THE OXYGEN DISSOCIATION CURVE OF VIPER (*VIPERA ASPIS*) HEMOGLOBIN: FUNCTIONAL SIMILARITY WITH HUMAN HEMOGLOBIN PORTLAND

Michel DUGUET and Roger ACHER
Laboratory of Biological Chemistry, 96 Bd Raspail, 75006, Paris, France

Received 13 October 1975

1. Introduction

Viper (*Vipera aspis*) hemoglobin is apparently composed of two types of α-chains and one type of β-chain [1]. The complete amino acid sequence of one α-chain has been determined [2]. When compared with all the vertebrate hemoglobin α-chains known to date, the substitution of leucine for His-122 (H5) appears unique. Because this His-122 is supposed to play a role in the Bohr effect [3], it was of interest to examine the oxygen dissociation curve of viper hemoglobin, and particularly the pH-dependence of the affinity for oxygen.

2. Materials and methods

Hemolysate was prepared from the blood as previously described [4,5]. Low mol. wt compounds and ions were removed by gel filtration on Sephadex G-25 according to Benesch et al. [6] by using a 0.05 M Tris buffer at the pH chosen for the measurements of oxygen equilibria. 'Stripped' hemoglobin was then diluted with the same buffer in order to obtain a concentration of 1.5–2.0 mg ml^{-1}. Fresh normal human hemoglobin was prepared in the same way for comparison. The solutions contained virtually no methemoglobin as judged by the ratio A_{576} to A_{522}. The oxygen dissociation curve of hemoglobin was determined by a spectrophotometric procedure [7] using an automatic recording technique.

3. Results

The oxygenation curve as a function of the partial pressure of oxygen was determined at pH 7.15 and at 37°C. The oxygen affinity of the viper hemoglobin in terms of P_{50}, the partial pressure of oxygen at half saturation, is high when compared with that of normal human hemoglobin under the same conditions (8 mm Hg instead of 21 mm).

The Hill plots of viper hemoglobin is shown on fig.1 (0.05 M Tris–HCl buffer pH 7.15 at 37°C). A co-operative oxygen binding is apparent. From this curve a Hill coefficient (n) of 2.6 can be calculated. This value is rather similar to that of normal human hemoglobin. However in contrast to human hemoglobin, the n value in the case of viper hemoglobin is pH-dependent: at pH 7.45, n = 2.3, at pH 8.00, n = 2.2 and at pH 9.00, n = 2.2. This may indicate a change in the conformation at pHs higher than 7.15.

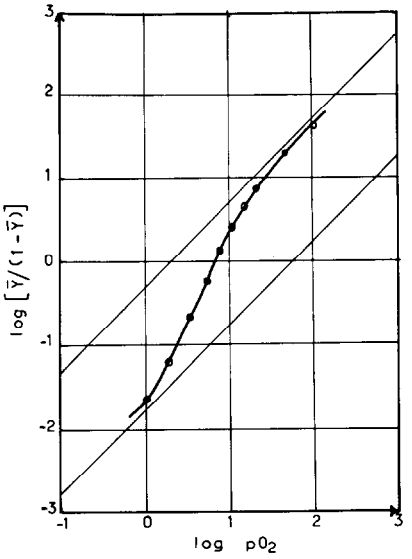

Fig.1. Hill plots of oxygen dissociation curve for viper hemoglobin (0.05 M Tris–HCl buffer pH 7.15, 37°C). Ȳ: fractional saturation. p_{O_2}: partial pressure of oxygen (mm Hg).

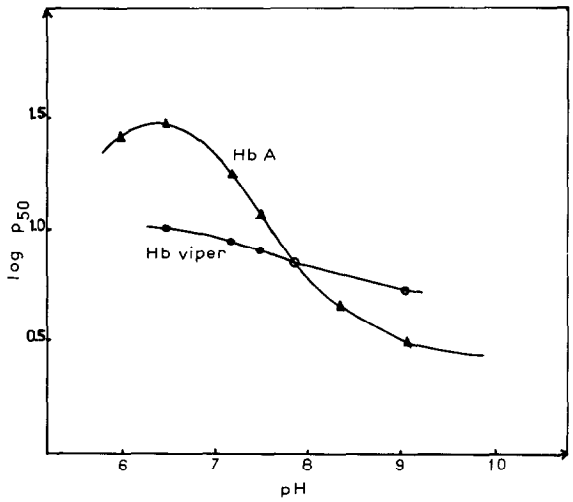

Fig.2. The pH dependence of the oxygen affinity (Bohr effect) of viper hemoglobin (●——●) and human hemoglobin (▲——▲); P_{50}: partial pressure of oxygen at half saturation.

The alkaline Bohr effect (influence of the pH on P_{50}) has been measured both for viper and normal human hemoglobins (fig.2). The intensity of the Bohr effect (Δ log P_{50}/Δ pH between pH 7.0–8.0) is clearly lower for the viper hemoglobin: 0.1 instead of 0.5 for human hemoglobin.

4. Discussion

Viper hemoglobin displays unusual functional properties and can be compared in some respects with abnormal human hemoglobin Portland 1 (ζ_2 γ_2) [8,9]. It has recently been shown that the ζ-chain of hemoglobin Portland has an α-chain structure [9]. In both hemoglobins, the residues of the α-chain involved in the 'vital' contacts between α_1 and β_2 chains [10] are preserved. The n value is about 2.0 for Hb Portland [8] and 2.6 for viper hemoglobin. However both hemoglobins have an α-type chain in which histidine is missing in position 122 (H_5); it is replaced either by leucine in viper [2] or by Asx [9] in Hb Portland. It is of interest to note that the alkaline Bohr effect is considerably reduced for both hemoglobins (this work and [8]) perhaps in relation with the absence of His-122. If Portland ζ-chain is very similar or identical to the early embryonic α-chain [11,12], the appearance of histidine in position 122 might be regarded as an evolutionary improvement in the function of the α-chain.

Acknowledgements

The authors are indebted to Professor Rosa for advice and laboratory facilities. This investigation was supported in part by grants from the Centre National de la Recherche Scientifique (E.R.A. 563) and the Délégation Générale à la Recherche Scientifique et Technique (n° 72-7-0802).

References

[1] Duguet, M., Chauvet, J. P. and Acher, R. (1971) FEBS Lett. 18, 185–188.
[2] Duguet, M., Chauvet, J. P. and Acher, R. (1974) FEBS Lett. 47, 333–337.
[3] Perutz, M. F. (1970) Nature 228, 726–739.
[4] Chauvet, J. P. and Acher, R. (1967) C. R. Acad. Sc. Paris 265, 2084–2087.
[5] Chauvet, J. P. and Acher, R. (1971) Int. J. Protein Research 3, 261–269.
[6] Benesch, R., Benesch, R. E. and Chi Ing Yu (1968) Proc. Nat. Acad. Sci. US 59, 526–532.
[7] Rossi-Fanelli, A. and Antonini, E. (1958) Arch. Biochem. Biophys. 77, 478–492.
[8] Tuchinda, S., Nagai, K. and Lehmann, H. (1975) FEBS Lett. 49, 390–391.
[9] Kamuzora, H. and Lehmann, H. (1975) Nature 256, 511–513.
[10] Perutz, M. F., Muirhead, H., Cox, J. M. and Goaman, L. C. G. (1968) Nature 219, 131–139.
[11] Melderis, H., Steinheider, G. and Ostertag, W. (1974) Nature 250, 774–776.
[12] Kamuzora, H., Jones, R. T. and Lehmann, H. (1974) FEBS Lett. 46, 195–199.