HEMOGLOBIN CONSTANT SPRING : HEMOGLOBIN SYNTHESIS IN HETEROZYGOUS AND HOMOZYGOUS STATES*

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SUMMARY

Thirteen adult and one newborn heterozygotes, and three homozygotes for hemoglobin Constant Spring were examined for globin chain synthesis. Reticulocytes from venous blood were incorporated with [^{3}H]-leucine in an incubation mixture for 3 hours. Globin prepared from the radioactive, washed red cells was fractionated by CM-cellulose chromatography in 8 M urea and the total radioactivity of each globin chain was determined. The mean of α/β ratio in the heterozygotes was 1.34 ± SD 0.08, which is significantly different from that of 1.07 ± SD 0.03 in eleven normal controls. The $\alpha/\beta+\gamma$ ratio in the heterozygous neonate was also 1.39 . The α/β ratios in the three homozygotes were around 1.6 . The α-Constant Spring chain appears to be over produced, but it may be unstable or labile, not fully available for conjugation with the non alpha chains.

INTRODUCTION

Hemoglobin Constant Spring (Hb Con Sp) is a unique α-chain variant with 31 amino acid residues elongating from the C-terminal of the normal α-chain(1). Recently, it has been shown that slow hemoglobin variants which had been previously described in Thailand as Hemoglobin Thai(2,3), in Greece as Hemoglobin Athens(4), in Malaysia as Hemoglobin χ (5) and a slow component found in Hong Kong, are identical to Hb Con Sp(6). The abnormal hemoglobin is present in very small quantities, usually less than 2 % in heterozygotes, and is easily denatured, causing technical problems to detect the abnormal pigment(7). Since either the Hb Con Sp or α-thalasse-

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mia$_2$ gene in interacting with α-thalassemia$_1$ gene results in the clinical syndrome of hemoglobin H disease, it suggests that the Hb Con Sp gene has equivalent effect to that of α-thalassemia$_2$(1,3,7). It is of interest to compare the hemoglobin synthesis in the individuals with Hb Con Sp with those who carry the α-thalassemia genes. This communication presents results of globin chain synthesis in heterozygous and homozygous states of Hb Con Sp.

MATERIALS AND METHODS

Subjects

The subjects were Thai and Chinese. Eleven healthy subjects with normal hematologic data, normal Hb A_2 and without known Hb H disease or Hb Bart's hydrops fetalis in their families were used as normal controls.

Thirteen asymptomatic subjects with Hb Con Sp trait were examined. Another heterozygote was a neonate of a woman who was homozygous for Hb Con Sp. The baby had Hb Con Sp in association with small amount of Hb Bart's besides the normal Hb A and Hb F in the cord blood. The cord blood of this newborn was studied for globin synthesis. Three cases with homozygosity for Hb Con Sp, designated on the basis of family studies, were also examined.

Hematologic studies

Routine hematologic examination were carried out by standard methods. Hemoglobin concentration was determined by cyanmethemoglobin. The red cells were counted in an electronic cell counter. Packed red cell volume was measured by microhematocrit technique. The starch gel electrophoresis was performed in tris-EDTA-borate buffer, pH 8.6 (8). Alkali resistant hemoglobin was quantitated by the one minute method of Singer et al (9). Abnormal hemoglobin components were quantitated by cellulose acetate electrophoresis(10)

Incorporation studies

About 40-50 ml of heparinized venous blood was collected and centrifuged at 5,000 rpm for 5 minutes, at 4°C in SorvallRC2-B refrigerated centrifuge. The plasma was then discarded. After washing three times with reticulocyte saline solution, the washed red cells were centrifuged at 15,000 rpm for one hour at 4°C, and 5 ml of reticulocyte-rich upper layer were collected. The incorporation study of intact reticulocytes was carried

out as previously described by Lingrel and Borsook(11), and by Weatherall et al (12). The incorporation time was three hours. Globin prepared from the radioactive packed red cells was fractionated by CM-cellulose chromatography. The fractions containing globin chains were separated, pooled and desalted by dialysis against four changes of 0.5 % formic acid for 48 hours. One ml of the dialysed globin solution was added into 10 ml of Bray's solution. The radioactivity was measured in a Packard Tri-Carb Liquid Scintillation spectrometer. The total radioactivity was obtained by multiplying the cpm/ml by its total volume. Finally, the total radioactivity of the α-globin chain against that of the β-globin chain was calculated as α/β ratio.

RESULTS

All the 13 Hb Con Sp heterozygotes had normal hematologic values with the exception of reduced mean corpuscular volume in some. The mean total radioactivity α/β ratio was 1.34 ± 0.08, which is significantly higher than 1.07 ± 0.03 found in 11 normal subjects (Fig. 1). The newborn heterozygote for Hb Con Sp had normal hematologic data at birth except the presence of Hb Con Sp and Hb Bart's. The radioactivity α/β+γ ratio of the cord blood was 1.39 which is in the range of the α/β ratios found in adult heterozygotes for this hemoglobin.

Three patients with homozygosity for Hb Con Sp were found to have mild jaundice, splenomegaly and definite thalassemic red cell changes. The hemoglobin levels were around 9-11 gm%. Hemoglobin electrophoresis revealed 3-5 % Hb Con Sp + Hb A + trace of Hb Bart's. The total radioactivity α/β ratios of the three were 1.52, 1.66 and 1.70 (Fig. 1). Family studies in two of these patients showed that all the parents had starch gel electrophoresis and α/β ratios compatible with the findings in Hb Con Sp trait.

DISCUSSION

Globin chain synthesis in peripheral blood reticulocytes has been reported by several authors (13-18). α- and β-globin chain production in normal persons has been found to be synchronous and the α/β ratio is nearly equal to one. In our laboratory the average α/β ratio is 1.07 ± 0.03 . Recently, we have reported the mean α/β ratios in obligatory α-thalassemia$_1$ and α-thalassemia$_2$ heterozygotes as 0.76 ± 0.04 and 0.92 ± 0.03 respec-

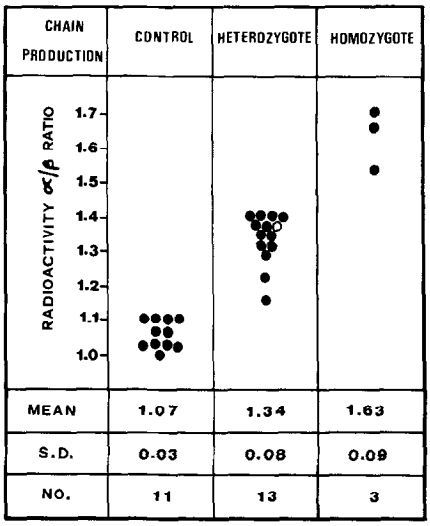

Figure 1. A summary of the mean total radioactivity α/β ratio of 11 normal controls, 13 heterozygotes and 3 homozygotes for Hb Con Sp. The total radioactivity α/β +γ ratio in cord blood incorporation study of a newborn heterozygote for Hb Con Sp is illustrated in open circle.

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tively (19). For cases with β-thalassemia trait our α/β ratios are 1.72 - 2.0 (20).

Against the above background our consistent findings of elevated α/β ratios in individuals with Hb Con Sp are unlikely to be attributable to errors, although Clegg and co-workers reported the α/β ratio in one Hb Con Sp heterozygote as 1.05 (1).

Since Hb Con Sp gene has an equivalent effect to α-thalassemia$_2$ gene, the finding of α/β ratio of 1.34 in Hb Con Sp trait is unexpected.

The α-chain of Hb Con Sp is elongated by 31 amino acids four which are leucine. The increased radioactivity in the α-chains can not be attributed to the radioactive leucine.in this extended portion of α-chain. For the radioactivity counted as α came from the normal α-chain peak only, $\alpha^{\text{Con Sp}}$ being not apparent in the chromatogram.

Hb Con Sp trait is associated with increased amounts of Hb Bart's in cord blood (21), and together with α-thalassemia$_1$ gene gives rise to Hb H disease. This would suggest that Hb Con Sp gene is associated with decreased α-chain synthesis. The findings of increased α/β ratios in Hb Con Sp heterozygotes and homozygotes are then in conflict with the expected

decrease in α-chain synthesis. The reason for this is not yet clear. It may be postulated that $\alpha^{Con\ Sp}$ is synthesized more rapidly than α^{A}, but $\alpha^{Con\ Sp}$ is unstable or labile, thus not fully available for γ- and β-chains. Due to gradual chopping off of the elongated portion, the $\alpha^{Con\ Sp}$ is shifted, in the chromatogram, toward and counted as α^{A} peak, causing the observed high α/β ratios.

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REFERENCES

1. Clegg, J.B., Weatherall, D.J.,and Milner, P.F. (1971) Nature 234, 337-340.
2. Wasi, P., Na-Nakorn, S., Pootrakul, S., Sookanek, M., Disthasongchan, P., Pornpatkul, M., and Panich, V. (1969) Ann. N.Y. Acad. Sci., 165, 60-82.
3. Wasi, P. (1970) J. Med. Ass. Thailand 53, 677-686.
4. Sofraniadou, K., Kaltsoya, A., Loukopoulos, D., and Fessas, Ph. (1968) Abstacts of the Simultaneous Sessions, XII. Congress Int. Soc. Hemat. New York, p. 56.
5. Lie-Injo, L.E. (1973) Acta Haemat. 49, 25-35.
6. Fessas, Ph., Lie-Injo, L.E., Na-Nakorn, S., Todd, D., Clegg, J.B., and Weatherall, D.J. (1972) Lancet i, 1308-1310.
7. Wasi, P., Na-Nakorn, S., Pootrakul, P., and Panich, V. (1972) Ann. Hum. Genet. 35, 467-470.
8. Smithies, O. (1959) Biochem. J. 71, 585-587.
9. Singer, K., Chernoff, A.I., and Singer, I. (1951) Blood 6, 413-428.
10. Wasi, P., Disthasongchan, P., and Na-Nakorn, S. (1968) J. Lab. Clin. Med. 71, 85-90.
11. Lingrel, J.B., and Borsook, H. (1963) Biochemistry 2, 309-314.
12. Weatherall, D.J., Clegg, J.B., Na-Nakorn, S., and Wasi, P. (1969) Brit. J. Haemat. 16, 251-267.
13. Heywood, D., Karon, M., and Weissman, S. (1965) J. Lab. Clin. Med. 66, 476-481.
14. Weatherall, D.J., Clegg, J.B., and Naughton, M.A. (1965) Nature 208, 1061-1065.
15. Bank, A., and Marks, P.A. (1966) Nature 212, 1198-1200.
16. Clegg, J.B., and Weatherall, D.J. (1967) Nature 215, 1241-1243.

17. Kan, Y.W., Schwartz, E., and Nathan, D.G. (1968) J. Clin. Invest. 47, 2515-2521.
18. Modell, C.B., Latter, A., Steadman, J.H., and Huehns, E.R. (1969) Brit. J. Haemat. 17, 485-501.
19. Pootrakul, S., Sapprapa, S., Wasi, P., Na-Nakorn, S., and Suwanik, R. (1974) Abstracts Part I, XV. Congress of The Int. Soc. Haemat. Jerusalem. p. 206.
20. Assawamunkong, S. (1974) Master of Science Thesis, Chulalongkorn University.
21. Pootrakul, S., Wasi, P., Na-Nakorn, S., and Pravatmuang, P. (1974) Abstracts, International Symposium on Abnormal Hemoglobins and Thalassemias, Istanbul, p. 14.