for-$\alpha$ exchange distinguishing Hb Portland-2 and Hb A alters hemoglobin O$_2$-transport capacity by increasing its $P_{50}$ and decreasing its Bohr effect. By comparison, the $\epsilon$-for-$\beta$ exchange distinguishing Hb Gower-2 and Hb A has little impact on these same functional parameters. Hb Gower-1, assembled entirely from embryonic subunits, displays an elevated $P_{50}$ level, a reduced Bohr effect, and increased 2,3-BPG binding compared to Hb A. The data support the hypothesis that Hb Gower-2, assembled from reactivated $\epsilon$ globin in individuals with defined hemoglobinopathies and thalassemias, would serve as a physiologically acceptable substitute for deficient or dysfunctional Hb A. In addition, the unexpected properties of Hb Gower-1 call into question a common hypothesis for its primary role in embryonic development. (Blood. 2001;97:1099-1105)

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assembled from αα- and he-globin subunits (αααα–he-he) displayed O2-binding properties similar to those of control Hb αααα–h-he-he. This result strengthened the hypothesis that physiologically important characteristics of fully human Hb Gower-2 (αααα–e-e-e) might be similar to those of Hb A (αααα–β-β-β-β). In contrast, substantial differences in the properties of Hbs h-he–mββ and he–mββ suggested that the physiologic characteristics of fully human Hb Portland-2 (ζζζζ–mββ) and Hb A might differ in several important respects. In addition to providing an estimate of the biochemical and physiological properties of the semi-embryonic hemoglobins, these studies also indicated the potential value of human embryonic globin subunits as substitutes for adult globin subunits in individuals with defined thalassemias or hemoglobinopathies.

The current study extends our previous work by assessing key biochemical and physiological properties of fully human semi-embryonic and embryonic hemoglobins purified from complex transgenic–knockout mice. We describe a strategy for generating mice expressing high levels of human Hbs Gower-1 (αααα–e-e–e), Gower-2 (αααα–e–e–e), and Portland-2 (ζζζζ–mββ), as well as specific methods for their rapid and efficient purification. The key biochemical characteristics of each hemoglobin are subsequently determined, including their O2 affinities, subunit cooperativities, and changes in O2 affinity in response to allosteric modifiers and variations in ambient pH. We also assess the stability of each of these hemoglobins in response to defined mechanical, chemical, and thermal stresses. Based on the data, we speculate on the evolutionary basis for hemoglobin switching and the potential value of these poorly understood hemoglobins to patients with congenital α- and β-globin chain defects.

**Materials and methods**

**Transgenic and knockout mice**

The generation and characterization of transgenic mice expressing high levels of human α, β, ζ, and ε globins have previously been described. Mice with heterozygous knockout of their endogenous αα−globin genes (genotype max−/−) or β-globin genes (genotype mβ−/−) were generously provided by Y. W. Kan and Judy Chang (University of California, San Francisco) and O. Smithies (University of North Carolina, Chapel Hill), respectively. All mouse husbandry and experimentation was performed using protocols approved by the IACUC of the University of Pennsylvania.

**Hemoglobin purification**

Whole blood was collected from decapitated mice in 200 μL phosphate-buffered saline (PBS)–heparin (20 U/mL) or PBS–EDTA (27 mM), and the hemoglobins were promptly converted to the carbonmonoxomy form by bubbling the sample with CO. Erythrocytes were subsequently washed twice with excess PBS–EDTA (27 mM), and the cell pellets were stored in PBS–heparin (20 U/mL) or PBS–EDTA (27 mM), and the supernatant was diluted 10-fold with developer solution 50°C and 4°C, respectively, and spun for 10 minutes on a desktop centrifuge. Aliquots (2 mL) were shaken for defined intervals at a setting of 2000 on a Maxi-Mix III type 65800 shaker (Thermolyne, Dubuque, IA), and denatured hemoglobins were precipitated by a 5-minute desktop spin. The soluble hemoglobin was determined by A 542 spectrophotometry of the supernatant.

**Electrophoretic analysis**

The identity and purity of each hemoglobin preparation was verified by denaturing Triton–acid–urea (4,25) and non-denaturing cellulose acetate electrophoresis (4,25) using methods recommended by the manufacturer (Helena Laboratories, Beaumont, TX).

**Oxygen equilibrium curves**

Purified CO-hemoglobins were resuspended to a final concentration of approximately 7.5 μM in P50 buffer (50 mM Bis-Tris, pH 7.4, 100 mM NaCl, 5 mM EDTA) and converted to the oxyhemoglobin form by photolysis under 100% O2 using an ice water–cooled rotary condenser. Conversion to the oxyhemoglobin form was judged complete by an A 540 :A 576 ratio of less than 0.95. Oxygen equilibrium curves were subsequently determined on a HEMOX analyzer (TCS, Southampton, PA) at 20°C. Studies of 2,3-bisphosphoglycerate binding (2,3-BPG; Sigma, St Louis, MO) were carried out in P50 buffer (pH 7.4), whereas Bohr effect studies were carried out in P50 buffer adjusted to defined pH values.

**Stability determinations**

**Mechanical.** Using a modified version of a previously described method, Hbs were diluted to approximately 13 μM with 10 mM potassium phosphate buffer (pH 8.0) and converted to the oxyhemoglobin form (see above). Aliquots (2 mL) were shaken for defined intervals at a setting of 2000 on a Maxi-Mix III type 65800 shaker (Thermolyne, Dubuque, IA), and denatured hemoglobins were precipitated by a 5-minute desktop spin. The soluble hemoglobin was determined by A 542 spectrophotometry of the supernatant.

**Chemical.** Purified hemoglobins were diluted to approximately 0.1 mM in buffer (0.1 mM Tris, pH 7.4) and converted to the oxyhemoglobin form as described above. Aliquots diluted 10-fold in prewarmed Tris buffer containing 17% (vol/vol) isopropanol were incubated at 37°C for 5 minutes. Precipitated hemoglobin were clarified by desktop centrifugation, and the A 540 of the supernatant was determined.

**Thermal.** Purified hemoglobins were diluted to approximately 50 μM in buffer (0.1 mM Tris, pH 7.4) and converted to the oxyhemoglobin form as described above. Test and control aliquots were incubated for 2 hours at 50°C and 4°C, respectively, and spun for 10 minutes on a desktop centrifuge. The supernatant was diluted 10-fold with developer solution [11.9 mM NaHCO3, 0.77 mM KCl, 0.61 mM K2Fe(CN)6, insoluble hemoglobins were precipitated by desktop centrifugation, and the A 540 of the supernatant was determined.

**Results**

**Generation of adult mice expressing high levels of human embryonic and semi-embryonic hemoglobins**

The construction of transgenes and the generation of mice expressing h-h, h-he, and h-he globins in definitive erythrocytes has previously been described. The high-level, developmental-stage inappropriate expression of transgenic h-h and he globins was achieved by linking their encoding genes to transcriptional control elements from the h-h and h-he globins, respectively (Figure 1A). Full-length genes encoding h-h and h-he globins, containing their native transcriptional control elements, were anticipated to be expressed at high levels in adult erythrocytes and consequently were not modified. Each transgene was linked to a micro-β-locus control region to insulate its high-level, integration position-independent expression. Single lines expressing high levels of each transgenic globin were identified by phenotypic screening of hemolysates using denaturing globin electrophoresis. These lines were used in the experiments described in the current work. A breeding strategy was designed to generate mice expressing high levels of human embryonic or semi-embryonic hemoglobins with minimal mouse globin background (Figure 1B). We had
nullizygous. a2 h1 rescued the viability of mice with homozygous-lethal deletions of mα1, whereas the viability of a rescue the viability of mice with homozygous-lethal deletions of mα1, which facilitated the task of hemoglobin purification, as did the fact that a transgenic–knockout mice as 37%, 24%, and approximately 70% transgenes increased substantially in mice carrying one or more knockout mutations of the related endogenous adult globin gene homologues.18,19 The level of hε and hε induction was sufficient to rescue the viability of mice with homozygous-lethal deletions of their endogenous mα-globin genes, whereas the viability of mice with homozygous-lethal deletions of the mβ-globin genes could be rescued by the expression of either transgenic hε or hβ globins.18,31,32 We reasoned that the assembly of human globin heterotetramers from transgenic human α-like and β-like globins would be similarly enhanced in mice carrying both mα- and mβ-globin knockout alleles.

Design of methods to purify human hemoglobins from transgenic hemolysates

A combination of genetic and biochemical strategies was used to facilitate the preparation of human Hbs from transgenic mice. We screened more than 125, 335, and 89 candidate pups expressing Hbs Gower-1, Gower-2, and Portland-2, respectively, without identifying any mαα/mββ mice expressing 100% of the desired human hemoglobins (data not shown). On the other hand, a substantial proportion of these pups displayed either mαα/mββ or mαα/mββ genotypes (more than 25 pups expressing each hemoglobin; data not shown). Hbs Gower-1 (εε), Gower-2 (αεε), and Portland-2 (εεεε) were expressed in these complex transgenic–knockout mice as 37%, 24%, and approximately 70% of total hemoglobin, respectively, corresponding to the expression of human hemoglobin in the range of approximately 20 to 80 mg/mouse (data not shown). These high levels of expression facilitated the task of hemoglobin purification, as did the fact that mαα/mββ or mαα/mββ mice each assembled only a single contaminant globin species (mαεε or mεεε).

A method was subsequently established for isolating each of the desired human hemoglobin heterotetramers using cation-exchange chromatography. Human Hbs Gower-1 (εεεε), Gower-2 (αεεε), and Portland-2 (εεεε) were purified from contaminant hybrid Hbs mαεεε, mεεεε, and mεεεε, respectively, in single-step processes using NaCl gradients at defined pH levels (Figure 2, and data not shown). The identities and purities of the eluted human hemoglobins were subsequently verified by non-denaturing 19 and denaturing 22,23 electrophoretic methods (Figure 2 and data not shown). The large quantities of high-purity human embryonic and semi-embryonic hemoglobins efficiently prepared by this method were sufficient to permit their detailed physiological and biochemical evaluation and to provide substantial banked product for future functional and structural studies.

Embryonic and semi-embryonic hemoglobins exhibit elevated O2 affinities

The O2-binding affinities of human Hbs εεεε, αεεε, and εεεε were determined on 3 or more occasions under standard conditions by
to Hb Gower-2), whereas an α-to-ε exchange (converting Hb A to Hb Portland-2) has a more substantial impact on both O₂ affinity and subunit cooperativity.

The P₅₀ values of embryonic and semi-embryonic hemoglobins display disparate responses to changes in pH and [2,3-BPG]

The effect of pH on the O₂-binding affinities of the 3 human embryonic and semi-embryonic hemoglobins was determined by measuring the P₅₀ values for each in a series of buffers with defined pH levels ranging from 6.0 to 8.2 (Figure 4A; Table 1). The 2 human hemoglobins containing ζ-globin subunits displayed attenuated Bohr effects [Hbs ζε₂ (−0.10 ΔlogP₅₀/ΔpH₂) and ζβ₁ (−0.25 ΔlogP₅₀/ΔpH₂)], whereas the Bohr effect of Hb Gower-1 (αε₂) and control Hb A (αβ₂) were nearly identical (∼0.51 vs ∼0.54 ΔlogP₅₀/ΔpH₂, respectively). The apparent binding constant of 2,3-BPG for each hemoglobin was estimated by establishing the half-saturation point using buffers with defined concentrations of the allosteric modifier (Figure 4B; Table 1). 2,3-BPG appears to bind to Hb Portland-2 (ζβ₂) and control Hb A with equal affinity (0.30 mM and 0.29 mM, respectively) while binding to Hbs Gower-1 (ζε₂) and Gower-2 (αε₂) with substantially higher avidity (0.09 mM and 0.17 mM, respectively). These results indicate that the inclusion of embryonic globin subunits may have an impact on the biochemical function of intact heterotetramers, permitting speculation on the evolutionary pressures favoring the conservation of globin gene switching.

Human embryonic and semi-embryonic hemoglobins display different physical stabilities

Hbs Gower-1, Gower-2, and Portland-2 were assessed for their stability in the setting of defined mechanical, chemical, and thermal stresses.²⁹ Hbs A and S were evaluated in parallel as stable and unstable hemoglobin controls, respectively (Figure 5). The stabilities of the various hemoglobins by each of the 3 methods were in general agreement: Hbs Gower-1 (ζε₂) and Portland-2 (ζβ₂) were equally or less stable than Hb S, whereas the stability of Hb Gower-2 (αε₂) was generally intermediate between the 2 control hemoglobins. Hence, the low stability of Hb Gower-1 appears to be well adapted to the short survival of primitive erythrocytes, whereas the higher stability of Hb Gower-2 may facilitate its expression in long-lived definitive erythrocytes.

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**Table 1. Properties of human hemoglobins**

<table>
<thead>
<tr>
<th>Hemoglobin</th>
<th>αβ₂</th>
<th>αε₂</th>
<th>ζε₂</th>
<th>ζβ₂</th>
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<tbody>
<tr>
<td>Hb Gower-1</td>
<td>1.4 ± 0.06</td>
<td>1.7 ± 0.24</td>
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<td>Hb Gower-2</td>
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<td>2.3 ± 0.02</td>
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<td>0.17</td>
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<tr>
<td>Portland-2</td>
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<td>0.30</td>
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<th>Stability</th>
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<th>2,3-BPG</th>
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*torr; †log[Y]/Y = Y/log P₅₀; ‡log P₅₀/ΔpH in the alkaline region; ††apparent binding constant (mM) calculated from half-saturation point.

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**Figure 3. Oxygen equilibrium curves and Hill coefficients for human embryonic and semi-embryonic hemoglobins.**

(A) Human Hb Gower-1 (ζε₂). (B) Human Hb Gower-2 (αε₂). (C) Human Hb Portland-2 (ζβ₂). (D) Human Hb Gower-1 (ζε₂). (E) Human Hb Gower-2 (αε₂). (F) Human Hb Portland-2 (ζβ₂). Hill plots constructed from OECs of human hemoglobins in panels A to C are illustrated along with control human Hb A (O). Hill coefficients derived from these curves are included in Table 1.

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**Figure 4. Biochemical properties of human embryonic and semi-embryonic hemoglobins.**

(A) Effect of pH on O₂ binding (Bohr effect). The P₅₀ of each Hb was measured in a series of buffers with defined pH levels ranging from 6.0 to 8.2. The Bohr effect of Hb Gower-1 (ζε₂) and control Hb A (αβ₂) were nearly identical (∼0.51 vs ∼0.54 ΔlogP₅₀/ΔpH₂, respectively). The apparent binding constant of 2,3-BPG for each hemoglobin was estimated by establishing the half-saturation point using buffers with defined concentrations of the allosteric modifier (Figure 4B; Table 1). 2,3-BPG appears to bind to Hb Portland-2 (ζβ₂) and control Hb A with equal affinity (0.30 mM and 0.29 mM, respectively) while binding to Hbs Gower-1 (ζε₂) and Gower-2 (αε₂) with substantially higher avidity (0.09 mM and 0.17 mM, respectively). These results indicate that the inclusion of embryonic globin subunits may have an impact on the biochemical function of intact heterotetramers, permitting speculation on the evolutionary pressures favoring the conservation of globin gene switching.

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**Figure 5. Biochemical properties of human embryonic and semi-embryonic hemoglobins.**

(A) Effect of pH on O₂ binding (Bohr effect). The P₅₀ of each Hb was measured in a series of buffers with defined pH levels ranging from 6.0 to 8.2. The Bohr effect of Hb Gower-1 (ζε₂) and control Hb A (αβ₂) were nearly identical (∼0.51 vs ∼0.54 ΔlogP₅₀/ΔpH₂, respectively). The apparent binding constant of 2,3-BPG for each hemoglobin was estimated by establishing the half-saturation point using buffers with defined concentrations of the allosteric modifier (Figure 4B; Table 1). 2,3-BPG appears to bind to Hb Portland-2 (ζβ₂) and control Hb A with equal affinity (0.30 mM and 0.29 mM, respectively) while binding to Hbs Gower-1 (ζε₂) and Gower-2 (αε₂) with substantially higher avidity (0.09 mM and 0.17 mM, respectively). These results indicate that the inclusion of embryonic globin subunits may have an impact on the biochemical function of intact heterotetramers, permitting speculation on the evolutionary pressures favoring the conservation of globin gene switching.

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The temporal overlap of embryonic and adult globin defined trisomies may express easily detectable levels of z-suggestion of developmental globin gene switching. Third, a direct role in human development or are simply incidental to developmental overlapping of globin gene expression, a full understanding of their properties, along with the properties of Hb Gower-1, would be valuable for several purposes. First, structure–function analyses of all 3 hemoglobins are likely to provide additional insight into the biochemical composition of abundant, thoroughly studied hemoglobins such as Hbs A and F. Second, knowledge of its properties would provide a context for assessing the role of Hb Gower-1 in normal development and would help focus speculation on the evolutionary pressures favoring absolute phylogetic conservation of developmental globin gene switching. Third, a direct application of the analyses of Hbs Gower-2 and Portland-2 stems from the possibility that reactivation of the ζ and ε genes would produce globins that could substitute for deficient or abnormal α- or β-globin chains, respectively, in individuals with defined thalassemias and hemoglobinopathies. The implications of the current work on each of these 3 issues are considered below.

Many well-established studies have identified functionally crucial amino acid residues in the α- and β-globin chains. Our data generally confirm the role of these residues, but they also emphasize the importance of distant residues on specific hemoglobin functions through their likely influence on the high-order structure of the globin molecule. In general, Hb Gower-2 and Hb A function similarly, suggesting that many of the 36 amino acid differences between the β- and ε-globin chains (of 146 total residues) cluster in functionally silent domains (Table 1). The 2 hemoglobins exhibit similar O₂-binding characteristics and display Hill coefficients consistent with normal hemoglobin function (Figures 3B, E), reflecting the conservation of 13 of 17 and 15 of 16 residues comprising the αβ1 and αβ2 interfaces, respectively. Similarly, the robust Bohr effect common to Hb Gower-2 and Hb A (Figure 4A) might be anticipated from the conservation of 82Lys, 143His, and 146His in both the ε- and β-globin chains. Unexpectedly, Hb Gower-2 and Hb A displayed markedly different affinities for 2,3-BPG (Figure 4B) despite their identity at all 4 residues believed to mediate this property (1Val, 2His, 82Lys, and 143His). Although the half-saturation method provides only an estimate of the strength of this interaction, it is likely that one or more differences in amino acid content elsewhere in the ε- and β-globin chains alter the spatial arrangement, and hence the function, of the 4 conserved residues. A less likely explanation is that a fifth, unrecognized residue, differing between the β- and ε-globin chains, directly participates in 2,3-BPG binding. Crystallographic evaluation of Hb Gower-2, available in quantity from the transgenic-knockout mice, would be useful in evaluating these 2 possibilities.

In comparison to the β-like globin chains, the 57 (of 141) amino acid differences between the ζ- and α-globin chains appear to occupy functionally sensitive regions (Table 1). Hb Portland-2 and Hb A, differing only in the identity of their α-like chains, display substantially different P₅₀ values and Hill coefficients (Figure 3C, F) despite sharing 14 of 16 and 15 of 15 residues at the αβ1 and αβ2 interfaces, respectively. Remarkably, 2 of the 3 interface substitutions are conservative, emphasizing the unpredictable functional effects of changes in high-order globin subunit structure resulting from spatially distant amino acid substitutions. Although both globins share a common 122His, the αVal-ζSer exchange would be expected to substantially blunt the Bohr effect exhibited by Hb Portland-2, a prediction that is experimentally observed (Figure 4A). A direct comparison of Hb Portland-2 function with its high-resolution structure, when available, may yield important new information relative to the function of human heterotetramers.

The observation that the properties of Hb Gower-1, comprising developmental stage-concordant ζ- and ε-globin subunits, are strikingly different from those of Hb A suggests a strong evolutionary basis for Hb switching (Figures 3A, 4A-B; Table 1). A widely held, yet largely unsubstantiated, explanation for this process suggests that embryonic and fetal hemoglobins have been evolutionarily selected to facilitate the trans-placental delivery of O₂ during intrauterine development. This hypothesis has been challenged by studies that do not detect the predicted elevated rates of fetal wastage in pregnant women with high-affinity hemoglobins. By demonstrating that the O₂-binding properties of Hb Gower-1

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**Discussion**

In spite of their developmental sobriquets, there is considerable overlap in the temporal expression of embryonic ζ and ε globins and adult α and β globins, respectively. Human α and β globins are expressed at moderate levels in developing embryos and fetuses, whereas varying amounts of embryonic ζ and ε globins and their encoding mRNAs can be detected in fetal erythrocytes and in adult-stage reticulocytes, respectively. The developmental integrity of embryonic globin gene expression is further compromised in patients with certain congenital genetic disorders. Low-level ζ-globin expression persists in adults heterozygous for the α-thalassemia —SEA deletion, whereas fetuses and infants with defined trisomies may express easily detectable levels of ε globin. The temporal overlap of embryonic and adult globin expression, particularly during intrauterine development, favors the assembly of Hbs Gower-2 and Portland-2. Remarkably few studies have directly addressed the biochemical and physiological properties of either of these hemoglobins or of the embryonic hemoglobin Gower-1, despite their assembly in normal primitive and definitive erythrocytes.

Although it is uncertain whether Hbs Gower-2 and Portland-2 play a defined role in human development or are simply incidental to developmental overlapping of globin gene expression, a full understanding of their properties, along with the properties of Hb Gower-1, would be valuable for several purposes. First, structure–function analyses of all 3 hemoglobins are likely to provide additional insight into the biochemical composition of abundant, thoroughly studied hemoglobins such as Hbs A and F. Second, knowledge of its properties would provide a context for assessing the role of Hb Gower-1 in normal development and would help focus speculation on the evolutionary pressures favoring absolute phylogetic conservation of developmental globin gene switching. Third, a direct application of the analyses of Hbs Gower-2 and Portland-2 stems from the possibility that reactivation of the ζ and ε genes would produce globins that could substitute for deficient or abnormal α- or β-globin chains, respectively, in individuals with defined thalassemias and hemoglobinopathies. The implications of the current work on each of these 3 issues are considered below.

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differ from those of Hb F, our data support the latter dissenting opinion. In contrast to Hb F, which exhibits a normal O$_2$ affinity (in the absence of 2,3-BPG), a relatively high Bohr effect, and poor 2,3-BPG binding, we have found that Hb Gower-1 displays a high O$_2$ affinity, a reduced Bohr effect, and relatively tight 2,3-BPG binding. Were their developmental roles strictly limited to O$_2$-transport, the O$_2$-binding properties of Hbs Gower-1 and F might be anticipated to be more similar. An alternative explanation for this difference is that the evolutionary basis for developmental hemoglobin switching reflects properties of Hb Gower-1 that are independent of its gaseous-exchange function. This possibility is underscored by recent reports indicating that in addition to its O$_2$-transporting function, Hb A may serve an important role in the regulation of vascular tone and blood flow.$^{54}$ It is clear that the functional properties of Hb Gower-1, reflecting poorly understood evolutionary demands and manifesting as phylogenic conservation of hemoglobin switching, merit additional study.

We have previously proposed that Hbs Portland-2 ($\zeta_b$ and $\epsilon_b$) and Gower-2 ($\epsilon_a\epsilon_b$) might be suitable substitutes for Hb A in individuals with defined hemoglobinopathies and thalassemias.$^{18,19}$ These 2 hemoglobins would assemble from existing adult globin chains in adults in whom the embryonic-globin genes were reactivated. The functional identity between Hb Gower-2 ($\epsilon_a\epsilon_b$) and Hb A ($\alpha_2\beta_2$) indicates that the former hemoglobin would act in a physiologically valuable manner in definitive erythrocytes. Evidence that the $\epsilon$- and $\gamma$-globin genes are independently regulated$^{55-58}$ raises the possibility that different classes of agents, with different toxicities, might be used to separately reactivate expression from the 2 genes. This consideration is particularly important in patients with severe $\beta$-thalassemia determinants in whom $\gamma$-globin reactivation by current agents is toxicity-limited.$^{59,60}$ The observation that Hb Gower-2 will serve a physiologically important role in individuals with any of several hemoglobinopathies and thalassemias should serve as an impetus to identify agents that might be useful in this regard.

Acknowledgments

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References


Expression, purification, and characterization of human hemoglobins Gower-1 (ζ^2 ε^2), Gower-2 (α^2 ε^2), and Portland-2 (ζ β^2) assembled in complex transgenic-knockout mice

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