



Bohr effect of avian hemoglobins: Quantitative analyses based on the Wyman equation



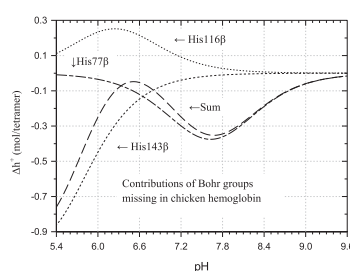
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HIGHLIGHTS

- The Wyman equation does not fit most mammalian hemoglobin Bohr effect data.
- Dog hemoglobin, like avian hemoglobins, does not have His77 β .
- The Wyman equation fits dog hemoglobin Bohr effect data.
- The Wyman equation fits avian hemoglobin Bohr effect data.
- pK_as of NH₃⁺ terminal group of Val1 α : 6.48 in the R and 7.76 in T quaternary structure.

GRAPHICAL ABSTRACT



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ABSTRACT

The Bohr effect data for bar-headed goose, greylag goose and pheasant hemoglobins can be fitted with the Wyman equation for the Bohr effect, but under one proviso: that the pK_a of His146 β does not change following the T \rightarrow R quaternary transition. This assumption is based on the x-ray structure of bar-headed goose hemoglobin, which shows that the salt-bridge formed between His146 β and Asp94 β in human deoxyhemoglobin is not formed in goose deoxyhemoglobin. When the Bohr data for chicken hemoglobin were fitted by making the same assumption, the pK_a of the NH₃⁺ terminal group of Val1 α decreased from 7.76 to 6.48 following the T \rightarrow R transition. When the data were fitted without making any assumption, the pK_a of the NH₃⁺ terminal group increased from 7.57 to 7.77 following the T \rightarrow R transition. We demonstrate that avian hemoglobin Bohr data are readily fitted with the Wyman equation because *avian hemoglobins* lack His77 β . From curve-fitting to Bohr data we estimate the pK_as of the NH₃⁺ terminal group of Val1 α in the R and T states to be 6.33 ± 0.1 and 7.22 ± 0.1 , respectively. We provide evidence indicating that these pK_as are more accurate than estimates from kinetic studies.

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1. Introduction

The Bohr effect is an important physiological phenomenon that is closely linked to the uptake of oxygen by hemoglobin from the lungs and its release to the metabolizing tissues in the body. Depending on the pH of the medium, it is associated with hemoglobin either ejecting protons into solution or absorbing protons from solution upon oxygen binding. The ejection of protons is

regarded as a positive contribution while the absorption of protons is considered a negative contribution to the Bohr effect. Considerable experimental and theoretical effort has been dedicated to attempts to gain a full understanding of this phenomenon (Antonini et al., 1965; Bailey et al., 1970; Beetlestone et al., 1976; Brygier et al., 1975; Fang et al., 1999; Kilmartin and Rossi-Bernadi, 1969; Kilmartin and Wootton, 1970; Lukin and Ho, 2004; Okonjo et al., 2014; Okonjo, 2015; Sun et al., 1997; Taylor et al., 1966; Wyman, 1948, 1964). A complete understanding at the molecular level requires accurate measurements of the Bohr effect, such as those employed in the pH-stat method (Bailey et al., 1970;

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Beetlestone et al., 1976; Brygier et al., 1975; Matsukawa et al., 1984; Rollema and Bauer, 1979). It also requires knowledge of the amino acid residues responsible for the Bohr effect, as well as their pK_a s of ionization in the T and R quaternary states. Such pK_a s have been accurately determined with the 1H NMR technique for thirteen histidine Bohr groups in human hemoglobin (Fang et al., 1999; Lukin and Ho, 2004; Sun et al., 1997). The only non-histidine Bohr group in human hemoglobin is the terminal NH_3^+ group of the α chain. There is as yet no method for determining the pK_a of this group that can match the accuracy of the 1H NMR technique employed for histidines. However, the pK_a of this group has been determined for human hemoglobin from kinetic studies (Garner et al., 1975; van Beek and de Bruin, 1980).

It is reasonable to assume that once all the groups responsible for the Bohr effect of a given hemoglobin are known, and their pK_a s in the R and T quaternary structures are accurately determined, it should be possible to fit accurately determined Bohr data with the Wyman equation for the Bohr effect (Wyman, 1948, 1964). We recently proved this assumption to be correct for an avian hemoglobin ... that of pigeon ... but not for four mammalian hemoglobins (Okonjo, 2015). Since the amino acid sequences of avian hemoglobins are closely similar (Kleinschmidt and Sgouros, 1987), especially with respect to the amino acid residues appearing at the Bohr group positions, it seems interesting to check whether the Bohr data for other avian hemoglobins can be fitted with the Wyman equation. Fortunately, the Bohr effect has been accurately determined for three additional avian hemoglobins: those of bar-headed goose, greylag goose and chicken (Brygier et al., 1975; Rollema and Bauer, 1979). More recently, a detailed oxygen binding study of two pheasant hemoglobins has been carried out as a function of pH (Grispo et al., 2012). From the results of this study we have extracted Bohr effect data. Our consideration of the Bohr data obtained for these hemoglobins led to the conclusion that, of the five, the Bohr data for bar-headed goose, greylag goose and the two pheasant hemoglobins (HbA and HbD) can be fitted with the Wyman equation, provided His146 β is assumed not to contribute to their Bohr effect. This assumption is based on the x-ray structure of bar-headed goose deoxyhemoglobin. Although the Bohr data for chicken hemoglobin can also be fitted with the Wyman equation, the fit requires that the pK_a of the NH_3^+ terminal group of the α -chain be higher in the R compared to the T quaternary structure, contrary to expectation.

2. Methods

In this paper we shall concern ourselves with data for stripped hemoglobins only. In reporting the Bohr effect of pigeon and other hemoglobins, Bailey et al. (1970) provided numerical data, as well as diagrams, for Δh^+ , the number of moles of H^+ ions released per mol of oxygen bound to deoxyhemoglobin. Unfortunately, the corresponding reports on chicken and goose hemoglobins (Brygier et al., 1975; Rollema and Bauer, 1979) contain only diagrams. Since we needed numerical data for our computational purposes, we proceeded as follows to extract numbers from the diagrams (Figs. 5 and 6 of Brygier et al. (1975); Fig. 4 of Rollema and Bauer (1979)). Using the 'Snipping tool' of Windows 10, we extracted each diagram and saved it as a JPEG document under 'Pictures'. After clicking on the extracted diagram, we expanded it to twice its original size. This two-fold expanded diagram was printed out and used as the source for extracting the Bohr effect data. This procedure faithfully reproduced the original graphical data, as we shall see later. All calculations were carried out with the Levenberg–Marquardt method, as previously reported (Okonjo, 2015), with programs written on a MicroMath Scientist software (Salt Lake City, Utah, USA). The starting points for all calculations were

the pK^R and pK^T values determined for the Bohr residues at 29 °C (Fang et al., 1999; Lukin and Ho, 2004; Sun et al., 1997), following corrections to their appropriate values at 25 °C (Okonjo, 2015), the temperature at which the Bohr data were collected (Bailey et al., 1970; Brygier et al., 1975; Rollema and Bauer, 1979).

We employed the same method as above to extract $\log_{10}P_{50}$ versus pH data for pheasant hemoglobins from Fig. 2 of Grispo et al. (2012). We converted these data to Δh^+ data as follows. The equation relating the oxygen partial pressure at half saturation, P_{50} , to pH is given by the linkage relation (Antonini et al., 1963; Craescu et al., 1986):

$$\log_{10}P_{50} = \text{constant} + \log_{10} \frac{(10^{-pH} + 10^{-pK_i^T})(10^{-pH} + 10^{-pK_j^T})}{(10^{-pH} + 10^{-pK_i^R})(10^{-pH} + 10^{-pK_j^R})} \dots \quad (1)$$

In Eq. (1) pK_i^T , pK_j^R ($i=1,2$) are the ionization constants of hypothetical oxygen-linked groups involved in the alkaline Bohr effect, and T and R stand for deoxy- and oxyhemoglobin, respectively. We fitted the extracted $\log_{10}P_{50}$ versus pH data with Eq. (1) to obtain values for pK_i^T and pK_j^R ($i=1,2$). We then generated a hypothetical Bohr effect by inserting these values into the Wyman equation for the Bohr effect (see Eq. (2) below).

3. Results

3.1. Validation of numerical data extracted from diagrams

We extracted numerical data from the Bohr effect diagrams for chicken and goose hemoglobins in Fig. 6 of Brygier et al. (1975) and Fig. 4 of Rollema and Bauer (1979). Before proceeding to analyze the results, it is important to demonstrate that these extracted numerical data are of sufficient accuracy to be used for our computations. In Table 1 we present the numerical data on the Bohr effect for human and chicken hemoglobin extracted from Figs. 5 and 6 of Brygier et al. (1975) and, for the goose hemoglobins, from Fig. 4 of Rollema and Bauer (1979). In reporting their Bohr data for human hemoglobin, Bailey et al. (1970) provided diagrams as well as numerical data for Δh^+ , the moles of H^+ ions produced per mol of oxygen bound by deoxyhemoglobin. To validate the numbers extracted from the Bohr effect diagrams of Brygier et al. (1975) and of Rollema and Bauer (1979), it is necessary to first compare the human hemoglobin data of Bailey et al. (1970) with those of Brygier et al. (1975). Bailey et al. (1970) presented their data as moles of H^+ ions released per mol of O_2 bound, whereas Brygier et al. (1975) presented theirs as moles of H^+ ions released per tetramer. Therefore, before comparing both sets of data we multiplied those of Bailey et al. (1970) by four. In Fig. 1 we present a comparison of the Bohr data for human hemoglobin obtained from the two sources (Bailey et al., 1970; Brygier et al., 1975). It is seen that the two sets of data are similar but are not identical. This is not surprising since Bailey et al. (1970) collected their data at an ionic strength of 50 mmol dm^{-3} , whereas Brygier et al. (1975) collected theirs at a KCl concentration of 100 mmol dm^{-3} . The small difference seen between the two sets of data (Fig. 1) is in the order expected for Bohr data collected at different ionic strengths. As shown in Fig. 5 of Rollema et al. (1975), the Bohr effect decreases with increasing ionic strength. This is exactly the trend seen in Fig. 1. We conclude that the results presented in Fig. 1 validate the numerical data extracted from the diagrams of Brygier et al. (1975) and of Rollema and Bauer (1979). Besides, the diagrams resulting from the extracted data are exact replicas of the original diagrams (see Figs. 2 and 3 below). As will be shown below (see Fig. 5), the $\log_{10}P_{50}$ versus pH diagram resulting from the extraction of data from Fig. 2 of Grispo et al. (2012) bears a close resemblance to the relevant part of that figure.

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