Complete Snapshots of the Hemoglobin Allosteric Transition in a Single Crystal Form

Hemoglobin was one of the first protein structures ever to be solved by X-ray crystallography. Two crystal structures of hemoglobin, tense (T) and relaxed (R), have shaped our view of protein allostery, but it is increasingly clear that the hemoglobin allosteric transition cannot be adequately described by just these two states. We determined a wide range of nine hemoglobin quaternary structures including previously unidentified intermediates by using a novel crystal form in which hemoglobin is free to adopt any structure, depending on the conditions. Our findings give a comprehensive picture of the equilibrium conformers and transition pathway for hemoglobin.

Human hemoglobin, an $(\alpha\beta)_2$ tetrameric hemoprotein (see Fig. 1, top), acts as an efficient O_2 carrier by changing its affinity for O_2 more than 100-fold during the four successive binding steps [1]. Such a cooperative interaction is generally thought to be attributed to an allosteric transition from a tense (T) to a relaxed (R) state that binds O_2 more strongly [2]. Earlier crystallographic studies on the deoxy unliganded T state and fully liganded R state of hemoglobin provided a simple view of this transition, which involves a rotation of one $\alpha\beta$ dimer ($\alpha1\beta1$) relative to the other ($\alpha2\beta2$) by approximately 15° [Fig. 1(a)] [3]. In contrast to this textbook description, later studies argued that there may be intermediate states in this transition, but capturing those structures proved difficult [4-6]. Thus, significant debate continues as to the sequence and nature of the allosteric transition.

We recently succeeded in fully capturing the hemoglobin allosteric transition by using three isomorphous hemoglobin crystals [Fig. 1(b)] [7]. We used metal substitution [8], a variety of conditions, and interdimer chemical cross-linking [9] to generate three kinds of hemoglobin crystals, namely, half-CO-liganded hemoglobin crystals with and without phosphate (referred to as HL+ and HL–, respectively) and fully-H_2O-liganded met-hemoglobin crystal with phosphate (referred to as FL+) [Fig. 2(a)]. Using X-ray crystallography, we found that each crystal contains three tetramers (A, B, and C) with different quaternary structures [Fig. 2(a)]. We also found that the conformational set of this crystal form varies with the samples, and thus we could identify nine distinct conformations in a single crystal form. Since the structure of the $\alpha1\beta1$ (or $\alpha2\beta2$) dimer remains nearly the same in all the tetramer conformations, each quaternary structure was characterized by a movement of its $\alpha2\beta2$ dimer as a whole (an RMSD in the Cα atoms in the $\alpha2\beta2$ dimer) with respect to the $\alpha2\beta2$ dimer of R-state hemoglobin after superposing the $\alpha1\beta1$ dimers of both structures [Fig. 2(b)], and also by a difference distance matrix of the $\alpha1\beta2$ subunits using R as a reference [Fig. 2(c)]. Significantly, the nine snapshot structures cover the entire conformational space of hemoglobin, ranging from T to R2 (the second relaxed quaternary structure) through R [Fig. 1(b) and Fig. 2(b), (c)].

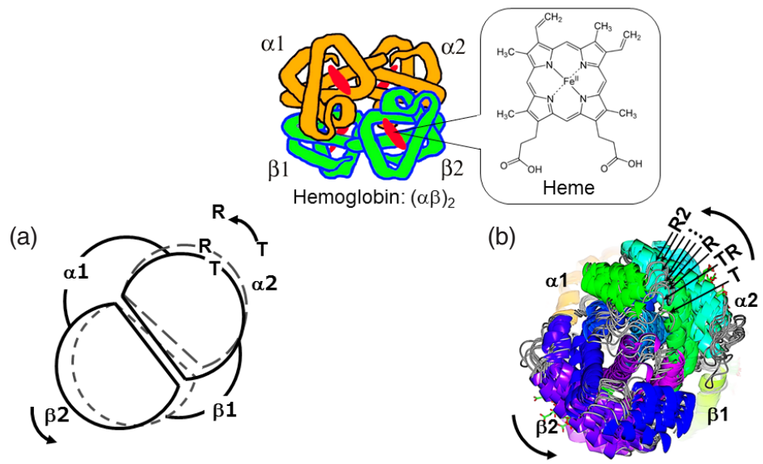

Figure 1: Hemoglobin allosteric transition. (a) Textbook description. (b) Our new view.

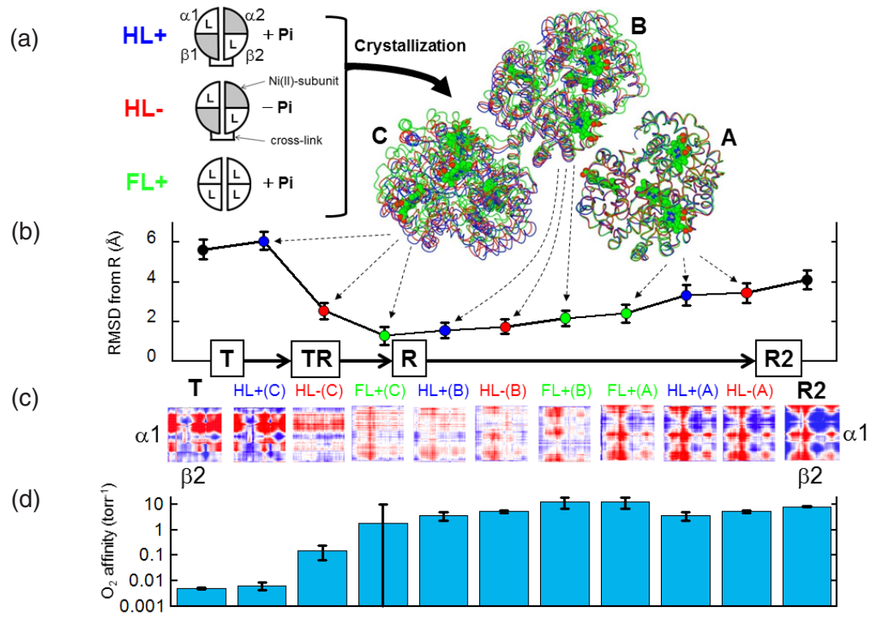

Figure 2: Nine hemoglobin structures in a single crystal form. (a) Three samples (HL+, HL–, and FL+) that crystallize in the same crystal form, each capturing three tetramers (A, B, and C) with different structures. (b) RMSD of the $\alpha2\beta2$ dimers after superposing the $\alpha1\beta1$ dimers of each structure and R. (c) Difference distance matrices of the $\alpha1\beta2$ subunits using R as a reference (red indicates closer than R and blue indicates the opposite). (d) O_2 affinity in the crystals.

Using microspectrophotometry, we next measured the O_2 equilibrium curves in the crystals to assess the O_2 affinity of each of the nine quaternary structures [Fig. 2(d)] [7]. As shown in Fig. 2, hemoglobin can assume various relaxed states from classical R to R2, all of which have a loosely packed dimer-dimer interface and bind O_2 with a similar high affinity to the isolated $\alpha\beta$ dimer, confirming the central role of quaternary (interdimer) constraints in the cooperativity of tetrameric hemoglobin. More importantly, we discovered a previously unidentified intermediate between T and R (called TR) with a truly intermediate O_2 affinity (Fig. 2), which has been sought by many research groups for a couple of decades [4-6]. While the O_2 affinity of deoxyhemoglobin is known to vary widely with solution conditions [1], so far all known deoxy T crystals contain essentially the same T quaternary structure [10] with a very low affinity for O_2 [11], suggesting that there is an as yet unidentified allosteric state with intermediate O_2 affinity. We now identify TR as a candidate for this new allosteric state, which may represent the missing link between hemoglobin structure and function that has remained elusive for several decades.

Our results illustrate the hemoglobin allosteric transition with a stepwise pattern in moving from the more tense-like state (classical T state) to the new intermediate TR to the relaxed-like states [Fig. 1(b) and see details in Fig. 2]. The results strongly suggest that hemoglobin exists in pre-existing equilibrium between multiple, not only two, conformations, providing an important reminder that allosteric proteins may have multiple quaternary structures that are structurally and functionally different.

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