FUNCTIONAL STUDIES OF Hb MALMÖ β97 (FG4) HIS → GLN

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1. Introduction

Hb Malmö β97 (FG4) His → Gln, a high oxygen affinity variant, was found by Lorkin et al. [1] in a polycythemic Swedish family and by Fairbanks et al. [2] in a large family of the midwestern United States. Electrophoretic and physiological properties of various propositi were described [3–5]. On the basis of the functional properties of the total hemolysate published by Boyer et al. [6], Zak et al. [7] have proposed an hypothesis for the stereochemistry of this hemoglobin.

A new case of Hb Malmö was found in a propositus native of France. This paper describes the functional properties of this abnormal hemoglobin isolated by preparative isoelectrofocusing. It shows an increased oxygen affinity, a decreased cooperativity, a decreased Bohr effect and a normal 2,3-DPG effect. These results are discussed with regard to the stereochemical hypothesis and Hb Malmö properties are compared with those of Hb Wood β97 (FG4) His → Leu.

2. Materials and methods

Routine hematological data were obtained using standard techniques.

Analytical isoelectrofocusing was performed according to Monte et al. [8] and preparative isoelectrofocusing by the procedure of Suzuki et al. [9].

The structural studies were performed according to the techniques used in the laboratory.

2.1. *Functional and physicochemical studies*

The oxygen affinity was measured by the discontinuous spectrophotometric technique of Benesch et al. [10] at 37°C. The red blood cells were suspended in isotonic phosphate buffer, pH 7.15. The purified hemoglobins were freed of 2,3-diphosphoglycerate (2,3-DPG) by passing through a Dintzis column [11] and studied in Tris or bis–Tris buffer 0.05 M.

The rate of spontaneous oxidation at atmospheric pressure was studied according to Jensen et al. [12].

Difference spectra of ferri HbA and ferri Hb Malmö as well as those of deoxy HbA and deoxy Hb Malmö were determined in the absence and in the presence of IHP as described by Perutz et al. [13,14] using a Cary 118C spectrophotometer.

3. Results

The abnormal hemoglobin was found during a screening of polycythemic patients and was detected by the abnormal oxygen affinity of its red blood cells. Electrophoretic and structural data, including manual Edman degradation, demonstrated that the abnormal hemoglobin was a new case of Hb Malmö : β97 His → Gln.

3.1. *Functional studies*

For functional studies, Hb Malmö was isolated by preparative isoelectrofocusing. Oxygen affinity of Hb Malmö is increased approx. 5-fold: P_{50} = 2.6 mm Hg compared to HbA for which P_{50} = 14 mm Hg when studied in the same conditions (pH 7.15, 37°C)

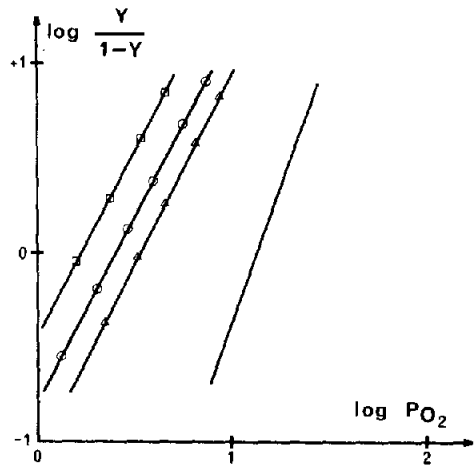

Fig.1. Hill plot of the oxygen dissociation curves of phosphate free hemoglobins A (——) and Malmö pH 6.45 (–△—△–) pH 7.15 (o—o—) and 7.45 (–□—□–).

(fig.1). The Hill coefficient of the pure fraction was reduced to 2.0 at all pH values studied from 6.45–7.45. The Bohr effect ($\Delta \log P_{50}/\Delta pH$) is decreased by approx. 30%. The right shift of the oxygen dissociation curve occurring in the presence of 2,3-DPG was normal.

The autoxidation rate of Hb Malmö was slightly decreased as compared to that of HbA treated in the same conditions.

The effect of IHP on the ferri-derivatives of HbA and Hb Malmö were studied by differential spectra. In the case of HbA, three prominent peaks at 512 nm, 600 nm and 649 nm were present. According to Perutz et al. [14] this result suggests a transition between the two quaternary forms R and T. In contrast, such peaks were not observed in the difference spectrum of Hb Malmö.

When the same kind of differential spectrum was performed on the deoxy forms, deoxy HbA did not show a difference spectrum with and without IHP since it is in both cases in the T form. Sometimes mutants remain in the R state even when fully deoxygenated and switch to the T state after addition of IHP. This is expressed by a difference spectrum. With deoxy Hb Malmö, the result was identical to that of HbA: there was no difference spectrum.

4. Discussion

The stereochemical hypothesis formulated by Zak et al. [7] was the following: they expected that

> 'the substitution of a glutaminyl residue for histidine in the β97 position permits bonding to take place between the -SH proton of β93 cysteine and the amide oxygen of β97 glutamine'.

This hypothesis fits well with our results concerning the high oxygen affinity and the decreased cooperativity of this hemoglobin but not for the Bohr effect.

They expected that the Bohr effect would be practically normal, but it was found 30% decreased. Nevertheless, it is notable that the β97 residue is close to the Asp β94 and the Val β98 residues which are implicated in the Bohr effect [15]. It is possible that the new glutamine residue prevents in the deoxy form the formation of the bridges between ASP β94 and His β146, Tyr β145 and Val β98, respectively.

The results of the difference spectra led us to conclude that Hb Malmö in its liganded form, cannot shift from the R to the T form in the presence of IHP. This is in accordance with the presence of a new bond between Gln β97 and Cys β93 which stabilizes the R form. In contrast, the deoxy form of Hb Malmö is in the T conformation which suggests that the bond is broken. These results could be compared to those obtained by Taketa et al. [16] with Hb Wood β97 His → Leu. Their experiments were performed on NO-Hb Wood and show that this liganded form cannot switch from R to T in the presence of IHP. Deoxy Hb Wood behaved like deoxy HbA in their EPR experiments.

Although, the β97 residue was in the $\alpha_1\beta_2$ contact, there was no indication that it plays any specific role in the allosteric mechanism. Nevertheless, one could note that the replacement of His β97 either by glutamine in Hb Malmö or by leucine in Hb Wood perturbs the R → T transition; but in the absence of X-ray crystallographic analysis of Hb Malmö or Hb Wood, it is difficult to deduce this specific role.

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