FUNCTIONAL PROPERTIES OF HEMOGLOBIN RAINIER*

G. AMICONI, K.H. WINTERHALTER**, E. ANTONINI
and Maurizio BRUNORI
C.N.R. Center for Molecular Biology, Institute of Biochemistry, University of Rome,

and

Libera Facoltà di Medicina e Chirurgia, Sora (Frosinone), Italy

Received 19 January 1972

1. Introduction

The molecular mechanisms forming the basis of the functional properties of hemoglobin are currently a subject of active investigation [1]. One possibility of gaining insight into the events occurring normally on binding of ligands is the careful analysis of the functional behaviour of hemoglobins with an abnormal primary structure. This approach is particularly fruitful when it is possible to correlate the findings on the functional properties of the molecule with the structural information derived from the analysis of the tridimensional structure [2].

Hemoglobin Rainier is a variant whose abnormality has been recently identified as the replacement of tyrosine HC2(145)β by cysteine [3]. Crystallographic studies have also shown that the new residue (cysteine) introduced as a result of the mutation forms a disulphide bridge with cysteine F9(93)β of the same β chain, and it has been suggested that the formation of this disulphide bridge interferes with the structural mechanism invoked for hemoglobin function [4].

In view of the large body of knowledge now available on hemoglobin Rainier it seemed important to reinvestigate in detail the equilibrium and kinetics of ligand binding by this abnormal protein. Previous studies [5] have been performed with whole blood; the few data on purified hemoglobin Rainier, quoted in reference [4], have not been described in extenso.

Particularly, for an unequivocal interpretation of the effects of the structural abnormality in hemoglobin Rainier, it would appear critical to know i) the exact magnitude of the Bohr effect, ii) the existence (if any) of heme–heme interaction, iii) the possible interference of intramolecular heterogeneity in determining the functional properties. This forms the object of the present paper.

2. Experimental

The hemolysate containing the abnormal protein was adsorbed onto a CM-Sephadex column (20 X 200 mm) equilibrated with a 0.01 M phosphate buffer pH 6.0. The column was eluted with a linear gradient in ionic strength going from the equilibrating buffer to the same buffer containing 0.5 M NaCl. Due to the absence of clearly different electrophoretic properties of Hb Rainier versus the normal hemoglobin, purity had to be evaluated by amino acid analysis.

The oxygen dissociation curves were determined by the method of Rossi et al. [6]. Stopped flow measurements were carried out with a Gibson–Durrum apparatus equipped with a 2 cm observation tube.

* A preliminary account of the results reported here has already been given in [1].

** Friedrich Miescher-Institut, Basel, Switzerland P.O. Box 273.

3. Results and discussion

The oxygen equilibrium curves of hemoglobin Rainier represented in fig. 1 show a value of n of exactly 1 at all pH values indicating complete loss of heme–heme interactions.

The oxygen affinity is more than 10 times higher than that of normal human hemoglobin at neutral pH.

The Bohr effect is still present although, in the alkaline range, is about $\frac{1}{2}-\frac{1}{3}$ lower than that of normal hemoglobin (fig. 2).

The kinetic experiments show that: i) the rate for CO combination is much higher (about 10 times) than the overall rate for normal hemoglobin; ii) the rate of O_2 dissociation, on the other hand, is lower (by a factor of about 5) (table 1). Both the combination with CO and the dissociation of O_2 correspond to a simple bimolecular and monomolecular process, respectively, and show no sign of kinetic heterogeneity.

The results with hemoglobin Rainier are similar to those obtained with other abnormal hemoglobins insofar as they show loss of heme–heme interactions,

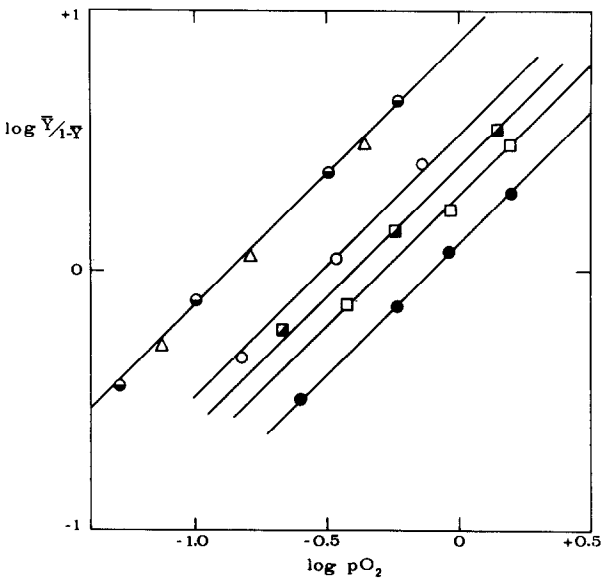

Fig. 1. Hill plot of the oxygen equilibrium at 20° and different pH values (□ = pH 5.4; ● = pH 6.5; ◪ = pH 7.5; ○ = pH 8.2; △ = pH 9.05; ○ = pH 9.7). Hemoglobin concentration ∿ 3 × 10^{-4} M in heme.

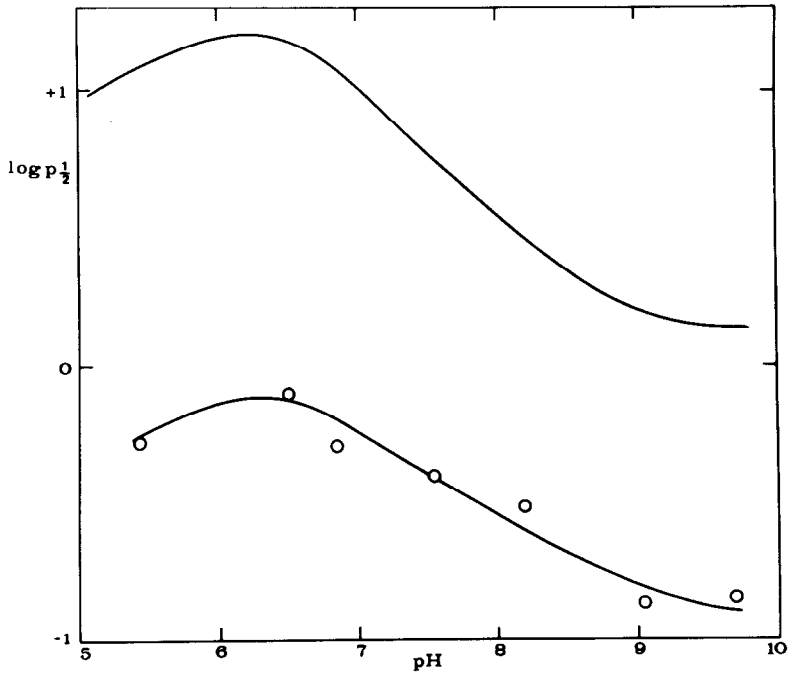

Fig. 2. Oxygen Bohr effect of hemoglobin Rainier (∿ 3 × 10^{-4} M in heme) at 20°, in 0.2 M phosphate and 0.05 M borate (over pH 9). Upper line indicates the Bohr effect of human hemoglobin A under the same conditions.

Table 1
Kinetic constants for the reaction of ligands with hemoglobin Rainier (at 20°).

k_{off}		l_{on}
pH 7	pH 9	pH 7
4.6 sec^{-1}	9.8 sec^{-1}	1×10^6 M^{-1} sec^{-1}

k_{off} = dissociation rate constant with oxygen.
l_{on} = combination rate constant for carbon monoxide.

high oxygen affinity and existence of a Bohr effect. Kinetically the changes in the ligand affinity are reflected both in a very large increase in the combination velocity and in a significant decrease in the dissociation velocity constant.

Of particular significance is the kinetic homogeneity which, together with the value of n equal 1, shows that all the hemes, even associated with normal chains, have similarly altered reactivity. The situation is similar to that observed for other abnormal or chemically modified hemoglobins [1]. Perhaps the most striking and specific feature of hemoglobin Rainier is the presence of a substantial Bohr effect and in this connection it represents another case of dissociation between heme–heme interaction and Bohr effect.

The results of the present work emphasize once again the important role of the C-terminal region of the β chains and particularly of tyrosine HC2(145)β in the ligand binding function of hemoglobin [4, 7]. In fact the general functional properties of hemoglobin Rainier are similar to those of normal hemoglobin digested by carboxy peptidase A [8].

References

[1] E. Antonini and M. Brunori, Hemoglobin and Myoglobin in Their Reactions with Ligands (North-Holland, Amsterdam, 1971).
[2] M.F. Perutz and M. Lehmann, Nature 219 (1968) 902.
[3] A. Hayaishi, G. Stamatoyannopoulos, A. Yoshida and J. Adamson, Nature New Biology 230 (1971) 264.
[4] J. Greer and M.F. Perutz, Nature New Biology 230 (1971) 261.
[5] J. Adamson, J.T. Parer and G. Stamatoyannopoulos, J. Clin. Invest. 48 (1969) 1376.
[6] A. Rossi Fanelli and E. Antonini, Arch. Biochem. Biophys. 77 (1958) 478.
[7] J.K. Moffat, S.R. Simon and W.M. Konigsberg, J. Mol. Biol. 58 (1971) 89.
[8] E. Antonini, J. Wyman, R. Zito, A. Rossi Fanelli and A. Caputo, J. Biol. Chem. 236 (1961) 60.