# Hb SETIF: Gl (94)α Asp → Tyr
# A NEW α CHAIN HEMOGLOBIN VARIANT WITH SUBSTITUTION OF THE RESIDUE INVOLVED IN A HYDROGEN BOND BETWEEN UNLIKE SUBUNITS

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## 1. Introduction

Bolton and Perutz [1] have demonstrated that the contact between hemoglobin unlike subunits ($\alpha_1\beta_2$) undergoes drastic changes during the transition from the oxy- to the deoxyform. The hydrogen bond between Asn G4 (102)$\beta_2$ and Asp G1 (94)$\alpha_1$ is the only polar interaction across the $\alpha_1\beta_2$ interface in the normal oxyhemoglobin conformation. The rest of the interactions are non polar. Therefore, among the hemoglobin variants localized on this contact, hemoglobin Kansas G4 (102)β Asn → Thr [2] and hemoglobin Richmond G4 (102)β Asn → Lys [3] are of particular interest. The consequences of the modification of this unique $\alpha_1\beta_2$ polar bond was thoroughly studied by Greer [4] in the case of these two abnormal hemoglobins.

A new example of disturbance of this hydrogen bond is reported in this paper: in Hb Setif, the residue Asp G1 (94)α is substituted by a tyrosine.

Moreover, the fact that the abnormality lies on the α chain and that the new residue is a tyrosine, bears a genetic interest.

## 2. Material and methods

Blood was collected on heparin and hemolysate prepared by routine procedures.

The abnormal component was isolated by Bio Rex 70 chromatography as previously described [5]. The hemoglobin was deheminized and the chains separated. After amino-ethylation, the protein was submitted to tryptic digestion. The peptides were separated and purified by ion exchange chromatography as described in [6].

The presence of an abnormal peptide, not clearly revealed on the elution pattern, was checked by the use of thin-layer analytical electrophoresis and chromatography. For this purpose, an aliquot of 1/50 of each of the 8 first zones of the chromatography was submitted to an electrophoresis on cellulose thin-layers in pyridine–acetic buffer pH 6.4 and to a chromatography on silica gel thin-layers in iso-amyl alcohol, pyridine, water (35/35/27).

All these runs were done simultaneously with the same zones of a normal α chain digest. Specific amino acid staining was performed on the thin layers. Amino acid composition was determined on a Beckman 120 C amino acid analyser and the sequence by the Edman-dansyl method of Gray [7], slightly modified, for ultra-micro procedure.

## 3. Results and discussion

An S-like moving component amounting to roughly 12% was found on starch gel electrophoresis at pH 8.8, as well as a very faint component slower than $A_2$, which is in favor of a mutated α chain.

This localization was proved by chain separation of the purified abnormal component.

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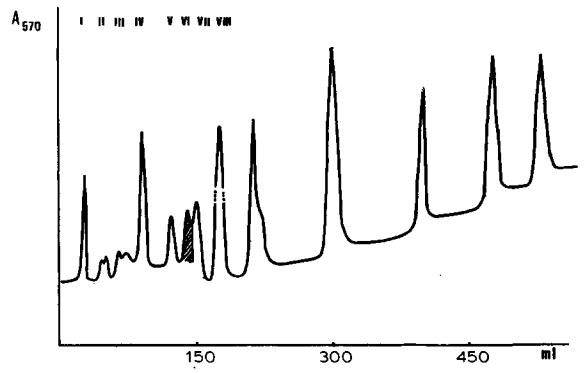


Fig. 1. Elution pattern of the $\alpha^{AE}$ chain tryptic digest of Hb Setif (three-fold electrical amplification). One supplementary peak can be observed in zone VI. When analytical thin-layer electrophoresis and chromatography were performed on zone IV, one peptide was lacking, the supplementary peptide of zone VI was characteristic of tyrosine (see text).

One supplementary peak was found on the elution pattern of the tryptic digest (fig. 1). Since the elution pattern of the amino ethylated α chain is rather complex, the presence of an abnormal peptide was also checked by analytical electrophoresis and chromatography on thin layers of the 8 first zones. In zone IV normally containing peptides $\alpha T_1$, $\alpha T_5$ and $\alpha T_{11}$, one peptide was lacking; in zone VI, a supplementary peptide giving specific staining for tyrosine, was found.

In view of the fact that the electrophoretic mobility was different and that all the peptides containing tyrosine were in their normal position, and considering the genetic code, the substitution was assumed to be Asp → Tyr in $\alpha T_{11}$.

This was proved by the amino acid composition of the purified supplementary peptide (table 1) and the sequence determination. The abnormality of Hb Setif is localized on site G1, one of the most important of the interface $\alpha_1\beta_2$. Two β chain variants have been described on the homologous site: Hb Kempsey G1 (99)β Asp → Asn [8] and Hb Yakima G1 (99)β Asp → His [9]. Only four α chain mutants with a structural abnormality localized on this contact have been described up to now:

Hb Chesapeake FG4 (92)α Arg → Leu [10].
Hb J Capetown FG4 (92)α Arg → Glu [11],
Hb Georgia G2 (95)α Pro → Leu [12], and
Hb Rampa G2 (95)α Pro → Ser [13].

Table 1
Amino acid composition of peptide $\alpha T_{11}$ Setif.

| | Abnormal peptide | Normal $\alpha T_{11}$ |
|---|---|---|
| Lysine | 1.1 | 1 |
| Aspartic acid | 1.0 | 2 |
| Proline | 1.1 | 1 |
| Valine | 1.7* | 2 |
| Tyrosine | 0.7* | 0 |
| Phenylalanine | 1.0 | 1 |

* The lower recovery of valine and tyrosine may be explained by the sequence Val–Tyr– not completely hydrolysed in 22 hr in 6 N HCl at 110°.

All of these abnormal hemoglobins present a more or less important modification of the three dimensional structure of the protein. Therefore, they are very useful models for the understanding of the structure–function relationship of hemoglobin. The fact that the modified residue of Hb Setif is involved in a bond changing during the oxy–deoxy transition confers a great interest to this model.

Our preliminary data give evidence for an unstable and functionally modified protein. Nevertheless the intraerythrocytic disorders are minor when compared to β chain mutant ones. This can easily be explained by the low percentage of the abnormal component due to the duplication of the α gene.

From the genetic point of view, two features have to be noticed: this mutant is an α chain mutant, and it is known that this category has a much lower frequency than β chain mutants [14]. This fact is explainable by the constraints of its successive pairing with different other chains. Secondly, the substitution of a tyrosine for an aspartic acid is most exceptional. It leads to a drastic change together in polarity and in steric hindrance. The genetic code prevents such substitutions from happening frequently [15]. It can be emphasized that transversions are rarely observed in the interior of the molecule, and that the mutation Asp → Tyr is due to a transversion of the first base from G to U.

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