Evidence for Posttranslational Control of Hb C Synthesis in an Individual With Hb C Trait and α-Thalassemia

By Stephen A. Liebhaber, Faith E. Cash, and Dennis B. Cornfield

The level of Hb C in the erythrocytes of individuals with Hb C trait decreases significantly in the presence of coexisting α-thalassemia. This relationship may result from the higher affinity of β than β for limiting amounts of α-globin during hemoglobin assembly. This mechanism would predict that the β and β synthetic capacity in α-thalassemic individuals with Hb C trait should be balanced despite the low levels of Hb C in their circulating erythrocytes. To directly test this prediction, we have measured the β and β synthetic capacity of reticulocyte RNA isolated from two individuals with Hb C trait, one with a normal α-globin genotype and one with α-thalassemia. The balanced expression of β and β in both cases supports the proposed posttranslational control over Hb C expression.

MATERIALS AND METHODS

Hemoglobin studies. Hemoglobin, mean corpuscular volume (MCV), and reticulocyte counts were carried out by routine procedures in a clinical laboratory. Hemoglobin A, C, A2, and F compositions were determined by cellulose acetate electrophoresis and confirmed by Triton-acid urea (TAU) electrophoresis (see Protein analysis) and isoelectric focusing (data not shown).

DNA isolation and analysis. DNA was isolated from theuffy coat of peripheral blood as previously described.11 The structure of the α-globin genes was analyzed by Southern blotting after single enzyme digests with the restriction endonucleases EcoRI, BglII, and BamHI, all as previously described.13 Restriction enzymes were purchased from New England Biolabs, Beverly, MA, and used according to the supplier’s suggestions.

RNA isolation and analysis. Isolation of RNA from acid-precipitated reticulocyte polysomes was carried out as previously described.14 This RNA was used without further purification. In vitro translations in micrococcal nuclease–treated rabbit reticulocyte lysate15 were carried out in 15 μL reaction volumes as previously described16 in the presence of [3H]leucine (L-[(4,5,5)-H]leucine, 130 Ci/mmol, Amersham, Arlington Heights, IL).

Protein analysis. To quantitate the levels of β and β-globin in circulating erythrocytes, 200 μL of packed cells were lysed in 1.5 vol of sterile water, clarified by centrifugation at 15,000 g, and analyzed by TAU gel electrophoresis.19 After electrophoresis, gels were fixed in 30% methanol and 7% acetic acid for one hour, stained in 0.3% Coumassie blue, and destained overnight. The relative intensity of each band was quantitated by soft-laser densitometry (Zeineh Soft Laser scanning densitometer; Biomed Instruments Inc, Fullerton, CA). The sample scanned was within the linear range of detection. After analysis, the gel was dried on Whatman (Maidenhead, England) 3M paper under vacuum. To quantitate the levels of β, β, and α-globin synthesized in the in vitro translation, 5 μL of the translation mix was analyzed on a TAU gel, fixed, enhanced, dried, and autoradiographed as previously detailed.18 The relative intensity of each band was quantitated by soft-laser densitometry of an autoradiograph, the exposure of which is within the linear range of detection.

RESULTS

The two individuals studied are both AC heterozygotes. F.C. is a 56-year-old white male of Italian ancestry who was noted to have Hb C trait on routine screening. He has normal...
hematologic values and no coexisting disease. M.M. is a 72-year-old black female with atherosclerotic cardiovascular disease and an evolving myeloproliferative disorder who was referred for evaluation of a microcytic anemia. The relevant hematologic data on these two individuals is listed in Table 1.

The α-globin genotype of each individual was established by Southern blot analysis using three separate restriction enzymes as shown in Fig 1. F.C. can be assigned a normal genotype of αα/αα based on the normal Southern map and normal hematologic parameters. The Southern map of M.M. is compatible with either α-/α- or --/α-. The α/-α genotype is preferred on the basis of gene dosage (correlation of signal strength on Southern with the amount of DNA loaded on the gel and the intensity of stainable DNA on the gel, data not shown) and on the basis of the extreme infrequency of --/α-chromosomes in the black population.21,22

To directly measure the β⁺ v β⁺ synthetic capacity in both individuals, we isolated their reticulocyte RNA and translated it in a nuclelease-treated rabbit reticulocyte lysate system containing 3H-leucine. The labeled protein products were separated by electrophoresis on a TAU gel, and the radiolabeled globins were quantitated by densitometric scanning (Fig 2, lanes 2 and 3). In addition, to accurately determine the level of erythrocyte β⁺ v β⁺ on the same gel system, we analyzed the clarified hemolysate from the peripheral blood of each individual on the TAU gel, stained the gel with Coomassie blue, and quantitated total β⁺⁺, β⁺⁻, and α-globin by densitometry (Fig 2, lanes 4 and 5; Table 2).

The results of these analyses demonstrate a number of points. First, the ratio of α to β (A + C) synthesis in the in vitro translation confirmed the presence of an α chain deficit (α-thalassemia) in M.M. The α/β (total) ratio for F.C. was within the normal limits for this assay and was 5.75-fold higher than that of M.M. This directly demonstrates the significant loss of α-globin synthetic capacity in M.M. Second, the analysis of the clarified hemolysates from each individual by TAU electrophoresis demonstrated approximately the same relative levels of β⁺⁺ and β⁺⁻ as was determined for Hb A and Hb C by cellulose acetate electrophoresis. This confirmed the relative deficiency of β⁺⁻ in the individual with α-thalassemia, M.M., as compared with F.C., the AC individual with a normal complement of α-globin genes. Third, the levels of β⁺⁺ and β⁺⁻ synthesized in vitro by their reticulocyte RNA were equivalent and balanced.

DISCUSSION

Structural mutations of the hemoglobin molecule are common in world populations. There are over 230 different β-globin variants presently described.1 Although the level of β-globin variants averages 40% to 50%, the individual values cover a wide range.1 The level at which each variant is expressed can be affected by the numerous variables that control gene expression and protein synthesis. Of particular interest in the case of a multisubunit protein such as hemoglobin is the potential for controlling production at the level of transcription.
of subunit assembly. If an amino acid mutation in a globin chain happens to alter the ability with which it can combine with the α-globin chains to form a hemoglobin tetramer, this change will be reflected in the final level of mutant hemoglobin tetramer produced. In the case of the three major β-globin variants, β^a, β^c, and β^e, the level of the corresponding hemoglobin tetramer, Hb S, Hb C, and Hb E, appears to be adversely affected by specific alterations at this step.\textsuperscript{12,23}

The demonstration of equal concentrations of β^a and β^s mRNAs in the reticulocytes of AS individuals with α-thalassemia is consistent with this proposed posttranslational control.\textsuperscript{24} In the present report we directly demonstrate that β^a and β^c chains are synthesized at equal rates by reticulocyte mRNA isolated from AC individuals with and without coexisting α-thalassemia. Because these individuals therefore have the same capacity to synthesize equal levels of β^a and β^c, the differences in the levels of Hb C in their circulating red cells directly support a posttranslational mechanism as the determinant of the relative levels of HbA and HbC in AC heterozygotes.

REFERENCES

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SA Liebhaber, FE Cash and DB Cornfield