A Recombinant Human Hemoglobin with Anti-sickling Properties Greater than Fetal Hemoglobin*

Received for publication, March 8, 2004, and in revised form, April 8, 2004

Published, JBC Papers in Press, April 14, 2004, DOI 10.1074/jbc.M402578200

Dana N. Levasseur‡§§, Thomas M. Ryan‡§§, Michael P. Reilly¶¶**, Steven L. McCune‡‡‡, Toshio Asakura*, and Tim M. Townes$$$•

From the 1Department of Biochemistry and Molecular Genetics, Schools of Medicine and Dentistry, University of Alabama at Birmingham, Birmingham, Alabama 35294 and the 2Department of Pediatrics and Department of Biochemistry and Biophysics, The Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, Pennsylvania 19104

A new recombinant, human anti-sickling β-globin polypeptide designated β₂AS₃ (βGly₁⁶ → Asp/βGluₘ₂ → Ala/βThrₙ₇ → Gln) was designed to increase affinity for α-globin. The amino acid substitutions at β₂2 and β₈₇ are located at axial and lateral contacts of the sickle hemoglobin (HbS) polymers and strongly inhibit deoxy-HbS polymerization. The β₁₈ substitution confers the recombinant β-globin subunit (β₂AS₃) with a competitive advantage over β₂ for interaction with the α-globin polypeptide. Transgenic mouse lines that synthesize high levels of HbAS₃ (α₂β₂AS₃) were established, and recombinant HbAS₃ was purified from hemolysates and then characterized. HbAS₃ binds oxygen cooperatively and has an oxygen affinity that is comparable with fetal hemoglobin. Delay time experiments demonstrate that HbAS₃ is a potent inhibitor of HbS polymerization. Subunit competition studies confirm that β₂AS₃ has a distinct advantage over β₂ for dimerization with α-globin. When equal amounts of β₂ and β₂AS₃-globin monomers compete for limiting α-globin chains up to 82% of the tetramers formed is HbAS₃. Knock-out transgenic mice that express exclusively human HbAS₃ were produced. When these mice were bred with knock-out transgenic sickle mice the β₂AS₃ polypeptides corrected all hematological parameters and organ pathology associated with the disease. Expression of β₂AS₃-globin should effectively lower the concentration of HbS in erythrocytes of patients with sickle cell disease, especially in the 30% percent of these individuals who coinherit α-thalassemia. Therefore, constructs expressing the β₂AS₃-globin gene may be suitable for future clinical trials for sickle cell disease.

Sickle cell disease (SCD) results from an A to T transversion at the sixth codon of the human β-globin gene on chromosome 11 (1, 2). The mutation of a single DNA base leads to the substitution of a valine for a glutamic acid in the β-globin polypeptide of sickle hemoglobin (HbS). The positioning of a hydrophobic residue at this position permits an interaction with a hydrophobic pocket on another hemoglobin tetramer (see Fig. 1). This interaction allows deoxy-HbS to polymerize in an entropy-driven process (3–5). The polymerization of deoxy-HbS leads to erythrocyte deformation from a biconcave morphology into the sickle shapes for which SCD is named.

Polymerization of deoxy-HbS is effectively inhibited by fetal hemoglobin (HbF), and individuals who are homozygous for the sickle mutation but also express high levels of HbF are typically asymptomatic (6–8). The level of HbF necessary to significantly reduce the symptoms of SCD ranges from about 20 to 25%; however, data showing an enhanced red cell survival with as little as 9% HbF have been reported (9). The efficacy of HbF in inhibiting HbS polymerization suggests that transduction of fetal globin genes into hematopoietic stem cells might be an effective strategy for sickle cell disease gene therapy. However, high levels of γ-globin gene expression are difficult to achieve in adult erythroid cells even in the absence of competition with the β-globin gene for locus control region (LCR) interactions (10, 11). Low level expression of the γ-globin gene apparently results from the absence of fetal-specific positive regulatory factors in adult cells. Our approach to overcome this deficiency has been to utilize the β-globin gene as a backbone and to introduce γ-globin amino acid substitutions into this construct. We demonstrated previously that a β-globin gene containing alanine at position 22 and glutamine at position 87 (β₈₇) significantly inhibited HbS polymerization (12). We have now introduced an additional modification into β₈₇ to form β₂AS₃, this modification increases the affinity of β₂-globin subunits for α-globin polypeptides. In this paper we demonstrate that β₂AS₃ has a competitive advantage over β₂ polypeptides for interaction with limiting α-globin subunits and that HbAS₃ dramatically inhibits deoxy-HbS polymerization.

MATERIALS AND METHODS

Construction and Microinjection of Anti-sickling β-Globin Genes—Plasmids were constructed by standard procedures (13). Mutagenesis was performed using the Altered Sites system (Promega, Madison, WI) (14) and megaprimer mutagenesis (15). The mutagenic oligonucleotides were as follows: β₁₆, GTGCCCTGTTGGACAGATGTACG; β₂₂, GTGAACGTTGATGTCGTTGAG; β₈₇, GCCACCTTTTGACTGATGACG; β₂AS₃, GCCACCTTTTGACTGATGACG.

Fragment preparation and microinjection were as described previously (16). Transgenic animals expressing high levels of human hemoglobin were identified by isoelectric focusing of hemolysates. Isoelectric focusing was performed using the Isothermal Controlled Electrophore-
sis system (Fisher) with precast agarose isoelectric focusing gels (Iso-lab, Akron, OH).

Analysis and Purification of Recombinant Human Hemoglobins—Initial analysis of hemoglobin tetramers was performed by anion exchange high performance liquid chromatography (HPLC) utilizing a Synchropak AN 2500 (4.6 × 25 mm) column (MICRA Scientific, Northbrook, IL) (17). Preparative isoelectric focusing was performed on 4% acrylamide gels with 2% Pharmalyte (Amersham Biosciences AB, Uppsala, Sweden) (pH 6.7–7.7). Bands of hemoglobin were sliced from the gel and eluted in 0.1 M potassium phosphate buffer, (pH 7.0) (18). Mouse and human globins were separated by reverse-phase HPLC using a Series 4500 HPLC system (Dionex, Sunnyvale, CA). Approximately 25–30 µg of hemoglobin was injected into a C4 reverse-phase (4.6 × 250 mm) column (Vydac, Hesperia, CA) and eluted with a linear gradient of acetonitrile and 0.3% trifluoroacetic acid (19, 20).

Functional Analysis of Recombinant Human Hemoglobins—Oxygen equilibrium curves were measured with a Hemox Analyzer (TCS Scientific, New Hope, PA) (21). The oxygen equilibrium curves were determined in 0.1 M potassium phosphate buffer (pH 7.0) at 20 °C with a hemoglobin concentration of 25 µM. Polymerization kinetics were determined in 0.1 M potassium phosphate buffer as described (22). Polymerization was initiated using the temperature jump method in which the temperature of deoxygenated hemoglobin solutions is rapidly changed from 0 to 30 °C, and aggregation is monitored turbidimetrically at 700 nm (22, 23).

Analysis of Hemoglobin Subunit Recombination—Monomeric α- and β-globin subunits were prepared as described (24). The hemolysates were treated with carbon monoxide prior to separation to reduce formation of methemoglobin. Isolated α- and β-globin monomers were allowed to combine at 0 °C for 1 h. The amounts of HbS and HbAS3 formed were determined by HPLC using a PolyCAT A cation exchange column (PolyLC, Columbia, MD). Hemolysates were eluted from the column with a linear gradient of Buffer A (35 mM BisTris, 1.5 mM KCN, 3 mM ammonium acetate, (pH 6.47)) and Buffer B (35 mM BisTris, 1.5 mM KCN, 16.85 mM ammonium acetate, 150 mM sodium acetate, (pH 7.5)). The flow rate was 1 ml/min with detection at 415 nm. The relative amounts of each hemoglobin were calculated by integration of the area under each peak.

Analysis of Heterotetramer Formation—Equimolar amounts of purified oxygenated HbS and HbAS3 or HbA were mixed and allowed to equilibrate overnight at 0 °C as described previously (17). The mixtures were analyzed by cation exchange chromatography as described above with the addition of 3 mM sodium dithionite to the elution buffers. Sodium dithionite was added to allow separation of hemoglobins under anaerobic conditions, which was necessary for detection of heterotetramers.

Production of HbAS3, Knock-out Transgenic Sickle Mice—Mice that express HbAS3 exclusively were produced by breeding the HbAS3 mice with mouse α- and β-globin gene knock-out animals (26, 27). These animals were subsequently bred with knock-out transgenic sickle mice (28) to obtain mice that were homozygous for mouse α- and β-globin gene knock-outs and contained the human LCR α, LCR γβ, and LCR βAS3 transgenes. These animals expressed approximately equal amounts of HbAS3 and HbS.

Hematological Indices and Histopathology—Blood was collected from anesthetized animals into Microtainer EDTA collection tubes. The red blood cell count was measured on a Hemavet 1500 hematology analyzer. Hemoglobin concentration was determined spectrophotometrically after conversion to cyanomethemoglobin with Drabkin’s reagent. Before determining the hemoglobin concentration, red cell membranes were formed into pellets at 14,000 rpm for 5 min in an Eppendorf centrifuge. Removal of the membranes inhibits artifactualy high values caused by membrane-bound, denatured hemoglobin. Hematocrit was measured with a JorVet J503 microhematocrit centrifuge. Reticulocyte count was determined by flow cytometry after staining with thiazole orange. Urine osmolality was measured after food and water were withheld from the mice for 4 h. Tissues were fixed in 70% alcoholic formalin, embedded in paraffin, sectioned, and stained with hematoxylin-eosin or Gomori’s iron stain by standard methods.

RESULTS AND DISCUSSION

Production of Recombinant Human Hemoglobin in Transgenic Mice—Fig. 1 illustrates the major contacts present in the HbS polymer. We reported previously amino acid substitutions (βGly72 → Asp and βThr75 → Gln) that inhibit HbS polymerization (12). To provide the modified β-globin polypeptide with a competitive advantage for interacting with α-globin chains, we made an additional amino acid substitution (βGly16 → Asp). Codon changes were introduced into the human β-globin gene by site-directed mutagenesis, and the modified sequences were inserted downstream of a 22-kb DNA fragment containing the DNase hypersensitive sites 1–5 (5’-HS 1–5) of the β-globin LCR (16). These constructs were injected into fertilized mouse eggs, and transgenic lines were established. Hemolysates obtained from several animals were analyzed by HPLC to quantitate the amounts of human, mouse, and hybrid hemoglobins (17). The purity of the human hemoglobins was assessed by denaturing reverse-phase HPLC, which separated the α- and β-globin subunits (19, 20).
ship is straightforward and is based primarily upon the charge of the α- and β-chains (34). Under physiologic conditions, the α-subunit is positively charged (pI 8.4), and the β-subunit is negatively charged (pI 6.7) (35). Mutations that increase the net negative charge on the β-subunit increase its ability to dimerize with the α-subunit. Similarly, mutations that decrease the negative charge on the β-subunit decrease its ability to dimerize with the α-subunit. The βS-subunit is particularly vulnerable to competition with a more negatively charged β-subunit because the sickle mutation, glutamic acid to valine, reduces the negative charge on the βS-chain. For this reason, erythrocytes in individuals with sickle trait do not contain 50% HbS and 50% HbA but contain ~42–45% HbS (36). Competition for the α-subunit, therefore, appears to be a potential method to increase the amount of anti-sickle hemoglobin in an erythrocyte at the expense of sickle hemoglobin.

The addition of the HbJ-Baltimore mutation causes the net charge on βAS3 to equal that of βA, the addition of an aspartic acid at β16 compensates for the loss of a glutamic acid at β32. Based strictly upon charge, βAS3 should have a competitive advantage over βS for dimerization with the α-subunit. When α, βS, and βAS3-globin subunits are combined at an α-globin: β-globin ratio of 1:1, HbAS3 comprises 59.5% of total hemoglobin. As the α-globin subunit becomes limiting, the proportion of HbAS3 increases dramatically, reaching a level of 75.1% of total hemoglobin at an α-globin:β-globin ratio of 0.5:1 and 82% of total hemoglobin at a ratio of 0.1:1. These results demonstrate that β-subunits with the HbJ-Baltimore mutation have a significant competitive advantage over βS for polymerization with α-subunits. The βAS3-subunit interacts with the α-subunit even more efficiently than would be expected on the basis of charge alone. Apparently, the specific combination of negative residues in βAS3-subunits improves the ability of this polypeptide to interact with α-globin chains.

Additionally, a recombinant therapeutic hemoglobin must be able to form heterotetramers with deoxy-HbS to achieve effective inhibition of polymerization. Earlier studies have shown that the inhibitory effect of HbF on the polymerization of deoxy-HbS is dependent on the formation of heterotetramers (αβSγγ) (37). A similar mechanism was proposed to explain the polymerization inhibitory effects of the naturally occurring minor hemoglobin, HbA2 (α2δ2) (38). Unlike the αβSβS heterotetramer, the αβSγγ and αβSδ heterotetramers are excluded from the sickle polymer, which accounts for the increased inhibitory effects of HbF and HbA2 relative to HbA. To determine whether recombinant HbAS3 could form heterotetramers (αβAS3γγ), we mixed oxygenated HbAS3 with oxygenated HbS. At equilibrium, a binomial (1:2:1) distribution of parent hemoglobins and heterotetramers is observed (Fig. 3). Similar results were obtained for oxygenated mixtures of HbA and HbS.

Correction of Abnormal Red Blood Cell Morphology and Hematological Parameters in Knock-out Transgenic Sickle/AS3 Mice—To determine whether HbAS3 would inhibit HbS polymerization in vivo, we bred mice that express HbAS3 with our knock-out transgenic sickle mice and obtained animals that were homozygous for mouse α- and β-globin gene knock-outs and contained the human LCR α, LCR γ-βS, and LCR βAS3 transgenes. Blood smears from sickle control and sickle/AS3 animals are illustrated in Fig. 4. Many sickled cells are observed in the sickle control; however, no sickled cells are observed in sickle/AS3 mice (two representative animals). The upper left panel is a wild-type control. The blood smears of sickle/AS3 animals also lack the anisocytosis, poikilocytosis, and polychromasia that are characteristic of erythrocyte morphology observed in SCD mice and HbS patients (28, 36). Fig. 5 compares hematological indices of control, sickle, and sickle/
AS3 mice. In sickle/AS3 animals, red blood cell counts are nearly doubled, hemoglobin levels are increased by 6 g/dl, hematocrit is normalized, and reticulocytosis is reduced to levels approaching those of wild-type mice. Sickled erythrocytes are not observed in blood smears or in tissues of sickle/AS3 mice. These data demonstrate that HbAS3 is a potent anti-sickling hemoglobin.

Amelioration of Spleen, Liver, and Kidney Pathology and Restoration of Kidney Function in Sickle/AS3 Mice—Histological sections of wild-type, sickle, and sickle/AS3 animals are shown in Fig. 6A. The spleens of sickle mice are characterized by a massive expansion of red pulp, dramatic pooling of sinusoidal erythrocytes, vaso-occlusion, and a complete loss of lymphoid follicular structure. In sickle/AS3 mice, normal splenic red and white pulp is observed, and virtually no pools of sickle erythrocytes or infarcts are evident. In addition, splenomegaly is substantially diminished in sickle/AS3 mice (Fig. 6C); sickle/AS3 spleens are ~0.6% of total body weight com-
**FIG. 4.** Correction of abnormal red blood cell morphology in sickle/AS3 mice. Shown is a blood smear of a sickle animal with characteristic sickled erythrocytes, anisopoikilocytosis, and a pronounced reticulocytosis (S). Two representative sickle/AS3 animals are shown (S/A #1 and S/A #2). No sickled cells were observed in any fields examined. WT, wild-type C57Bl6 control.

**FIG. 5.** Hematological parameters of sickle/AS3 corrected mice. Shown are hematological indices of control (n = 5), sickle (n = 5), and sickle/AS3 (n = 5) mice. Red blood cell (RBC) counts of sickle/AS3 mice are nearly doubled, hemoglobin levels are increased by 6 g/dl, hematocrit is normalized, and reticulocytosis is reduced to levels approaching those of wild-type mice.
pared with sickle spleens that are almost 4% of body weight.

Livers of sickle animals are characterized by focal areas of necrosis and pronounced congestion of the intrahepatic vasculature with pooling of sickled red blood cells. Large aggregates of erythroid progenitors are evident in the sinusoids, and this extramedullary hematopoiesis is indicative of severe anemia.

Fig. 6. Normalization of spleen, liver, and kidney pathology in sickle/AS3 mice. A, spleen, liver, and kidney sections were analyzed at high magnification (×100), and kidney was examined at low (×10, bottom three panels) magnification. In sickle/AS3 mice, normal splenic red and white pulp is observed, and no pools of sickle erythrocytes or infarcts are evident. In livers of sickle/AS3 animals, focal areas of necrosis and aggregation of sickled erythrocytes are not observed; also, extramedullary hematopoiesis and hemosiderin deposition are absent. Kidneys of sickle/AS3 mice appear normal and free of the disruptive vascular red blood cell pooling and hemosiderin deposits observed in mock treated animals. B, urine concentrating ability is restored to wild-type levels in sickle/AS3 animals. n = 5 for wild-type, sickle, and sickle/AS3, respectively. C, correction of splenomegaly in sickle/AS3 mice. n = 5 for wild-type, sickle, and sickle/AS3.
There is also abundant hemosiderin deposition subsequent to Kupffer cell erythropagocytosis. In sickle/AS3 animals, focal areas of necrosis and aggregation of sickled erythrocytes are not observed; also, extramedullary hematopoiesis and hemosiderin deposition are absent.

In the kidneys of mock transduced mice, engorgement and occlusion of blood vessels results in vascular, tubular, and glomerular changes. Sequestration and occlusion are most obvious at the corticomedullary junction where dilated capillaries are easily observed in this region of reduced oxygen tension. Reduced medullary blood flow in HbS patients causes extensive tubular damage that results in hypostenuria, and this same loss of urine-concentrating ability is observed in the sickle mice. Kidneys of sickle animals also accumulate abundant hemosiderin in the cortical region, and these aggregates are easily visualized with Gomori’s iron staining (data not shown). In contrast, kidneys of sickle/AS3 mice appear normal and free of the disruptive vascular red blood cell pooling and hemosiderin deposits observed in mock treated animals. Most importantly, urine-concentrating ability is completely restored in sickle/AS3 mice (Fig. 6B).

Expression of βAS3-globin should effectively lower the concentration of HbS in erythrocytes of patients with SCD, especially in the 30% of these individuals who coinherit α-thalassemia (39). We recently corrected our knock-out transgenic mouse model of SCD using lentiviral transduction of the βAS3 anti-sickling gene into purified hematopoietic stem cells (40). These results suggest that stem cell- and genetics-based therapies using recombinant βAS3-globin may be able to be transplanted to human sickle patients.

Acknowledgment—We thank members of the Townes laboratory for helpful discussions.

REFERENCES
1. Ingram, V. M. (1956) Nature 178, 792–794
2. Ingram, V. M. (1957) Nature 180, 329–328
3. Wishner, B. C., Ward, K. B., Lattman, E. E., and Love, W. E. (1975) J. Mol. Biol. 98, 179–194
4. Padlan, E. A., and Love, W. E. (1985) J. Biol. Chem. 260, 8280–8291
5. Dykes, G. W., Crepeau, R. H., and Edelstein, S. J. (1979) J. Biol. Chem. 254, 451–472
6. Brittenham, G., Loroff, B., Harris, J. W., Maysan, S. M., Miller, A., and Huismann, T. H. (1979) J. Histochem. Cytochem. 47, 307–313
7. Wood, W. G., Pembrey, M. E., Serjeant, G. R., Perrine, R. P., and Weatherall, D. J. (1986) Br. J. Haematol. 63, 431–445
8. Noguchi, C. T., Rodgers, G. P., Serjeant, G., and Schechter, A. N. (1988) N. Engl. J. Med. 318, 338–39
9. Platt, O. S., Brambilla, D. J., Rosse, W. F., Milner, P. F., Castro, O., Steinberg, M. H., and Klug, P. P. (1994) N. Engl. J. Med. 330, 1639–1644
10. Behringer, R. R., Ryan, T. M., Palmiter, R. D., Brinster, R. L., and Townes, T. M. (1990) Genes Dev. 4, 380–389
11. Exner, T., Raisch, N., Ebens, A. J., Papayannopoulou, T., Costantini, F., and Stamatoyannopoulos, G. (1990) Nature 344, 309–312
12. McCune, S. L., Reilly, M. P., Chomo, M. J., Asakura, T., and Townes, T. M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8852–8856
13. Sambrovik, J., Fritsch, E., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, pp. 390–401, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York
14. Lewis, M. K., and Thompson, D. V. (1990) Nucleic Acids Res. 18, 3439–3443
15. Sarkar, G., and Sommer, S. S. (1990) BioTechniques 8, 404–407
16. Ryan, T. M., Townes, T. M., Reilly, M. P., Asakura, T., Palmiter, R. D., Brinster, R. L., and Behringer, R. R. (1990) Science 247, 566–568
17. Ip, C. Y., and Asakura, T. (1986) Anal. Biochem. 156, 345–353
18. Behringer, R. R., Ryan, T. M., Reilly, M. P., Asakura, T., Palmiter, R. D., Brinster, R. L., and Townes, T. M. (1989) Science 245, 971–973
19. Adachi, K., and Asakura, T. (1987) J. Chromatogr. 419, 303–307
20. Schroeder, W. A., Shelton, J. B., Shelton, J. R., Sun, H., and Teplov, D. B. (1985) Hemoglobin 9, 461–482
21. Asakura, T., and Reilly, M. P. (1986) in Oxygen Transport in Red Blood Cells (Nicolau, C., ed) Pergamon Press, New York
22. Adachi, K., and Asakura, T. (1979) J. Biol. Chem. 254, 7765–7771
23. Adachi, K., Segal, R., and Asakura, T. (1980) J. Biol. Chem. 255, 7595–7603
24. Turci, S. M., and McDonald, M. J. (1985) J. Chromatogr. 343, 168–174
25. Kutlar, A., Kutlar, F., Gu, L. G., Mayson, S. M., and Huismann, T. H. (1990) Hum. Genet. 85, 106–110
26. Ciavatta, D. J., Ryan, T. M., Farmer, S. C., and Townes, T. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9259–9263
27. Paszty, C., Mohandas, N., Stevens, M. E., Loring, J. F., Liebhaber, S. A., Brion, C. M., and Rubin, E. M. (1995) Nat. Genet. 11, 11–19
28. Ryan, T. M., Ciavatta, D. J., and Townes, T. M. (1997) Science 278, 873–876
29. Altay, C., Schroeder, W. A., and Huismann, T. H. (1977) Am. J. Hematol. 1, 1–14
30. Hofrichter, J., Ross, D. P., and Eaton, W. A. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 4864–4868
31. Baglioni, C., and Weatherall, D. J. (1963) Biochim. Biophys. Acta 70, 637–644
32. Mrabet, N. T., McDonald, M. J., Turci, S., Sarkar, R., Szabo, A., and Bunn, H. F. (1986) J. Biol. Chem. 261, 5222–5228
33. McDonald, M. J., Turci, S. M., Mrabet, N. T., Himelstein, B. P., and Bunn, H. F. (1987) J. Biol. Chem. 262, 5951–5956
34. Bunn, H. F. (1987) Blood 69, 1–6
35. Bunn, H. F., and McDonald, M. J. (1983) Nature 306, 498–500
36. Bunn, H. F., and Forget, B. G. (1986) Hemoglobin: Molecular, Genetic, and Clinical Aspects, Saunders Press, Philadelphia
37. Sokolov, R. M., Nagel, R. L., and Balazs, T. (1975) Nature 256, 667–668
38. Nagel, R. L., Sokolov, R. M., Johnson, L., Libe, D., Wajcman, H., Isaac-Sodey, W. A., Honig, G. R., Schliro, G., Crookston, J. H., and Matsumoto, K. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 670–672
39. Higgins, D. R., Vickers, M. A., Wilke, A. O., Pritchard, I. M., Jarman, A. P., and Weatherall, D. J. (1989) Blood 73, 1091–1104
40. Levasseur, D. N., Ryan, T. M., Pawlik, K. M., and Townes, T. M. (2003) Blood 102, 4312–4319
A Recombinant Human Hemoglobin with Anti-sickling Properties Greater than Fetal Hemoglobin
Dana N. Levasseur, Thomas M. Ryan, Michael P. Reilly, Steven L. McCune, Toshio Asakura and Tim M. Townes

J. Biol. Chem. 2004, 279:27518-27524.
doi: 10.1074/jbc.M402578200 originally published online April 14, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M402578200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 36 references, 16 of which can be accessed free at http://www.jbc.org/content/279/26/27518.full.html#ref-list-1