THE HEMOGLOBIN P-GALVESTON–Hb-C CONDITION IN MEMBERS OF A BLACK FAMILY FROM SOUTH CAROLINA

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Received 26 July 1978

1. Introduction

Structure and properties of Hemoglobin (Hb) P-Galveston or $\alpha_2\beta_2$ (117 (G19) His → Arg) were first described in 1969 by Schneider et al. [1] who observed this variant in a 32 year old black woman and her 10 year old son. Both persons showed slight morphological abnormalities, i.e., hypochromia, aniso- and poikilocytosis, and some target cells. A second report appeared in 1975 by Di Iorio et al. [2] who found this variant in 7 members of a Swiss family, 5 having a Hb P-Galveston heterozygosity and 2 with the Hb P-Galveston–β^0-thalassemia condition. Hematological data on the simple heterozygotes were normal. The functional properties of isolated Hb P-Galveston were (nearly) identical to those of Hb A.

This communication reports the observation of Hb P-Galveston in association with Hb C ($\alpha_2\beta_2$ 6 (A3) Glu → Lys) in two black females originating from Charleston, SC. The quantity of Hb P-Galveston exceeded that of Hb C in these persons by a factor of 1.5–1.8; this observation initiated a review of similar data on subjects with a combination of Hb C (or Hb S) and any other β chain variant, and an attempt to explain variations by assuming differences in post-translational rates of formation of the appropriate $\alpha\beta^X$ dimers and $\alpha_2\beta_2{}^X$ tetramers.

2. Materials and methods

2.1. *Blood samples*

Blood was collected in vacutainers with EDTA as anticoagulant and transported in ice to Augusta, GA. Hematological data were collected with a Coulter Counter Model S. Hemolysates were prepared from washed erythrocytes after addition of 1 vol. water and 0.4 vol. carbon tetrachloride.

2.2. *Hemoglobin analyses*

Hemoglobin variants were initially identified by starch gel electrophoresis, at pH 8.9 [3], by citrate agar gel electrophoresis [4], and by a solubility test [4]. The quantities of the hemoglobins in freshly-prepared red cell lysates were determined by DEAE-cellulose chromatography [5].

2.3. *Structural analyses*

Hb P-Galveston was isolated by preparative DEAE-cellulose chromatography [5], converted into globin [6], and the aberrant β^X chain was isolated by CM-cellulose chromatography [7]. The isolated material was aminoethylated with ethylenimine [8] and ~75 mg AE-β^X chain was digested with trypsin (Worthington Biochem. Corp; TPCK trypsin) at room temperature for 3 h, at pH 9.0 in a pH stat. The resulting peptides were separated on a 0.9 × 60 cm column of chromobead type P resin (Technicon Instruments) at 40°C using pyridine–acetic acid and volatile buffers [9]. Isolated peptides were hydrolyzed with 6 M HCl in vacuo at 110°C for 24 h. Amino acid analyses were made with a Spinco Model 121M automated amino acid analyzer equipped with high-sensitivity cuvettes and an integrator.

3. Results

3.1. *Case report*

The proposita, S.D., was a healthy 21 year old black

Elsevier/North-Holland Biomedical Press

female with a medical history not pertinent to the presence of any hemoglobin abnormality. The liver and spleen were not palpable. Her sister, L.B., was 28 years old, married, and had 3 children 2 of whom were available for studies. In addition, the mother, L. D., who was 55 years old, donated a blood sample for analysis. Past clinical histories on these persons were unremarkable.

3.2. *Hematological analyses*

Hematological data on the proposita S.D. and her sister A.B., both with the Hb C–P-Galveston condition, the mother L.D. and her grandchild Y.B., both with the Hb P-Galveston heterogeneity, and a second grandchild with Hb C trait are given in table 1. All data were essentially normal; examination of peripheral blood smears of these 5 subjects showed no significant changes from normal except for a slight microcytosis and for target cells in blood of the child with the Hb C heterozygosity.

The amount of Hb P-Galveston in the 2 heterozygotes averaged 49% and was the same as that of Hb A (48%). However, the level of Hb P-Galveston in the 2 persons with the CP combination was considerably higher (av. 61%) while that of Hb C (including Hb A_2) averaged only 38%.

3.3. *Structural analysis of the variant*

All peptides were recovered from the tryptic digest of the aminoethylated β^X chain, and all except fragment T 12^b (residues 113–120, inclusive) had the expected amino acid composition. Peptide T 12^b was recovered in 2 fragments; a pentapeptide with the composition His 1.07 (2), Arg 0.87 (0), Ala 1.18 (1), Val 1.05 (1), Leu 0.82 (1) corresponded to a fragment of the β chain involving the residues in positions 113–117, inclusive, while a tripeptide with the composition Lys 1.00 (1), Gly 1.02 (1), Phe 0.85 (1) corresponded to the residues in positions 118–120 (values between parentheses indicate the number of residues in corresponding fragments of the β^A chain). These data indicate a His → Arg substitution in position 117 of the β chain which is the same as the substitution found in Hb P-Galveston [1,2].

The identity of the second β chain variant, i.e., Hb C, in the proposita and her sister was based on its electrophoretic properties at alkaline pH and at acid pH in citrate agar gel electrophoresis, as well as on its behavior in anion-exchange chromatography [4].

4. Discussion

The finding of another family with the relatively rare β chain variant Hb P-Galveston is not of great significance and our observations on the simple Hb P-Galveston heterozygotes are comparable to those in [1,2]. However, the relatively high level of Hb P-Galveston in the persons with the Hb C–P-Galveston combination is of considerable interest and made us survey the literature and our own files of unpublished cases for comparable data on β chain variants occurring in combination with Hb S or Hb C. Initially this search was limited to SX combinations but was later extended to include CX combinations because it is generally known that the percentages of Hb S and Hb C in

Table 1
Hematological and hemoglobin composition data

Subject (age)	Hb type	Hb (g/dl)	PCV (l/l)	RBC (10^{12}/l)	MCV (fl)	MCH (pg)	MCHC (%)	A_2[b] (+C) (%)	P[b] (%)	A[b] (%)	F[b] (%)
L.D. (55)	AP[a]	12.2	0.336	4.62	75	26.5	34.6	2.1	46.5	50.2	1.2
S.D. (21)	CP	13.0	0.356	4.44	80	29.5	36.5	34.9	62.5	0	2.6
L.B. (28)	CP	11.8	0.338	4.22	83	28.3	33.4	40.7	59.3	0	0
Y.B. (7)	AP	12.6	0.362	4.77	78	26.7	33.3	2.8	51.5	45.7[c]	<1[d]
T.B. (5)	AC	13.0	0.362	5.23	72	25.1	34.4	39.7	0	60.3[c]	<1[d]

[a] P refers to Hb P-Galveston
[b] Determined by DEAE-cellulose chromatography [5]
[c] Includes small amounts of Hb F
[d] By alkali denaturation [4]

persons with SC disease are essentially the same (for references, see [4]). Data that were obtained with methods other than chromatographic procedures and electrophoretic methods in which the percentages are obtained after elution of the hemoglobin components were excluded from this comparison.

Acceptable data were found for the following β chain variants: Hb C-Harlem (6 Glu → Val; 73 Asp → Asn), Hb J-Baltimore (16 Gly → Asp), Hb E (26 Glu → Lys), Hb N-Baltimore (95 Lys → Glu), Hb P-Galveston (117 His → Arg), Hb O-Arab (121 Glu → Lys), Hb Camden (131 Gln → Glu), Hb Leslie (131 Glu, deleted) and Hb Hope (136 Gly → Asp). References describing the structural variations and some physico-chemical properties can be found in [4]; Hb C-Harlem has the sickling properties of Hb S while the Hbs E, Leslie and Hope are slightly unstable using the common instability tests [4]. The data collected pertained to the percentages of the variant in simple heterozygotes and in persons with combinations of Hb X with either Hb S or Hb C; besides own data, information from [1,2,10–23] has been included.

Figure 1 shows the results of this survey; open circles and squares refer to percentages of Hb X in simple heterozygotes and closed circles and squares to these in persons with SX or CX combinations. The accuracy of the data is not always optimal because in some instances (Hb Camden, Hb Hope) the separation of the variant and Hb A is somewhat incomplete while for 3 hemoglobins (C-Harlem, O-Arab and E) the quantity of the variant includes that of Hb A_2. Despite these limitations 3 groups are evident. In the first group of 4 variants (the Hbs N-Baltimore, P-Galveston, J-Baltimore and Camden) the quantities of Hb A and Hb X in heterozygotes are similar, but the level of Hb X is considerably increased in persons with the SX or CX condition. These differences do not exist in the second group of 3 variants (the Hbs Hope, C-Harlem and O-Arab) and the hemoglobins A and X or S and X are present in about the same amounts in the simple heterozygotes and in the persons with the SX combination, respectively. The variants Hb Leslie and Hb E form a third group; irrespectively of the presence of Hb S, Hb C or Hb A the percentage of Hb X is always $\leqslant 30\%$.

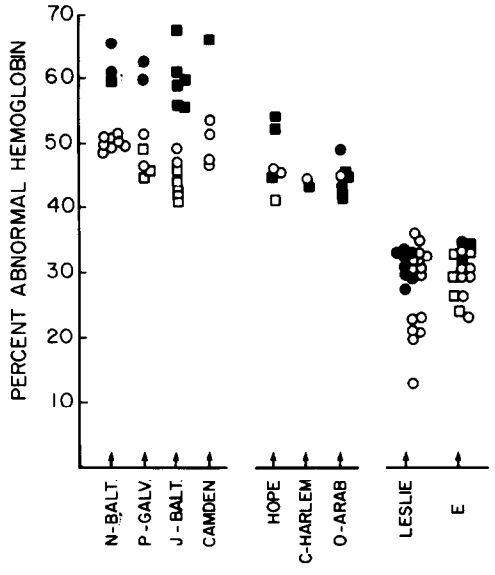

Fig.1. The relative amounts of various β chain abnormal hemoglobins in heterozygotes (open circles and squares) and in persons with the SX or CX combination (closed circles and squares). Data given by open or closed circles are from cases studied in our laboratories, while those indicated by open or closed squares are from [1,2,10–23].

Evidence has been presented previously that the number of active α chain structural loci determines to some extent the relative quantities of hemoglobin types such as Hb S, Hb C, Hb Leslie, a.o. [24]. However, family studies have shown that this effect is relatively small in persons with the SX (or CX) combination [24], thus, other factors must play a role. Recent in vitro analyses involving the relative affinities of certain β^X chains for normal α chains have shown considerable differences which can be interpreted to indicate that the affinity of β chains for α chains is a major post-translational control mechanism which determines to a great extent the level of the variant in heterozygotes or in persons with the SX (or CX) combination. Table 2 presents some of the data that were obtained, and lists the percentages of the variants that were formed when different amounts of α chains were combined with a mixture of equal amounts of β^A and β^X chains. Considerable differences can be observed; the formation of Hb N-Baltimore, for instance, was identical to that of Hb A, that of Hb Leslie was decreasing rapidly in conditions of relative α chain deficiency, while the values for Hb S were intermediate between those of Hb N and Hb Leslie. The differences between the 3 Hb types are best illustrated at an $\alpha/\beta^A + \beta^X$ ratio of 0.75 showing the formation of about 52% Hb N, 42%

Table 2
The in vitro formation of β chain variants from mixtures of isolated α chains and $\beta^A + \beta^X$ chains (from [25])[a]

Hb variant	Ratio of $\alpha/(\beta^A + \beta^X)$[b]				
	0.25	0.50	0.75	1.0	2.0
N-Baltimore	55.9	54.4	51.5	–	–
S	30.6	35.3	42.4	48.5	50.4
Leslie	–	0	32.1	39.5	42.8

[a] Data are given in percent
[b] The ratio β^A/β^X was one in all experiments

Hb S and 32% Hb Leslie. These data are indeed consistent with the observations presented in fig.1; thus, specific structural variations in the β^X chains, and subsequent changes in secondary and/or tertiary structures of these proteins, dictate the rates of recombination with α chains to form the $\alpha\beta^X$ dimer and ultimately the $\alpha_2\beta_2{}^X$ tetramer. Apparently the 4 hemoglobins of the first group are preferentially formed over Hb S (or Hb C) but not over Hb A, and the person with 1 of these SX (or CX) combinations resembles a simple Hb S (or Hb C) heterozygote. This is not the case for the hemoglobins of the other 2 groups, and the preferential formation of Hb S tetramers over that of Hb Leslie (or Hb E) tetramers results in excessive percentages of Hb S (or Hb C) in persons with the S Leslie (or C Leslie) and SE (or CE) combinations.

Acknowledgements

This research was supported by US Public Health Service Research Grants HLB-05168 and HLB-15158. The author acknowledges the expert collaboration of his coworkers Ms Marsha Gravely, Mr J. B. Wilson, Mr H. L. Lam, Ms R. N. Wrightstone, Mr H. Harris and Mrs J. C. P. Spray.

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