Hb SERBIA (α112 (G19) HIS → ARG), A NEW HAEMOGLOBIN VARIANT FROM YUGOSLAVIA

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Received 9 August 1975

1. Introduction

A new variant, Hb Serbia α112 (G19) His → Arg, was found in a 40 year old Yugoslavian woman and her 12 year old daughter, during a haematological examination. Both carriers were mildly anaemic with haemoglobin concentrations of 11.2 and 10.0 g/dl, and target cells were observed.

2. Methods

Electrophoretic analysis of the haemolysates was carried out on paper in Tris buffer pH 8.9 and on starch gel in Tris–EDTA–borate buffer pH 8.6 according to standard techniques [1]. Electrophoresis of the dissociated globin chains was carried out on gelatinised cellulose acetate (Cellogel) in 6 M urea containing either 0.025 M sodium barbiturate pH 8.0 or 0.05 M phosphate pH 6.5. The abnormal haemoglobin was isolated for structural analysis by chromatography on DEAE Sephadex A50 using Tris–HCl buffers [2]. The isolated haemoglobin was converted to globin with 1.5% v/v conc. HCl in acetone and separated into α and β chains by chromatography on CM cellulose in 8 M urea-phosphate buffers pH 6.7 [3]. The isolated α-chain was split specifically at Cys α104 with 2-nitro-5-thiocyanobenzoic acid and the fragments were separated by gel filtration on Sephadex G-75 in 0.2 M acetic acid [4]. Fingerprints of a tryptic digest of α1–103 and a peptic digest of α104–141 were prepared by standard techniques and stained with specific reagents for histidine, arginine, tyrosine, tryptophan and sulphur-containing amino acids [1]. Peptic peptides were isolated by preparative fingerprinting [5] and the peptides were eluted with 6 N HCl and hydrolysed in sealed evacuated tubes at 108°C for 24 h prior to amino acid analysis.

3. Results

Paper electrophoresis at pH 8.9 of haemolysates from the two carriers revealed a new component (Hb Serbia) migrating between Hb A and Hb A_2. Electrophoresis of globin on cellulose acetate in 6 M urea, revealed a new α-chain which migrated more slowly towards the anode than the α^A chain at pH 9.0 but did not separate at pH 6.5. These findings indicated that Hb Serbia had an abnormality in the α-chain, probably a substitution of histidine by either lysine or arginine, which would cause a difference in electric charge only at alkaline pH values where the imidazole group is uncharged, but not at acid and neutral pH values where it would be positively charged. Only the His → Arg substitution is compatible with a single point mutation in the Genetic Code.

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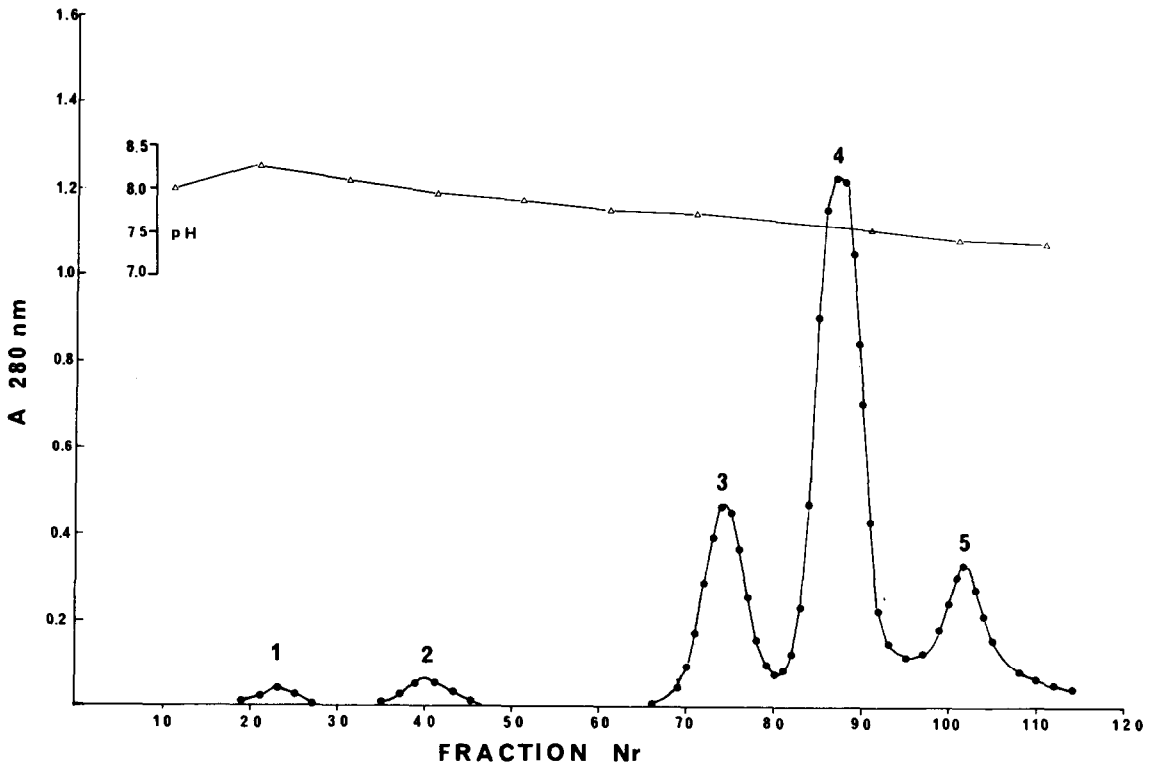

Fig.1. Chromatographic separation of a haemolysate containing Hb A and Hb Serbia on DEAE Sephadex in Tris–HCl buffer. Peak 1, Hb Serbia$_2$ ($\alpha_2{}^{Serb}\delta_2$) 1.4%, probably containing some carbonic anhydrase. Peak 2, Hb A_2 2.7%. Peak 3, Hb Serbia 22.2%. Peak 4, Hb A + Hb Serbia$_3$ 59.1%. Peak 5, Hb A_3 16.6%. Estimated proportion of Hb Serbia + Hb Serbia$_3$ = 26.6%.

Hb Serbia separated from Hb A on chromatography on DEAE-Sephadex with Tris–HCl buffer (fig.1) and amounted to 26.6% of the total haemoglobin. A minor fraction (1.4%) probably corresponding to Hb Serbia$_2$ ($\alpha_2{}^{Serb}\delta_2$) was detected but was not investigated further. A fingerprint of a tryptic digest of the globin of isolated Hb Serbia did not differ significantly from that of Hb A, indicating that the abnormality was probably in the insoluble 'core' of the α-chain. In order to investigate this the isolated α-Serbia chain was cleaved specifically at cysteine 104 with 2-nitro-5-thiocyanobenzoic acid. The sequence α104–141 of Hb A containing the 'core' peptides has two histidine residues at positions α112 and α122. In the fingerprint of the peptic digest of α104–141 of Hb Serbia (fig.2) peptide α110–116 gave a positive reaction for arginine instead of histidine, indicating that the predicted His → Arg substitution was at position α112 (fig.3). A second arginine-containing peptide was identified as α110–113 (table 1). All other peptic peptides had amino acid compositions similar to those reported by Casey and Lang [4].

4. Discussion

Hb Serbia is the third variant to be reported with a substitution of His α112, the others being Hb Hopkins-II (His → Asp) and Hb Dakar (His → Gln). Hb Hopkins-II has been found in an American Negro family [6,7,8] and also in a Scottish woman [9] and Hb Dakar has been found in Senegal. Both variants were reported to show a mild thermal instability. In Hb A the imidazole group of His α 112 (G19) probably forms a salt bridge with the carboxyl group

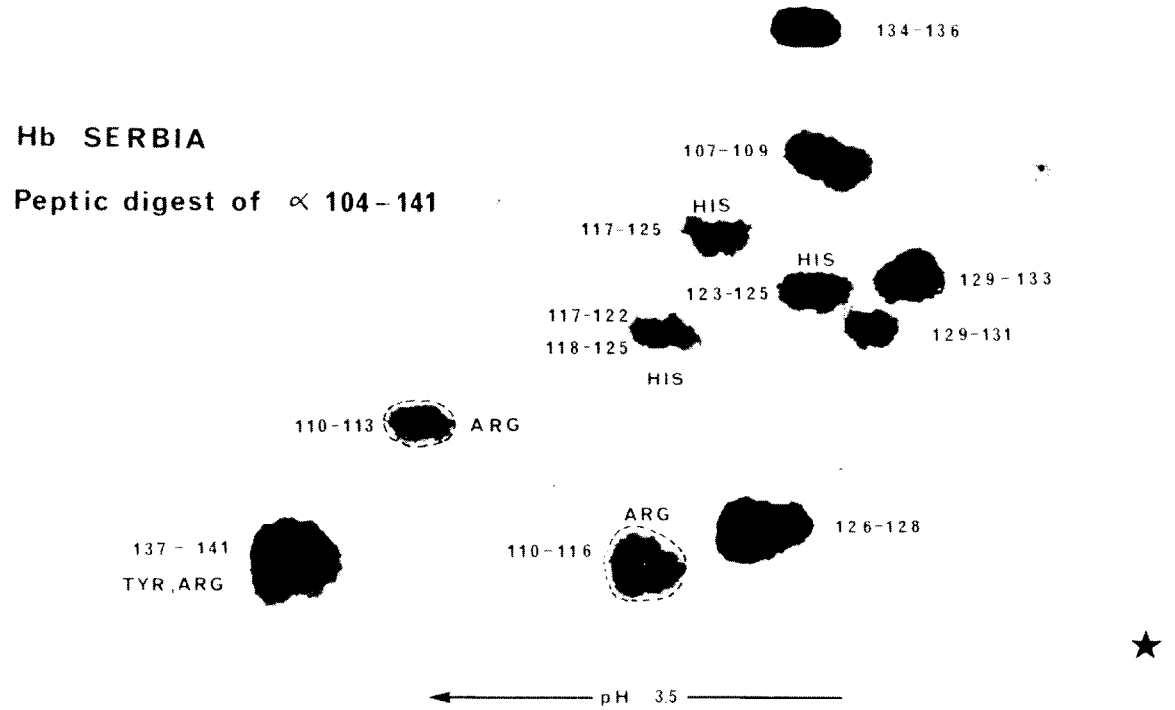

Fig.2. Fingerprint of peptic digest of α104–141 of Hb Serbia; electrophoresis at pH 3.5, chromatography ascending in amyl alcohol–pyridine–water. The new arginine-containing peptides of Hb Serbia are indicated by the broken circles (⋆) origin.

of Glu α27 (B8), which helps to hold the B and G helices together. In Hb Hopkins-II, two negatively charged groups are opposed and would repel each other and weaken the structure. Similarly, there should be some weakening of the structure in Hb Dakar, though perhaps to a lesser extent than in Hb Hopkins-II because there is no electrostatic repulsion. The guanidinium group of the side chain of Arg α112 in Hb Serbia is positively charged and should be capable of forming the salt bridge, consequently the variant should have a normal stability. It is not clear whether the

Table 1
Amino acid compositions of abnormal peptic peptides of Hb Serbia showing yields in nmol and molar ratios observed and (in parentheses) expected for the corresponding sequences from Hb A

	α110–116		α110–113	
	nmoles	molar ratio	nmoles	molar ratio
Glu	21.4	1.1 (1)		
Pro	13.7	0.7 (1)		
Ala	59.3	3.0 (3)	18.0	1.9 (2)
Leu	19.6	1.0 (1)	9.6	1.0 (1)
His	–	0.0 (1)	–	0.0 (1)
Arg	19.5	1.0 (0)	10.2	1.1 (0)

HB	110	111	112	113	114	115	116
A	Ala	Ala	His	Leu	Pro	Ala	Glu
Serbia			Arg				
Dakar			Gln				
Hopkins-2			Asp				

Fig.3. Amino acid sequence of α110–116.

slight anaemia in the carriers is directly attributable to the presence of the variant or to some other cause. Position G19 is occupied by histidine in most mammalian α-chains [11] and by tyrosine in carp α-chain. The homologous position in the non-α-chains is more variable, being occupied by either histidine, arginine or asparagine in the β- and γ-chains of various mammalian species and by lysine or arginine in myoglobins. Hb Serbia is homologous with the β-chain variant Hb P Galveston β117 (G19) His → Arg [12] found in an American Negro woman and her son. Both carriers were reported to be healthy but with slight morphological abnormalities in the red cells (hypochromia), anisocytosis and target cells) but it was not certain that they were related to the presence of the variant.

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