OXYGEN DISSOCIATION CURVE OF HAEMOGLOBIN PORTLAND

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1. Introduction

Haemoglobin (Hb) Portland [1,2] consists of two pairs of unlike chains and can be described as $\zeta_2\gamma_2$ [3,4]. An analysis of the tryptic peptides of the ζ-chain suggested a resemblance to the α-chain [5]. If the ζ-chain is indeed an α-like chain, then Hb Portland, unlike Hb H (β_4) or Hb Bart's (γ_4) should show a co-operative oxygen binding function.

2. Materials and methods

Haemolysate was prepared from the blood of an infant with Hb Bart's hydrops foetalis, the homozygous state for a-thalassaemia, type 1. In this condition the red cells contain Hb Bart's (γ_4) as the major fraction with Hb Portland ($\zeta_2\gamma_2$) as a minor fraction [3,4] and some traces of Hb H (β_4). Carboxy (CO) Hb Portland and CO Hb Bart's were separated by paper electrophoresis at pH 8.9 [6]. A normal haemolysate was treated similarly to obtain Hb A. The Hb bands were eluted and the eluates concentrated in a Sartorious collodion bag by ultrafiltration. CO was removed [7] from each sample and it was found that Hb A contained 2% met Hb, and the Hb Bart's sample 35%. All samples were treated with the reductase system [8]. This reduced more than 99% of a met Hb A sample freshly prepared with potassium ferricyanide. The Hb Bart's sample still contained more than 10% 'met Hb' as measured by the ratio of A_{576} to A_{508}. Hb Bart's is unstable and the failure to obtain a fully reduced Hb is presumed to be due to hemichrome formation [9].

The Hb Portland sample was dansylated by Mr H. Kamuzora [10] and failed to yield a derivative of valine indicating within the limits of the method the absence of normal α-chains. The met Hb content was not measured accurately because the solution was too dilute. However, an identically treated control sample showed three days later less than 10% met Hb.

The oxygen equilibrium curves of fresh normal haemolysate, Hb A after electrophoresis, and of the Hb Portland sample, were measured using an automatic recording technique [11]. As only a small amount of Hb Portland was available, measurements were carried out with 0.0049% Hb A and 0.0044% Hb Portland in 0.1 M potassium phosphate buffer at 432 μm, 25°C. Except for Hb Portland at pH 6.4 all equilibrium curves reproduced well, the same samples being measured three times: on deoxygenation, reoxygenation and again deoxygenation. This showed clearly that a substantial amount of met Hb could not have been formed during the measurement of Hb A and Hb Portland.

3. Results

The Hill plots of Hb Portland and Hb A before and after electrophoresis are shown in fig. 1. Paper electrophoresis of CO Hb did not alter the oxygen

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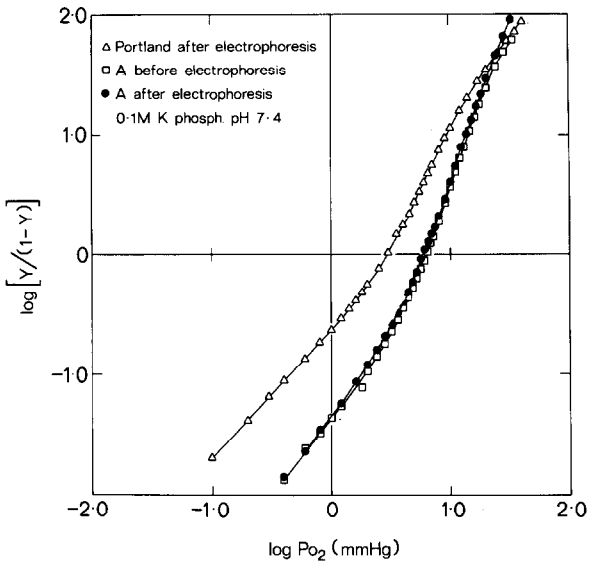

Fig. 1. Hill plots of oxygen dissociation curves for Hb Portland and Hb A. Y = fractional saturation.

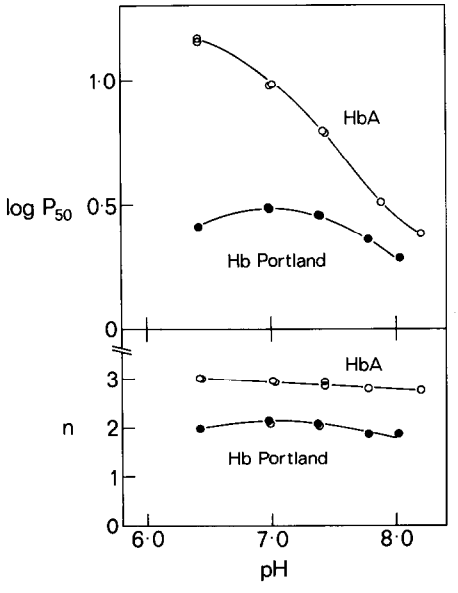

Fig. 2. The n value and the Bohr effect of Hb Portland and Hb A.

binding properties of Hb A. The Hill plot of Hb Portland indicates co-operative oxygen binding.

The partial oxygen pressure at 50% saturation (P_{50}) and Hill's coefficient (n) of Hbs A and Hb Portland are shown in fig. 2. Compared with Hb A, the oxygen affinity of Hb Portland is higher, the n value smaller, and the alkaline Bohr effect measured as $\Delta \log P_{50}/\Delta$ pH is nearly halved.

4. Discussion

The present communication presents evidence for co-operative function of Hb Portland, and this might explain, as indicated earlier [5], why infants with severe α-thalassaemia who are unable to produce α-chains, nevertheless can survive at all, and sometimes even for a few hours after birth.

In the α-chain, residue 122 His is considered to be responsible for part of the alkaline Bohr effect [12]. Alignment of the tryptic peptides [5] suggests that the ζ-chain has no His at position 122, and this might explain the diminished alkaline Bohr effect in Hb Portland, and possibly even the difference in oxygen affinity.

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