A New Model of Hemoglobin Oxygenation

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Abstract: The study of hemoglobin oxygenation, starting from the classical works of Hill, has laid the foundation for molecular biophysics. The cooperative nature of oxygen binding to hemoglobin has been variously described in different models. In the Adair model, which better fits the experimental data, the constants of oxygen binding at various stages differ. However, the physical meaning of the parameters in this model remains unclear. In this work, we applied Hill’s approach, extending its interpretation; we obtained a good agreement between the theory and the experiment. The equation in which the Hill coefficient is modulated by the Lorentz distribution for oxygen partial pressure approximates the experimental data better than not only the classical Hill equation, but also the Adair equation.

Keywords: cooperative binding of ligands; oxyhemoglobin dissociation curve; Hill equation; Hill coefficient; allosteric interactions

1. Introduction

Cooperativity in allosteric systems such as oligomeric proteins [1,2] provides the ability to rapidly regulate their functional activity. Recent work has shown that allosteric interactions expand the ability of biological systems to store information [3]. It has also been demonstrated that cooperative interactions lead to an entropy reduction in the system [4]. In particular, the cooperation between the subunits of ion channels during nerve impulse generation potentiates the ability of neurons and nerve networks to process information [5].

In this connection, the study of cooperative interactions is of general biological importance [6–8]. Of particular relevance is the study of the cooperativity of hemoglobin oxygenation [9]. This macromolecule is a convenient model for studying the mechanisms of the enzymatic reactions of oligomers because its active center is similar to the catalytic center of the enzyme [10]. The ability of hemoglobin to hold a ligand in the active center for a long time makes it possible to study its structural state; e.g., the short-term state of the enzyme–substrate complex [11,12]. This probably played a role in the choice of hemoglobin molecule by M. Perutz to study the spatial structure of complex proteins [13].

Over 100 years ago, Hill proposed an empirical equation to describe the hemoglobin oxygenation curve, which has found an application not only in describing the sigmoid shape of the oxygenation curve, but also in a number of other fields from enzymology [14,15], toxicology [16] and pharmacology [17] to problems of modeling gene transcription regulation [18,19] as well as in models describing potential-dependent ion channel transitions [20,21] among others.

At the same time, Hill’s approach may be more than just a descriptive theory characterizing the cooperative effect. The equation demonstrates interesting explanatory and mechanistic properties in direct connection with the law of mass action [22–24].
Previously, we proposed a mathematical model for hemoglobin oxygenation \([25,26]\) and ascertained that our model fits better to experimental oxyhemoglobin dissociation curves when compared with the classical Hill equation and is comparable with the Adair equation (Adair–Klotz) \([26]\). However, for oxygen partial pressure levels below 3 mm Hg, the proposed equation is inferior to the Adair equation in accuracy approximation.

Thus, considering the importance of evaluating the cooperative interactions in the macromolecule at the initial stage of its oxygenation, we further analyzed the previously proposed equation and searched for a more accurate approximating model.

2. Materials and Methods

The subjects of this study were the Hill model and a set of experimental data obtained by Winslow et al. \([27]\) and Severinghaus \([28]\). The optimization of the model parameters was performed by the generalized reduced gradient (GRG) method \([29]\), where the target function was the sum of least squares (LS) method \([30]\). The evaluation of the degree of model fit to the experimental data was performed through the coefficient of determination \([31]\). Confidence and prediction bands for the studied non-linear models were calculated at \(\alpha = 0.0001\) \([32]\). The necessary calculations were performed in MS Excel.

3. Results

3.1. The Hill Equation and Its Limitations

The Hill equation, in general terms, assumes the simultaneous binding of ligand molecules by all oligomer subunits:

\[
y = \frac{p^h}{p_{50}^h + p^h}
\]

where, in the case of oxygenation, \(y\) is the degree of saturation of hemoglobin by oxygen, \(p\) is the partial pressure of \(O_2\), \(p_{50}\) is the oxygen pressure at which half of the macromolecules are saturated by the ligand and \(h\) is the Hill coefficient.

Currently, this equation, which can formally be deduced from the law of mass action, is viewed primarily as a formal description of the interaction of ligands with the receptor sites of the protein and is defined by the corresponding microscopic constants of the equilibrium reactions. Their product is a macroscopic constant equal to \((p_{50})^{-h}\). However, it is not possible to correlate the values of these microscopic constants in the Hill equation. For the hemoglobin tetramer, this expression can be represented as follows:

\[
y = \frac{k_1 p \cdot k_2 p \cdot k_3 p \cdot k_4 p}{1 + k_1 p \cdot k_2 p \cdot k_3 p \cdot k_4 p} = \frac{k_1 \cdot k_2 \cdot k_3 \cdot k_4 p^4}{1 + k_1 \cdot k_2 \cdot k_3 \cdot k_4 p^4} = \frac{k^4 p^4}{1 + k^4 p^4} = \frac{K p^4}{1 + K p^4} = \frac{p^h}{p_{50}^h + p^h},
\]

where \(k_1, k_2, k_3\) and \(k_4\) are the equilibrium reaction constants for each of the subunits; \(k^4\) is the product of constants \(k_1\)–\(k_4\); and \(K\) is the macroscopic constant.

A similar situation arises when analyzing the Adair (Adair–Klotz) equation, which is a development of the Hill equation, considering the product of these microscopic constants for each of the oxygenation stages:

\[
y = \frac{k_1 p + 2 \cdot k_1 k_2 p^2 + 3 \cdot k_1 k_2 k_3 p^3 + 4 \cdot k_1 k_2 k_3 k_4 p^4}{4 \cdot (1 + k_1 p + k_1 k_2 p^2 + k_1 k_2 k_3 p^3 + k_1 k_2 k_3 k_4 p^4)} = \frac{K_1 p + 2 K_2 p^2 + 3 K_3 p^3 + 4 K_4 p^4}{4(1 + K_1 p + K_2 p^2 + K_3 p^3 + K_4 p^4)}.
\]

However, in the Adair–Klotz equation, the microscopic constants can be determined from the corresponding calculated values of the macroscopic constants. It should also be noted that the equilibrium reaction constants of the above equation are apparent and it is not clear from them what the value of \(p_{50}\) is \([33]\). As this equation consists of four components and is given by the same number of fitting coefficients \((K_1–K_4)\), it is expected to have a higher approximation capability relative to the Hill and Bernard equations \([25]\).
For the Hill equation describing hemoglobin oxygenation, there is a pronounced discrepancy between the theoretical value of the degree index $h$, corresponding with the number of subunits, and the calculated value $h$, which makes it difficult to understand the physical meaning of this value [26,34–36].

This equation is also characterized by a poor approximation of the experimental data at the calculated value of $h$ in the range of the partial oxygen pressures where the degree of saturation of hemoglobin with the ligand is outside the range of 20–80%, especially for low $p$-values. This circumstance indirectly points to the variability of the Hill coefficient during oxygen binding by hemoglobin [37].

3.2. Phenomenological Model of Oligomer Liganding

We proposed a hypothetical model of ligand binding by the subunits of a macromolecule, which was defined by the following conditions:

1. Each subunit of the oligomer could be either vacant for the ligand or liganded and also in different conformational states;
2. In these conformational states, the oligomer subunits experienced coordinated perturbation by neighboring subunits;
3. The current superposition of the conformational states of the subunit states could change the affinity of a subunit for an attachable or already attached ligand;
4. Ligand binding reconfigured the interaction between the subunits and created a new self-consistent level of cooperativity between them, which determined the nature and strength of the interaction of the attachable or already attached ligand according to a feedback mechanism.

Thus, based on this model (Figure 1), the Hill coefficient could be viewed as a function of the number of interacting subunits and the degree of cooperativity between them modulated by the degree of the vacancy filling with the corresponding ligand.

![Figure 1. Phenomenological model of oligomer liganding. I, initial (unliganded) state of oligomer; II/a–d, liganding steps (hemoglobin oxygenation); III, terminal state (complete liganding). Digits indicate: 1, ligand; 2, resultant interaction of oligomer with ligand; 3, oligomer; 4, interaction component of the liganded subunit; 5, interaction component of the conjugated subunit; 6, mediated interaction component through the conjugated subunit. Color of arrows and subunits denotes from inhibition (warm) to promotion (cold) of oxygenation.](image-url)
3.3. The Hill Coefficient as a Function of Oxygen Partial Pressure

Based on the proposed model (Figure 1), we suggested considering the Hill coefficient as a function of the partial pressure of oxygen conjugated with the given number of interacting subunits. The dependence of this coefficient upon the partial pressure of oxygen was demonstrated in classical works \[37,38\] by calculating $h$ using the known values of the degree of hemoglobin oxygen saturation, the partial pressure of $O_2$ and the value of $p_{50}$. However, a function allowing a fit with the empirical oxygenation curve has not yet been proposed \[38\].

Earlier \[26\], we attempted to develop a new mathematical model of hemoglobin oxygenation. This model has a better approximation of experimental data on hemoglobin oxygenation relative to Hill’s equation and is comparable with Adair’s equation; however, it describes hemoglobin oxygenation at its initial stages worse than the latter. It should also be noted that there are several publications in which macromolecule-binding constants are also considered as functions of macromolecule filling by ligands \[25,39–41\].

We presented the conclusion and justified the equations that were used to calculate the Hill coefficient as a function of the partial pressure of oxygen.

For the original Hill equation, the value of $h$ is independent of the partial pressure of oxygen $p_{O_2}$ and appears as:

$$ h = h_i = h_{\text{max}} = \frac{h_{\text{max}}}{p_i} = \text{const}, $$

where $h_{\text{max}}$ is the maximum possible value of the Hill coefficient in a given interval of $p_{O_2}$, $h_i$ is the Hill coefficient at a certain $p_{O_2}$ and $\bar{h}$ is its average value.

Assuming that $h_i$ cannot be greater than $h_{\text{max}}$, but depends on $p_i$, the dependence of $h_i$ on $p_i$ ($p_i > 1$) can be represented by the relation:

$$ h_i = \frac{h_{\text{max}}}{p_i}, $$

where $p_i$ is the partial pressure of oxygen at the $i$-th point of the measurement.

For $h_i$, we could find the value of $p_i$ at which $h_i = h_{\text{max}}$, with $p_i - p_{\text{max}} = 1$ (the value of the partial pressure of oxygen at which $h_{\text{max}}$ could be detected; the parameter $p_{\text{max}} \geq 0$ shifted the function plot along the horizontal axis):

$$ h_i = \frac{h_{\text{max}}}{p_i - p_{\text{max}}}. $$

The value of $h_i$ must be greater than 0 for any $p_i - p_{\text{max}}$. Equation (6) would then be represented as the ratio of $h_{\text{max}}$ to the square of the difference of $p_i$ and $p_{\text{max}}$:

$$ h_i = \frac{h_{\text{max}}}{(p_i - p_{\text{max}})^2}. $$

In the range of values where $(p_i - p_{\text{max}})^2$ belongs to an open interval from 0 to 1, the value $h_i > h_{\text{max}}$ and is undefined when the square of this difference is zero.

Representing $(p_i - p_{\text{max}})^2$ as the exponent on any basis greater than unity, Equation (7) could then be presented as follows, where $(p_i - p_{\text{max}})^2 = 0$ and $h_i = h_{\text{max}}$:

$$ h_i = \frac{h_{\text{max}}}{\exp(p_i - p_{\text{max}})^2}. $$

Using an arbitrary factor $a$ to $(p_i - p_{\text{max}})^2$ only scales the argument and does not eliminate the discontinuity of the presented function at the zero point for a given difference:

$$ h_i = \frac{h_{\text{max}}}{a(p_i - p_{\text{max}})^2}. $$
However, by representing the denominator as a sum, we could also obtain $h_i = h_{\text{max}}$ at $(p_i - p_{\text{max}})^2 = 0$ in the same way as Equation (8):

$$h_i = \frac{h_{\text{max}}}{1 + (p_i - p_{\text{max}})^2}. \quad (10)$$

In Equations (9) and (10), we introduced a scale parameter $s$ along the horizontal axis:

$$h_i = \frac{h_{\text{max}}}{\exp\left((p_i - p_{\text{max}})/s\right)^2}. \quad (11)$$

$$h_i = \frac{h_{\text{max}}}{1 + (\ln(p_i/p_{\text{max}})/s)^2}. \quad (12)$$

As the experimental dependence of the Hill coefficient on the logarithm of the partial oxygen pressure is bell-shaped, Equations (11) and (12) could be transformed:

$$h_i = \frac{h_{\text{max}}}{\exp\left(\ln(p_i/p_{\text{max}})/s\right)^2}. \quad (13)$$

$$h_i = \frac{h_{\text{max}}}{1 + [\ln(p_i/p_{\text{max}})/s]^2}. \quad (14)$$

As the Hill coefficient in the Hill equation is equal to one in the absence of cooperativity, Equations (13) and (14) could be reduced to the following form, thus shifting the statistical distribution along the vertical axis by this unity:

$$h_i^{(-1)} = \frac{h_{\text{max}}^{(-1)}}{\exp\left(\ln(p_i/p_{\text{max}})/s\right)^2} + 1, \quad (15)$$

$$h_i^{(-1)} = \frac{h_{\text{max}}^{(-1)}}{1 + [\ln(p_i/p_{\text{max}})/s]^2} + 1. \quad (16)$$

The substitution of the Hill equation:

$$y_i = \frac{p_i^{h_i}}{p_{50}^{h_i} + p_i^{h_i}}, \quad (17)$$

instead of $h_i$ could transform Equation (15) or (16) based on Gauss and Lorentz distributions, respectively, to two models describing hemoglobin oxygenation, where $y_i$ is the degree of ligand saturation at $p_i$.

Each of the models has four adjustable parameters: $p_{50}$; $h_{\text{max}}^{(-1)}$; $\ln p_{\text{max}}$; and $s$ ($s_G$ and $s_L$ are scale parameters for the Gaussian and Lorentz distributions, respectively).

### 3.4. Evaluation of the Effectiveness of Approximation of Experimental Data by Modified Hill Equations

According to the experimental data of Winslow et al. [27] and Severinghaus [28] (Figure 2), approximating oxyhemoglobin dissociation curves were obtained using the classical (Hill classic) and modified Hill equations with Gauss (Hill/G) and Lorentz (Hill/L) distributions and the Adair equation.

We determined the coefficient of determination ($r^2$) for the given equations and ranked them in descending order of $r^2$ (Tables 1 and 2).

As the tables show, the modified Hill equation with the Lorentz distribution (Hill/L) demonstrated the best approximation results for the two sets of experimental data.

As hemoglobin oxygenation in the range of partial gas pressure up to 3 mm Hg was of particular interest in our studies, we analyzed the ODC at low $pO_2$ values (Figures 3–6).
As the tables show, the modified Hill equation with the Lorentz distribution (Hill/L) demonstrated the best approximation results for the two sets of experimental data. According to the experimental data of Winslow et al. [27] and Severinghaus [28],

\[ r^2_{\text{Hill/L}} = 0.999951 \]

\[ r^2_{\text{Hill/G}} = 0.999944 \]

\[ r^2_{\text{Hill classic}} = 0.999603 \]

\[ r^2_{\text{Adair}} = 0.999953 \]

This discrepancy could indicate that, at low oxygen partial pressures, the degree of ligand saturation at the site of maximal oxygenation is less than that predicted within the classical model. We determined the coefficient of determination \( r^2 \) for the data sets of Winslow et al. [27] and Severinghaus [28].

As the Hill coefficient in the Hill equation is equal to one in the absence of ligand, the subunits in the macromolecule were calculated for these values. The Adair equation (Figure 4) has four adjustable parameters and relies on the assumption of sequential ligand binding, which is phenomenologically more reasonable. In this case, at a confidence probability of 0.9999, the experimental points of the curve did not go beyond the confidence interval. This discrepancy could indicate that, at low oxygen partial pressures, the degree of cooperativity of the subunits in the macromolecule calculated for these values was lower than that predicted within the classical model.

The substitution of the Hill equation:

\[ s_i = h_i \cdot \text{exp}(a \cdot p_{O2} - b) \]

\[ s_i = h_i \cdot \text{exp}(a \cdot p_{O2} - b) \]

into the empirical equation for the ODC (Equation (15)) or (16) instead of the classical Hill equation:

\[ s_i = h_i \cdot \text{exp}(a \cdot p_{O2} - b) \]

\[ s_i = h_i \cdot \text{exp}(a \cdot p_{O2} - b) \]

We concluded that the modified Hill equation with the Lorentz distribution (Hill/L) demonstrated the best approximation results for the two sets of experimental data.

Figure 2. Experimental data points of the hemoglobin dissociation curve: 1, according to Winslow et al. [27] and 2, according to Severinghaus [28].

Figure 3. Approximation of the experimental oxygenation curve, represented in logarithmic coordinates, by the classical Hill equation: (a) from the data set of Winslow et al. [27] and (b) from the data set of Severinghaus [28]. Legend: 1, experimental data points; 2, approximation; 3 and 4, upper and lower bounds of the confidence intervals, respectively; 5 and 6, upper and lower bounds of the prediction intervals, respectively. For all types of intervals, the confidence probability was 0.9999.

Table 1. Approximating functions for the ODC, the coefficient of determination \( r^2 \), which was calculated from the experimental data of Winslow et al. [27].

| Equation   | \( r^2 \)       |
|------------|-----------------|
| Hill/L     | 0.999951        |
| Adair      | 0.999953        |
| Hill/G     | 0.999944        |
| Hill classic | 0.999603      |
The Hill equation modulated by the Gauss distribution (Hill/G) that we obtained did not satisfactorily approximate the experimental data in the region of low oxygen partial values because of the shape of this distribution conditioned by the exponential dependence (Equation (8); Figure 5).

Thus, the proposed Hill/L equation preserved and extended the physical content of the application of the Lorentz distribution obtained the best results (Figure 6).

Table 2. Approximating functions for the ODC, the coefficient of determination $r^2$, which was calculated from the experimental data of Severinghaus [28].

| Equation       | $r^2$     |
|----------------|-----------|
| Hill/L         | 0.999951  |
| Hill/G         | 0.999943  |
| Adair          | 0.999907  |
| Hill classic   | 0.999446  |

Figure 4. Approximation of the experimental oxygenation curve, represented in logarithmic coordinates, by the Adair equation: (a) from the data set of Winslow et al. [27] and (b) from the data set of Severinghaus [28]. Legend: 1, experimental data points; 2, approximation; 3 and 4, upper and lower bounds of the confidence intervals, respectively; 5 and 6, upper and lower bounds of the prediction intervals, respectively. For all types of intervals, the confidence probability was 0.9999.

Figure 5. Approximation of the experimental oxygenation curve, represented in logarithmic coordinates, by the Hill equation with $h$ modulated by the Gauss distribution: (a) from the data set of Winslow et al. [27] and (b) from the data set of Severinghaus [28]. Legend: 1, experimental data points; 2, approximation; 3 and 4, upper and lower bounds of the confidence intervals, respectively; 5 and 6, upper and lower bounds of the prediction intervals, respectively. For all types of intervals, the confidence probability was 0.9999.
Calculating the parameters of this equation, in contrast to Adair’s equation, produced a stable result when optimizing the search for the best approximation parameters [43,44]. It should also be noted that the Hill/G equation had similar properties to the Hill/L equation. The classical Hill equation, as noted in [42], did not satisfactorily approximate the experimental data in the range of low $pO_2$ values (Figure 3).

This discrepancy could indicate that, at low oxygen partial pressures, the degree of cooperativity of the subunits in the macromolecule calculated for these values was lower than that predicted within the classical model.

The Adair equation (Figure 4) has four adjustable parameters and relies on the model of sequential ligand binding, which is phenomenologically more reasonable. In this case, at a confidence probability of 0.9999, the experimental points of the curve did not go beyond the confidence interval.

The Hill equation modulated by the Gauss distribution (Hill/G) that we obtained did not satisfactorily approximate the experimental data in the region of low oxygen partial pressures because of the change in this distribution conditioned by the exponential dependence (Equation (8); Figure 5).

The application of the Lorentz distribution obtained the best results (Figure 6).

Thus, the proposed Hill/L equation preserved and extended the physical content of the classical Hill equation. With the same number of fitting parameters as the Adair equation, the Hill/L equation had a higher approximation ability than the former. Calculating the parameters of this equation, in contrast to Adair’s equation, produced a stable result when optimizing the search for the best approximation parameters [43,44]. It should also be noted that the Hill/G equation had similar properties to the Hill/L equation, except for a weak approximation of the curve in the section where the partial pressure of oxygen did not exceed 3 mm Hg.

3.5. Fitting and Derived Parameters in the Modified Hill Equations

The fitting and derived parameters in the Hill/G and Hill/L equations enabled a more complete description of the oxyhemoglobin dissociation curve and an assessment (albeit formal) of the cooperativity of oxygen molecule binding. However, such generally accepted constants as $p_{50}$ ($K_h$), $K_m$, EC50 and IC50 [45] are convenient, but also formal, estimates of the half-maximum value of the corresponding function. In principle, they could be replaced in the corresponding equations [46–49] with any constants expressed as a percentage; for

![Figure 6](image-url). Approximation of the experimental oxygenation curve, represented in logarithmic coordinates, by the Hill equation with $h$ modulated by the Lorentz distribution: (a) from the data set of Winslow et al. [27] and (b) from the data set of Severinghaus [28]. Legend: 1, experimental data points; 2, approximation; 3 and 4, upper and lower bounds of the confidence intervals, respectively; 5 and 6, upper and lower bounds of the prediction intervals, respectively. For all types of intervals, the confidence probability was 0.9999.
example, \( p_{95} \) (the full saturation tension presented as a constant) or in fractions of \( 1/e \), \( 1/\pi \), etc. [45–49].

The fitting parameter \( p_{50} \) corresponds with a similar value for the classical Hill equation. The mean value of the Hill coefficient \( h \) was calculated for all uniformly distributed points of the model curve. From the fitting parameter \( h_{\text{max}}(-1) \), the maximum value of the Hill coefficient \( h_{\text{max}} \) was calculated using the following formula:

\[
h_{\text{max}} = h_{\text{max}}(-1) + 1.
\]  (18)

Using Equation (19), we could estimate the maximum possible relative cooperativity of the subunit interaction [50]:

\[
h_{\text{max}} = \theta(n)(n - 1) + 1,
\]  (19)

where \( \theta(n) \) is the relative cooperativity coefficient and \( n \) is the number of oligomer subunits.

The value of the Hill coefficient \( h_i \) for any \( p_i \) could be found through the variable \( h_i(-1) \) similar to \( h_{\text{max}} \) (18):

\[
h_i = h_i(-1) + 1.
\]  (20)

The value of \( \Delta h_i \), as the difference between \( h_{\text{max}} \) and \( h_i \), provides an idea of the dispersion of cooperativity as a function of the partial pressure of oxygen.

The value of the partial pressure of oxygen, \( p_{\text{max}} \), corresponds with the highest value of the Hill coefficient \( h_{\text{max}} \) and is determined by the fitting parameter \( \ln p_{\text{max}} \). The value of \( p_{\text{max}} \) determines the degree of hemoglobin oxygen saturation \( \text{HbO}_2 \), % (for \( p_{\text{max}} \)).

The difference between the degree of oxygen saturation at \( p_{\text{max}} \) and its 50% saturation is \( \Delta \text{HbO}_2 \), %, which can also be used when analyzing the oxygen-binding properties of hemoglobin. The difference between \( p_{\text{max}} \) and \( p_{50} \), \( \Delta p_{\text{max}} \), estimates the shift of the maximum cooperativity and requires a physical interpretation.

The fitting parameter \( s \) (\( s_g \) for Hill/G and \( s_l \) for Hill/L) makes it possible to obtain \( p_{O_2\text{low}} \) and \( p_{O_2\text{high}} \) using the following formulae:

\[
p_{O_2\text{low}} = \exp \left[ \ln p_{\text{max}} - s_g (-\ln \omega)^{1/2} \right],
\]  (21)

\[
p_{O_2\text{high}} = \exp \left[ \ln p_{\text{max}} + s_g (-\ln \omega)^{1/2} \right],
\]  (22)

\[
p_{O_2\text{low}} = \exp \left[ \ln p_{\text{max}} - s_l (\omega^{-1} - 1)^{1/2} \right],
\]  (23)

\[
p_{O_2\text{high}} = \exp \left[ \ln p_{\text{max}} + s_l (\omega^{-1} - 1)^{1/2} \right],
\]  (24)

where \( p_{O_2\text{low}} \) and \( p_{O_2\text{high}} \) are the lower and upper limits of oxygen partial pressure values, respectively, beyond which \( h_i(-1) < \omega h_{\text{max}}(-1) \) and \( \omega \) is the fraction expressed from 0 to 1 (for 0.5, more commonly known as half-width at half-maximum or HWHM).

The range of partial pressure \( \Delta p_{O_2} \) values within which \( h_i(-1) < \omega h_{\text{max}}(-1) \) could be determined by the difference:

\[
\Delta p_{O_2} = p_{O_2\text{high}} - p_{O_2\text{low}}.
\]  (25)

### 3.6. Calculation of Fitting and Derived Parameters in the Modified Hill Equations

The values of \( p_{95} \) and \( h \) were calculated for the classical Hill equation and were 28.82 mm Hg and 2.52, respectively, according to Winslow et al. [27] and 26.38 mm Hg and 2.65, respectively, according to Severinghaus [28].

The values of the fitting and derived parameters of the Hill/G and Hill/L equations characterizing the oxygenation process for the experimental curves according to Winslow et al. [27] and Severinghaus [28] are presented in Tables 3 and 4, respectively.
Table 3. Values of fitting parameters and their derivatives from the Hill equation with various modifications (from the experimental data of Winslow et al. [27]).

| No. | Equation Parameters | Hill/G Equation | Hill/L Equation |
|-----|---------------------|-----------------|-----------------|
| 1   | $p_{50}$, mm Hg     | 29.08           | 29.11           |
| 2   | $h$                 | 2.30            | 2.30            |
| 3   | $h_{\text{max}(-1)}$ | 1.66            | 1.68            |
| 4   | $h_{\text{max}}$ ($h_{\text{max}(-1)} + 1$) | 2.66            | 2.68            |
| 5   | $\Delta h$ ($h_{\text{max}} - h$) | 0.36            | 0.38            |
| 6   | $p_{\text{max}}$, mm Hg | 52.92           | 47.82           |
| 7   | $s_g$               | 3.33            | —               |
| 8   | $s_l$               | —               | 1.80            |
| 9   | $pO_2\text{low}$ (for $\omega = 0.99$), mm Hg | 37.92           | 36.36           |
| 10  | $pO_2\text{high}$ (for $\omega = 0.99$), mm Hg | 73.87           | 62.89           |
| 11  | $\Delta pO_2$ ($pO_2\text{high} - pO_2\text{low}$), mm Hg | 35.95           | 26.53           |
| 12  | HbO$_2$, % (for $p_{\text{max}}$) | 83.12           | 79.09           |

Following on from Tables 3 and 4, the calculated fitting parameters and their derivatives made it possible to analyze the oxygen-binding properties of this heme protein in more detail.

It should be noted that the values of $p_{50}$ calculated according to the Hill/G and Hill/L equations almost completely corresponded with the values of $p_{50}$ obtained by means of the classic Hill equation. The mean values of the Hill coefficients of the modified equations were comparable with or somewhat lower than those from the classical equation.

There was a good agreement between the values of parameters 1–5 and 12 within the same data set (Tables 3 and 4). The group of parameters 6 and 9–11 was more labile and was associated with differences in the exponential and hyperbolic distributions. For the Hill/L equation, the parameter $\Delta pO_2$ was expected to have lower values than for the Hill/G equation because of the more peaked distribution.

It is noteworthy that the position of the cooperativity maximum shifted toward higher values of the partial oxygen pressure for all data sets and approximating models.

Earlier [26], we suggested that this could be due to a certain physiological role of the hemoglobin macromolecule because it is a heterotetramer whose subunits, despite their structural symmetry, possess a functional asymmetry. This was confirmed by the difference in the equal weight constants of oxygen binding for these subunits [51]. The use of a model with a higher approximation capability (Hill/L) relative to the previous one (Hill/G) also strengthened these assumptions.

Moreover, based on the analysis of the parameters of the Hill/G approximating model in our previous work [26], we believed that the functional asymmetry of this macromolecule...
could not be effectively realized in the case of identical subunits (for example, hemoglobin H, which consists of only four β-subunits) [52].

4. Discussion and Conclusions

The Hill equation did not approximate well with the experimental points of the oxygenation curve if the Hill coefficient was equal to the number of oligomer subunits. The best fit with the experimental data was achieved when the Hill coefficient was smaller than the number of interacting monomers. At the same time, the value of this coefficient was non-integer and the model itself correctly described the oxygenation curve only in the range from 20–30 to 70–80% of hemoglobin saturation with oxygen.

The introduction of the parameter “relative coefficient of cooperativity $\theta(n)$”, which coupled the Hill coefficient and the number of subunits, made it possible to explain the nature of the non-integer value of $h$, but it did not solve the problem of the quality of the approximation of the experimental data approximations [50].

The oxygenation equation proposed by Adair provided the best results in describing the course of the experimental hemoglobin dissociation curve because it has four fitting parameters in terms of the number of ligand-binding constants. However, this equation does not provide an insight into such important parameters of enzymatic kinetics as protein half-saturation with ligands ($K_m$, $p_{50}$) and the degree of cooperative interactions of subunits ($h$).

We considered oxygenation characterized by $p_{50}$ and $h$ as a function of oxygen partial pressure. Note that the very idea of such a dependence, in which macromolecule-binding constants are considered to be a function of macromolecule filling with ligands, is not new [25,39–41].

The modified Hill’s equation [26] we proposed earlier had four fitting parameters (as with Adair’s equation) and the advantages of the original model; it approximated the experimental data better. However, in the range of values of partial pressure below 3 mm Hg, it was inferior in approximation to the Adair equation although, in general, it is comparable with it.

An analysis of the previously obtained approximating equation based on the Gauss distribution (Hill/G) showed a few limitations in its application. The proposed version of the modified Hill equation based on the Lorentz distribution (Hill/L) showed the best results in the approximation of the experimental data. The fitting parameters of both the Hill/L and Hill/G equations, along with the derived parameters from them, allowed a more complete description of the nature of hemoglobin oxygenation. Thus, it was shown that the maxima of cooperativity ($p_{\text{max}}$) for the considered sets of experimental data lay around higher partial pressures of oxygen relative to the $p_{50}$ value.

In conclusion, the proposed model for hemoglobin oxygenation certainly needs to be improved, both in terms of its physical content and mathematical description, given the fact that it provides a very general description of the oxygenation process. Considering the role of the hemoglobin macromolecule as a generalizing physical model for cooperativity in biophysical studies, the proposed equation for oxygenation could be considered in a wider context from tasks of enzymology and pharmacology [36,53] to the modeling of gene transcription regulation [54,55] as well as various dose–response relationships [56,57], i.e., where the classical Hill equation is already traditionally applied. At the same time, starting from the classical models of MWC [58] and KNF [59], evolving to modern models of cooperative ligand binding [60], repeated attempts have been made to improve these models in order to fully and most accurately match the experimental results [61–64]. In our forthcoming work, we aim to describe the main mechanisms of the cooperative binding of hemoglobin with oxygen as well as to establish a connection between the different models.
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References

1. Cui, Q.; Karplus, M. Allosterity and cooperativity revisited. Protein Sci. 2008, 17, 1295–1307. [CrossRef]
2. Lisi, G.P.; Loria, J.P. Solution NMR Spectroscopy for the Study of Enzyme Allosteroy. Chem. Rev. 2016, 116, 6323–6369. [CrossRef]
3. Biddle, J.W.; Martinez-Coral, R.; Wong, F.; Gunawardena, J. Allosteric conformational ensembles have unlimited capacity for integrating information. elife 2021, 10, e65498. [CrossRef]
4. Aristov, V.V.; Buchelnikov, A.S.; Nechipurenko, Y.D. The Use of the Statistical Entropy in Some New Approaches for the Description of Biosystems. Entropy 2022, 24, 172. [CrossRef]
5. Nikitin, E.S.; Malyshnev, A.Y.; Balaban, P.M.; Volgushev, M.A. Physiological Aspects of the Use of the Hodgkin–Huxley Model of Action Potential Generation for Neurons in Invertebrates and Vertebrates. Neurosci. Behav. Physiol. 2017, 47, 751–757. [CrossRef]
6. Aagni, I.F.; Guidolin, D.; Leo, G.; Fuxe, K. A boolean network modelling of receptor mosaics relevance of topology and cooperativity. J. Neural Transm. 2007, 114, 77–92. [CrossRef]
7. Gruber, R.; Horovitz, A. Unpicking allosteric mechanisms of homo-oligomeric proteins by determining their successive ligand binding constants. Philos. Trans. R. Soc. Lond. B Biol. Sci. 2018, 373, 20170176. [CrossRef]
8. Pabis, A.; Risso, V.A.; Sanchez-Ruiz, J.M.; Kamerlin, S.C. Cooperativity and flexibility in enzyme evolution. Curr. Opin. Struct. Biol. 2018, 48, 83–92. [CrossRef]
9. Bellolli, A. Hemoglobin and cooperativity: Experiments and theories. Curr. Protein Pept. Sci. 2010, 11, 2–36. [CrossRef]
10. Brunori, M. Hemoglobin is an honorary enzyme. Trends Biochem. Sci. 1999, 24, 158–161. [CrossRef]
11. Gell, D.A. Structure and function of haemoglobins. Blood Cells Mol. Dis. 2018, 70, 13–42. [CrossRef]
12. Premont, R.T.; Singel, D.J.; Stamler, J.S. The enzymatic function of the honorary enzyme: S-nitrosylation of hemoglobin in physiology and medicine. Mol. Asp. Med. 2022, 84, 101056. [CrossRef]
13. Perutz, M.F.; Rossmann, M.G.; Cullis, A.F.; Muirhead, H.; Will, G.; North, A.C. Structure of haemoglobin: A three-dimensional Fourier synthesis at 5.5-A. resolution, obtained by X-ray analysis. Nature 1960, 185, 416–422. [CrossRef]
14. Weiss, J.N. The Hill equation revisited: Uses and misuses. FASEB J. 1997, 11, 835–841. [CrossRef]
15. Prinz, H. Hill coefficients, dose–response curves and allosteric mechanisms. J. Chem. Biol. 2010, 3, 37–44. [CrossRef]
16. Bounias, M. Algebraic potential of the Hill equation as an alternative tool for plotting dose (or time)/effects relationships in toxicity: A theoretical study. Fundam. Clin. Pharmacol. 1989, 3, 1–9. [CrossRef] [PubMed]
17. Gesztelyi, R.; Zsuga, J.; Kemeny-Beke, A.; Varga, B.; Juhasz, B.; Tosaki, A. The Hill equation and the origin of quantitative pharmacology. Arch. Hist. Exact Sci. 2012, 66, 427–438. [CrossRef]
18. Santillan, M. On the Use of the Hill Functions in Mathematical Models of Gene Regulatory Networks. Math. Model. Nat. Phenom. 2008, 3, 85–97. [CrossRef]
19. Shi, X. A Hill type equation can predict target gene expression driven by p53 pulsing. FEBS Open Bio. 2021, 11, 1799–1808. [CrossRef]
20. Zimmerman, A.; Baylor, D. Cyclic GMP-sensitive conductance of retinal rods consists of aqueous pores. Nature 1986, 321, 70–72. [CrossRef]
21. Ruiz, M.; Brown, R.L.; He, Y.; Haley, T.L.; Karpen, J.W. The Single-Channel Dose–Response Relation Is Consistently Steep for Rod Cyclic Nucleotide-Gated Channels: Implications for the Interpretation of Macroscopic Dose–Response Relations. Biochemistry 1999, 38, 10642–10648. [CrossRef] [PubMed]
22. Guldberg, C.M.; Waage, P. Studies concerning affinity. CM Forh. Vidensk. Selsk. Christ. 1864, 35, 1864.
23. van’t Hoff, J.H. Die grenzeneben, ein Beitrag zur kenneniss der esterbildung. Ber. Dtsch. Chem. Ges. 1877, 10, 669–678.
24. McLean, F.C. Application of the law of chemical equilibrium (law of mass action) to biological problems. Physiol. Rev. 1938, 18, 495–523.
25. Lavrinenko, I.A.; Vashanov, G.A.; Sulin, V.Y.; Nechipurenko, Y.D. An Analysis of Models of Cooperative Oxygen Binding by Hemoglobin. Biophysics 2021, 66, 905–912. [CrossRef]
26. Lavrinenko, I.A.; Vashanov, G.A.; Nechipurenko, Y.D. New Mathematical Model to Describe Hemoglobin Oxygenation. *Biophysics* 2022, 67, 347–352. [CrossRef]

27. Winslow, R.M.; Swenberg, M.L.; Berger, R.L.; Shrager, R.I.; Luzzana, M.; Samaja, M.; Rossi-Bernardi, L. Oxygen equilibrium curve of normal human blood and its evaluation by Adair’s equation. *J. Biol. Chem.* 1977, 252, 2331–2337. [CrossRef]

28. Severinghaus, J.W. Simple, accurate equations for human blood O2 dissociation computations. *J. Appl. Physiol.* 1979, 46, 599–602. [CrossRef]

29. Winslow, R.M.; Swenberg, M.L.; Berger, R.L.; Shrager, R.I.; Luzzana, M.; Samaja, M.; Rossi-Bernardi, L. Oxygen equilibrium curve of normal human blood and its evaluation by Adair’s equation. *J. Biol. Chem.* 1977, 252, 2331–2337. [CrossRef]

30. Lavrinenko, I.A.; Vashanov, G.A.; Nechipurenko, Y.D. Cooperative Oxygen Binding with Hemoglobin as a General Model in Molecular Biophysics. *Biophysics* 2022, 67, 327–337. [CrossRef]

31. Katz, M.H. *Multivariable Analysis: A Practical Guide for Clinicians and Public Health Researchers*, 3rd ed.; Cambridge University Press: Cambridge, UK, 2011.

32. Härdle, W.; Müller, M.; Sperlich, S.; Werwatz, A. *Nonparametric and Semiparametric Models*; Springer: Berlin/Heidelberg, Germany, 2004; Volume 1.

33. Lavrinenko, I.A.; Vashanov, G.A.; Buchelnikov, A.S.; Nechipurenko, Y.D. Cooperative Oxygen Binding with Hemoglobin as a General Model in Molecular Biophysics. *Biophysics* 2022, 67, 327–337. [CrossRef]

34. Coval, M.L. Analysis of Hill interaction coefficients and the invalidity of the Kwon and Brown equation. *J. Biol. Chem.* 1970, 245, 6335–6336.

35. Hofmeyr, J.-H.S.; Cornish-Bowden, A. The reversible Hill equation: How to incorporate cooperative enzymes into metabolic models. *Bioinformatics* 1997, 13, 377–385.

36. Goutelle, S.; Maurin, M.; Rougier, F.; Barbaut, X.; Bourguignon, L.; Ducher, M.; Maire, P. The Hill equation: A review of its capabilities in pharmacological modelling. *Fundam. Clin. Pharmacol.* 2008, 22, 633–648. [CrossRef] [PubMed]

37. Cantor, C.R.; Schimmel, P.R. *Biophysical Chemistry: Part III: The Behavior of Biological Macromolecules*; WH Freeman: New York, NY, USA, 1980.

38. Tyuma, I.; Imai, K.; Shimizu, K. Analysis of oxygen equilibrium of hemoglobin and control mechanism of organic phosphates. *Biochemistry* 1973, 12, 1491–1498. [CrossRef] [PubMed]

39. Tanford, C. *Physical Chemistry of Macromolecules*; Wiley: Hoboken, NJ, USA, 1961.

40. Podrabinek, P.A.; Kamenskii, I.I. Relation between the oxygenation of hemoglobin and its molecular changes. *Biofizika* 1967, 12, 983–988. [PubMed]

41. Nechipurenko, Y.D. Analysis of binding of ligands to nucleic acids. *Biophysics* 2014, 59, 6–27. [CrossRef]

42. Konkoli, Z. Safe uses of Hill’s model: An exact comparison with the Adair-Klotz model. *Theor. Biol. Med. Model.* 2011, 8, 10. [CrossRef]

43. Purich, D.L.; Allison, R.D. *Handbook of Biochemical Kinetics: A Guide to Dynamic Processes in the Molecular Life Sciences*; Elsevier: Amsterdam, The Netherlands, 1999.

44. Ingalls, B.P. *Mathematical Modeling in Systems Biology: An Introduction*; MIT press: Cambridge, MA, USA, 2013.

45. Chang, J.B.; Quinnies, K.M.; Realubit, R.; Karan, C.; Rand, J.H.; Tatonetti, N.P. A novel, rapid method to compare the therapeutic windows of oral anticoagulants using the Hill coefficient. *Sci. Rep.* 2016, 6, 29387. [CrossRef] [PubMed]

46. Hüfner, G. Über die Bedeutung der in der vorigen Abhandlung vorgetangenen Lehre für die Spectroskopie und Photometrie des Blutes. *Arch. Physiol. des Blutes.* 1890, 1, 28–30.

47. Hill, A.V. The possible effects of the aggregation of the molecules of hæmoglobin on its dissociation curves. *J. Physiol.* 1910, 40, i–vii.

48. Michaelis, L.; Menten, M.L.; Johnson, K.A.; Goody, R.S. The original Michaelis constant: Translation of the 1913 Michaelis-Menten paper. *Biochemistry* 2011, 50, 8264–8269. [CrossRef] [PubMed]

49. Sebaugh, J.L. Guidelines for accurate EC50/IC50 estimation. *Pharm. Stat.* 2011, 10, 128–134. [CrossRef] [PubMed]

50. Lavrinenko, I.A.; Vashanov, G.A.; Nechipurenko, Y.D. New Interpretation of the Hill Coefficient. *Biophysics* 2022, 67, 171–174. [CrossRef]

51. Ilgenfritz, G.; Schuster, T.M. Kinetics of Oxygen Binding to Human Hemoglobin: Temperature Jump Relaxation Studies. *J. Biol. Chem.* 1974, 249, 2999–2973. [CrossRef] [PubMed]

52. Galanello, R.; Cao, A. Alpha-thalassemia. *Genet. Med.* 2011, 13, 83–88. [CrossRef]

53. Bordbar, A.K.; Saadati, Z.; Sohrabi, N. Analysis of ligand binding process using binding capacity concept. *Acta Biochim. Pol.* 2004, 51, 963–970. [CrossRef]

54. Li, J.; Zhu, X.; Byrnes, M.; Nelson, J.W.; Chang, S.H. Site-directed mutagenesis of rabbit muscle phosphofructokinase cDNA. Mutations at glutamine 200 affect the allosteric properties of the enzyme. *J. Biol. Chem.* 1993, 268, 24599–24606.

55. Aramaki, H.; Kabata, H.; Takeda, S.; Itou, H.; Nakayama, H.; Shimamoto, N. Formation of repressor-inducer-operator ternary complex: Negative cooperativity of d-camphor binding to CamR. *Genes Cells* 2011, 16, 1200–1207. [CrossRef]

56. Ding, S.; Sachs, F. Single channel properties of P2X2 purinoceptors. *J. Gen. Physiol.* 1999, 113, 695–720. [CrossRef]

57. Srinivasan, S.; Waghu, F.H.; Idicula-Thomas, S.; Venkatesh, K.V. A steady-state modeling approach for simulation of antimicrobial peptide-cell membrane interaction. *Biochim. Biophys. Acta (BBA)-Biomembr.* 2020, 1862, 183242. [CrossRef]
58. Monod, J.; Wyman, J.; Changeux, J.-P. On the nature of allosteric transitions: A plausible model. *J. Mol. Biol.* 1965, 12, 88–118. [CrossRef]

59. Koshland, D.E., Jr.; Némethy, G.; Filmer, D. Comparison of experimental binding data and theoretical models in proteins containing subunits. *Biochemistry* 1966, 5, 365–385.

60. Eaton, W.A.; Henry, E.R.; Hofrichter, J.; Bettati, S.; Viappiani, C.; Mozzarelli, A. Evolution of allosteric models for hemoglobin. *IUBMB Life* 2007, 59, 586–599. [CrossRef]

61. Henry, E.R.; Mozzarelli, A.; Viappiani, C.; Abbruzzetti, S.; Bettati, S.; Ronda, L.; Bruno, S.; Eaton, W.A. Experiments on Hemoglobin in Single Crystals and Silica Gels Distinguish among Allosteric Models. *Biophys. J.* 2015, 109, 1264–1272. [CrossRef] [PubMed]

62. Nagatomo, S.; Nagai, Y.; Aki, Y.; Sakurai, H.; Imai, K.; Mizusawa, N.; Ogura, T.; Kitagawa, T.; Nagai, M. An Origin of Cooperative Oxygen Binding of Human Adult Hemoglobin: Different Roles of the α and β Subunits in the α2β2 Tetramer. *PLoS ONE* 2015, 10, e0135080. [CrossRef]

63. Henry, E.R.; Harper, J.; Glass, K.E.; Metaferia, B.; Louis, J.M.; Eaton, W.A. MWC allosteric model explains unusual hemoglobin–oxygen binding curves from sickle cell drug binding. *Biophys. J.* 2021, 120, 2543–2551. [CrossRef]

64. Horovitz, A.; Mondal, T. Discriminating between Concerted and Sequential Allosteric Mechanisms by Comparing Equilibrium and Kinetic Hill Coefficients. *J. Phys. Chem. B* 2021, 125, 70–73. [CrossRef]