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K562 cells: a source for embryonic globin chains

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ABSTRACT

A combination of DEAE-cellulose chromatography and reversed-phase high-performance liquid chromatography (HPLC) has been used to devise a method for generating large quantities of embryonic as well as fetal globin chains. The identity of these globin chains was further confirmed by their tryptic peptide mapping. This technique could, therefore, provide a reliable source for these polypeptides for both analytical and immunological purposes. Moreover, the study of human hemoglobin switching, particularly embryonic to fetal, has been greatly hampered by the absence of a suitable model. K562 cells, due to their potential for differential induction of embryonic and fetal hemoglobin synthesis, can thus be used for this purpose and the various hemoglobins produced can then be effectively monitored using this method.

INTRODUCTION

Normal erythropoiesis has been a useful system for defining various aspects of eukaryotic cell differentiation and the regulation of gene expression. Investigations using normal erythropoietic cells are, however, limited by several factors, including the inability to establish long-term cultures of erythroid precursor cells. Erythroleukemia cells have, therefore, proven to be valuable tools for such purposes. One such human cell line, the K562, has been established from the pleural effusion of an adult with chronic myelogeneous leukemia in blast crisis [1]. These cells can be induced by hemin and a variety of other agents [2–5] to produce fetal and embryonic hemoglobins, but not adult hemoglobins [6–8].

The biochemical analyses of hemoglobins synthesized by these cells have so far involved methods such as fingerprinting of tryptic peptides [9,10] and radioimmunoassay [8]. Clegg and Gagnon [11] determined the amino acid sequence of the ζ-globin chain following cyanogen bromide cleavage of Gower I hemoglobin obtained from lysates of hemin-induced K562 cells. Approximately one half of the amino acid sequence of the human ε-globin chain has also been described [12].

A method is described here to obtain milligram quantities of pure ε- and

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ζ-globin chains from large-scale cultures of K562 cells. The identity of these globin chains was confirmed by amino acid analysis of their tryptic peptide fragments.

EXPERIMENTAL

Growth of K562 cells

K562 cells were grown in RPMI 1640 medium supplemented with heat-inactivated fetal calf serum in roller bottles. For the induction of hemoglobin synthesis, the cells were cultured in the presence of 50 μM hemin for three days. The cells were then pelleted by low-speed centrifugation (300 *g*) and washed three times with cold saline. The packed cell pellet was lysed in an equal volume of distilled water containing 0.1% KCN. The cellular debris was removed by ultracentrifugation at 90 000 *g* at 4°C for 1 h. The supernatant (hemolysate) was then washed twice with distilled water and concentrated by positive pressure (1034 Torr) in a Diaflo ultrafiltration unit using a YM10/62 mm filter.

Separation of hemoglobin fractions of K562 cell lysate by DEAE-cellulose chromatography

Hemolysate containing about 100 mg of hemoglobin was applied to a 300 mm × 20 mm I.D. column of DEAE-cellulose (Whatman DE-52) equilibrated with 0.2 *M* glycine containing 0.01% KCN, and 0.005 *M* NaCl. The chromatograms were developed at room temperature with a flow-rate of 30 ml/h. A linear gradient of 0.005 to 0.06 *M* NaCl was applied, using a 500-ml mixing flask [13].

Fractions of 10 ml each of the effluent were collected, and their absorbances were determined spectrophotometrically both at 415 nm (for hemoglobin concentration) and 280 nm (for total protein content). The results were plotted and each individual peak was collected separately and concentrated in the Diaflo ultrafiltration unit.

High-performance liquid chromatographic (HPLC) separation of isolated hemoglobin fractions

Each DE-52-isolated peak was further analyzed by HPLC on a 250 mm × 4.6 mm I.D. Vydac C_4 column. Mobile phases A and B were 0.1% trifluoroacetic acid (TFA) in a mixture of 60:40 and 20:80 (v/v) acetonitrile–water, respectively. The elution was carried out by increasing the concentration of mobile phase A (from 50 to 60%) and a corresponding decrease in mobile phase B (from 50 to 40%) resulting in an acetonitrile gradient (from 40 to 44%) for a period of 120 min. This was followed by a gradient of 60–80% mobile phase A (44–52% acetonitrile) applied for an additional 60 min. The column was finally purged with 100% mobile phase A (60% acetonitrile) for 5 min and then reequilibrated for 10 min with 50% acetonitrile. The flow-rate was maintained at 1.0 ml/min and the absorbance monitored at 220 nm (for peptides).

The large-scale isolation of the globin chains in the various DE-52-isolated hemoglobin fractions was done by reversed-phase HPLC utilizing a BIO-SIL TSK-ODS-120T C_{18} column (300 mm × 21.5 mm I.D.), as described by Huisman [14]. The chromatograms were obtained at a flow-rate of 2.0 ml/min using an acetonitrile–water gradient with 0.1% TFA. The globin chains of the isolated hemoglobin fractions were separated in the order: α–Aγ T–Gγ–ε–Aγ I–ζ. Appropriate fractions were then freeze-dried for structural analyses.

Reversed-phase HPLC analyses of soluble tryptic peptides of the globin chains

The soluble peptides were separated by reversed-phase HPLC as described by Wilson *et al.* [15]. A 5–10 mg amount of the globin polypeptide chains, isolated as above, was dissolved in 10 ml of distilled water with 3–6 mg of NH_4HCO_3 added for buffering. The pH was adjusted to 8.5 with 0.5 *M* NaOH. The polypeptide chains in the above solution were then digested with 100 μl of a stock TPCK-trypsin (specific activity: 225 U/mg of protein) solution (1 mg/ml) with constant stirring at 22–25°C for 24 h. The reaction was stopped by the addition of a drop of 4 *M* HCl. The reaction mixture was lyophilized and finally dissolved in 10% acetic acid. Solutions containing approximately 1 mg of dissolved tryptic peptides were then analyzed by reversed-phase HPLC using a μBondapak C_{18} column (300 mm × 3.9 mm I.D.) with an ammonium acetate–water–acetonitrile gradient. The mobile phases were (A) 50% acetonitrile in 0.01 *M* ammonium acetate, pH 5.7, and (B) 0.01 *M* ammonium acetate, pH 5.7. The solvent program used for developing the chromatograms was T-1, 10 min (1% A) gradient curve 0; T-2, 120 min (1–60% A, resulting in an acetonitrile gradient of 0.5–30%) gradient curve 1; T-P (purge), 10 min (100% A). The flow-rate was maintained at 1.5 ml/min.

Amino acid analysis

Each isolated fraction from the above tryptic peptide analysis was dried under nitrogen and hydrolyzed *in vacuo* using 6 *M* HCl with phenol (9 mg per 100 ml of 6 *M* HCl) at 110°C for 24 h. The amino acid composition of the resulting hydrolysate was determined by a Beckman Spinco Model M-121 automated amino acid analyzer equipped with a Hewlett-Packard Model 3390 A integrator.

RESULTS AND DISCUSSION

The DE-52 isolation of the K562 cell lysate with five peaks, A–E, are shown in Fig. 1. Fig. 2 represents the chromatograms obtained from the analytical reversed-phase HPLC analyses, showing the presence of varying quantities of α, Aγ T, Gγ, ε, Aγ I and ζ chains in the different DE-52-isolated fractions. A larger quantity of the putative ε- and ζ-chains were isolated from peak B of the DE-52-isolated hemoglobin fractions using the preparative BIO-SIL TSK-ODS-120T C_{18} column (Fig. 3). Approximately 1.2 mg of hemoglobin obtained from DE-52 column (peak B) was applied to the preparative HPLC column, and the material

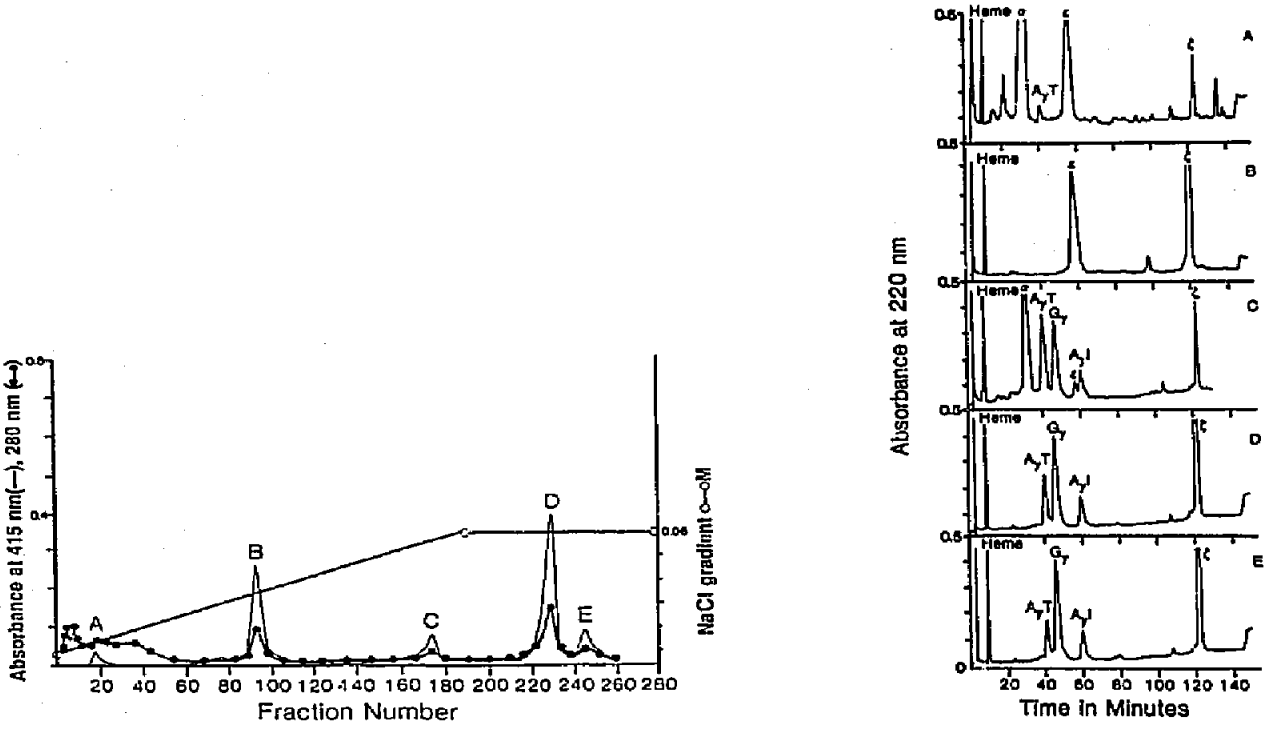

Fig. 1. Isolation of hemoglobin components from K562 cell lysate by preparative chromatography on a 300 mm × 20 mm I.D. column of DEAE-cellulose.

Fig. 2. Separation of the globin chains by reversed-phase HPLC on a large-pore Vydac C_4 analytical column (250 mm × 4.6 mm I.D.) of DE-52-isolated (Fig. 1) K562 cell lysate fractions. (A) Zone A; (B) zone B; (C) zone C; (D) zone D; (E) zone E. Flow-rate: 1.0 ml/min.

from eight such chromatograms (yielding 1–2 mg of the polypeptides) were combined for the structural analyses. Peptides from tryptic digests of the putative ε- and ζ-chains (peaks A and C of Fig. 3, respectively) were then separated and isolated by reversed-phase HPLC (Fig. 4), and their amino acid composition was determined by amino acid analyzer. The method, however, does not allow effec-

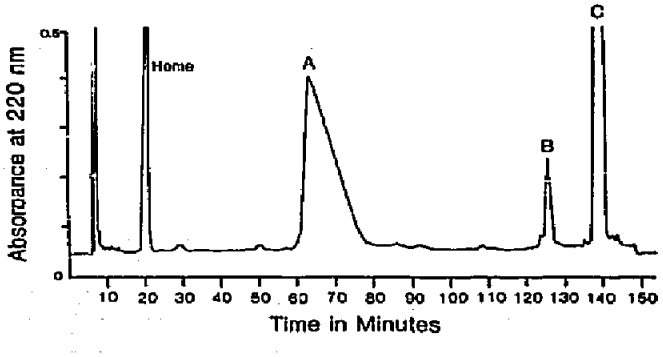

Fig. 3. Isolation of globin chain components on a BIO-SIL TSK-ODS-120T C_{18} preparative column (300 mm × 21.5 mm I.D.) from DE-52-separated (Fig. 1) zone B of K562 cell lysate. Flow-rate: 2.0 ml/min.

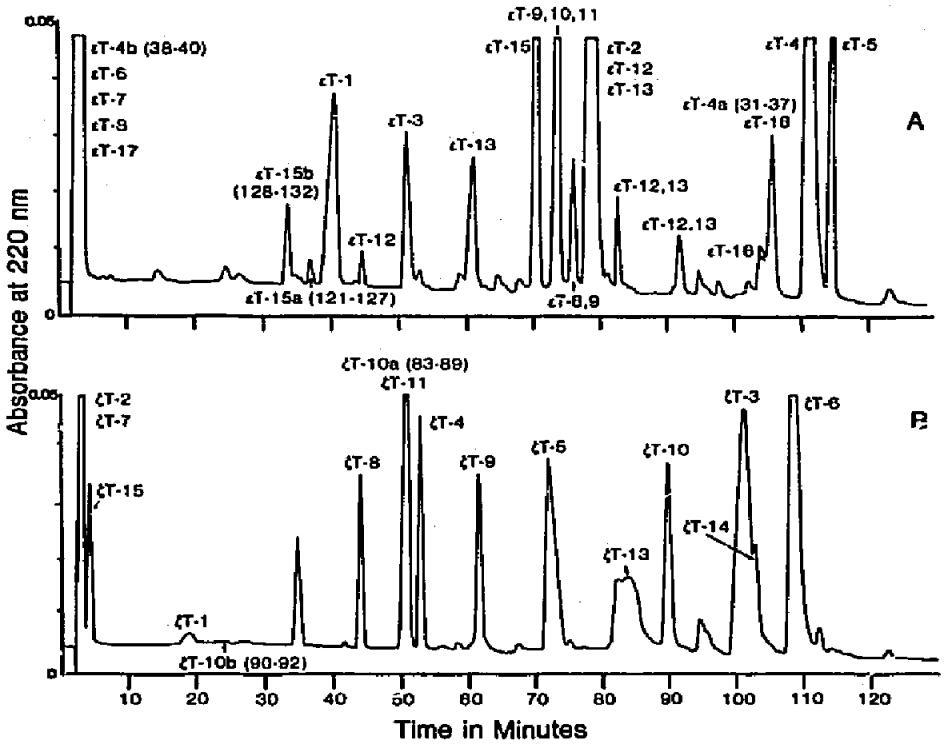

Fig. 4. Reversed-phase HPLC elution profile of tryptic digest of the putative ε-and ζ-globin chains (peaks A and B from Fig. 3). (A) Peak A; (B) peak C. The chromatograms were obtained with a μBondapak C_{18} analytical column (300 mm × 3.9 mm I.D.) with acetate–acetonitrile as mobile phase at pH 5.7. Flow-rate: 1.5 ml/min.

tive quantitation of sulphur-containing amino acids or tryptophan as they are easily destroyed by the acid hydrolysis procedure used. These data, summarized in Table I and II, offer convincing evidence that the polypeptide recovered from peaks A and C of Fig. 3 are, indeed, ε- and ζ-chains. This was further clarified by assessing their amino acid analysis data (Tables I and II) in light of the respective sequence predicted from the published nucleic acid sequences [16,17].

The method can similarly be used for the generation of large quantities of the isolated fetal globin chains, *viz.* Aγ T, Gγ and Aγ I, which are present in varying quantities in the different DE-52-isolated fractions (Fig. 2).

CONCLUSIONS

The K562 cell line has been extensively used as a model for studying gene regulation [18–24]. The method presented here shows its potential for generating large quantities of embryonic globin chains, ε- and ζ-, as well as α- and the various γ-chains. The various inducers of hemoglobin synthesis in K562 cells have been found to produce preferential induction of the different globin chains [25], which

TABLE I

AMINO ACID COMPOSITION OF SOLUBLE TRYPTIC PEPTIDES OF THE ε-CHAIN

Values are moles per peptide. Values in parentheses are expected number of residues.

Amino acid	T-1	T-2	T-3	T-4[a]	T-4a[a]	T-4b	T-5	T-6	T-7	T-8
Aspartic acid			1.28 (1)				3.34 (3)			
Threonine	0.95 (1)	1.10 (1)		1.00 (1)		0.95 (1)				
Serine		1.98 (2)					3.66 (4)			
Glutamic acid	2.19 (2)		3.11 (3)	1.06 (1)		1.11 (1)				
Proline				0.92 (1)	0.86 (1)		1.79 (2)			
Glycine			2.71 (3)				1.77 (2)		1.05 (1)	
Alanine	0.96 (1)	1.83 (2)	1.80 (2)				0.92 (1)		1.00 (1)	
Valine	0.76 (1)	0.94 (1)	0.95 (1)	1.18 (2)	1.17 (2)			1.00 (1)		
Isoleucine							0.88 (1)			
Leucine		1.19 (1)	1.15 (1)	2.13 (2)	2.18 (2)		1.94 (2)			
Tyrosine				0.92 (1)	0.95 (1)					
Phenylalanine	0.95 (1)						2.96 (3)			
Histidine	0.62 (1)								(1)	
Lysine	0.95 (1)	0.97 (1)					0.93 (1)	(1)	(1)	1.00 (1)
Arginine			0.98 (1)	0.88 (1)		0.95 (1)				
Methionine			(1)							
Tryptophan		(1)		(1)						
Residues in ε-chain	1–8	9–17	18–30	31–40	31–37	38–40	41–59	60–61	62–65	66

	T-8,9	T-9,10,11	T-12	T-13	T-15	T-15a	T-15b	T-16	T-17
Aspartic acid	1.19 (1)	4.82 (4)	1.32 (1)	2.14 (2)					
Threonine	1.06 (1)	0.94 (1)			1.04 (1)	0.98 (1)			
Serine	1.09 (1)	0.91 (1)	1.05 (1)			1.12		1.32 (1)	
Glutamic acid			1.14 (1)	1.10 (1)	4.12 (4)	3.71 (3)	1.38 (1)		
Proline		0.79 (1)		0.92 (1)	1.03 (1)	0.99 (1)			
Glycine	0.88 (1)	0.87 (1)							
Alanine	1.11 (1)	2.88 (3)			1.77 (2)		1.83 (2)	3.72 (4)	
Valine	0.97 (1)	0.82 (1)		0.91 (1)	1.02 (1)	1.23 (1)		1.98 (2)	
Isoleucine	0.86 (1)	0.88 (1)						1.09 (1)	
Leucine	1.21 (1)	2.26 (2)	1.73 (2)	1.15 (1)				2.32 (2)	
Tyrosine									1.09 (1)
Phenylalanine	0.92 (1)	2.01 (2)		0.92 (1)	1.00 (1)	0.80 (1)			
Histidine			0.76 (1)	0.74 (1)				0.90 (1)	0.91 (1)
Lysine	1.72 (2)	3.02 (3)	0.98 (1)	1.12 (1)	1.03 (1)		1.17 (1)	1.08 (1)	
Arginine									
Methionine		(1)							
Tryptophan						(1)			
Cystine			(1)						
Residues in ε-chain	66–76	67–87	88–95	96–104	121–127	128–132	133–144	145–146	

[a] Valine value is low due to Val–Val bonds.

TABLE II

AMINO ACID COMPOSITION OF SOLUBLE TRYPTIC PEPTIDES OF ζ-CHAIN

Values are moles per peptide. Values in parentheses are expected number of residues.

Amino acid	T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8[a]
Aspartic acid				1.11 (1)		1.14 (1)		1.16 (1)
Threonine	1.01 (1)	0.97 (1)	1.03 (1)	3.84 (4)	1.03 (1)	1.06 (1)		
Serine	0.99 (1)		1.00 (1)	0.94 (1)	1.03 (1)	1.07 (1)	1.00 (1)	
Glutamic acid		1.02 (1)		3.15 (3)	1.11 (1)	1.12 (1)		
Proline					1.01 (1)	2.04 (2)		
Glycine				1.02 (1)		1.07 (1)	1.01 (1)	1.01 (1)
Alanine			0.97 (1)	0.95 (1)		1.04 (1)	1.09 (1)	2.92 (3)
Valine			1.05 (1)					2.83 (4)
Isoleucine			1.27 (2)	1.97 (2)				
Leucine	1.05 (1)			1.03 (1)	2.08 (2)	2.08(2)		
Tyrosine						0.79		
Phenylalanine						0.92 (1)	1.84 (2)	
Histidine					0.82 (1)	1.72 (2)	0.83 (1)	
Lysine	0.96 (1)		0.99 (1)		0.99 (1)		1.07 (1)	0.90 (1)
Arginine		1.01 (1)		0.99 (1)		1.03 (1)		
Methionine			(1)					
Tryptophan			(1)					
Residues in ζ-chain	1–4	5–7	8–16	17–31	32–40	41–56	57–61	62–71

	T-9	T-10	T-10a	T-10b	T-11	T-13[b]	T-14	T-15
Aspartic acid	2.23 (2)				2.20 (2)	2.80 (2)		
Threonine						1.42 (1)	1.04 (1)	
Serine	1.94 (2)	1.08 (1)	1.05 (1)				3.08 (3)	
Glutamic acid		0.80 (1)	1.19 (1)			1.77 (1)	1.43 (1)	
Proline					0.99 (1)	1.57 (1)		
Glycine	2.02 (2)							
Alanine	1.08 (1)	1.06 (1)	0.97 (1)			4.97 (5)		
Valine					1.92 (2)		2.31 (3)	
Isoleucine	1.84 (2)	0.87 (1)		0.97 (1)				
Leucine	0.92 (1)	2.94 (3)	2.05 (2)	1.03 (1)			1.57 (2)	
Tyrosine		0.12 (1)	0.96 (1)					1.19 (1)
Phenylalanine					0.95 (1)	2.03 (2)	1.15 (1)	
Histidine		1.04 (1)	0.83 (1)			0.99 (1)		
Lysine	0.98 (1)				0.95 (1)	1.35 (1)	0.83 (1)	
Arginine		1.20 (1)		1.00 (1)				0.81 (1)
Tryptophan						(1)		
Residues in ζ-chain	72–82	83–92	83–89	90–92	93–99	113–127	128–139	140–141

[a] Valine value is low due to Val–Val bond.

[b] Impure fraction.

could very well be used as a strategy for obtaining the globin chains of choice. Suitable DE-52-isolated fractions can then be used as a source for the desired globin chains.

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