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Fitting Parameters of a Modified Hill's Equation and Their Influence on the Shape of the Model Hemoglobin Oxygenation Curve

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Abstract: Oxygen binds to hemoglobin cooperatively, and a correct description of this binding is relevant not only for understanding the mechanisms of involved molecular processes but also for clinical purposes. Hill's equation, derived over a hundred years ago, is still the simplest and most efficient way to perform this description. However, in terms of accuracy, it is inferior to Adair´s equation, which contains more parameters. We proposed to use a modified Hill equation and showed that it allows a more accurate description of the binding of oxygen to hemoglobin than Adair's equation. At the same time, unlike Adair's equation, our model retains the physical meaning given to the original Hill equation. We considered and analyzed the influence of the equation parameters on the course of the oxygenation curve and presented the relationship between the fitting parameters and other parameters derived from them in the form of a diagram-graph, which, in our opinion, simplifies the perception of these estimates and can be useful in solving a number of problems for which the traditional way of analyzing the degree of cooperative interaction was via the Hill equation. We suggest that the newly proposed parameter h_{max} introduced in our model should be regarded as crucial for a better description of the oxygenation curve.

Keywords: cooperativity; oxygenation; oxygenation curve; oxyhemoglobin dissociation curve; Hill equation; Hill coefficient

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1. Introduction

The accumulation of oxygen in Earth's atmosphere as a consequence of the appearance of photosynthesizing organisms has become a determining factor for the development of more complex forms of life [1]. The use of oxygen as electrons' end-acceptor in the respiratory chain allowed more efficient means for obtaining energy from organic compounds as compared to anaerobic respiration [2,3]. With multicellular organisms' increasing size and, as a consequence, the appearance of diffusion constraints, diverse transport systems were developed, the oxygen-transporting system being the most important [4–6]. For vertebrates and for humans as well, oxygen transport is performed by hemoglobin in red blood cells, and its saturation level (SaO_2) becomes a significant physiological and health indicator of arterial blood oxygenation [7–9]. For a wide range of oxygen partial pressures, the degree of blood oxygenation is assessed by the oxyhemoglobin dissociation curve (ODC) [10,11].

The study of oxygen binding by hemoglobin is of interest for assessing the degree of blood oxygenation, which is of clinical relevance [12,13]. It is important for us to understand the molecular mechanisms underlying the cooperative binding of oxygen to

hemoglobin [14,15]. The phenomenon of cooperativity is characteristic of many processes in biology at various levels of the organization of living systems [16–18].

The availability of hemoglobin, the ease of its isolation from whole blood, its important physiological role, and the issues of interpretation of experimental oxygenation curves have determined the unfading interest of researchers in this macromolecule [19]. Ultimately, this protein has actually become the starting point in the study of cooperative effects in biology, primarily in enzymology [20]. Hemoglobin became an "honorary enzyme" and one of the first objects of study of the spatial structure of proteins [21,22]. It became possible to study the phenomenon of cooperativity, taking into account the knowledge of the mutual position of atoms in the macromolecule [23,24]. Subsequently, the accumulated knowledge in this area formed the basis for studies of the interaction of proteins with low molecular weight compounds, and in the applied area, it served as the basis for drug design in pharmaceuticals [25,26]. Due to the fact that hemoglobin has been studied in great detail, and also thanks to its significance as an object of research, it became possible to formulate a paradigm for the study of cooperative systems in biology [27–29].

Since hemoglobin reversibly binds oxygen, the study of oxygenation and cooperativity can be carried out from the idea of C.-L. Berthollet about reverse reaction and chemical equilibrium [30]. C. Guldberg and P. Waage, as well as J. van't Hoff, developed these ideas and formulated the law of mass action, which is a consequence of the second law of thermodynamics [31,32]. Based on this theoretical basis, G. Hüfner proposed the first equation for oxygenation, which, however, could not satisfactorily describe the known experimental data [33]. A. Hill introduced his equation with a reasonable approximation to experimental data, as well as with the assumption that the hemoglobin molecule is capable of aggregation, whereas oxygenation is realized through the simultaneous binding of oxygen by an aggregate of several protein molecules [34]. Further studies of oxygenation and cooperative effects began to rely on the mathematical apparatus of statistical physics already developed by that time [29].

Nevertheless, thanks to its simplicity, good approximation capability, and clear-cut meaning of approximated parameters (the half-saturation of ligand binding by the oligomer—p_{50}/EC_{50}—and cooperativity coefficient h), Hill's equation is commonly accepted for a large number of biomedical tasks and applications [35,36].

Based on the results of his own research, which later received its confirmation in the experiments of T. Svedberg [37], G. Adair proposed an equation that is based on the established fact that the hemoglobin molecule has four binding centers as well as the idea of their sequential oxygenation [38]. According to these conceptions, Adair's equation includes four parameters to be estimated—the apparent binding constants (K_1–K_4)—and this allows a better description of the hemoglobin oxygenation curve in comparison to Hill's equation [39]. Thus, Adair´s equation became, at that time, the most accurate in describing the oxyhemoglobin dissociation curve [39]. However, it is not clear how it is possible, using Adair's equation, to directly assess the value of hemoglobin half-saturation, as well as to characterize cooperativity in the oligomer [40].

Later, based on the Wyman-Allen hypothesis, which assumed the simultaneous binding of two oxygen molecules by hemoglobin, S. Bernard proposed his oxygenation equation, which, although it has an easily interpretable parameter p_{50}, does not make it possible to evaluate the cooperativity of this process [41]. Exhibiting a higher approximating capability compared to Hill's equation but being inferior in this regard to Adair's equation, this equation has not received such recognition as previous mathematical models [36,42].

It should be noted that, apparently, a compromise option (Bernard's equation) is not always the optimal solution in describing the oxygen-binding properties of hemoglobin. In all likelihood, the priority for researchers is either convenience in estimating the cooperative properties of a molecule (Hill's equation) or a good approximation of the experimental results (Adair's equation).

Taking into account the possible options for the spatial position of oxygen binding centers, L. Pauling rethought Aider's equation from the standpoint of biophysical chem-

istry [43]. The model in the form of a tetrahedron, which is close to the natural structure of the heme protein, has led to the most accurate solution to this problem. Later, I. Klotz considered the possibility of applying the law of mass action to describe the binding of a large number of ligands to a protein macromolecule and performed the de-convolution of the constants in Adair's equation to the level of microscopic constants (the Adair-Klotz equation) [44]. Subsequently, D. Koshland, G. Némethy, and D. Filmer, based on data on the structural rearrangement of oligomers, refined the mechanism of ligand binding using a macro-molecule proposed by Pauling and developed their own phenomenological model and oxygenation equation based on the induced fit hypothesis (Pauling/KNF model, sequential model) [45]. However, the large number of coefficients in this equation significantly complicates its application to the approximation of experimental data [46] and is noted by dubbed researchers as an "algebraic morass" [29]. Since the equation of the Pauling/KNF model is based on the Adair equation, the "Koshland constants" can be easily converted to "Adair constants" [40].

Taking into account the data of X-ray diffraction analysis of hemoglobin, J. Monod, J. Wyman, and J.-P. Changeux proposed a phenomenological model (MWC model, concerted model) for oxygen binding by hemoglobin and the oxygenation equation arising from this conception [47]. The Pauling/KNF model was developed later than the MWC and included a concerted transition of subunits from one conformational state to another (concerted Pauling/KNF), similar to the MWC model. At the same time, the MWC model, as well as the Pauling/KNF model, is not without drawbacks in terms of its application to the approximation of experimental data since the equation parameters of this model are correlated with each other and sensitive to the data-fitting method [46].

New experimental data with a higher spatial resolution of the hemoglobin molecule, data on the nanosecond kinetics of ligand binding, as well as a number of other discovered facts, required the development of new models of cooperative interaction: the "Cooperon" model by M. Brunori et al. [48], the models of A. Szabo and M. Karplus (SK model) [49], based on the stereochemical mechanism of M. Perutz, which was further generalized and revised by A. Lee and M. Karplus (SKL model) [50], the tertiary two-state model (TTS model) developed by E. Henry et al. [51].

Despite the differences in the interpretation of oxygenation mechanisms, the considered equations have a general similarity since they are based on a power law. At the same time, to describe the oxygen-binding properties of hemoglobin, a number of authors also proposed equations based on the exponential function [39]. However, as the results of our studies have shown, these equations are inferior in their ability to approximate experimental data on hemoglobin oxygenation [39]. In addition, the idea underlying the construction of these equations, in our opinion, does not agree very well with the crystallographic data of this protein or the kinetics of ligand binding [24,52].

Taking this into account, we proposed a new mathematical model (Hill/L-model), which is based on Hill's equation (we will refer to this equation as the "Hill classic" in the rest of this document below) [34]:

$$y = \frac{p^h}{p_{50}^h + p^h}, \tag{1}$$

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

In our model, this case, the cooperativity coefficient in the Hill/L equation is modulated by the Lorentz distribution as a function of oxygen's partial pressure:

$$h = \frac{h_{\max} - 1}{1 + [\ln(p/p_{\max})/s]^2} + 1. \tag{2}$$

Thus, this Hill/L model uses the following fitting parameters: p_{50}; h_{max} (as the maximum value of the Hill coefficient); ln p_{max} is the logarithm of the oxygen partial pressure value at which h_{max} is determined, and s—is a scale parameter for the Lorentz distribution.

The Hill/L equation contains the same number of fitting parameters as Adair's equation (four) [38]. Nevertheless, unlike the latter, it hopefully allows, in our opinion, to better describes the ODC. Also, the Hill/L model retains a higher approximation capability, as assessed by the determination coefficient r^2 compared to Adair's equation [53]. Another important virtue of the Hill/L model is its compatibility with Hill's classical equation as per parameters p_{50} and h (for Hill classic) or h_{max} (for Hill/L) since for s $\rightarrow \infty$, $h_{max} - h \rightarrow 0$.

Thus, the proposed equation includes four parameters to be fitted, retaining all the above-mentioned advantages of Hill's equation and yielding a better fit to experimental data (Figure 1) [54].

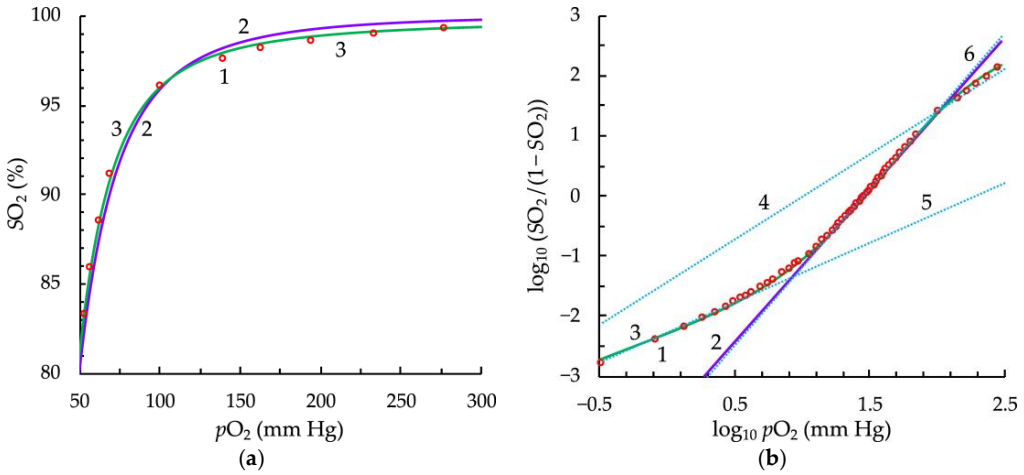

Figure 1. Oxyhemoglobin dissociation curve (ODC): (**a**) linear scale for the x- and y-axes; (**b**) logarithmic scale for both the x-axis and y-axis (Hill's plot); 1, experimental data points for oxygenation curve (from the data set of Winslow et al. [54]); 2, approximation with the Hill's equation (Hill classic); 3, approximation with the modified Hill's equation (Hill/L); 4, 5, dotted lines correspond to asymptotes, which are not exactly parallel, but with slopes very close to one; 6, the dotted line is tangential to the point p_{50}; axes: pO_2, partial pressure of oxygen; SO_2, oxygen saturation.

In the present work, we analyzed the influence of different parameters from the Hill/L equation on the aspect of the model oxygenation curve and also represented the interconnection between these parameters and others derived from them in a graphic scheme.

2. Materials and Methods

The object of our study was our previously proposed modification of Hill's equation [53], as well as a set of experimental data obtained by Winslow et al. [54]. Optimization of the model's parameters was performed via the generalized reduced gradient (GRG) method [55], with the target function being the sum of minimal squares (LS method) [56]. Corresponding computations and graphical representations were performed with MS Excel. An example of approximation of experimental data by the modified Hill equation is presented in Supplementary Materials.

3. Results

3.1. Fitting and Derived Parameters from the Modified Hill's Equation

Figure 2 represents the relations between Hill/L-equation fitting parameters (as well as those derived from them) during ODC.

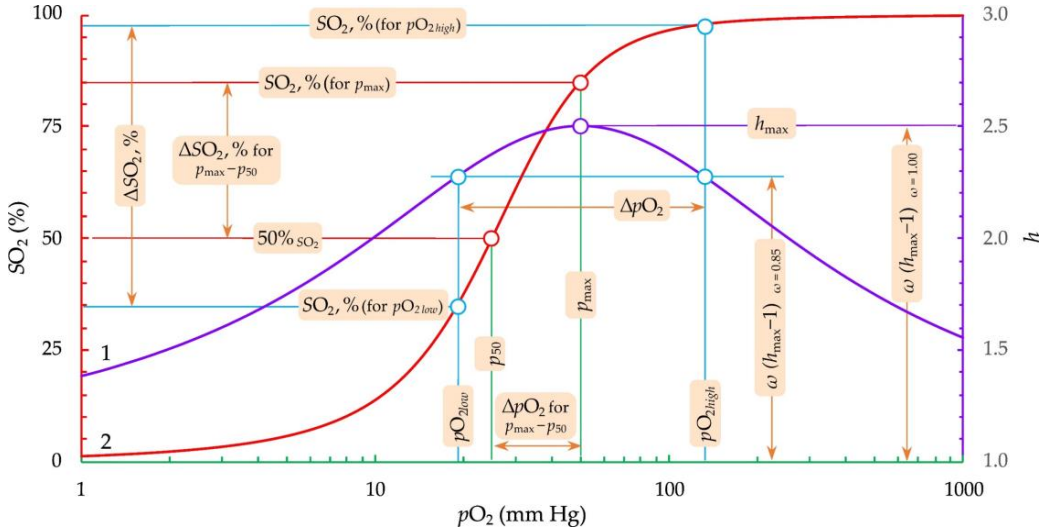

Figure 2. Relation between model fitting parameters and others derived from them, which describe the oxyhemoglobin dissociation curve (ODC). Legend: 1, curve representing Hill's coefficient dependence with respect to oxygen partial pressure; 2, oxyhemoglobin dissociation curve (ODC); axes: pO_2, partial pressure of oxygen; SO_2, oxygen saturation; h, Hill coefficient.

Besides parameter p_{50}, which characterizes the value of half-saturation of hemoglobin by oxygen (50% SO_2), p_{max} indicates the value of partial pressure for which Hill's coefficient reaches its maximum. For p_{max} from Equation (2), it is possible to find the degree of hemoglobin oxygenation SO_2,% (for p_{max}). Also, from these parameters, it is possible to obtain the following differences: ΔpO_2 (for $p_{max} - p_{50}$), as a measure of the departure of the maximal cooperativity coefficient respective to the point p_{50} in the x-axis, as well as ΔSO_2 (for $p_{max} - p_{50}$) corresponding to the difference in the oxygenation degree respect to hemoglobin's 50% saturation. The parameter h_{max} allows for the assessment of the maximal value of Hill's coefficient.

The model parameter s in Equation (2) allows obtaining the lowest (pO_{2low}) and highest (pO_{2high}) values of oxygen partial pressure for $h - 1 = \omega(h_{max} - 1)$, where ω—is the fraction of $h_{max} - 1$ expressed from 0 to 1 (for $\omega = 0.5$, more commonly known as half-width at half-maximum or HWHM):

$$p_{O_2 low} = \exp\left[\ln p_{max} - s_l\left(\omega^{-1} - 1\right)^{1/2}\right], \quad (3)$$

$$p_{O_2 high} = \exp\left[\ln p_{max} + s_l\left(\omega^{-1} - 1\right)^{1/2}\right]. \quad (4)$$

Hence, it is possible to find out the partial pressures range (ΔpO_2), as the difference between pO_{2high} and pO_{2low}, where $h - 1$ is not below the corresponding fraction ω from $h_{max} - 1$ (for example, in Figure 2, it is represented as $\omega = 0.85$, $h_{max} - 1 = 1.5$, $\omega(h_{max} - 1) = 1.275$).

From these parameters, the value of the degree of saturation of hemoglobin with oxygen for pO_{2high} —SO_2, % (for pO_{2high}) and for pO_{2low} —SO_2, % (for pO_{2low}), as well as the difference between them—ΔSO_2, % can be found. In addition, according to Equations (1) and (2), the lower and upper limits of the ranges of partial oxygen pressures and the degree of saturation of hemoglobin with oxygen, as well as the corresponding differences obtained based on h_{max} (not shown in Figure 2), can be determined.

Thus, the presence of four adjustable parameters makes it possible to obtain a combination of values that allows a more complete characterization of the course of the oxyhemoglobin dissociation curve. The most significant parameters, in our opinion, should include: p_{50}, p_{max}, and h_{max}.

3.2. Influence of Fitting Parameters of the Modified Hill Equation on the Model Oxygenation Curve Course

Figure 3a,c considers the option when p_{50} takes values from 5 to 50 mm Hg, with a step of 5 mm Hg, while the parameter $h_{max)}$ is equal to one. Then the first term in Equation (2) vanishes, and consequently, the parameters p_{max} and s do not determine the course of the curve. In this case, the equation proposed by us does not differ from the Hüfner [33] and Michaelis-Menten [57] equations.

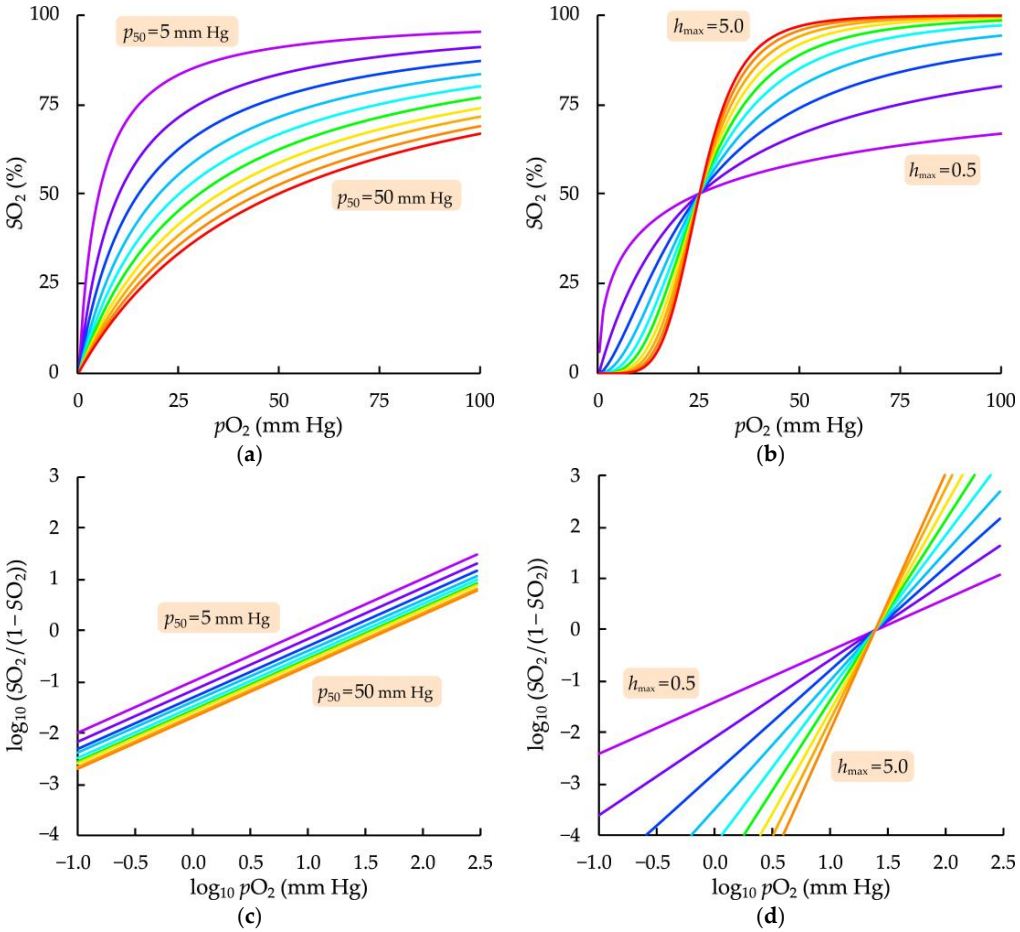

Figure 3. Dependence of the model oxyhemoglobin dissociation curve: (**a**,**c**) at constant parameters: h_{max}, p_{max}, s, and variable p_{50}; (**b**,**d**) at constant parameters: p_{50}, p_{max}, s, and variable h_{max}; axes: pO_2, partial pressure of oxygen; SO_2, oxygen saturation.

At a constant value of the parameters p_{50} (in this case equal to 25 mm Hg), p_{max} = 50 mm Hg, s = 1000, and keeping the parameter h_{max} greater than zero, the oxygenation curve practically does not differ from the curve constructed according to the classical Hill equation (Figure 3b,d). It should be noted that for $s \geq 1000$, the value of p_{max}, which is in the range from 0.01 mm Hg to 1000 mm Hg, has almost no effect on the course of the model oxygenation curve. Figure 3b,d shows model curves, with h_{max} taking values from 0.5 to 5.0 in steps of 0.5.

For the variant when p_{50}, h_{max}, and s are kept constant (in this case, they take the values of 25 mm Hg, 4, and 0.75, respectively), and the parameter p_{max} varies (in this case from 5 to 50 mm Hg; Figure 4a,c,e); one can note the advantage of approximation by the Hill/L equation relative to the Hill´s classic equation. Thus, by adjusting the parameter p_{max}, it is possible not only to improve the quality of the approximation by redistributing the position of the maximum of the cooperativity coefficient along the x-axis (indicating oxygen partial pressure) but also to interpret this parameter as having a certain physical

meaning. Thus, it was previously shown [53,58] that for the experimental ODC $p_{max} > p_{50}$, which may indicate a certain physiological significance for the fact that the maximum of the cooperativity coefficient h does not fall on the value of p_{50} but lies in the region of higher oxygen partial pressures.

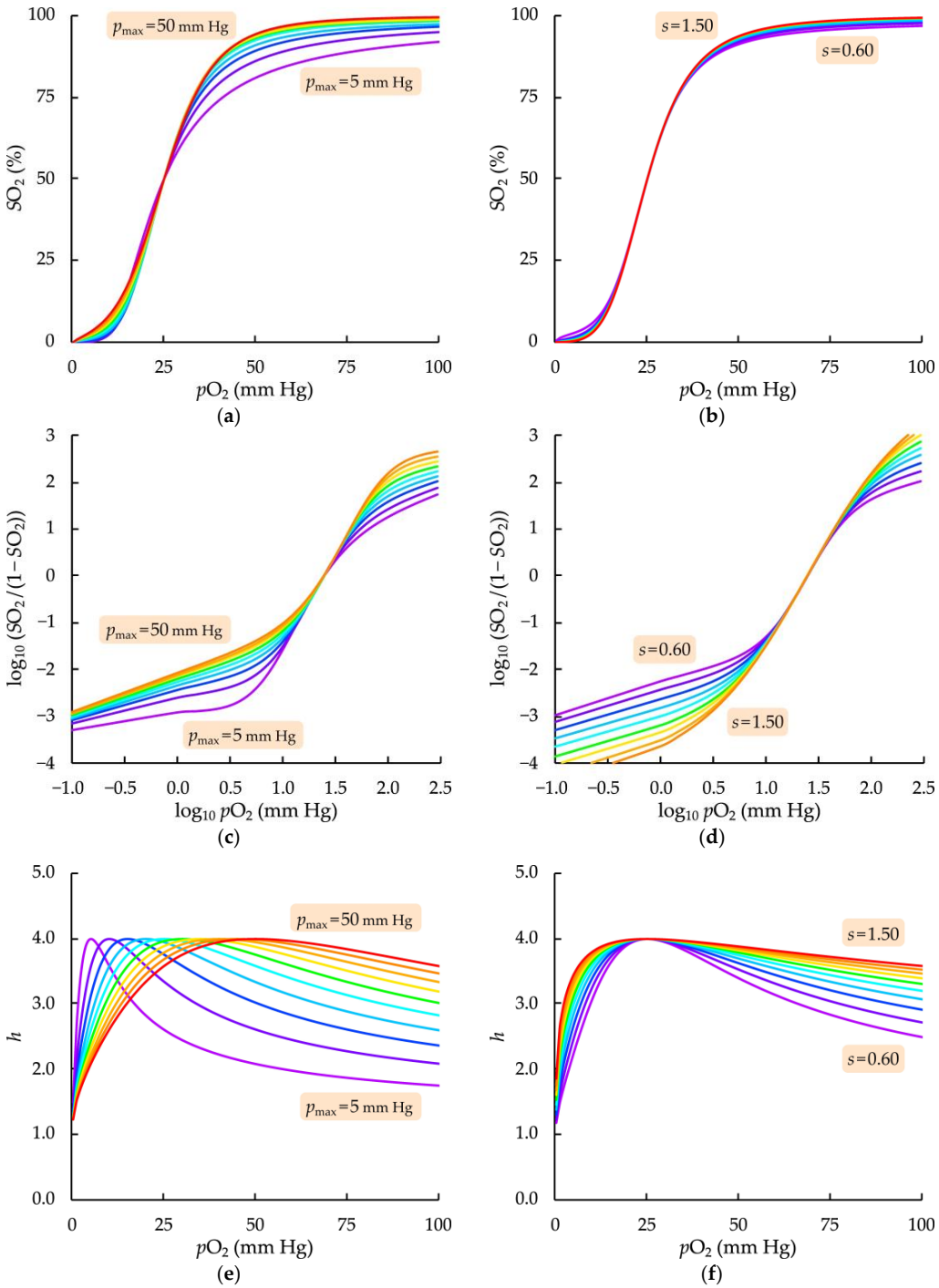

Figure 4. Dependencies with respect to oxygen partial pressure for oxyhemoglobin dissociation (left panels), and for the Hill coefficient (right panels); (**a**,**b**) with constant parameters: p_{50}, h_{max}, s and variable p_{max}; (**c**,**d**) with constant parameters: p_{50}, p_{max}, h_{max}, and variable s; (**e**,**f**) axes: pO_2, partial pressure of oxygen; SO_2, oxygen saturation; h, Hill coefficient.

By varying the parameter s at constant parameters p_{50}, h_{max}, and p_{max}, it is possible to determine the width of the region of oxygen partial pressures in which the Hill

coefficient varies insignificantly. It is then possible to find the lower and upper limits of the range of the corresponding level of hemoglobin oxygenation by setting the value of ω in Equations (3) and (4). Figure 4b,d,f shows the model dependences of the degree of oxygenation and the function of the Hill coefficient on the oxygen partial pressure for the parameters p_{50} = 25 mm Hg, p_{max} = 50 mm Hg, and h_{max} = 4; the parameter s varies from 0.60 to 1.50 with steps of 0.1. Figure 4b,d,f also shows that as the value of the parameter s increases, the model curve tends to the curve described by the classical Hill equation.

4. Discussion

Hill's equation, despite its lower approximating ability relative to Adair's equation [59], has become widespread not only as a convenient empirical measure for estimating the degree of oxygenation of hemoglobin [60], depending on the partial pressure of oxygen. This equation has found applications in other areas of biomedical research: enzymology [61,62], pharmacology [63,64], toxicology [65,66], analysis of various dose-effect relationships [67], a number of other applications related to modeling the regulation of gene transcription [68,69], analysis of conjugated ion transport, etc. [70–72].

Thus, if Hill's coefficient is represented as an abstract compound "indicator" for cooperativity, then we represent this coefficient as a function of oxygen partial pressure, whose maximum value h_{max} is reached at a partial pressure value defined as p_{max}. This approach, even when shifting this abstraction towards a new level, can, nevertheless, retain a certain physical meaning.

Thus, we previously showed [53] that h_{max} does not coincide with the point of half-saturation of hemoglobin by oxygen. Instead, it is located in the region of higher partial pressures. This, in our opinion, may indeed characterize the asymmetric cooperativity for a symmetric tetramer, as it was previously discussed by G. Ackers and J. Holt [73]. The analysis of a greater bulk of experimental data with the Hill/L model could specify the efficacy of cooperativity assessment in the molecule via h_{max} and p_{max}, as well as its application to physiological experiments as well as to clinical and diagnostic investigations.

At the same time, a more accurate fit to experimental data with parameters from the Hill/L equation, together with the possibility to assess Hill's coefficient over the whole range of the oxygenation curve, allows to carry out a comparative analysis of these curves as well as the curves of Hill's coefficient dependence respect to oxygen partial pressure.

The better fit obtained with the Hill/L equation with respect to the classical Hill equation in the region of low oxygen partial pressures might be useful for the analysis of mechanisms of cooperative ligand binding at the initial stages of this process. Since oxygen partial pressure in interstitial fluid as well as inside the cell is below 40 mm Hg, the analysis of oxygen binding properties of hemoglobin in this region embroils both physiological as well as clinical relevance, e.g., during the investigation of tissue oxygenation and microcirculatory blood flow for optimal hemodynamic patient management [74].

Another important aspect of the Hill/L equation application may be the study of the curve of dependence of the Hill's coefficient with respect to oxygen partial pressure for different values of temperature, pH, 2,3-DPG, as well as for the case of study of oxygenation of different mutational variants of the hemoglobin. Thus, S. Edelstein [75] showed that Hill's coefficient in the classical equation is a dependent parameter with respect to pH, 2,3-DPG, and other oxygenation conditions.

It should be noted that studies of hemoglobin oxygenation were also carried out in the field of developing structural and functional models of the ligand binding of this molecule. Thus, the tetrameric structure of hemoglobin, which was shown by G. Adair [38] and corroborated by T. Svedberg [37], became the basis for understanding the nature of the mutual allocation of iron atoms in this molecule. L. Pauling [43] re-examined Adair's equation from the viewpoint of biophysical chemistry and attempted to link this equation with the hemoglobin molecule's structure (about which very little was known at that time). The research started by M. Perutz [21] to determine the hemoglobin structure using X-ray diffraction analysis laid the foundation for classical structure-functional models of

hemoglobin oxygenation: MWC and Pauling/KNF [45,47]. Later on, the MWC model was further developed in the works by W. Eaton and other researchers [28,29,49,51,76], where the main task became the study of the physical bases for cooperative ligand binding and corresponding conformational changes in the macromolecule. Nevertheless, the fullest understanding of the oxygenation process demands the application of computational methods, able to evaluate changes in the macromolecule at the atomic scale together with the assessment of interaction energy of the corresponding structural elements [14,20].

At the same time, our current work is aimed at improving the descriptive parameters for cooperative systems.

Thus, in this work, we considered and analyzed the influence of the equation's parameters on the course of the oxygenation curve. We also clearly presented the relationship between the fitting parameters and their derivatives in the form of a diagram-graph, which, in our opinion, simplifies the perception of these estimates and can be useful in solving a number of problems where the traditional way of analyzing the degree of cooperative interaction was via considering the parameters EC_{50}/p_{50} and h. Of the parameters proposed by us for assessing the course of the oxygenation curve, in addition to p_{50}, p_{max}, and h_{max}, should be referred to as the most significant.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/oxygen3010007/s1, Table S1: Approximation of experimental data with the modified Hill's equation (template and example) (Hill(L)_Example.xlsx).

Author Contributions: Conceptualization, I.A.L.; methodology, I.A.L.; software, I.A.L.; validation, I.A.L.; formal analysis, I.A.L.; investigation, I.A.L. and Y.D.N.; resources, I.A.L.; data curation, I.A.L.; writing—original draft preparation, I.A.L.; writing—review and editing, I.A.L., Y.D.N., J.L.H.C. and G.A.V.; visualization, I.A.L.; supervision, Y.D.N.; project administration, Y.D.N.; funding acquisition, Y.D.N. All authors have read and agreed to the published version of the manuscript.

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