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John Edsall and ligand-linked subunit interactions in hemoglobin

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Ever since my days as a graduate student in Berkeley, John Edsall provided a model for scientific rigor. In the 1960s, the authoritative volume by Edsall and Wyman, *Biopyhsical Chemistry*, was one of the rare books that covered a wide range of the topics of interest in this discipline, and its authors took on the aura of founding fathers. In addition, my thesis adviser, Howard Schachman, often referred to John Esdall's high standards and would invoke his hypothetical reactions to help chart an appropriate course with respect to issues of scientific ethics. At the level of a poignant detail, I was struck by the multi-authored 1966 article on carbonic anhydrase from the Esdall laboratory [1] in which the different results for the enzyme's sedimentation coefficient were presented with the name and date of the author responsible. The values differed only slightly, but the paper made a lasting impression on me as the only example I could recall of a multi-authored paper in which individual responsibility was assigned. It would be tempting to speculate on whether the widespread adoption of this practice might have changed the face of biomedical research.

After the completion of my thesis, while working in Paris as a post-doctoral fellow at the Pasteur Institute in the laboratory of Jacques Monod, I met Jeffries Wyman and he invited me to Rome for an extended visit, where I spent several weeks early in 1968. That began a lengthy acquaintance, nourished by long walks in various countries over the years, during which he often mentioned his friend John Edsall. I finally had the occasion to meet John Edsall in the mid-1970s during a visit to Harvard, but it was only in the mid-1980s that circumstances led to a period of active interactions between him and myself. At that time, I had been on the faculty of Cornell University for many years and was enjoying a sabbatical leave in Paris, when Dominique Bonne, a colleague from my tubulin collaborations, asked my opinion about a paper by Gregorio Weber that had just appeared in the Proceedings of the National Academy of Sciences [2]. I had not seen the paper, but quickly found it and discovered that Gegorio Weber discussed one of the subtle features of the Monod–Wyman–Changeux model that I had been aware of for some time, but which he interpreted as a flaw that disqualified the MWC model as a description for hemoglobin.

By then, the MWC model [3] that provided an explanation of the cooperative binding of oxygen based on only two states, T and R (corresponding respectively to deoxy- and oxy-hemoglobin) had been shown to provide a suitable representation of

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the properties of hemoglobin under a wide range of conditions [4–7]. The T state, with a relatively low affinity for oxygen, was assumed to be favored over the R state in the absence of ligand because of stronger interactions between subunits ('quaternary constraint'). However, the R state was assumed to be present at a low incidence even in the absence of oxygen (the $T_0 \rightleftarrows R_0$ equilibrium was defined by the allosteric constant, $L_0 = [T_0/R_0]$, where the subscript refers to the number of ligands bound, zero in this case), in marked contrast to the alternative formulation involving an 'induced' conformational change [8]. According to the MWC model, as oxygen is added to the system, the R state—with a relatively high affinity for oxygen, but with weaker interactions between subunits, i.e. the 'relaxed' state—becomes progressively favored and at saturation with oxygen predominates over the small fraction of molecules in the T state (see Fig. 1). The issue raised by Gregorio Weber, as discussed below, revolved around the extent to which the dissociation of hemoglobin $\alpha_2\beta_2$ T state tetramers into $\alpha\beta$ dimers is modified by the addition of ligand, a subject that was of keen interest to me and that had begun to be addressed by Gary Ackers and his colleagues [9,10].

Although the concepts of 'quaternary constraint' and 'relaxed state' in the MWC model applied to quaternary interactions for any hypothetical allosteric homooligomer, hemoglobin, with its $\alpha_2\beta_2$ structure, had to be considered as a special case. The cooperative $\alpha_2\beta_2$ tetramers dissociate into $\alpha\beta$ dimers that are devoid of cooperativity, as Quentin Gibson and I had demonstrated soon after my joining the faculty at Cornell in 1968, by performing parallel sedimentation and flash photolysis experiments that I will now describe.

Although cooperativity is generally characterized as an increase in affinity for oxygen as a function of oxygen binding, leading to the classical sigmoidal curve, even more dramatic manifestations of cooperativity are produced in kinetic experiments. Such experiments can be achieved by rapid mixing, or more conveniently by flash photolysis with an appropriate ligand. For hemoglobin, the ligand carbon monoxide (CO) has generally been employed for flash photolysis

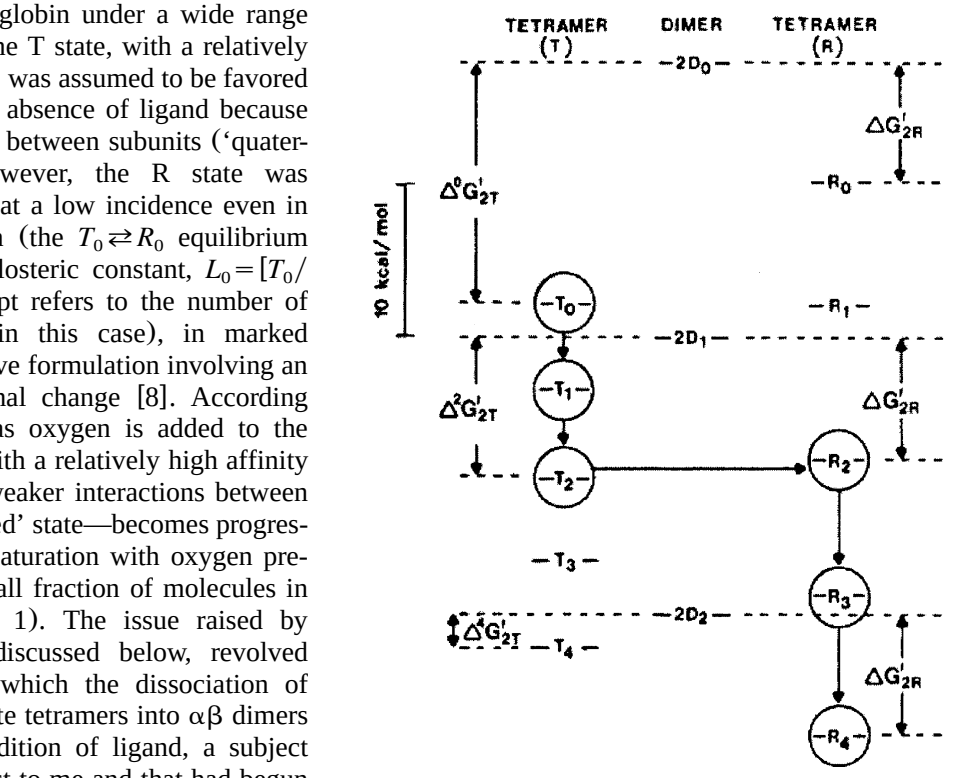

Fig. 1. The various states of hemoglobin tetramers (R and T) and dimers on a scale of Gibbs free energy. For dimers (D) and tetramers in the R and T states, the integer after the letter corresponds to the number of ligand molecules bound. The basic ligand binding properties of the T and R states are represented by their ladders of equally spaced binding steps, $-RT \ln K_T$ and $-RT \ln K_R$, respectively. The lower position of T_0 compared to R_0 corresponds to the enhanced stability of the T state in the absence of ligand, $-RT \ln L_0$. The circled species indicate the major states in the sequence of ligand binding events. Energies of tetramer stabilization relative to the corresponding dimer species are indicated on the far left for the T state and on the far right for the R state. While the stabilization of the R state ($\Delta G'_{2R}$) remains constant relative to the dimers, the stabilization for the T state relative to the corresponding dimer state diminishes with increasing numbers of ligand molecules bound, as indicated for the T tetramers with 0, 2 or 4 ligands bound, ($\Delta^0 G'_{2T}$, $\Delta^2 G'_{2T}$, or $\Delta^4 G'_{2T}$, respectively). For simplicity, only the subunit interaction energies for T and R state tetramers with an even number of ligands bound are presented. The subunit interaction energies are calculated using 'association' constants, e.g. $\Delta G'_{2R} = RT \ln ({}^R K_{4,2})^{-1}$.

because its bond to the heme iron is more sensitive to light than the corresponding bond for oxygen (O_2). Although CO has a much higher affinity

than O_2 for hemoglobin, the cooperativity of its equilibrium binding is similar to oxygen's and therefore it is a suitable substitute for oxygen in kinetic experiments.

Pioneering experiments by Quentin Gibson prior to our collaboration had established a number of important foundations for the exploration of the MWC model for hemoglobin. For example, in a remarkably prescient article published in 1959 [11], he was able to demonstrate that HbCO subjected to a long flash produced 'normal' molecules of deoxyhemoglobin that recombined slowly with CO, whereas HbCO subjected to a short flash produced previously undetected 'exceptional' molecules that recombined rapidly with CO. These exceptional molecules, designated Hb*, also displayed an absorption spectra with a lower maximum in vicinity of the deoxyhemoglobin peak at 430 nm. In the context of the MWC model, these results were subsequently interpreted as indicating that the long flash produced slowly-reacting unliganded T state, whereas the short flash produced rapidly-reacting unliganded (or partially liganded) R state. In effect, this demonstrated that molecules of unliganded (or partially liganded) R, a major premises of the MWC model, do indeed exist. Two additional important observations were reported in this extraordinary article: the fraction of rapidly-reacting molecules increased when the concentration of the HbCO was decreased, as well as when the mercurial *p*-chloromercuribenzoate (PCMB) was added. In fact, it was subsequently established that both dilution and PCMB favor dissociation of the HbCO into αβ dimers.

Our parallel sedimentation and kinetic experiments demonstrated that αβ dimers of hemoglobin were non-cooperative, with R state-like properties, i.e. rapid ligand binding kinetics. Specifically, we were able to show that the rapidly-reacting form following a flash represented an increasingly large fraction of the molecules as the HbCO solutions were diluted, producing a curve that overlapped precisely with the fraction of dimers in the solutions. Both properties ('weights and rates'), therefore, corresponded to the tetramer–dimer dissociation constant for liganded hemoglobin (R state), with a characteristic value of ${}^{R}K_{4,2} \sim 10^{-6}$ M [12,13]. As a result, the issue of quaternary constraint was focused on the tetramer–dimer equilibrium, since all cooperative interactions arose from interactions at this level.

With my first doctoral student, John Thomas, a method was developed that exploited the high affinity of the αβ dimers for CO to measure the tetramer–dimer dissociation constant of the T state (deoxyhemoglobin), providing a value of ${}^{T}K_{4,2} \sim 10^{-12}$ M [14]. Since both T and R state tetramers are in equilibrium with R-like αβ dimers, the intrinsic allosteric constants, L_0, governing the equilibrium between unliganded T and R states could be equated to the ratio of the tetramer–dimer dissociation constants of the two states ($L_0 = {}^{R}K_{4,2}/{}^{T}K_{4,2}$). The value of this ratio, $\sim 10^6$ was in agreement with estimates of L_0 from fitting ligand-binding equilibrium curves [5]. These data confirmed the hypothesis that the T and R states were distinguished by enhanced quaternary constraint for T and relaxed quaternary interactions for R, as formulated initially [3].

The point raised by Gregorio Weber was the extent that ${}^{T}K_{4,2}$ varied as a function of the number of ligands bound. Assuming that the T-state represented a single, well-defined conformational state, he argued that its subunit dissociation constant should be independent of the number of ligands bound. Since measurements had been made on the tetramer–dimer equilibria by Gary Ackers and his colleagues for mono- and di-liganded hemoglobin in the T state that revealed higher dissociation constants than for deoxyhemoglobin [9,10], Gregorio Weber concluded that the MWC model was incompatible with the properties of hemoglobin [2].

My view at the time was that the increased tetramer–dimer dissociation constant of T-state molecules with increased ligand occupancy was required by a full linkage analysis of the MWC model, as a consequence of the high affinity of the αβ dimers [5]. Since dimers display an affinity for ligands approximately 100-fold stronger than T-state tetramers, for each ligand bound, the tetramer–dimer dissociation constant should increase by ~ 100, in close correspondence to the values that had been observed [10]. The story became more complex subsequently with distinctions proposed for symmetric and asymmetric partially

liganded molecules [15]. The dénouement of this story has involved considerable reverberations [16–19] that we have discussed in detail elsewhere [20], but from all points of view it is agreed that ligand binding favors dissociation of the T state.

After reading Gregorio Weber's article, my reaction was to write to him to suggest that a more complete linkage analysis should be considered. However, my arguments did not convince him that the subject needed to be reopened. Since Gregorio Weber had thanked John Edsall for his advice on an earlier version of the manuscript, I sent John Edsall a copy of the correspondence with Gregorio Weber and asked for his advice about how best to proceed. After reviewing the matter, he replied that he was in agreement with my arguments and suggested that I put them into form for publication as an article for the Proceedings of the National Academy that he would contribute. As communications continued, he made extensive and incisive suggestions for presenting this material that led me to invite him to join me as a co-author on the publication. In this way our joint publication [21] was initiated.

The principal figure of our article was an energy diagram that provided a graphic representation of the changes in T-state tetramer–dimer dissociation with ligand binding (Fig. 1). For individual T and R tetramer states, the quaternary stability is presented with respect to dissociation into the corresponding dimers. Since the affinity for ligand is identical for R tetramers and αβ dimers, the tetramer–dimer interaction energy for the R state is independent of ligand binding, yielding a free energy indicated by $\Delta G'_{2R}$. However, in the case of the T tetramers, ligand binding favors dissociation to αβ dimers because of the higher affinity of the latter, yielding decreasing energies of stabilization, $\Delta^0 G'_{2T} < \Delta^2 G'_{2T} < \Delta^4 G'_{2T}$. Therefore, the enhanced dissociation of the T tetramers with increased ligand binding is a direct consequence of the principles of the MWC model.

Although my opportunity to work with John Edsall occurred at a time when his attention had turned mainly to historical aspects of biochemistry, he launched whole-heartedly into our collaboration. I was moved by his enthusiasm, as our collaboration advanced by mail and, after my return to Cornell, by telephone, including an excited weekend call from John to make a point that he did not want to hold until Monday. Several years later, while chairing a session on hemoglobin at a meeting at the NIH, I was able to call upon him to discuss our paper and we were both delighted that this occasion enabled him to participate again in an active scientific debate.

References

[1] J.M. Armstrong, D.V. Myers, J.A. Verpoorte, J.T. Edsall, Purification and properties of human erythrocyte carbonic anhydrase, J. Biol. Chem. 241 (1966) 5137–5149.
[2] G. Weber, Order of free energy couplings between ligand binding and protein subunit association in hemoglobin, Proc. Natl. Acad. Sci. USA 81 (1984) 7098–7102.
[3] J. Monod, J. Wyman, J.-P. Changeux, On the nature of allosteric transitions: A plausible model, J. Mol. Biol. 12 (1965) 88–118.
[4] S.J. Edelstein, Extensions of the allosteric model for hemoglobin, Nature 230 (1971) 224–227.
[5] S.J. Edelstein, Cooperative interactions of hemoglobin, Annu. Rev. Biochem. 44 (1975) 209–232.
[6] R.G. Shulman, J.J. Hopfield, S. Ogawa, Allosteric interpretation of hemoglobin properties, Quart. Rev. Biophys. 8 (1975) 325–420.
[7] J. Baldwin, C. Chothia, Haemoglobin: the structural changes related to ligand binding and its allosteric mechanism, J. Mol. Biol. 129 (1979) 175–220.
[8] D.E. Koshland, G. Némethy, D. Filmer, Comparison of experimental binding data and theoretical models in proteins containing subunits, Biochemistry 5 (1966) 365–385.
[9] F.C. Mills, M.L. Johnson, G.K. Ackers, Oxygenation-linked subunit interactions in human hemoglobin: experimental studies on the concentration dependence of oxygenation curves, Biochemistry 15 (1976) 5350–5362.
[10] G.K. Ackers, M.L. Johnson, Linked functions in allosteric proteins: Extension of the concerted (MWC) model for ligand-linked subunit assembly and its application to human hemoglobin, J. Mol. Biol. 147 (1981) 559–582.
[11] Q.H. Gibson, The photochemical formation of a quickly reacting form of haemoglobin, Biochem. J. 71 (1959) 293–303.
[12] S.J. Edelstein, M.J. Rehmar, J.S. Olson, Q.H. Gibson, Functional aspects of the subunit association–dissociation equilibria of hemoglobin, J. Biol. Chem. 245 (1970) 4372–4381.
[13] S.J. Edelstein, Q.H. Gibson, Weights and rates in hemoglobin: properties of the α-β dimer, in: B. Chance,

(Ed.), Symposium on Heme and Heme Proteins, Academic Press, New York, 1971, pp. 417–429.
[14] J.O. Thomas, S.J. Edelstein, Observation of the dissociation of unliganded hemoglobin, J. Biol. Chem. 247 (1972) 7870–7874.
[15] G.K. Ackers, M.L. Doyle, D. Myers, M.A. Daughtery, Molecular code for cooperativity in hemoglobin, Science 255 (1992) 54–63.
[16] S.J. Edelstein, An allosteric theory for hemoglobin incorporating asymmetric states to test the putative molecular model for cooperativity, J. Mol. Biol. 257 (1996) 737–744.
[17] E.R. Henry, C.M. Jones, J. Hofrichter, W.A. Eaton, Can a two-state MWC allosteric model explain hemoglobin kinetics?, Biochemistry 36 (1997) 6511–6528.
[18] N. Shibayama, H. Morimoto, S. Saigo, Reexamination of the hyper thermodynamic stability of asymmetric cyanomet valency hybrid hemoglobin, (alpha+CN-beta+CN-)(alpha beta): no preferentially populating asymmetric hybrid at equilibrium, Biochemistry 36 (1997) 4375–4381.
[19] G.K. Ackers, J.M. Holt, Y. Huang, Y. Grinkove, A.L. Klinger, L. Denisov, Confirmation of a unique intra-dimer cooperativity in the human hemoglobin alpha(1)beta(1)half-oxygenated intermediate supports the symmetry rule model of allosteric regulation, Proteins 4 (2000) 23–43.
[20] M.C. Marden, L. Kiger, C. Poyart, S.J. Edelstein, Identifying the conformational state of bi-liganded haemoglobin, Cell. Mol. Life Sci. 54 (1998) 1365–1384.
[21] S.J. Edelstein, J.T. Edsall, Linkage between ligand binding and the dimer-tetramer equilibrium in the Monod-Wyman-Changeux model of hemoglobin, Proc. Natl. Acad. Sci. USA 83 (1986) 3796–3800.