Imbalance in α and β Globin Synthesis Associated with a Hemoglobinopathy

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ABSTRACT In contrast to findings in the thalassemia syndromes, studies of globin synthesis in subjects with structurally abnormal hemoglobins have generally revealed equal production of α and β polypeptide chains. However, in the present investigation of globin biosynthesis in vitro in blood and marrow from two subjects heterozygous for unstable hemoglobin Leiden, $\beta 6$ or 7 Glu $\rightarrow$ O, a significant excess of α-chain production was revealed. A mother and daughter of northern European ancestry with mild compensated hemolytic anemia were found to have 25% hemoglobin Leiden. Increased hemolysis occurred after the ingestion of a sulfonamide and during infections. Normal levels of hemoglobin A_2, 3.0 and 2.7%, and hemoglobin F, 0.8 and 0.6%, were found in the two subjects. Similar percentages of the minor hemoglobins were demonstrated in other family members without hemoglobin Leiden. After incubation of peripheral blood with [^{3}H]-leucine, the β^A/β^{Leiden} synthesis ratio was 1.3, and the specific activity of β^{Leiden} was 1.3–2 times β^A. These results indicate preferential destruction of the unstable hemoglobin Leiden. However, in contrast to previous studies of other unstable hemoglobins, there was excess synthesis of α-chains. The total β/α synthesis ratio was 0.47–0.63 in peripheral blood and 0.82 in marrow. A pool of free α-chains was demonstrated by starch gel electrophoresis and DEAE column chromatography. The synthesis of globin chains was balanced in family members without hemoglobin Leiden. This degree of predominance of α-chain synthesis in subjects with hemoglobin Leiden resembles the findings in heterozygous β-thalassemia. However, the relatively normal hemoglobin content of the cells with this abnormal hemoglobin suggests the possibility of an absolute excess α-chain production in the hemoglobin Leiden syndrome.

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INTRODUCTION

Normal human hemoglobin synthesis is characterized by equal α- and β-chain production. In the thalassemia syndromes, studies of globin biosynthesis in vitro have consistently revealed deficits in the production of one of the two polypeptide chains of hemoglobin (1). In contrast, in the absence of a gene for thalassemia, the structural hemoglobinopathies are not generally associated with an imbalance in α- and β-globin synthesis. Equal synthesis occurs, despite the fact that many of the abnormal globins are produced in much smaller amounts than the normal chains (2).

The present report describes in vitro studies of hemoglobin biosynthesis performed on peripheral blood and bone marrow specimens from two subjects with the unstable hemoglobin Leiden, $\beta 6$ or 7, Glu $\rightarrow$ O (3). These experiments demonstrated an imbalance in α/β-globin synthesis with excess α-chain production.

METHODS

Handling of blood samples. Venous blood and bone marrow samples for hemoglobin biosynthesis studies were collected with heparin as the anticoagulant. Specimens were placed on ice, and the experiments were begun within $\frac{1}{2}$ h after phlebotomy.

Hemoglobin synthesis in vitro. The technique employed for incubation of blood and bone marrow samples in the

TABLE I
Hematological Data

Subject	Hb	Hct	Erythrocytes	MCV	MCH	MCHC	Leiden	A_2	F	Reticulocytes	Fe
	g/100 ml	%	$\times 10^6$	μm^3	*pg*	%	%	%	%	%	*μg/100 ml*
I-1 (husband)	16.0	45.3	5.09	88	31.5	35.4	—	3.0	0.5	1.0	
I-2 (proposita)	12.6	36.6	4.42	82	28.5	34.6	23.7	2.8	0.8	4.0	100
II-1 (son)	13.0	39	4.2	93	30.5	33	—	3.0	0.5	0.6	
II-2 (daughter)	12.2	35.8	4.72	75	25.9	34.2	23.6	2.7	0.6	3.2	98
II-3 (daughter)	12.7	36.1	4.36	82	29.3	35.4	—	2.9	0.9	1.0	

MCH, mean corpuscular hemoglobin, MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume.

presence of radioactive amino acid was the same as previously described, except that no attempt was made to concentrate the reticulocytes before incubation (4). After incubation with [^{3}H]leucine, the cells were washed with 0.13 M NaCl, 0.005 M KCl, and 0.0074 M $MgCl_2$, and then lysed with distilled water. Specimens intended for immediate globin chain separation on carboxymethylcellulose (CMC) [1] were dialyzed overnight at 4°C against distilled water, treated with acid-acetone to remove heme without prior removal of membranes, and lyophilized. Specimens intended for hemoglobin chromatography on DEAE cellulose or Sephadex G-75 were dialyzed against the initial chromatography buffer and then centrifuged at 20,000 rpm for 30 min to sediment the stroma.

Globin chain chromatography. Up to 200 mg of heme-free globin were applied to a 1.5 × 28-cm column of CMC. A gradient of 275 ml of 0.008 M sodium phosphate versus 275 ml of 0.02 M sodium phosphate was followed by a gradient of 100 ml of 0.02 M sodium phosphate versus 100 ml of 0.03 M sodium phosphate. All buffers were adjusted to pH 6.7 and contained 8 M urea and mercaptoethanol (5).

Hemoglobin chromatography. Hemoglobin α, A_2, Leiden, A, and A_3 were separated by column chromatography on DEAE. The columns were equilibrated with 0.003 M Tris-phosphate, pH 8.6. Hemoglobins α and A_2 were eluted with 0.01 M Tris-phosphate, pH 8.6. A gradient of 0.01 M Tris-phosphate versus 0.01 M Tris-phosphate containing 0.01 M NaCl was used to elute Hb Leiden. A buffer containing 0.02 M Tris-phosphate, pH 8.6, and 0.02 M NaCl was used to elute hemoglobin A. Hemoglobin A_3 was removed from the column with 0.3 M Tris-phosphate, pH 6.5. All buffers contained 100 mg/liter KCN.

Sephadex G-75 gel filtration of membrane-free lysates was performed with 0.01 M Tris-phosphate, pH 8.6, with 100 mg/liter KCN.

Measurement of radioactivity. The CMC columns were monitored for radioactivity in the eluates by adding 1 ml of each fraction to 10 ml of Aquasol, New England Nuclear (Boston, Mass.) and counting in a Beckman LS-250 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.). The DEAE and Sephadex columns were monitored in the same manner except that color quenching was reduced in the fractions containing high concentrations of hemoglobin by counting only 50–100 μl of eluate diluted in 1 ml of H_2O. Specific activities, calculated as counts per minute per milligram of protein, were determined on water solutions of dialyzed, lyophilized globin samples by a combination of liquid scintillation counting and biuret protein determination.

[1] *Abbreviation used in this paper:* CMC, carboxymethyl cellulose.

RESULTS

Clinical data and case report. The proposita (I-2, Table I), a 32-yr-old Virginia woman of Dutch-English ancestry, was hospitalized because of the sudden onset of jaundice associated with dark urine. 3 days before admission, she had begun taking a sulfonamide preparation for a urinary tract infection. There had been several previous episodes of slight jaundice of unknown etiology associated with mild right upper quadrant abdominal discomfort. Prior administration of sulfonamides was without definitive clinical sequellae. The patient knew of chronic anemia of unknown etiology for several years. The family history was negative for jaundice. The patient's hematocrit value was 30% and the reticulocyte count was 7.6%. The peripheral blood film exhibited very slight anisopoikilocytosis. The serum indirect bilirubin was 7.3 mg/100 ml, and the direct-reacting bilirubin was 4.5 mg/100 ml. The jaundice subsided spontaneously; an X-ray examination of the gallbladder later revealed several nonopaque stones. After discharge, the patient's hematocrit value rose to 36.6%, while the reticulocyte count was maintained at about 4% (Table I). The red cell half-life was 19.2 days by the ^{51}Cr method.

Table I shows the results of the hematological examination of the patient's immediate family. A daughter, II-2, also had a persistently elevated reticulocyte count with slightly decreased mean corpuscular volume and mean corpuscular hemoglobin (Table I).

Hemoglobin electrophoresis on starch gel at pH 8.6 revealed an abnormal component migrating in a position similar to hemoglobin S in the hemolysates from the proposita, I-2, and her daughter, II-2 (Fig. 1). The abnormal fraction was approximately 25% of the total hemoglobin when quantitated by starch block and cellulose acetate electrophoresis, and by DEAE cellulose chromatography. The other family members had normal electrophoretic patterns (Fig. 1). HbF and HbA_2 levels were normal in all family members (Table I).

Hemoglobin instability was demonstrated in the blood of subjects I-2 and II-2 by the formation of multiple intraerythrocytic inclusion bodies after 2 h of incuba-

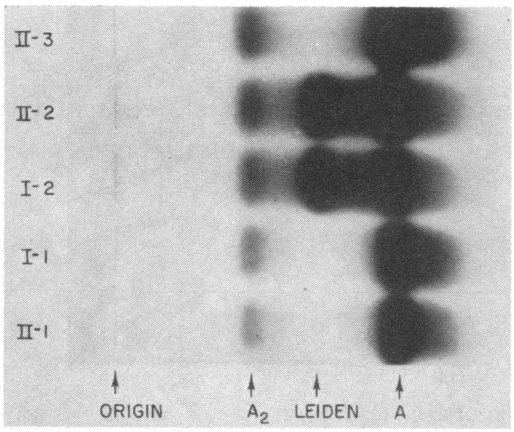

FIGURE 1 Starch gel electrophoresis of hemolysates from the family members. The abnormal hemoglobin is seen migrating between hemoglobin A and A_2. Benzidine stain.

tion with new methylene blue. No inclusions formed in the cells of other family members.

Fingerprint and amino acid analysis. Globin was prepared from the hemolysate of the proposita and analyzed by column chromatography on CMC in 8 M urea. An abnormal β-chain was eluted after β^A (Fig. 2). Fingerprints of the abnormal chain revealed that tryptic peptide I ($\beta T_1 I$) was missing from its customary position and that a new peptide was present in a more cathodic position. Amino acid analysis of the abnormal peptide by Dr. J. B. Clegg revealed that one of the two glutamic acid residues normally present at positions 6 and 7 was absent. No new amino acid residue was detected. Staining for tyrosine, phenylalanine, methionine, and cysteine also gave negative results. The abnormal hemoglobin thus has a deletion of one glutamic acid residue at position 6 or 7 and is identical to hemoglobin Leiden, previously reported in a single Dutch family (3).

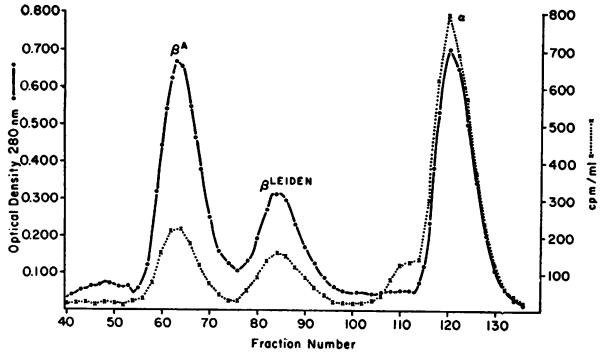

FIGURE 2 CMC column chromatography of whole globin prepared from peripheral blood of the proposita, I-2, after incubation for 60 min in the presence of [^{3}H]leucine. β-Leiden elutes from the column between β^A and α.

Hemoglobin synthesis. Hemoglobin Leiden represented only approximately 25% of the total hemoglobin in hemolysates from the affected subjects. An in vitro study of hemoglobin synthesis was therefore performed to determine whether the small percentage of the abnormal hemoglobin was due to reduced synthesis or an increased turnover relative to hemoglobin A.

Peripheral blood specimens from the proposita, I-2, and the affected daughter, II-2, were incubated with [^{3}H]leucine and portions were removed after 10, 30, and 60 min. After hemolysis and heme removal, each entire sample, including membranes, was subjected to globin chain separation on CMC (Fig. 2). The total radioactivity incorporated into each globin fraction and the specific activities of the separate globin chains were determined (Table II). At all time points, the specific activity of β-Leiden was greater than that of β^A. The total synthesis of β^A was 1.2–1.4 times that of β-Leiden. Since the amount of hemoglobin A in blood was almost three times that of hemoglobin Leiden, these results suggest that the unstable hemoglobin is destroyed more rapidly than hemoglobin A during erythrocyte maturation.

In addition, when the total synthesis of β^A plus β-Leiden was compared to total α synthesis, a marked imbalance in globin chain production with α-chain excess was noted: the β/α ratio varied from 0.48 to 0.57 (Table II). The specific activity of the α-chain was greater than that of either β^A or β-Leiden, suggesting a rapid turnover of the excess α-chains (Table II).

Similar results were obtained when peripheral blood of the proposita was incubated with [^{3}H]leucine for 3 min and for 6 min (Table II). A study of globin synthesis in a bone marrow specimen from the proposita revealed a lesser degree of imbalance in β/α-globin synthesis; the β/α synthesis ratio was 0.82 (Table II). No evidence of unequal α- and β-globin synthesis was found in blood samples from other family members (Table I).

To verify that the radioactivity appearing in the various globin fractions obtained by CMC chromatography was truly incorporated into hemoglobin protein, blood from subject I-2 was incubated with [^{3}H]leucine for 30 min. A dialyzed, membrane-free hemolysate was prepared and applied to a Sephadex G-75 column, and the eluate was monitored by measuring both the optical density at 280 and 540 nm and the radioactivity in the fractions (Fig. 3).

The void volume from the Sephadex chromatography contained a heme-free radioactive component (Peak I, Fig. 3). When this material was mixed with nonradioactive hemoglobin, converted to globin, and chromatographed on CMC in 8 M urea, all the radioactivity appeared in the rapidly eluting nonglobin fraction.

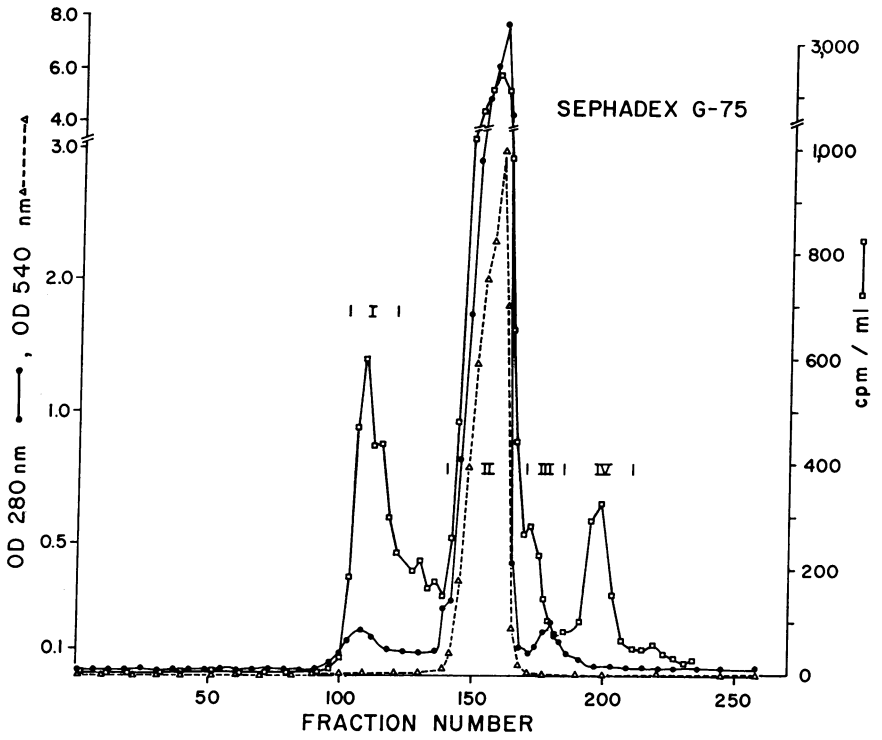

FIGURE 3 Column chromatography on Sephadex G-75 of the membrane-free hemolysate prepared after 30 min of incubation of the proposita's blood with [^{3}H]leucine.

Peak II from the Sephadex G-75 column (Fig. 3) contained the bulk of the radioactivity and all the heme-absorbing material. After treatment with acid-acetone, this material was also subjected to CMC chromatography (Fig. 4). There was a significant imbalance in β/α radioactivity in this component; the ratio of total β to α radioactivity was 0.7. Peak III from the Sephadex column had little total radioactivity and appeared similar to Peak II when subjected to CMC chromatography with nonradioactive carrier globin. Peak IV proved to be almost exclusively free α-chains. These studies confirmed the existence of a significant excess

TABLE II
Hemoglobin Synthesis in Vitro

Subject	Time	Total radioactivity			Radioactivity ratios			Specific activity		
		β^A	β^L	α	β^A/α	β^L/α	β^A/β^L	β^A	β^L	α
	min		*cpm*						*cpm/mg*	
I-2	10	2,576	1,947	8,471	0.3	0.23	1.32	116.8	165.4	232.2
	30	5,480	4,303	20,444	0.27	0.21	1.27	214.7	327.6	503
	60	10,727	8,818	39,612	0.27	0.22	1.22	355.3	572.3	864.2
	3	4,750	2,148	9,574	0.45	0.22	2.0	149.8	137.7	249.2
	6	8,253	4,572	22,010	0.37	0.21	1.8	249.8	260.1	466.4
	30*	91,700	75,405	205,696	0.45	0.37	1.22	8532	12588	12773
II-2	10	2,505	1,925	7,786	0.32	0.25	1.3	86.5	139.5	159.2
	30	4,469	3,434	14,195	0.31	0.24	1.3	159.1	276.1	362.6
	60	7,705	5,562	25,423	0.30	0.22	1.39	247.4	416.8	598.8
I-1	60	7,049	—	5,943	1.18	—	—	—	—	—
II-I	60	2,692	—	3,013	0.89	—	—	—	—	—
II-3	60	10,312	—	9,209	1.12	—	—	—	—	—

* Bone marrow.

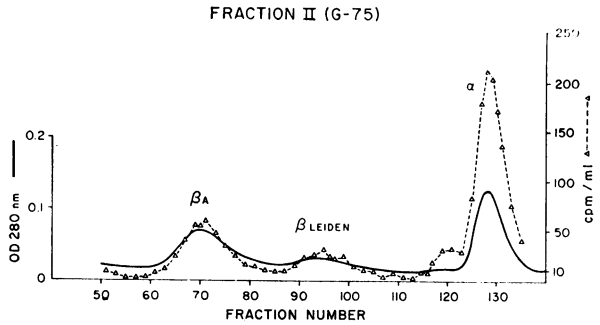

FIGURE 4 Globin chain chromatography on CMC of peak II from the Sephadex column shown in Fig. 3.

of α-chain synthesis compared to β-chain synthesis in the cells with hemoglobin Leiden.

The distribution of the radioactive α-chains between hemoglobin A and hemoglobin Leiden was next examined. Erythrocytes were incubated with [^{3}H]leucine for 30 min, and a portion of the specimen was immediately hemolyzed (30-min sample). The remaining cells were washed and reincubated for 22 h in nonradioactive medium in the presence of the antibiotic puromycin (2.5 mM), an inhibitor of protein synthesis, and then hemolyzed (22-h specimen). A portion of each of the two specimens was subjected to hemoglobin chromatography on DEAE cellulose. A portion of each specimen was also directly analyzed by globin chromatography on CMC in 8 M urea. Finally, the hemoglobin fractions eluted from the DEAE columns were further analyzed by globin chromatography on CMC.

Fig. 5 shows the chromatography of the 30-min sample on the DEAE column. Equal amounts of radioactivity were found in hemoglobin A and hemoglobin Leiden (Table III). Significant radioactivity was also noted in the hemoglobin α and hemoglobin A_3 peaks (Fig. 5, Table III) although the protein content of these minor peaks was only 0.15 and 9.2%, respectively, of the whole sample (Table III). CMC analysis of the minor fractions obtained from the DEAE column was carried out in the presence of nonradioactive "carrier" globin. All the radioactivity in the hemoglobin α peak migrated with authentic α-chains. Approximately 70% of the radioactivity in the hemoglobin A_3 peak was in α-chains and the rest in nonglobin protein. No radioactive peak was detected in the β globin region. As a result of chromatography on CMC, it was determined that the β/α radioactivity ratios in hemoglobin Leiden and hemoglobin A were 0.38 and 1.48, respectively (Table III and Figs. 6 and 7). The total β/α synthesis ratio, calculated from all the fractions in the material applied to the DEAE column, was 0.47 (Table III).

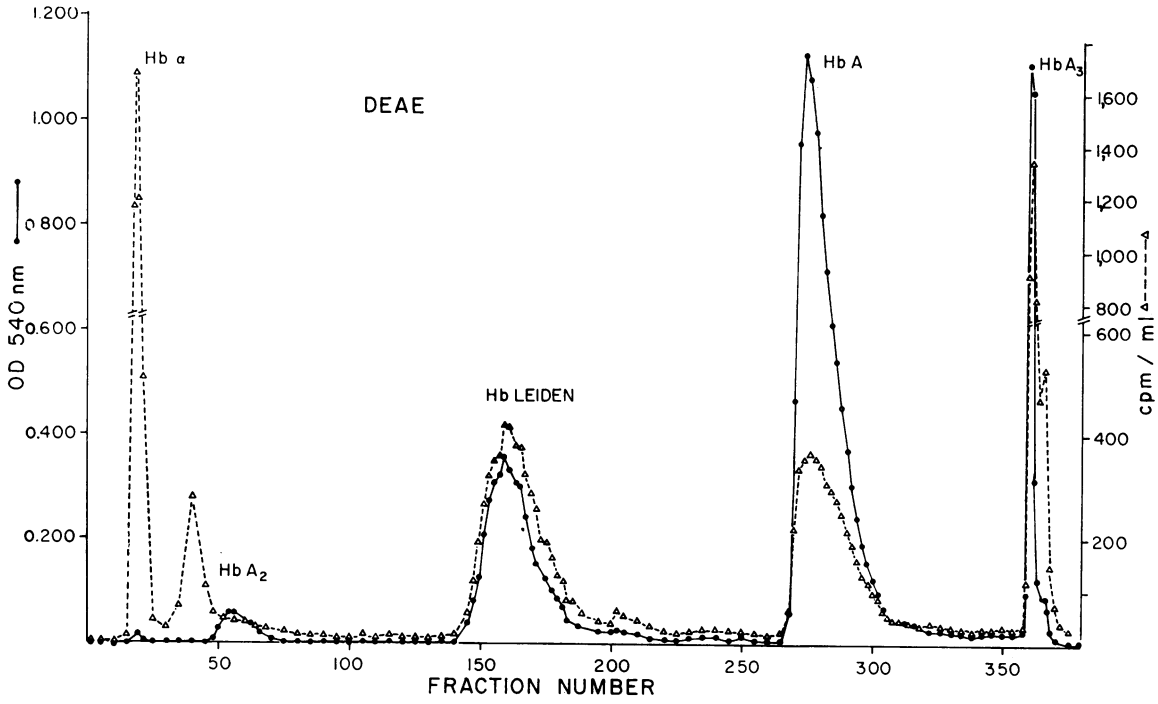

FIGURE 5 Column chromatography on DEAE of a hemolysate prepared from the proposita's blood after 30 min of incubation in the presence of [^{3}H]leucine.

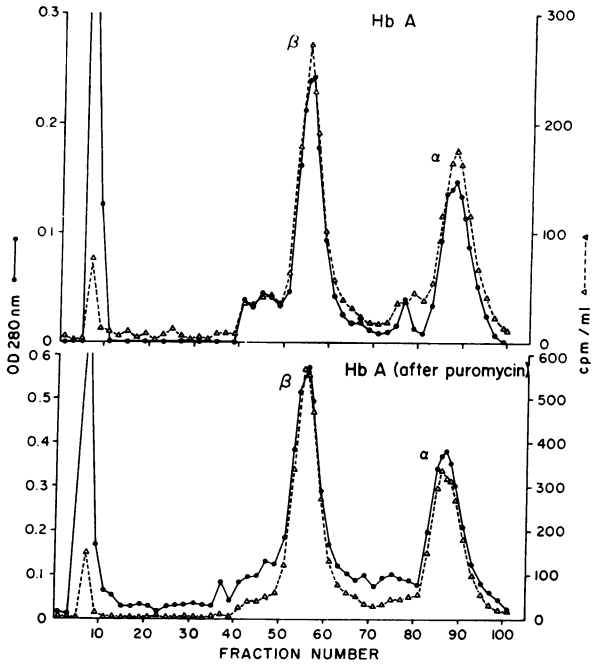

FIGURE 6 Globin chain chromatography on CMC of hemoglobin A, purified by DEAE chromatography, from the 30-min (upper panel) and 22-h (lower panel) incubation samples.

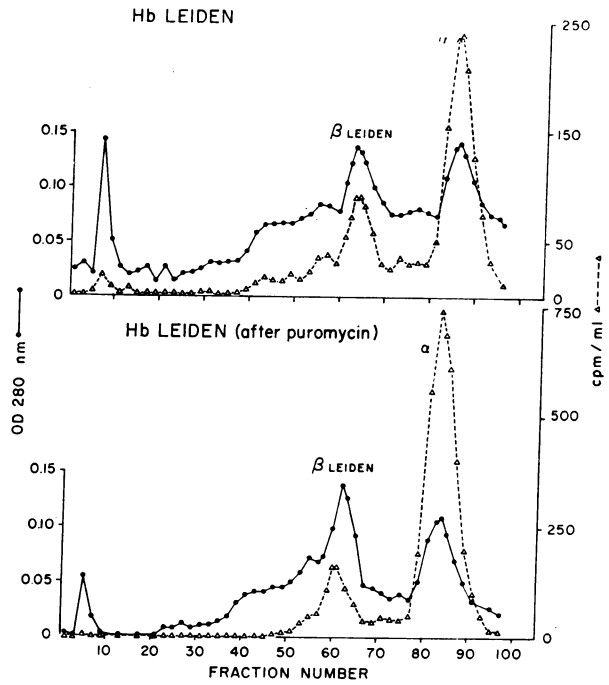

FIGURE 7 Globin chain chromatography on CMC of hemoglobin Leiden, prepared by DEAE chromatography, from the 30-min (upper panel) and 22-h (lower panel) incubation samples.

When the 22-h sample was analyzed, 53.3% of the radioactivity was in hemoglobin Leiden (Table III). The percentage of radioactivity in hemoglobin A was constant at 35%, while the free α-chain and A_3 fractions had declined in radioactivity (Table III). The distribution of the radioactivity in the constituent chains of hemoglobin A in the 22-h sample was the same as in the 30-min sample (Table III, Fig. 6). The increase in radioactivity in hemoglobin Leiden was almost exclusively in the α-chains associated with the abnormal hemoglobin (Table III, Fig. 7). The β/α radioactivity ratio in hemoglobin Leiden had decreased to 0.26. There was a doubling of the specific activity of the α-chains in the 22-h sample of the abnormal hemoglobin. The inhibition of protein synthesis during

TABLE III

Radioactivity In Hemoglobins Separated by DEAE Chromatography

Incubation time	Hb	Hb	Radioactivity Total		β/α	α	β	Specific activity β	α
min		%	*cpm*	%		*cpm*		*cpm/mg*	
30	α	0.15	26,665	9.3		26,665			
	A_2	2.5		<1					
	Leiden	25.7	99,010	34.6	0.38	71,852	27,158	1,752	3,137
	A	62.4	97,465	34.0	1.48	39,288	58,167	1,813	1,459
	A_3	9.2	63,115	22.0		43,511			
30*	α		6,213	1.8		5,712			
	A_2			<1					
	Leiden		183,861	53.5	0.26	145,921	37,940	1,929	6,513
	A		120,944	35.2	1.4	50,393	70,550	1,574	1,017
	A_3		32,782	9.5		18,536			

* Followed by 22 h in presence of puromycin.

the incubation with puromycin was indicated by the lack of a significant increase in the specific activities of the β- and α-chains of hemoglobin A and the β-chains of hemoglobin Leiden. In the absence of protein synthesis, the increase in specific activity of the α-chains of hemoglobin Leiden must have been the result of exchange of α subunits between the unstable hemoglobin and the highly radioactive minor fractions, hemoglobin α and hemoglobin A_2. The total β/α ratio in all the fractions from the 22-h sample was 0.48 (Table III).

The blockade of protein synthesis by puromycin was also confirmed by the analysis of the portions of the 30-min and 22-h whole globin samples which had been applied directly to CMC. The β^A, β-Leiden, and α-specific activities were measured as 1,390, 1,529 and 2,833 cpm/mg, respectively, in the 30-min sample, and 1,370, 1,989 and 2,975 in the 22-h sample. These analyses yielded total β/α synthesis ratios of 0.49 and 0.55 for the 30-min and 22-h samples, respectively. The experiments clearly demonstrated that the putative excess α-chain radioactivity noted previously was in a pool of authentic α-chains that could exchange freely between unstable hemoglobin Leiden and the minor fractions, hemoglobin α (free α-chains), and hemoglobin A_2.

DISCUSSION

The present study revealed an imbalance in the relative synthesis in vitro of the α and β polypeptide chains of hemoglobin in peripheral blood and bone marrow from subjects with hemoglobin Leiden. α-chain synthesis exceeded β-synthesis. The imbalance was evident in blood from the proposita and her daughter. Equal synthesis of α- and β-chains was noted in other family members without hemoglobin Leiden. Thus, there was no evidence in this family of a gene for β-thalassemia segregating independently of the gene for β-Leiden.

Attempts to uncover an artifactual basis for the elevated levels of α-chain radioactivity observed in these experiments were unsuccessful. After passage through Sephadex G-75 and DEAE, the purified hemoglobin components continued to exhibit excess total α-chain radioactivity when analyzed by CMC chromatography. Thus, it is unlikely that a nonglobin protein was contaminating and spuriously increasing the radioactivity in the α-chain fraction. In addition, in the experiment utilizing puromycin, the excess α-chain radioactivity behaved like authentic α-chains with respect to initial appearance in the free α-chain pool and subsequent exchange with the α-chains of the unstable hemoglobin Leiden. The presence of a free α-chain pool into which newly synthesized radioactive α-chains are diluted accounts for the observed lower specific activity of the α-chains of hemoglobin A compared to β^A. The marked increase in the specific activity of the α-chains of hemoglobin Leiden, after incubation in the presence of puromycin, indicated direct exchange of radioactive α-chains from the free pool with preformed hemoglobin Leiden. Such exchange has previously been demonstrated in studies of other unstable hemoglobinopathies (4).

Loss of a portion of the total β-chain radioactivity as a result of rapid denaturation and disappearance of newly synthesized unstable β-Leiden could result in depression of the apparent total β/α synthesis ratio. Loss of newly synthesized chains of unstable hemoglobins has been reported previously for hemoglobin Köln (6) and hemoglobin Genova (7). In those studies, there was a progressive decline in the ratio of specific activities of the abnormal β compared to β^A during incubation. If such a mechanism were operating in the present experiments, one would expect a decline in both the total β/α and β-Leiden/α radioactivity ratios and a rise in the β^A/β-Leiden ratio with increasing length of incubation. No such significant and consistent changes in the ratios were noted in these experiments. In specimens sampled as early as 3 min after the introduction of radioactive amino acid, imbalance was evident. No loss of β-Leiden radioactivity was noted after 22 h of incubation in the presence of puromycin. In addition, loss of β-Leiden radioactivity would not explain the low β^A/α ratios found. In most experiments the β^A/α ratio was approximately 0.3. In one instance a value of 0.45 for β^A/α was recorded in blood, and 0.45 in one experiment on bone marrow. β^A/α ratios of 0.5 or greater would be expected if the low total β/α ratio was solely due to loss of β-Leiden. One possibility that cannot be completely excluded would be a situation in which β-Leiden was synthesized in amounts greater than β^A and very rapidly degraded. Again, since progressive loss of β-Leiden radioactivity was never evident, it would appear that in these experiments, there was a deficit in total β^A and β-Leiden synthesis relative to α synthesis.

No studies of hemoglobin synthesis in vivo were performed on the patients in this study, as the administration of a radioactive amino acid was not felt to be justifiable. Therefore, it is conceivable that the results obtained from the experiments in vitro may not truly reflect the status of globin chain synthesis in vivo. However, the large experience obtained by many laboratories utilizing the in vitro incubation technique indicates that it is a valid model for hemoglobin synthesis.

The inequality in the production of the constituent chains of human hemoglobin found in the present study is reminiscent of the thalassemia syndromes. In β-thalassemia, the excess α-chains are a reflection of the

deficient synthesis of β-chains associated with a decreased cellular content of the messengerRNA for β-globin (8–11). A similar imbalance in globin chain synthesis has been noted in heterozygotes for hemoglobin Lepore Boston (12, 13). In the hemoglobin Lepore syndrome, synthesis of the δ-β fusion chain is markedly reduced in comparison to β-chain synthesis. This results in a low $\delta + \beta/\alpha$ ratio and α-chain excess. In both the thalassemia and hemoglobin Lepore syndromes, the defect in hemoglobin synthesis results in red cells with decreased hemoglobin content. The coexistence of a gene for mild β-thalassemia with the gene for β-Leiden would be expected to result in a deficit in β-chain production with α-chain excess. However, no corroborative evidence for β-thalassemia was detected in any of the subjects with hemoglobin Leiden or in the other members of the family.

Studies of globin chain synthesis in subjects with other structural anomalies of hemoglobin have generally failed to reveal marked imbalance in chain synthesis. Analyses of radioactivity incorporated into individual polypeptide chains in subjects with the β-chain mutants hemoglobin S (2, 14, 15), hemoglobin Hammersmith (16), hemoglobin Bristol (17), hemoglobin Riverdale-Bronx (15), and hemoglobin Gun Hill (4) indicated balanced synthesis. An occasional patient with sickle-cell anemia has been reported to exhibit greater synthesis of α than β-chains (2).

The slight excess in α-chain synthesis reported in association with hemoglobin Köln (18) may be a reflection of the extremely rapid loss of the newly synthesized β-Köln (6). Balanced synthesis has been noted in studies of the α-chain mutants hemoglobin I (19) and hemoglobin Hasharon.[3] Although heterozygotes for hemoglobin Constant Spring possess only 0.5–1.2% of this α-chain variant (20, 21), globin chain production is apparently balanced (20).

Imbalance in α/β synthesis with α-chain excess has been reported in patients with certain types of sideroblastic anemia (22).

Significant imbalance in globin chain synthesis has been described in a study of the unstable α-chain mutant hemoglobin Ann Arbor (23). However, in contrast to the present study, hemoglobin Ann Arbor was associated with a relative depression of synthesis of the globin chain opposite in type to the abnormal chain; in affected subjects total β-chain synthesis was less than α-chain synthesis. A feedback inhibition of new β-chain production caused by β-chains released from degraded hemoglobin Ann Arbor was postulated. A similar inhibition of β-chain synthesis by released β-Leiden chains is conceivable.

[3] B. Tatsis and R. F. Rieder. Unpublished observations.

Imbalance in globin chain synthesis with α-chain preponderance has recently been described in a form of congenital dyserythropoietic anemia (24, 25). Affected individuals exhibit normochromic anemia with marked abnormalities of size and shape of the red cells. Some patients had mild elevations of hemoglobin A_2 (24), or hemoglobin F (25). Erythroid precursors in the bone marrow contained cytoplasmic inclusion bodies and marked nuclear abnormalities. Because of the normal degree of hemoglobinization of the peripheral erythrocytes, an absolute excess of α-chain production has been postulated to explain the in vitro hemoglobin synthesis findings. Patients with hemoglobin Leiden also have erythrocytes with normal mean corpuscular volume and mean corpuscular hemoglobin content. Thus, an absolute excess in α-chain synthesis is possible in these individuals. However, the relatively normal peripheral blood and marrow morphology clearly differentiates the subjects with hemoglobin Leiden from those with the syndrome of congenital dyserythropoietic anemia and unbalanced chain synthesis.

The basis for the excess α-chain production in patients with hemoglobin Leiden is not known. Little is understood about normal mechanisms that regulate the rate of production of individual chains, influence the relative proportions of structurally different chains of the same class, or assure balanced production of α- and β-chains. Hemoglobin Leiden is structurally unique among the abnormal hemoglobins studied in that there is a deletion close to the N-terminal end. It is conceivable that the structural alteration disturbs the initiation of transcription or translation. Measurements of translation times for the α, β^A, and β-Leiden chains and assay of α and β mRNA content may provide further understanding of the present observations.

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