DEFECT IN HEMOGLOBIN SYNTHESIS POSSIBLY DUE TO A DISTURBED ASSOCIATION

Josée PAGNIER, Henri WAJCMAN and Aominique LABIE
Institut de Pathologie Moléculaire, 24, rue du faubourg Saint-Jacques, 75014 Paris, France*

Received 18 June 1974

1. Introduction

In heterozygous patients, the amount of some human β-chain variants in peripheral blood is much lower than that of the normal component A. This is mostly the case of unstable hemoglobins, in relation with protein destruction [1]. The abnormal component is also diminished in heterozygous patients with hemoglobin E, usually ranging from 25 to 35%. According to Rieder, this decrease could be explained by both a disturbed synthesis and an instability of hemoglobin E [2].

We had recently the opportunity of studying hemoglobin E in various kinds of association (heterozygous A, E and E-β° thalassemia) and in homozygous E, and demonstrated that the structural abnormality, located in a contact area between subunits, gave rise to some discrete functional disorders [3].

In this work, we describe the synthesis of hemoglobin in the same patients. Our data give evidence for a decrease synthesis as proposed by Rieder [2]. Nevertheless, the specific activity ratios β^E/β^A measured on the heterozygous patient at various times of incubation are not consistent with an instability of the protein as it was suggested by Rieder [2]. Gel filtration experiments bring arguments for the presence of a defect in the association of the newly synthesized subunits in the homozygous patient.

* Groupe U 15 de l'Institut National de la Santé et de la Recherche Médicale, Laboratoire Associé au Centre National de la Recherche Scientifique.

2. Materials and methods

Blood was collected in heparinized tubes and the reticulocyte count was increased by centrifugation. The in vitro synthesis of hemoglobin was studied according to Borsook [4] with slight modifications, using [^{3}H]leucine as a marker. The globin was prepared by acid acetone and the polypeptide chains fractionated [5]. The radioactivity was determined in each fraction by liquid scintillation counting of aliquots; the total radioactivity incorporated into the chains was estimated and the specific activity expressed as the ratio of counts per min, per ml and per optical density unit at 280 nm. After a 60 min incubation, a gel filtration was run immediately as previously described [6].

3. Results and discussion

3.1. *E-β° thalassemia hemoglobin studies*

After a 1 hr incubation of the cells from the E-β° thalassemic patient, the total synthetic ratio $\beta + \gamma / \alpha$ chains was equal to 0.46. The percentage of radioactivity incorporated into β^E chains was only 20 per cent and the total activity ratio β^E/α was equal to 0.28. As usually described in such cases, a high level of fetal hemoglobin was found (40%) and the γ chain incorporation amounted to 12% of the total labelling; the total activity ratio γ/α was equal to 0.17 (table 1). By gel filtration, free α chains amounting to 16% were found.

These data are in good agreement with those published by Weatherall et al. [7] and can be compared

Table 1
Total radioactivity incorporated into the chains

Patient	Incubation time (hr)	Total radioactivity cpm				Total synthetic ratio		
		γ	β^A	β^E	α	$\frac{\gamma}{\alpha}$	$\frac{\beta}{\alpha}$	$\frac{\beta+\gamma}{\alpha}$
E-β° Thalassemia	1	39 065		63 970	223 370	0.17	0.28	0.46
E/E	1			48 150	54 000		0.90	
E/E	4			240 480	336 510		0.74	
A/E	1		11 000	3 775	14 120		1.05	

The total synthetic ratio shows an unbalanced synthesis for the E-β thalassemic patient. After a 1 hr incubation, the α and β chain synthesis is balanced for both heterozygous AE and homozygous E.

to studies of heterozygous S-β° thalassemia. In these latter cases, the total activity ratio β^S/α ranged from 0.43 to 0.62 [7–10], and 31% of the total incorporation was found in β^S chains [7]. Our results can suggest a defective synthesis of β^E chains.

3.2. *Hemoglobin E studies*

The mechanism of disturbed synthesis was studied on cells containing only hemoglobin E. After 60 min of incubation, the total activity ratio was equal to 0.9: this value is in the lower normal limits of a balanced synthesis. When the incubation was continued for 4 hr, the ratio decreased to 0.74 (table 1).

Two radioactive peaks were observed after gel chromatography on Sephadex G-100 (fig. 1). In the major peak, eluted with hemoglobin, the total activity ratio β^E/α was 1.2 (fig. 2A); the minor one, corresponding to a molecular weight of about 30 000, was demonstrated to be pure α chains; it contained 12% of the total radioactivity (fig. 2B). In normal controls, the free α chains pool, expressed in radioactivity is 1 or 2%. The defect of newly synthesized α chains observed in the tetrameric fraction corresponds exactly to this α dimers peak. On the other hand, the excess of labelled β chains could be explained by the existence of abnormal β^E_4 tetramers, having the same electrophoretical mobility as hemoglobin E.

A large pool of free α chains has been described in β-thalassemia [7,11] where β chain synthesis is decreased, and in some unstable hemoglobins [1,12], in relation with an enhanced degradation. The results are not completely explained by a decreased synthesis since we observed an almost balanced synthesis between β^E and α chains.

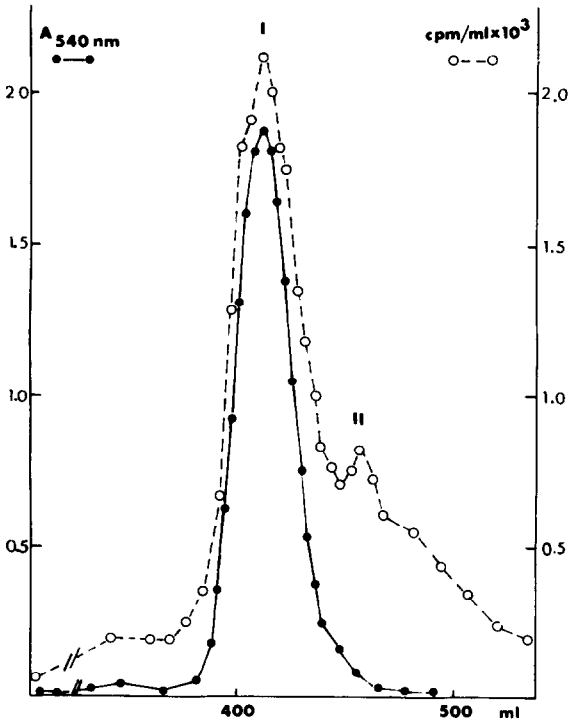

Fig. 1. Red blood cell homolysate of an homozygous patient E/E. Elution pattern on Sephadex G-100. Peak 1 corresponds to hemoglobin and peak II to a molecular weight of about 30 000.

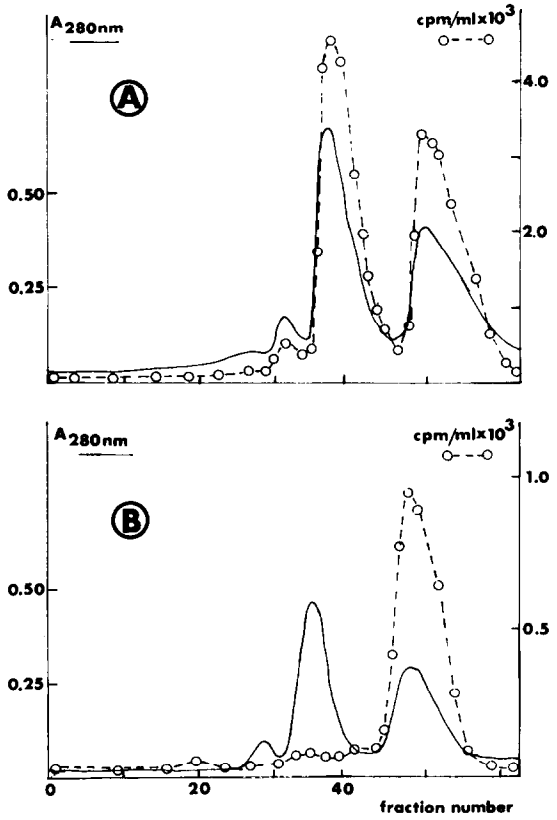

Fig. 2. A. Chain separation of peak I: the ratio of total radioactivity β^E/α is equal to 1.2; B. Chain separation of peak II after addition of carrier hemoglobin. Only the α chains are labelled.

3.3. *Hemoglobin AE studies*

In order to investigate the possible instability of hemoglobin E, the heterozygous patient's reticulocytes were incubated for various times ranging from 15–120 min. The low incorporation made shorter incubations impossible. The specific activity ratio was found constant and always inferior to 1 (table 2). These results differ from what has been described for unstable hemoglobins [1]. Moreover, no abnormal heat stability could be demonstrated.

These data favor a decreased synthesis of hemoglobin E.

To summarize our results, we found obvious similarities between the homozygous E/E and the doubly heterozygous E-β° thalassemic patients, the most striking feature being the abnormal large pool of free α chains. Some disparities have been noticed between the synthetic ratios observed in E-β° thalassemia and S-β° thalassemia. It is postulated that in heterozygous β-thalassemia a regulatory mechanism exists in the nucleated cells accounting for an apparent balanced synthesis of the subunits in the bone marrow [8,13]. If we assume a decreased or delayed association of α and β^E chains, and also a diminished synthesis of β^E chains, a similar mechanism could be evoked to explain the various results. In the heterozygous patients, there may be some compensatory increase in the production of β^A from the normal β^A gene, together with a reduction of the total globin synthesis. In the homozygous patient, the compensation is only at the expense of total production of hemoglobin; it gives rise to an almost balanced synthesis after 1 hr, but would not be more efficient after a long incubation. In the double heterozygous E-β° thalassemic patient, the production of γ chains is relatively high, but insufficient nevertheless to compensate for the defect of both β^A and β^E genes. This patient is anemic and hypochromic.

The regulatory effect of free α chains on its own synthesis has been demonstrated [14]. It could be involved as a factor in the overall regulation of globin chain synthesis.

Table 2
Study of the specific activity ratio β^E/β^A

Incubation time (min)	Specific activity cpm/ml/O.D. unit		Specific activity ratio
	β^E	β^A	β^E/β^A
15	208	260	0.80
30	380	505	0.75
60	635	890	0.71
120	1020	1325	0.77

Only slight modifications are observed over a period of time ranging from 15 to 120 min. This value is always smaller than 1, indicating a β^E chain defective synthesis.

Acknowledgements

We are thankful to Dr A. Najman who kindly provided the patients' blood samples.

This work was supported by grants from the 'Institut National de la Santé et de la Recherche Médicale', the 'Centre National de la Recherche Scientifique', the Délégation Générale à la Recherche Scientifique et Technique' and the 'Assistance Publique'.

References

[1] Cohen-Solal, M. and Labie, D. (1973) Biochim. Biophys. Acta 295, 67–76.
[2] Feldman, R. and Rieder, R. F. (1973) Blood 42, 783–791.
[3] Gacon, G., Wajcman, H. and Labie, D. (1974) FEBS Letters 41, 147–150.
[4] Lingrel, J. B. and Borsook, H. (1963) J. Lab. Clin. Med. 64, 668–674.
[5] Clegg, J. B., Naughton, M. A. and Weatherall, D. J. (1966) J. Mol. Biol. 19, 91–108.
[6] Pagnier, J., Labie, D. and Schapira, G. (1973) Biochimie 55, 1245–1252.
[7] Weatherall, D. J., Clegg, J. B., Na-Nakorn, S. and Wasi, P. (1969) Brit. J. Haematol. 16, 251–257.
[8] Nienhuis, A. W., Canfield, P. H. and Anderson, W. F. (1973) J. Clin. Invest. 52, 1735–1745.
[9] Bank, A., Dow, L. W., Farace, M. G., O'Donnell, J. V., Ford, S. and Natta, C. (1973) Blood 41, 353–357.
[10] Forget, B. G., Baltimore, D., Benz Jr., E. J., Housman, D., Lebowitz, P., Marotta, C. A., McCaffrey, R. P., Skoultchi, A., Swerdlow, P. S., Verma, I. M. and Weissman, S. M. Ann. N.Y. Acad. Sci. (in press).
[11] Modell, C. B., Latter, A., Steadman, J. H. and Huehns, E. R. (1969) Brit. J. Haematol. 17, 485–501.
[12] White, J. M. and Brain, M. C. (1970) Brit. J. Haematol. 18, 195–209.
[13] Natta, C., Banks, J., Niazi, G., Marks, P. A. and Bank, A. (1973) Nature New Biol. 244, 280–281.
[14] Blum, N., Maleknia, N. and Schapira, G. (1970) Bull. Soc. Chim. Biol. 52, 1279–1286.