COMPARISON BETWEEN HUMAN NORMAL AND SETIF HAEMOGLOBINS BY CIRCULAR DICHROISM AND DIFFERENTIAL ABSORPTION STUDIES

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1. Introduction

Hb Sétif is characterized by an $\alpha 94$ (G_1) Asp → Tyr substitution [1]. This change is associated with some abnormal physico-chemical properties of the molecule especially an altered oxygen affinity [2]. Moreover the oxy-form has a strong tendency to polymerize, causing sickling of erythrocytes in vitro [3,4].

The lowered oxygen affinity of Hb Sétif was discussed by Debray et al. [2]. They pointed out that the stabilization of the oxy-form of HbA by the hydrogen bonding between Asp $\alpha 94$ (G_1) and Asn $\beta 102$ (G_4) [5] could not occur in Hb Sétif and that there might be an increased dissociation of Hb Sétif involving the formation of identical $\alpha_1\beta_1$ and $\alpha_2\beta_2$ dimers. They did not observe such a dissociation and concluded that the differences between HbA and Hb Sétif are due to a more complex mechanism.

We thought it of interest to examine the effect of the Asp → Tyr substitution on the secondary structure. From the predictive rules of Chou and Fasman [6], and the recent values of the structural parameters [7], we concluded that the Asp → Tyr substitution induces a conformation change of the sequence around it, from a random coil in HbA to a β-sheet in Hb Sétif. Since β-sheet structures are well known to give rather stable hydrophobic interactions, this could result in abnormal binding between sub-units or molecules of Hb. Such a β-sheet–β-sheet interaction in Hb Sétif would cause aggregation between two α-chains, leading to an $\alpha_1\alpha_1$ contact in Hb Sétif instead of the $\alpha_1\beta_2$ contact in HbA. Since hydrophobic interactions increase with temperature, we tried to confirm our hypothesis using differential ultraviolet and visible absorption and circular dichroism studies at different temperatures with HbA and Hb Sétif. The results agree well with our predictions.

2. Materials and methods

2.1. *Preparation and purification of haemoglobins*

Pure HbA and Hb Sétif were obtained simultaneously from the total haemoglobin of a heterozygous patient by DEAE–Sephadex chromatography according to [8]. The haemoglobins were then dialysed for 20 h at 4°C against 0.05 M phosphate buffer, pH 7.4. All physical experiments were carried out less than 10 h after dialysis.

2.2. *Prediction of the secondary structure*

The secondary structure was predicted using the rules given by Chou and Fasman [6]. α-Helical, β-sheet and reverse β-turn regions can be predicted using the conformational parameters in [7] based on the occurrence frequency of each amino acid residue in each of the three possible structures.

2.3. *Spectroscopic and circular dichroism studies*

Differential ultraviolet and visible absorption studies were carried out on a Cary 118 C spectrophotometer fitted with a device allowing sample and reference cells to be at different temperatures. The reference cell was at 25°C and the temperature of the

sample cell was increased by 5°C steps from 25–50°C. A spectrum could be recorded in 15 min and 5 min was allowed between each step for temperature stabilization. The CD spectra were recorded with a Jobin Yvon R. J. Mark III dichrograph between 190 nm and 600 nm. The cells were 0.1 cm and 0.5 cm thick. The temperature was measured with a platinum probe placed directly in the solutions. The time required for temperature equilibration was 5 min and 45 min were needed to record a CD spectrum. The concentrations used for both types of measurement were about 1 mg/ml and were determined by ultraviolet absorption, taking A_{276} 34.4 [8]. All manipulations were carried out under dry N_2.

3. Results and discussion

3.1. *Prediction of the secondary structure*

The secondary structures of the α-chains of HbA and Hb Sétif in the region 75–117 were predicted. For both haemoglobins, two helical regions are predicted for residues 79–89 and 100–113. This is in good agreement with the X-ray crystallographic data of Perutz et al. for horse oxyhaemoglobin [5] and with Dickerson's model of sperm whale myoglobin [9]. Between the two helical regions, an unordered sequence is predicted for HbA but a β-sheet for residues 92–96 is predicted for Hb Sétif (table 1).

The presence of Pro 95 in this β-sheet region is considered to be possible as it is the only β-sheet breaker out of the five residues 92–96, whereas there are three very good β-sheet formers: Val_{93}, Tyr_{94} and Val_{96} in this sequence. A previously predicted β-sheet sequence in HbS, β1–6, contains a proline residue at β5 [10]. The difference in the predicted conformations of the α-92–96 sequences of HbA and Hb Sétif is because Tyr is a strong β-sheet former (H β) with $P_\beta = 1.47$ while Asp is a β-sheet breaking residue (B β) with $P_\beta = 0.54$.

3.2. *CD and differential absorption studies*

At 25°C no differences can be seen in the far ultraviolet CD spectra of the two haemoglobins. This is not unexpected since only five residues (1.7% of the total) are involved in the altered secondary structure. The values of ellipticities at different wavelengths and temperatures are given for HbA (fig.1) and for Hb Sétif (fig.2). The wavelengths, 574 nm, 418 nm, 260 nm, 222 nm and 207 nm, were chosen to correspond to the α band and the Soret γ band of the haemoglobins, aromatic amino acids residues and the $n \rightarrow \pi^*$ and the $\pi \rightarrow \pi^*$ bands of the peptide chromophore respectively.

The shape of all these bands is characteristic of a denaturation either of the secondary structure (222 nm and 207 nm) or the tertiary structure (574 nm, 418 nm and 260 nm). For each haemoglobin the transition temperature is the same for the five wave-

Table 1
Amino acids sequence and conformational prediction the 92–96 region of α-chain of human normal and Sétif haemoglobins

Haemoglobin	92	93	94	95	96
α-chain	R	V	D/Y	P	V

	HbA α-chain		Hb Sétif α-chain	
α-Potential	h_2IiB^a	$P<\alpha> = 0.94$	h_2ibB^a	$P<\alpha> = 0.87$
β-Potential	$H_2iB_2{}^a$	$P<\beta> = 1.08$	H_3iB^a	$P<\beta> = 1.27$
Predicted conformation	Random coil[b]		β-sheet	

[a] H, strong former; h, former; I, weak former; i, indifferent; b, breaker; B, strong breaker
[b] The sequence 92–96 in HbA was taken as a random coil and not as a β-sheet region because it exists two strong breakers out of the five residues

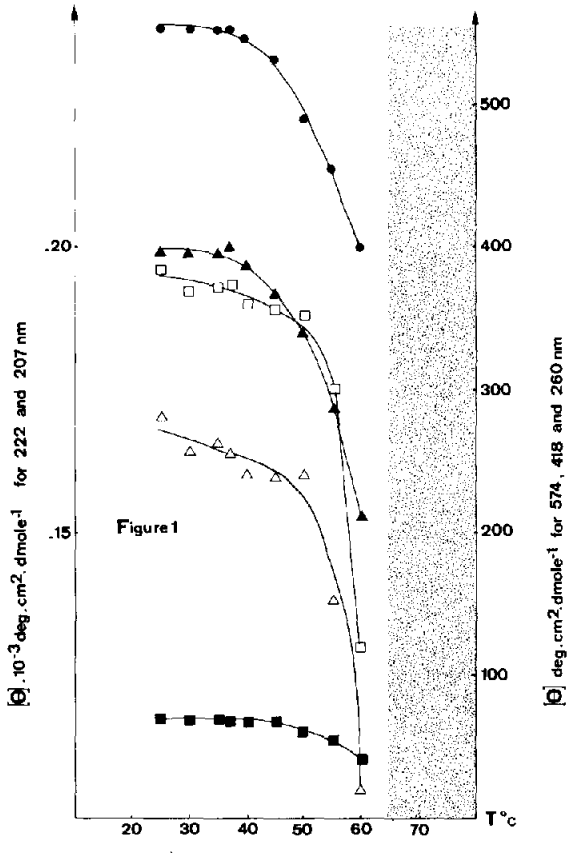

Fig.1. Ellipticities [θ] in degree.cm^2.dmol^{-1} versus temperature for HbA at 574 nm (■), 418 nm (●), 260 nm (□), 222 nm (▲) and 207 nm (△). Above 65°C a precipitation occurs (▒).

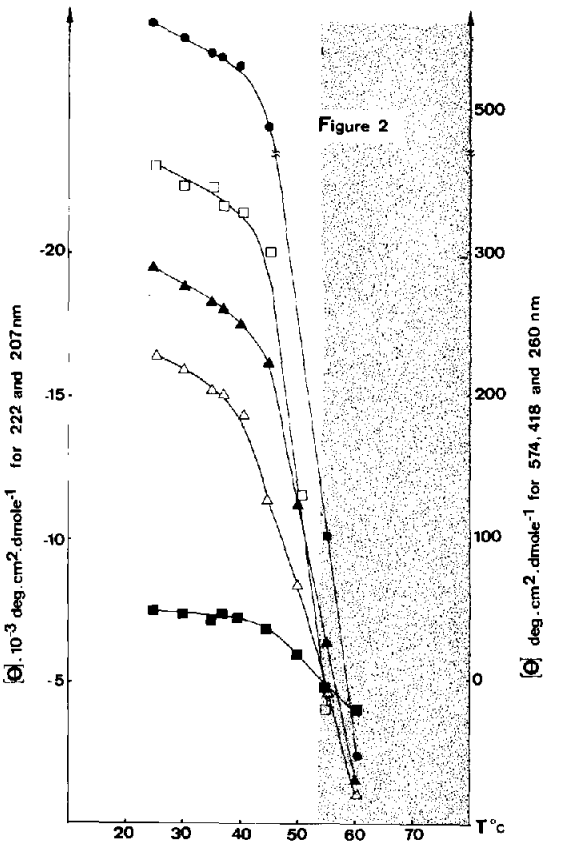

Fig.2. Ellipticities [θ] in degree.cm^2.dmol^{-1} versus temperature for Hb Sétif at 574 nm (■), 418 nm (●), 260 nm (□), 222 nm (▲) and 207 nm (△). Above 55°C a precipitation occurs (▒).

lengths considered, 58°C for HbA and 50°C for Hb Sétif. Precipitation occurs at 55°C for Hb Sétif and at 65°C for HbA.

The ultraviolet and visible spectra have the same shape and the same intensities for HbA and Hb Sétif (data not shown). As expected the maxima at 574 nm, 534 nm and 411 nm are due to α band β band and Soret γ band.

The differential absorption spectra were recorded for HbA and Hb Sétif at these three wavelengths at different temperatures below 50°C, to avoid a precipitation. For HbA (fig.3) the thermal denaturation is a linear function of temperature between 25°C and 50°C. For Hb Sétif (fig.4), the bands centered at 574 nm and 534 nm decreased linearly with the temperature but for the other band a change in the angle of the curve occurs at 37°C. The differential absorption study showed a new band which appears at 432 nm taking the place of the band at 411 nm. This band (γ Soret) is due to the lack of oxygen in Hb Sétif. As we worked in the same conditions for the two haemoglobins, this lack of oxygen seems to be directly related to the conformational change imposed by the presence of the Asp → Tyr substitution. The predicted conformational change random coil → β-sheet would explain the altered oxygen affinity and the instability of Hb Sétif as well as the sickling phenomenon observed in vitro. Indeed there

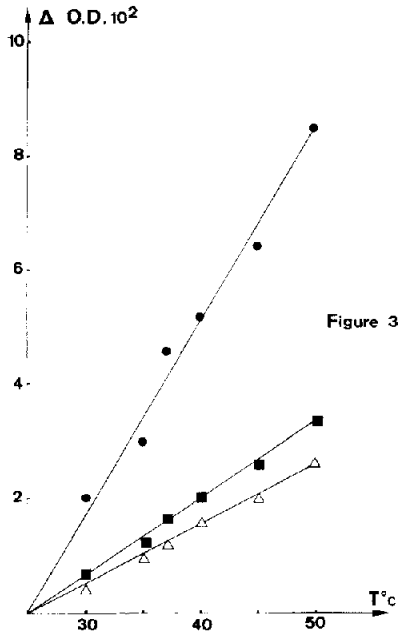

Fig.3. Differential absorption ($\triangle A$) versus temperature for HbA at 574 nm (■), 534 nm (△) and 411 nm (●).

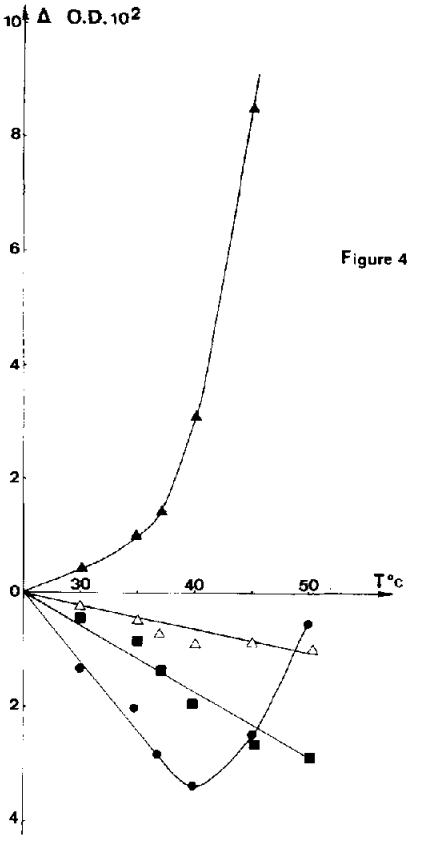

Fig.4. Differential absorption ($\triangle A$) versus temperature for Hb Sétif at 574 nm (■), 534 nm (△), 432 nm (▲) and 411 nm (●).

may be a change from the intramolecular $\alpha_1\beta_2$ contact to an intermolecular $\alpha_1\alpha_1$ contact in Hb Sétif.

Acknowledgements

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