Equal Synthesis of α- and β-Globin Chains in Erythroid Precursors in Heterozygous β-Thalassemia

YUET WAI KAN, DAVID G. NATHAN, and HARVEY F. LODISH

From the Division of Hematology, Department of Medicine, Children's Hospital Medical Center and the Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115, and the Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

ABSTRACT In patients with heterozygous β-thalassemia, the β/α synthetic ratio in marrow erythroid cells incubated in vitro is 1, whereas in reticulocytes the ratio is 0.5. These ratios reflect the equal synthesis of the two chains on the polyribosomes of the bone marrow and unequal synthesis on the polyribosomes of the peripheral blood reticulocytes. α- and β-chain synthesis is also equal in marrow cells in vivo. Equal synthesis is probably due both to a decrease in α-chain synthesis and an increase in β-chain synthesis in bone marrow erythroid cells and may contribute to the absence of overt hemolysis due to excess α-globin chain accumulation in heterozygous β-thalassemia.

INTRODUCTION

In thalassemia, there is imbalance in the synthesis of the globin chains. This imbalance can be demonstrated in the peripheral blood reticulocytes by measurement of the incorporation of radioactive amino acids into the globin chains (1–5). In homozygous β-thalassemia, the imbalance is severe and the β/α synthetic ratio ranges from 0 to 0.2. In heterozygous β-thalassemia, this ratio is usually about 0.5. In the homozygous state, the imbalance can also be demonstrated in the bone marrow (6), although the β/α ratio has been found to be somewhat higher than that in the peripheral blood. In the heterozygous state, however, Schwartz has reported that no imbalance in globin chain synthesis can be demonstrated in marrow cells (7).

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The absence of measurable imbalance in the bone marrow of patients with heterozygous β-thalassemia may be due to equal rates of synthesis of the α- and β-chains, loss of the excess newly synthesized α-chains, or an artifact of the in vitro incubation system when applied to the bone marrow. In the experiments described in this report, we examined these possibilities, and we conclude that the β/α ratio of 1 in the bone marrow cells of patients with heterozygous β-thalassemia is indeed due to equal rates of synthesis of α- and β-chains on the polyribosomes.

METHODS

Three patients with heterozygous β-thalassemia (A. H., B. A., and R. G.) were studied. They are each the parent of at least one child who has homozygous β-thalassemia of sufficient severity to require regular blood transfusion. Furthermore, the child of one of the heterozygotes (R. G.) did not show any β-chain synthesis in her peripheral blood reticulocytes. An additional patient (A. R.) who had sickle β-thalassemia was included in this study. She had a chronic hemolytic anemia with 13% hemoglobin F, 6% hemoglobin A_2, trace (< 1%) of hemoglobin A detected by agar gel electrophoresis, and 80% hemoglobin S. Three individuals, who had no evidence of thalassemia and who required bone marrow examination for other indications, served as controls.

Peripheral blood and whole bone marrow were incubated with leucine-^{14}C for 2 hr and the globin chains were separated on carboxymethyl cellulose columns as previously described (5). Specific activities were expressed as cpm/OD after correction for the difference in absorbance of the α- and β-chains at 280 nm. The ratios of these specific activities were then determined (7, 8).

The ratio of nascent α- and β-chains attached to the polyribosomes was determined in patient A. R. who had sickle β-thalassemia. She was chosen for this study because she has a hemolytic anemia, a high reticulocyte count, and erythroid hyperplasia in the bone marrow. Such conditions were necessary for enough radioactivity to be incorporated into the polyribosomes. It will be shown below that in this patient a similar discrepancy between the bone

TABLE I

Peripheral Blood and Bone Marrow β/α Ratios

Patients		Peripheral blood			Bone marrow		
		β	α	Ratio, β/α	β	α	Ratio, β/α
		cpm/OD*			cpm/OD*		
Heterozygous β-thalassemia	A. H.	259.6	494.6	0.52	5137.6	4965.1	1.03
	B. A.	94.6	159.5	0.59	594.2	691.3	0.86
	R. G.	74.4	139.5	0.53	2047.5	2278.3	0.90
	A. R.‡	585.6	997.8	0.59	15431.3	13308.6	1.16
	Mean ±SD			0.56±0.03			0.99±0.13
Nonthalassemic controls	T. L.	6435.1	6245.2	1.03	19551.5	17973.2	1.09
	X. B.	355.5	399.8	0.89	7313.8	7554.3	0.97
	K. G.	80.4	73.0	1.10	7358.4	7077.3	1.04
	Mean ±SD			1.01±0.10			1.03±0.05

* Corrected for difference in absorbance of α- and β-chain.

‡ Patient with sickle β-thalassemia: only β^S-chain was used for calculation because the contribution by β^A-chain was insignificant.

marrow and peripheral blood β/α ratios exists. The details of the methods by which polyribosomes were isolated and characterized have been reported elsewhere (9). Briefly, peripheral blood or bone marrow was incubated with methionine-^{35}S of high specific activity for 15 min. 6 ml of the stroma-free lysate was spun in a 20–50% sucrose density gradient in a Beckman (Beckman Instruments, Inc., Fullerton, Calif.) SW27 rotor at 26,500 rpm for 6 hr. The gradient was fractionated in a Gilford spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) and the fractions containing the polyribosomal peaks were pooled. Methionine-^{3}H-labeled human globin from a nonthalassemic patient was added both to the polyribosomal fraction and to the supernatant hemoglobin, and the protein was then digested with trypsin which had been treated with L-(tosylamido 2-phenyl) ethyl chloromethyl ketone (Worthington Biochemical Corp., Freehold, N. J.). The methionine-containing peptides: α-T5 and β-T5 were separated by high voltage paper electrophoresis and the ^{35}S/^{3}H ratios for each peptide determined. From these ratios the β/α ratios were obtained both for the supernatant hemoglobin and the polyribosomal-bound nascent chains.

The in vivo synthesis of α and β-chains was studied in patient R. G. After full explanation to the patient and with informed consent, 100 μCi of leucine-^{14}C was injected intravenously. Venous samples were obtained daily for 7 days and then at longer intervals for 50 days. The α- and β-chains were separated by carboxymethyl cellulose chromatography in 8 M urea. The chains were passed over a Sephadex G-100 column equilibrated with 0.5% formic acid to remove urea and sodium phosphate, and they were then lyophilized. A weighed sample of the lyophilized globin chain was combusted in a Packard oxidizer.[1] The trapped $^{14}CO_2$ was counted in a Packard liquid scintillation counter and the cpm/mg of α- and β-chain calculated. The daily increments in specific activity for each chain for the first 7 days were derived from these values.

[1] We are indebted to Mr. James Passmore, Packard Instrument Co., Inc., Downers Grove, Ill., for the use of the Tri-Carb oxidizer model 305.

RESULTS

The β/α specific activities of peripheral blood and bone marrow in the four patients with heterozygous β-thalassemia are shown in Table I. As reported by Schwartz (7), the bone marrow ratios were double those of the peripheral blood. This was also true of the ratios in patient A. R. with sickle thalassemia. Thus the same mechanism also applies in this patient. In contrast, no difference in ratios was found between the peripheral blood and the bone marrow cells in the nonthalassemic controls.

The ratios shown in Table I were determined from stroma-free hemolysates. Since uncombined α-chains are known to be very unstable (10, 11), it is possible that newly synthesized free α-chains might have rapidly precipitated and become attached to the membrane. However, the membrane-rich button when treated in the same way as the hemolysate had the same ratios as the supernatant hemoglobin.

To determine whether a putative proteolytic enzyme might have rapidly removed excess newly synthesized α-chains as soon as they were released from the ribosomes in the marrow, the ratio of polyribosome-bound nascent α- and β-chains was compared to the ratio found in soluble hemoglobin. The sucrose density gradient separations of peripheral blood and marrow cells into polyribosome and supernatant soluble hemoglobin fractions in patient A. R., together with the ratios of synthesis of their associated β- and α-chains are shown in Fig. 1. The polyribosomes and the soluble hemoglobin had nearly identical ratios in both marrow and peripheral blood indicating no significant destruction of newly synthesized labeled chains. The sucrose density gradient obtained from the marrow did reveal definite evidence

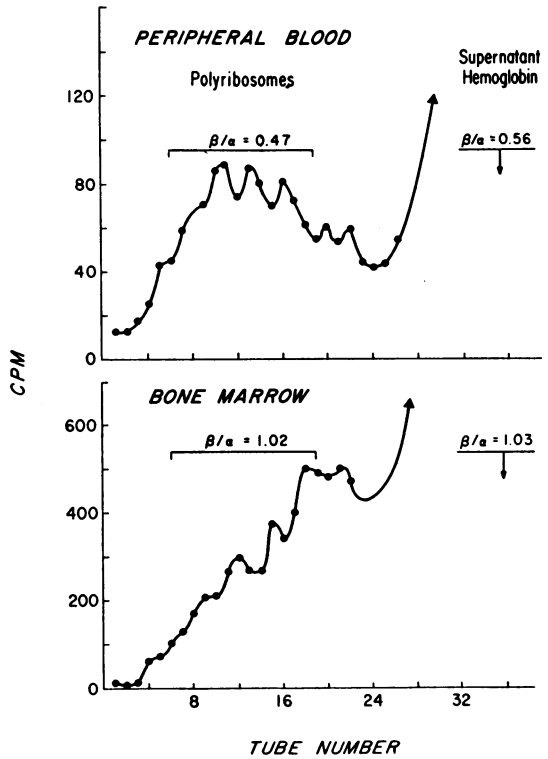

FIGURE 1 Sucrose density gradient separation of peripheral blood and bone marrow of patient A. R. 1-ml fractions were collected. 0.2-ml portions were counted in 10 ml of Aquasol (New England Nuclear Corp., Boston, Mass.). Tubes 6-19 were pooled as the polysome fractions, and tube 34 as the supernatant fractions for both the peripheral blood and bone marrow.

of polyribosome degradation probably due to release of nuclease activity during cell lysis, but the polysomes themselves were easily distinguished and the radioactivities of their attached nascent chains were clearly determined.

There is evidence by radioautography that a population of the red cell precursors of the bone marrow, the basophilic normoblasts, selectively incorporates most of the radioactive amino acid during in vitro incubation (12). If the synthesis of β-chains equals that of α-chains at this stage of development of the red cell, but decreases rapidly in the later stages, and if in vitro incubation preferentially labels the basophilic normoblasts, an overall difference in the synthesis of the two chains might escape detection. If, however, the precursor amino acid is administered in vivo, the cells of each stage of incubation sequentially emerge from the marrow carrying the synthetic ratio which reflects the particular maturation stage (13). Fig. 2*A* shows that when R. G. blood was labeled in vivo, the specific activities of the two chains were equal as they emerged from the marrow into the peripheral blood and remained equal. Furthermore, the peak increment in specific activities of the two chains occurred on the same day (Fig. 2*B*) showing that there was no asynchrony in the production of the two chains.

DISCUSSION

These experiments were designed to determine if the β/α ratio of 1 in the bone marrow of patients with heterozygous β-thalassemia is due to equal synthesis of the two chains. Our bone marrow and peripheral blood β/α ratios confirmed the findings of Schwartz (Table I). The polyribosome-bound nascent polypeptide chain ratio of 1 proved conclusively that these two chains are synthesized equally in the bone marrow (Fig. 1). The study in R. G. showed that the synthetic ratios of the two chains are equal in the bone marrow in vivo and that there was no asynchrony of their synthesis during maturation in the bone marrow (Figs. 2*A* and *B*).

The equal synthesis of the α- and β-chains in the marrow of patients with heterozygous β-thalassemia could be due to a compensatory increase in β-chain synthesis, to a decrease in production of α-chains, or to both. The mean corpuscular hemoglobin (MCH) of these patients sheds some light on the mechanism. If the equal rates were entirely due to compensation by the unaffected β-gene the red cells of these patients should not be hypochromic or microcytic since most of the red cell hemoglobin is made in the bone marrow. On the other hand, if the equal rates were entirely due to concomitant α-chain depression, the MCH of these patients' cells would be half of normal, viz about 15 pg/cell. In fact, the MCH of the red cells of these patients is usually about 22 pg/cell (8) indicating that the amount of hemoglobin per cell is about 70% of normal. Hence, we conclude that β-chain synthesis in the bone marrow in the patients is probably about 70% of normal and α-chain synthesis must be decreased to the same level in order to give a β/α ratio of 1. This compensatory increase in β-chain synthesis is probably directed by the nonthalassemic gene, since compensation also occurs in patient R. G., whose child with the homozygous disease had no β-chain synthesis, indicating complete suppression of synthesis by the affected gene. Furthermore in the sickle thalassemia patient A. R., who has no significant β^A production, the compensation in the bone marrow was entirely due to the β^S-chains. A similar conclusion was drawn by Schwartz from a study of a patient who had sickle thalassemia and some hemoglobin A synthesis (14). The mechanism of this mutual compensation is not understood at the present, but it may well be an important feature of the thalassemia problem. In β-thalassemia, the excess α-chains are believed to account for the large inclusions in the red blood cells. Some of the excess

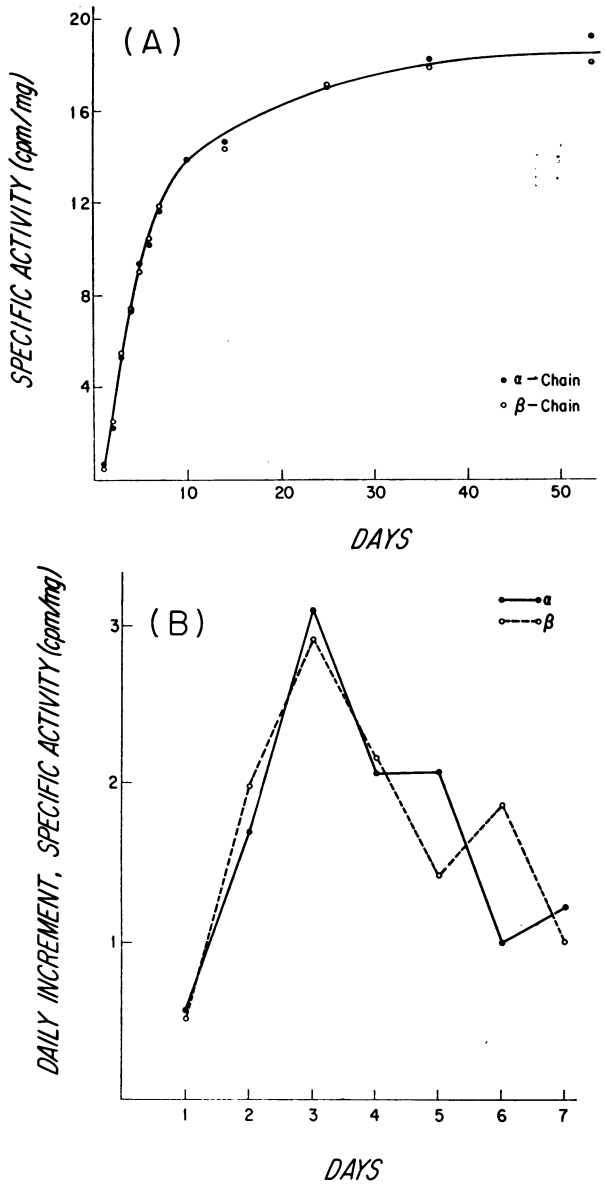

FIGURE 2 (*A*) Specific activities of α- and β-chains in R. G. peripheral blood after administration of 100 μCi of leucine-^{14}C. (*B*) Daily increments of specific activities derived from Fig. 2*A*.

α-chains are metabolized, but those that form inclusions are responsible for the ineffective erythropoiesis and hemolytic anemia (15–17). Patients with heterozygous β-thalassemia would probably suffer from hemolytic anemia due to uncombined α-chain precipitation if this regulatory system were not present.

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