Globin Synthesis in Iron-deficiency Anemia

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The α to β globin chain ratio has been determined in the peripheral red cells of 11 patients with iron deficiency anemia. The mean ratio was found to be 0.74 ± 0.07, which is significantly lower than the ratio of 0.97 ± 0.07 obtained in normals. When the stroma-free hemolysates were purified prior to globin preparation the α to β ratio did not change. On the other hand, globin extracted from whole iron-deficient cells, including the stroma, had a higher α to β ratio, 0.88 ± 0.04, but still significantly lower than normal. These results suggest that in iron deficiency there is a decrease in α-chain synthesis relative to β-chain and that there are membrane-bound globin chains, but no excessive increase in the free α-chain pool. Similar findings have been reported previously in other states of heme deficiency like sideroblastic anemia and lead poisoning.

THE AVAILABILITY OF iron for the synthesis of heme, or heme itself, is essential for the normal maturation and globin synthesis in erythroid cells. In iron deficiency, profound morphologic[1] and biochemical[2] disturbances have been found, the most striking being the marked decrease in globin synthesis.[3-5]

In addition to the general suppression of globin synthesis in iron deficiency, a selective effect on the different globin chains has been suggested. This is based on observations in heterozygotes for hemoglobin E,S and β-thalassemia who become iron deficient.[6-10] Furthermore, diminished synthesis of α-chains relative to β-chains has been found in patients with defects in heme synthesis such as sideroblastic anemia and lead poisoning.[11-13]

The purpose of the present study was to obtain data on the synthesis of α and β globin chains in iron deficiency anemia, which is the most common clinical state of heme deficiency.

MATERIALS AND METHODS

Patients

Eleven patients with uncomplicated chronic iron deficiency due to chronic blood loss were studied. All had hypochromic anemia, low serum iron level, and a high unsaturated iron-binding capacity. All were studied prior to iron therapy, and two patients were studied also during and after iron replenishment.

Methods

Routine hematologic studies were carried out as described by Dacie and Lewis.[14] Globin-chain ratios were determined according to the method of Clegg et al.,[15] with slight modifications as described previously.[16] Globin was prepared from stroma-free hemolysates by cold acid-acetone precipitation. In some cases, globin was also prepared from purified hemoglobin or from whole cells including stroma. Purified hemoglobin from the stroma-free hemolysates was prepared ac-

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Submitted January 9, 1974; accepted March 19, 1974.

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Table 1. The α to β Ratio of Globin Prepared From Stroma-free, Purified, and Whole Red Cell Hemolysates of Iron Deficiency Anemia Patients

Case	Hemoglobin (g/100 ml)	Reticulocytes (%)	Serum iron (μg/100 ml)	α to β Ratio		
				Stroma-free	Purified	Whole Cell
1	7.6	1.0	26	0.77	0.77	
2	11.0	3.5	28	0.69	0.71	
3	6.0	0.4	4	0.67	0.61	
4	9.0	1.5	30	0.78	0.82	
5	7.8	1.5	4	0.69		
6	9.6	0.4	43	0.70		0.87
7	6.0	1.4	8	0.87		0.96
8	6.5	2.8	30	0.84		0.89
9	8.5	1.5	16	0.67		0.86
10	9.3	3.4	26	0.75		0.86
11	5.6	1.8	11	0.72		0.86
			Mean ± SD	0.74 ± 0.07	0.72 ± 0.09	0.88 ± 0.04
			Controls	0.97 ± 0.07*	—	1.03†

*Mean ± SD in nine normal individuals.

†Mean in three normal individuals.

cording to the method of Winterhalter et.al.,[17] which allows only soluble tetramers of hemoglobin to elute off the Sephadex CM-50 column, any free globin chains not associated as hemoglobin being retained. Whole cell globin was prepared by adding the washed red cells directly to acid-acetone.[11] Globin chains were separated by column chromatography on CM-cellulose using a linear gradient of 0.005-0.5 *M* phosphate buffer, pH 6.7, containing 8 *M* urea and mercaptoethanol or dithiothreitol. The specific activities of the separated chains were determined in the peak tubes and the α to β ratio calculated.[16]

RESULTS

The α to β globin chain ratios determined in peripheral blood cells of 11 patients with iron deficiency anemia are given in Table 1. They ranged between 0.67 and 0.87 with a mean of 0.74 ± 0.07. In nine normal individuals the α to β globin chain ratio ranged between 0.90 and 1.12 with a mean of 0.97 ± 0.07 which is significantly different ($p < 0.0005$) from the ratio in iron deficiency.

The disturbed globin synthesis could not be correlated to the degree of the anemia, its duration, or to the serum iron levels: The addition of an iron salt (10^{-5} *M* ferriammonium sulfate) to the incubation mixture caused an increase in total protein synthesis in two out of three experiments but did not change the α to β globin chain ratio in any of the experiments. We have not yet studied the effect of in vitro heme supplementation.

In two patients hemoglobin synthesis was restudied during and after iron therapy (Table 2). In both, the α to β globin ratio was completely corrected after full recovery.

Table 2. The α to β Globin Ratio of Red Cells in Iron Deficiency Anemia Before and After Iron Therapy

Case	α to β Ratio (Stroma-free Hemolysates)			
	Before Therapy	After Therapy 1 wk	2 mo	7 mo
1	0.67	0.67	0.98	—
2	0.67	—	—	1.08

The observed disturbed α to β globin ratio could be affected by several factors, including (1) the presence of a large intracellular pool of free α-chains which will dilute out the radioactive chains, thus decreasing the specific activity of the α-chains, and (2) precipitation and attachment of α-chains on the cell membrane, thus creating an α-chain deficiency in the intracellular phase.

A partial answer to these questions was obtained from experiments in which globin prepared from purified or whole cell hemolysates was studied in parallel to standard globin extracted from stroma-free hemolysates (Table 1). In four experiments, the α to β ratio of globin prepared from column-purified hemolysates was the same as that of the stroma-free hemolysate globin preparations. On the other hand, in each of the six studied cases the α to β ratio of the whole-cell globin preparations, which includes stroma-bound globin chains, was found to rise to a mean of 0.88 ± 0.04, approaching the expected ratio of unity, yet it was still significantly lower than normal ($p < 0.005$).

DISCUSSION

Hemoglobin synthesis in the erythroid cell is a well regulated and balanced process where heme and the different globin chains are synthesized in equal proportions. Hemin has been shown to have a regulating effect on this complex process, exerting its effects on several stages of hemoglobin synthesis and assembly. The control by hemin of the initiation of hemoglobin synthesis has been extensively studied. Present evidence indicates that the action of hemin in reticulocyte lysates and cell-free systems is to prevent the formation of two inhibitors of protein initiation.[18,19] Recently it has also been shown that hemin enhances the synthesis of all proteins in reticulocyte lysates, including those programmed by added mRNA.[20] In addition to the possible effects at the initiation stage, heme seems to influence the assembly of the globin chains. Travill et al.[21] proposed a model of hemoglobin biosynthesis in which the effects of heme are explained by enhanced polyribosome formation and synthesis of both α- and β-chains, promotion of the assembly of the newly synthesized α- and β-chains, and conversion of $\alpha\beta$ dimers to hemoglobin tetramers with the elimination of an $\alpha\beta$ pool.

While Tavill et al. found that the ratio of α- to β-chains synthesized in reticulocytes of iron-deficient rabbits is normal, we found that in iron-deficient human reticulocytes there appears a diminished α-chain synthesis. This is shown by the significant reduction of the α- to β-chain ratio found in globin prepared from whole cell hemolysates. As globin made from whole cells had an α to β ratio higher than that obtained from stroma-free hemolysates (Table 1), it appears that in iron deficiency there is a precipitation on or an attachment to the cell membrane of α-globin monomers possibly with some $\alpha\beta$ dimers. The results found with the purified hemolysates indicate that iron deficiency does not cause a significant enlargement of the normally present small free α-chain pool. Since we did not measure the total radioactivity, we cannot relate our results to the findings of an excessive $\alpha\beta$ dimer pool in iron deficiency as reported both by Tavill et al.[21] and White et al.[11]

The finding of an apparent decrease in α-chain in iron deficiency is in complete agreement with the studies by White et al. in states of heme deficiency,

i.e., sideroblastic anemia and lead poisoning, and with his unpublished observations on iron deficiency.[11-13] The different findings in human and rabbit iron-deficient reticulocytes could be due to species differences or to methodology.

White et al.[11] suggested that the diminished α-chain synthesis may be explained by the observations of Blum et al.[22,23] that heme-depleted α- or β-chains specifically inhibit the synthesis of the homologous chain in a cell-free system and that the addition of α-chain stimulated β-chain synthesis, but not the converse.

Iron deficiency may also affect differentially other globin chains, as it has been shown that hemoglobin A_2 levels decrease in normal individuals and those with heterozygous β-thalassemia.[6,7] Wasi et al.[8] reported that hemoglobin E levels decrease in heterozygotes with concomitant iron deficiency. Similar observations were reported on sickle cell-trait carriers.[9,10] So it is conceivable that δ, β^S, and β^E are more suppressed than the other globin chains in the iron-deficient cells. Recently O'Brien[24] reported the effect of iron deficiency on the expression of hemoglobin H: Iron deficiency was associated with a complete suppression of hemoglobin H in the peripheral blood. A similar observation has been previously reported by Pearson and McFarland.[25] It seems that in α-thalassemia where there is already a marked decrease in α-chain production, the superimposed iron deficiency would affect mainly the synthesis of the β-chains. Alternatively, the decreased soluble hemoglobin H in that patient may have been due to more rapid precipitation and removal of excess β-chains resulting from the further decrease in α-chain synthesis. Only synthetic ratio studies will clarify the mechanism.

The failure of iron added in vitro to correct the globin synthesis and the late restoration by iron therapy (Table 2) are probably explained by the findings of Hershko et al.[2] of marked biochemical anomalies in the erythroid precursors in chronic iron deficiency. Only upon the establishment of an entirely new normal population does the globin-synthetic apparatus become normal.

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