ISSN: 2161-1009
Biochemistry & Analytical Biochemistry

OPEN ACCESS Freely available online

Review Article

De-Constructing the ^{T}State/^{R}State Structure Change of Human Hemoglobin

Francis Knowles*, Douglas Magde

Department of Chemistry and Biochemistry, University of California, San Diego, USA

ABSTRACT

The dimensionless equilibrium constant for the allosteric structure change is shown to be comprised of: (i) An endothermic change in structure, from ^{T}state to ^{R}state, of 24.3 kJ/mol; (ii) Exothermic conversion of ^{T}state $^{T}\alpha O_2$-chains to ^{R}state $^{R}\alpha O_2$-chains of-13.8 kJ/mol; (iii) Exothermic binding of BPG by R-states. Equation (1) defines the component steps whereby the ^{T}state structure is converted to the Rstate structure. $\Delta G°(^{R}(Hb_4)$, BPG) describes the endothermic decomposition of the binary complex, $^{T}Hb_4$/BPG into $^{R}Hb_4$ and BPG, equal to +33.7 kJ/mol (DeBruin et al. (1973). J. Biol. Chem. 248, 2774-2777). $\Delta G°$ of the equilibrium constant for $\Delta G°$ (K_Δ) and $\Sigma\,\Delta G°$ for binding of O_2 by the pair of equivalent ^{T}state α-chains, $\Delta G°(^{T}\alpha^{*}O_2)$, +9.41 kJ/mol and-49.6 kJ/mol, respectively, are determined by fitting of O_2 equilibrium binding data to the Perutz-Adair equation.

Keywords: Hemoglobin; Allosteric structure; E-molecules; Standard free energy change; Structure (protein) changes; Allosteric mechanisms

INTRODUCTION

ΔG^{o} for reaction of a pair of equivalent ^{R}state α-chains with O_2, $\Delta G°(^{R}\alpha O_2)$, was estimated from the known affinity of myoglobin for O_2 at 37°C. (Biochem. Z., 268, 73-81),-63.4 kJ/mol. The unknown quantity, $\Delta G°(^{R}(HbO_2)_4$/BPG), was obtained by solving Equation (1), being-10.5 kJ/mol, ^{K} $(HbO_2)_4$/BPG)=58.4 L/mol. The value of the equilibrium constant for binding BPG to the R-state structure represents 0.0073% of the value of the binding constant of BPG to the ^{T}state structure: 800,000 L/mol. The value of K_Δ; (i) Accounts for the ability of O_2 to escape, virtually unhindered from red blood cells and (ii) Provides a biophysical basis for manifestation of high resting rates of metabolism in warm blooded species. The Perutz/Adair equation of state, Equation (1), imposes the elements of the Perutz stereochemical model on the Adair equation. The Perutz/Adair equation, comprised of only three unknown quantities, accurately predicts equilibrium binding curves of whole blood with both O_2 and CO.

$$F = \frac{(2\,K_{\hat{a}}[O_2]\,(1 + K_{\hat{a}}[O_2]\,(1 + K_\Delta\,(1 + K_{\hat{a}}[O_2]\,(3 + 2\,K_{\hat{a}}[O_2])))))}{(4\,(1 + K_{\hat{a}}[O_2](2 + K_{\hat{a}}[O_2]\,(1 + K_\Delta\,(1 + K_{\hat{a}}[O_2]\,(2 + K_{\hat{a}}[O_2])))))} \quad \text{.....(1)}$$

These observations are particularly relevant insofar as they define the properties of haemoglobin in human RBCs *in vivo*, in the presence of the naturally occurring E-molecule, BPG. The result obtained with the Perutz/Adair equation permits unambiguous assignment of structural states, R and T, to the subunits in each intermediate species in the reaction sequence, Equation (a). Species I and II, as encountered in rbcs of whole blood, are ^{T}state. Species III and IV, as encountered in red blood cells of whole blood, are ^{R}state. Species l is in a special category insofar as it does not exist *in vivo*. Species I, however, is readily prepared in the laboratory [1,2].

The value of the equilibrium constant for the structural change, K_Δ=0.02602, is of particular interest. It accounts for the ability of molecules of O_2 to escape, virtually unhindered, from RBCs. This single insight, by itself, provides strong justification for the model underlying the Perutz-Adair equation. Upon release of O_2 atoms from β-chains, species III and IV, 98% of species III revert to species II. Species II is unable to bind O_2 molecules to ^{T}state β-chains, precisely defining the boundary condition of the molecular mechanism releasing O_2 from arterial blood into the systemic circulation. The value of K_Δ provides a biophysical basis for manifestation of high resting rates of metabolism in warm blooded species.

$$\underset{I}{^{T}(\alpha^{*}\bullet\beta^{*})} \rightarrow \underset{II}{^{T}(\alpha^{*}O_2\bullet\beta^{*})} \rightarrow \underset{III}{^{R}(\alpha^{*}O_2\circ\beta^{*})} \rightarrow \underset{IV}{^{R}(\alpha^{*}O_2\circ\beta^{*}O_2)} \quad \text{.....(a)}$$

The endothermic ^{T}state → ^{R}state structure change is coupled with: (i) exothermic conversion of $^{T}\alpha O_2$-chains (species II, Equation (a)) to $^{R}\alpha O_2$-chains (species III, Equation (a)) and (ii) marked decrease in affinity of the ^{R}state for BPG. $\Delta G°$ for formation of $^{T}Hb_4$/BPG is-33.7 kJ/mol at 25°C. The ^{R}state structure forms a less stable

Correspondence to: Francis Knowles, Department of Chemistry and Biochemistry, University of California, San Diego, USA, E-mail: fknowles@ucsd.edu

Received: 29-Dec-2022, Manuscript No. BABCR-23-19474; **Editor assigned:** 02-Jan-2023, Pre QC No. BABCR-23-19474 (PQ); **Reviewed:** 17-Jan-2023, QC No. BABCR-23-19474; **Revised:** 25-Jan-2023, Manuscript No. BABCR-23-19474 (R); **Published:** 01-Feb-2023, DOI: 10.35248/2161-1009.23.12.479

Citation: Knowles F, Magde D (2023) De-Constructing the ^{T}State/^{R}State Structure Change of Human Hemoglobin. Biochem Anal Biochem.12:479

binary complex with BPG. The Perutz/Adair equation is based on the assumption that: (i) ^{T}state protein structures, species I and II (Equation (a), bind BPG with the exactly the same high affinity and (ii) ^{R}state protein conformations, species III and IV (Equation (a), bind BPG with exactly the same relativity low affinity, in comparison with ^{T}state structure. Nevertheless, the diminished affinity of ^{R}state structures for BPG is large enough to be exothermic, estimated to be-10.5 kJ/mol. These reactions, endothermic and exothermic, taken together, account for the observed value of KC, 0.02602. Equation (2) and (3) define the reactions predicted to account for the observed value of the equilibrium constant for the ^{T}state to ^{R}state structure change [3,4].

$\Delta G°(K_{\Delta C})$ = endothermic structure change + exothermic binding of BPG to ^{R}state structures + exothermic conversion of $^{T}\alpha^{*}O_2$ chains to $^{R}\alpha^{*}O_2$ chains .....(2)

$\Delta G°(K_{\Delta C}) = \Delta G°(^{R}Hb_4,BPG)) + \Delta G°(^{R}HbO_2)_4/BPG)) + (\Sigma \Delta G°(^{R}\alpha^{*}O_2) - \Sigma \Delta G°(^{T}\alpha^{*}O_2))$ .....(3)

LITERATURE REVIEW

Determination of Unknown Quantities in Eq. (3): Calculation of the Values of $\Delta G°$ ($K_{\Delta C}$) and $\Delta G°$ ($^{T}\alpha^{*}O_2$) at 37°C

These values are returned by curve fitting of O_2-binding date of whole blood, under standard conditions, to the Perutz/Adair equation:

$\Delta G°(K_{\Delta C})$=9.41 kJ/mol; $\Delta G°(^{T}\alpha^{*}O_2)$=-49.6 kJ/mol. Results are summarized in Table 1.

Estimation of the Value of $\Delta G°(^{R}\alpha^{*}O_2)$ at 37°C. The model described in Figure (1) does not permit determination of $\Delta G°$ for binding of a molecule of O_2 to equivalent ^{R}state ($^{R}\alpha^{*}$)-chains. Molecules of O_2 have only equivalent ^{T}state deoxy-($^{T}\alpha^{*}$) chains to form equivalent ($^{T}\alpha^{*}O_2$) chains. ($^{R}\alpha^{*}O_2$) chains are formed directly from $^{T}(\alpha^{*}O_2)$ when the ^{T}state → ^{R}state structure change relaxes proximal strain. One can estimate $\Delta G°$ for formation of $^{R}\alpha O_2$ in two ways: (i) assume that $\Delta G°$ (MbO_2) is similar to $\Delta G°$ ($^{R}\alpha O_2$); (ii) assume that $\Delta G°$ ($^{R}\alpha O_2$) for E-free hemoglobin is an upper limit [5-7]. Theorell elaborated O_2-binding data for horse heart myoglobin at 37O, with half-saturation of myoglobin occurring at a partial pressure of O_2 in the gas phase of approximately 3.5 Torr, corresponding to a concentration of O_2 of 4.60 µmol/L. This allows an assignment of 2.2 x 105 L/mol as the O_2-binding equilibrium constant of ^{R}state ($^{R}\alpha^{*}O_2$) chains: $\Delta G°$=-63.4 kJ/mol. Using equilibrium constants obtained for E-free hemoglobin at 20°C Results are summarized in Table 1.

Table 1: Equilibrium constants and $\Delta G°$ for the sequence of reactions comprising the Perutz-Adair equation, 37°C.

Reactions	K L/mol	$\Delta G°$ kJ/mol
$^{R}Hb_4$+DPG → ^{T}Hb/DPGa	K_{DPG}=8 x 105	-33.69
In Whole Blood		
O_2+$^{T}(\alpha)_2$ → $^{T}(\alpha O_2)$ $^{T}(\alpha)$	$2K_{\alpha}$=30,180	-26.6
O_2+$^{T}(\alpha O_2)$ $^{T}(\alpha)$ → $^{T}(\alpha O_2)_2$	$K_{\alpha}/2$ = 7,545	-23.02
2 O_2+2 $^{T}(\alpha)$ → $^{T}(\alpha O_2)_2$		-49.62
^{T}State → ^{R}State	$K_{\Delta C}$=0.02602b	9.41
O_2+$^{R}((\alpha^{*}O_2)\bullet(\beta^{*}))$ → $^{R}((\alpha^{*}O_2)\bullet((\beta O_2)\beta)$	2 K_{β}=787,800	-35.01
O_2+$^{R}((\alpha^{*}O_2)\circ((\beta O_2)\beta)$ → $^{R}(\alpha^{*}O_2\circ\beta^{*}O_2)$	$K_{\beta}/2$=196,950	-31.43
Horse heart myoglobin		
$Mb+O_2$ → MbO_2 For two molecules of Mb	K=2.20 x 105	-63.4
Whole Blood Standard Conditions		
	K_{α}=15,090	
4 O_2+$^{T}(\alpha^{*}\bullet\beta^{*})$→	K_C=9.41	-106.7
$^{R}((\alpha^{*}O_2)\circ(\beta^{*}O_2))$	K_{β}=393,900	
E-Free Electrolyte	K_{α}=789,000	
0.05 M BisTris, pH 7 with HCl, 20OC	K_{β}=272,000	-134.6

Assignment of the value of $\Delta G°(^{T}HbO_2)_4/BPG)$ for the endothermic change, ^{T}state to ^{R}state, in protein structure

BPG, a potent E-molecule, binds avidly to $^{R}Hb_4$. In the process of forming the binary complex, the structure of $^{R}Hb_4$ changes to that of $^{T}(Hb_4/BPG)$. In contrast to the great stability of $^{T}Hb_4$/BPG, the equilibrium constant for the binary complex of BPG, yielding the ^{R}state binary complex, $^{R}(HbO_2)_4/BPG$, is low. $\Delta G°$ for reversing the binding of BPG to $^{R}Hb_4$, $\Delta G°$ ($^{R}(Hb)_4$, BPG), is +33.7 kJ/mol at 25°C. A correction due to the increase in temperature was not applied. $\Delta G°$ for binding of BPG by ^{R}state conformations, $\Delta G°(^{R}(HbO_2)_4/BPG)$, can be calculated directly from Equation (3) since all other values are known, estimated, or assigned. This procedure computes a value of $\Delta G°$ for binding of BPG to an ^{R}state structure, $\Delta G°(^{R}state/BPG)$=-10.5 kJ/mol, corresponding to an equilibrium constant for binding of BPG to R-state conformations, $^{K}(^{R}(HbO_2)_4/BPG)$=58.9 L/mol. O_2-Equilibrium binding curves at pH 9.1 demonstrate binding of IHP to R-state conformations.

DISCUSSION

$^{R}Hb_4$, free of E-molecules, such as BPG or 0.10 M chloride ions demonstrates a high affinity for O_2, being half saturated with O_2 in 2 µM O_2/L. Addition of stoichiometric amounts of BPG to solutions of $^{R}Hb_4$ results in formation of a binary complex with markedly diminished affinity for O_2. The product is a ^{T}state binary complex. The reaction can be written as follows, where BPG is indicated by a bullet: and superscript * indicates equivalent binding by a pair of subunits.

$$(^{T,1}\alpha, ^{R,2}\beta_2)(^{T,2}\alpha, ^{R,1}\beta_2) + \bullet \underset{\Delta G^{O} = -33.7\ kJ/mol}{\overset{K_{\bullet} = 8 \times 10^{5}\ L/mol}{\rightleftharpoons}} {}^{T}(\alpha^{*}\bullet\beta^{*}) \quad .....(b)$$

The reaction of BPG with $^{R}Hb_4$ is exothermic: $\Delta G°$=-33.7 kJ/mol. The history of observations of the properties of human hemoglobin, until approximately 1967, were conducted without knowledge of the effect of BPG on the properties of hemoglobin. Analysis of the O_2 equilibrium binding curve of E-free preparations of human Hb_4 reveals a pair of equivalent cooperative dimers: ($\alpha_1\beta_2$) and ($\alpha_2\beta_1$). It may be incorrect to describe these cooperative dimers as being ^{R}state. An ^{R}state β-chain regulates an α-chain of diminished affinity.α-Chains in cooperative dimers of $^{R}Hb_4$, then, are not ^{R}state until an ^{R}β-chain binds O_2. The cooperative dimer, nevertheless,

demonstrates a much higher affinity for O_2 than does $^T(\alpha^* \bullet \beta^*)$, requiring 35 μmol O_2/L for half saturation. Significant allosteric structure is present in $^RHb_4$. Addition of BPG to $^RHb_4$ results in: (i) formation of $^T(Hb_4)$/BPG; (ii) increased proximal strain in $^T\alpha$-chains; (iii) imposition of steric hindrance to O_2-binding by β-chain heme moieties. β-chain heme moieties in $^T(Hb_4/BPG)$ may be ^{R}state in reactivity and simply unable to gain access to an O_2 molecule due to distal side steric hindrance. If it was not possible to reverse the effects of binding BPG on ^{T}β-chain reactivity, the O_2 equilibrium binding curve would be described by a simple equation of state accounting only for species I and II of Equation (a).

Imposition of the stereochemical model on the Adair (1925) sequence of four O_2-binding reactions results in an equation of state acknowledging the existence of a ^{T}state → ^{R}state structure change. The discussion concerning binding of BPG to $^RHb_4$ suggests that an exothermic input of as much as-33.7 kJ/mol would be required. It is unlikely that BPG actually separates from the globin moiety in the ^{T}state → ^{R}state structure change. Our procedure is viewed from the perspective of thermodynamics: assuming release of BPG from a ^{T}state intermediate and rebinding to form an ^{R}state intermediate. The molecule of BPG bound to ^{T}state conformation remains, in all likelihood, attached to the globin moiety throughout the ^{T}state → ^{R}state transition. The physical realty of an equilibrium constant for a ^{T}state → ^{R}state change is supported by the virtual identity of the values for K_Δ returned by equilibrium binding curves for both CO and O_2, in whole blood, as well as purified human hemoglobin for O_2 in the presence of 0.1 M NaCl: 0.0574; 0.02602; 0.03252; respectively Following the ^{T}state → ^{R}state change, step II to step III, there are only equivalent O_2-binding reactions by ^{R}state β-chains, these reactions further stabilizing the ^{R}state conformation. Binding of O_2 to ^{R}β-chains and release of O_2 from $^R\beta O_2$-chains is free of changes in elements of allosteric structure. A ^{T}state structure is entirely different than an ^{R}state. ^{T}state and ^{R}state structures are, in fact, different molecules [8-14].

Since the curve fitting procedure is based on a model in which a pair of α-chains are equivalent and a pair of β-chains are equivalent, there can only be only be one ^{T}state → ^{R}state change and, therefore, only two structures: ^{R}state and ^{T}state. That single ^{T}state → ^{R}state transition occurs between species II and species III, Equation (a). Although there are two structural states, the model which underlies the Perutz/Adair equation is incompatible with the two state models in which the reactant, product, and all intermediate species are capable of being in either of the two states. These conclusions do extend to hemoglobin in whole blood from other species. These conclusions, however, do not address the validity of the two state models in other systems.

The value of K_Δ, 0.02602, establishes the ability of the binary complex of hemoglobin and DPG in red blood cell to release O_2 from arterial blood. The release of O_2 from the equivalent ^{R}state $^\beta O_2$-chains is, kinetically, first order. Recapture of O_2 by species III is extinguished by the thermodynamically favoured conversion of species III to species II, which is not capable of binding O_2 to β-chains. If O_2 depleted β-chains remained as ^{R}state haemoglobin molecules, the ability of the red blood cell to release O_2 would be dramatically impaired, O_2 molecules being subject to recapture before diffusing through the red blood cell membrane, thereby escaping from the RBC. The efficiency of O_2 transport to mitochondria of the components of the systemic circulation from arterial blood is dependent upon the conversion of species III to species II. Using the Perutz/Adair equation to fit equilibrium binding date, the value of $\Delta G°(K_\Delta)$ is revealed to be remarkably well fitted to the purpose of O_2 transport from arterial blood to mitochondria.

CONCLUSION

Variation in values of K_Δ across the spectrum of mammals, birds and lizards offers new insights into comparative respiratory physiology. A significant increase in the value of K_Δ would lower the rate at which rbcs could deliver O_2 to mitochondria. The Perutz/Adair equation of state is the first probe of respiratory function to reveal insight into the mechanism of enhanced transport of O_2 from the lungs to the respiratory tissues. The picture, however, is incomplete without consideration of the consequences of the cyclic variation in the pH of the interior of red blood cells. Doing so is the subject of another communication.

ACKNOWLEDGEMENT

Francis Knowles would like to acknowledge the help of Professor Glen Lo at Nicholls State University in providing assistance with curve fitting procedures and providing copies of XYCALC. Francis Knowles would to thank Professor Quentin Gibson and the Department of Biochemistry at Cornell University for their hospitality and assistance in elaborating instrumentation for determination of equilibrium binding curves of hemoglobin for O_2 (1972-1977). Francis Knowles would like to thank the Department of Chemistry and Biochemistry at UCSD for continuing appointments as a Lecturer and Professors Douglas Magde and Michael Tauber for hosting the author as a Visiting Scholar..

REFERENCES

1. Knowles F, Doyle SJ, Magde D. Architecture of allosteric structure. rate equations, rate constants, and equilibrium constants for reaction of: Hb4 with O_2 and (HbO_2) 4 with dithionate, in the presence of 2, 3-Bisphosphoglycerate.
2. Perutz MF, Fermi G, Luisi B, Shaanan B, Liddington RC. Stereochemistry of cooperative mechanisms in hemoglobin. Cold Spring Harb Symp Quant Biol. 1987 ;20(9): 309-321.
3. Perutz MF. Stereochemistry of cooperative effects in haemoglobin. Nature. 1970; 228(5273): 726-739.
4. Adair GS, Bock AV, Field Jr H. The hemoglobin system: VI. The oxygen dissociation curve of hemoglobin.J Biol Chem. 1925;63(2): 529-545.
5. Kernohan JC, Roughton FJ. The precise determination of the bottom of the oxyhemoglobin dissociation curve of human blood. 1972: 54-64.
6. Roughton FJ, Deland EC, Kernohan JC, Severinghaus JW. Some recent studies of the oxyhemoglobin dissociation curve of human blood under physiological conditions and the fitting of the Adair equation to the standard curve. 1972.
7. Severinghaus JW, Roughton FJ, Bradley AF. Oxygen dissociation curve analysis at 98.7–99.6% saturation. 1972:63.
8. Roughton FJ. The equilibrium of carbon monoxide with human hemoglobin in whole blood. Ann N Y Acad Sci. 1970;174(1): 177-188.
9. de Bruin SH, Janssen LH. The interaction of 2, 3-diphosphoglycerate with human hemoglobin: effects on the alkaline and acid bohr effect. J Biol Chem. 1973;248(8): 2774-2777.
10. Garby L, DE Verdier CH, Gerber G. Binding of 2, 3-diphosphoglycerate and adenosine triphosphate to human haemoglobin A. Eur J Biochem. 1969;10(1): 110-115.
11. Knowles FC. Binding of organic phosphates by human hemoglobin at

alkaline pH-values. Biochem Biophys Res Commun. 1980;92(3): 1060-1065.

12. Knowles F, Magde D. Resolution of the equilibrium constant for the t state r state conformational change of human hemoglobin into endothermic and exothermic component reactions.1934.

13. Chanutin A, Curnish RR. Effect of organic and inorganic phosphates on the oxygen equilibrium of human erythrocytes. Arch Biochem Biophys. 1967;121(1): 96-102.

14. Monod J, Wyman J, Changeux JP. On the nature of allosteric transitions: a plausible model. J Mol Biol. 1965;12(1): 88-118.