Artificial allosteric systems

Iwao Tabushi*
Department of Synthetic Chemistry, Kyoto University
Sakyo-ku, Kyoto 606, Japan

Abstract - The artificial allosteric molecule, gable porphyrin metal complexes, were prepared by connecting two independent metal porphyrin molecules by covalent bond. The author would like to discuss mostly on allosteric mechanism of the artificial allosteric systems. Our gable porphyrin system behaves similarly to natural Hb, binding small molecules like CO, O₂, or base, cooperatively. The original structure of the dimeric porphyrin, especially when they are very strongly cooperative, usually needs chemical strain between two porphyrin rings and this strain is released by the first binding of small molecule which causes coordination change of the metal porphyrin.

INTRODUCTION

Artificial Hb/Mb type O₂ transport system as man-made allosteric molecules

The basic structural difference between Hb and Mb is in their quarternary structures, tetrameric and monomeric protein, respectively. The plausible process to prepare Mb and Hb is "evolution", in which a common protein probably similar to the present Hb is converted to Hb and Mb by relaxing amino acid residues. The adsorption isotherms given by Hb based on the structural difference as listed in Table 1.

One of the great advances in mechanistic elucidation in connection with the structural feature, was made by assuming unequal equilibrium constants for the first, second, third and fourth O₂ binding to the tetrameric protein in spite of the fact that the four subunits have very similar local structures (ref. 2b,c). This progress was immediately followed by the new, greater progress made by Monod (ref. 2d), who first proposed a possibility of significant conformation change in the subunit protein, from T- (tense) to R- (relaxed) states. After the addition of some sophistication (stepwise T/R conversion instead of the one-step conversion originally proposed) by Koshland (ref. 2e,f), the present concept of "allosteric" mechanism became complete.

<table>
<thead>
<tr>
<th>TABLE 1 Proposed mechanisms in cooperativity</th>
</tr>
</thead>
<tbody>
<tr>
<td>cooperativity and allosteric system</td>
</tr>
<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td>Hill (1910)</td>
</tr>
<tr>
<td>Adair (1925)</td>
</tr>
<tr>
<td>Pauling (1935)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Monod, Wymand and Changeux (1965)</td>
</tr>
<tr>
<td>Koshland, Nemethy and Filmer (1966)</td>
</tr>
<tr>
<td>Conway and Koshland (1968)</td>
</tr>
</tbody>
</table>

* Manuscript completed by Prof. Iwao Tabushi before he died on 22 March 1987. Typed and submitted by Dr. Shin-ichi Kugimiya

581
SPECTROSCOPIC EVIDENCE SUPPORTING ALLOSTERIC MECHANISM

Subunit-subunit interaction is clearly shown by the detailed X-ray analysis of Hb (ref. 3). As already mentioned, there are many amino acid residues (aa) in the single subunit interacting with amino acid residues (aa') in the other subunit. The subunit-subunit interaction given by the sum of the elementary aa-aa' interactions is usually too strong to be broken even in very dilute solution. The interaction keep Hb as a tetramer even in a dilute solution. The strong subunit interaction makes each Hb subunit considerably "twisted" (T-form) as predicted by Monod. Among the conformation changes induced pull down movement of the "F"-chain seems to be very important. This causes imidazole 5th ligand for the porphyrinato Fe(II) center (see Fig. 1) to move apart from the porphyrinato Fe(II) reaction center (see Fig. 2). The actual change in Fe(II)-imidazole distance (ca 0.1 Å) is clearly seen from the X-ray crystallographies (Fig. 3) (ref. 3).

![Diagram](image1)

**Fig. 1.** Ferrous porphyrin reaction center in a monomeric O₂ binding protein in its deoxy form. Compare this schematic structure to that of deoxy-Hb in Fig. 2.

![Diagram](image2)

**Fig. 2.** Ferrous porphyrin reaction center in deoxy Hb.

![Diagram](image3)

**Fig. 3.** Schematic representation of the results of X-ray crystallographies of (a) deoxy Hb and (b) deoxy Mb, from ref. 3b with modification; CH₄, CH₂ mean meso positions of porphyrin ring.

ARTIFICIAL ALLOSTERIC MOLECULAR SYSTEM

An allosteric system must be provided i) smooth conformational change of a subunit from the T- to R-state, (ii) considerably lower O₂ affinity of the T- than the R-state, (iii) "information transfer" from subunit 1 to subunit 2, (etc.), (iv) "local configuration activity change" by information transferred. It looks a quite challenging target for us to synthesize artificial allosteric molecules.

Rebek et al have been trying to prepare artificial allosteric systems 1-2 by applying the principle of metal-crown binding-conformation change to the high affinity/low affinity conversion. In this system, "chemical information" is really transferred from one site to the other, intra-molecularly (ref. 4, 5, 6).
Traylor et al. has taken an entirely different approach to the artificial allosteric system (ref. 7) consisting of two subunits. In his model, subunit association, one of the important characteristics of the natural allosteric systems, is taken more seriously into account.
It may be a good time for us to examine more carefully what is going on in Hb, the sophisticated natural molecular system. We have tried to mimic the Hb/Mb allosteric system. In Fig. 4, a sketchy description about a series of physicochemical phenomena involved in the Hb allosteric effect is given. Looking at Fig. 4, one must notice that two crucial keys are missing in the previous molecular designs. One is the existence of breakable subunit bridges and the other is the T/R conversion. By picking up these two missing keys, a design of a new molecular system may be carried out as shown in Fig. 5. T-form via tight subunit interaction.

(1) local sites in first \( \text{O}_2 \)
very similar induced configuration change in
structure and Fe-O function bond (T \( \rightarrow \) R)
formation (Fe-L distance) (T \( \rightarrow \) R)

(1') slightly different

\( T_1, T_2 \)

(4) cleavage of (5) total (6) indirect (7) enhanced
intersubunit conformation induced second
bridge change in binding
unbound subunit change
(T \( \rightarrow \) R)
(T \( \rightarrow \) R in
unbound subunit

Fig. 4. Simplified Hb allosteric mechanism
*: This makes allosteric effect much larger (ref. 5g)

Fig 5 Possible artificial allosteric system Of Hb type

The stable T/R couples were independently prepared by changing the length of the arm (in Fig. 6, the number of CH\(_2\) unit). For N,N-diimidazolyl-methane, for example, a typical T-complex was formed judging from the resonance Raman (32 cm\(^{-1}\) shift) and other spectroscopies. These T- and R-complexes adsorb various coordinating guests — such as \( \text{O}_2\), CO, or coordinating bases strongly and reversibly. The reversible \( \text{O}_2 \) adsorptions to the complex 1 (CO, base) was ascertained by repeated appearance and disappearance of the electronic absorptions characteristic of \( \text{P} \cdot \text{Fe(II)} \cdot \text{O}_2 \cdot \text{B (oxy) and P} \cdot \text{Fe(II)} \cdot \text{B (deoxy) complexes on repeated O}_2 \) application and app evacuation.

Fig 6 Allosteric molecules of Tabushi.
The T-complex showed much lower affinities toward \( \text{O}_2, \text{CO} \) or \( B' \) than the corresponding R-complexes and also than the corresponding monomeric coordination ligand (see Table 2). Therefore, we are really really mimicking Hb in (i) a subunit bridge \((B(\text{CH}_2)_2)B\) linked two "locally identical" subunits to give the T-complex and (ii) the T-complex thus formed has lower affinity than the corresponding R-complex. In order to prepare corresponding R-complex model having the same local structure as the T-complex, \( a,N,N\)-diimidazolylpropane, for example, was used as an intersubunit bridge. The R-complex thus formed showed "normal" affinity toward \( \text{O}_2 \).

In the rather wide concentration range of bisimidazolymethane remarkable positive cooperativity in the \( \text{CO} \) (ref. 9), base (ref. 10) or \( \text{O}_2 \) (ref. 11) binding was observed. The second guest binding to the allosteric molecular system was by a factor of 31-16 stronger than the first guest binding, thus showing typical sigmoid adsorption characteristics (Fig 7).

---

**TABLE 2**  
Affinity differences between T-complexes and R-complexes in native and artificial systems

<table>
<thead>
<tr>
<th></th>
<th>CO</th>
<th>1MI</th>
<th>( \text{O}_2 )</th>
<th>ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>deoxy Hb</td>
<td>( K_1 )</td>
<td>10</td>
<td>0.007</td>
<td>8a</td>
</tr>
<tr>
<td></td>
<td>( K_4 )</td>
<td>149</td>
<td>0.67</td>
<td>8a</td>
</tr>
<tr>
<td>monomer Hb</td>
<td>625</td>
<td>2.94</td>
<td></td>
<td>8b</td>
</tr>
<tr>
<td>deoxy Mb</td>
<td>83.3</td>
<td>1.42</td>
<td></td>
<td>8b</td>
</tr>
<tr>
<td>Gable\cdot\text{M}_2\cdot\text{DIM}</td>
<td>( K_1 )</td>
<td>0.8(^a)</td>
<td>300(^b)</td>
<td>0.002(^c)</td>
</tr>
<tr>
<td></td>
<td>( K_2 )</td>
<td>18(^a)</td>
<td>11000(^b)</td>
<td>0.018(^c)</td>
</tr>
<tr>
<td>Gable\cdot\text{Fe}_{2}\cdot\text{DIP}</td>
<td>330(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPP\cdot\text{M}\cdot\text{1MI}</td>
<td></td>
<td>45000(^b)</td>
<td>0.025(^c)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\): \( M=\text{Fe}^{II}, \text{DMF}, 25^\circ\text{C}, \text{mmHg}^{-1} \);  
\(^b\): \( M=\text{Zn}^{II}, \text{benzene}, 24^\circ\text{C}, \text{M}^{-1} \);  
\(^c\): \( M=\text{Co}^{II}, \text{DMF}, -20^\circ\text{C}, \text{mmHg}^{-1} \)

---

![Fig. 7. \( \text{O}_2 \) adsorption isotherms in the artificial system and mechanism of allosteric \( \text{O}_2 \) binding (Tabushi's model)](image)
Hill's coefficient estimated was 1.9, 1.7 and 1.5, for CO, N-MeIm, and O₂ adsorption, satisfactorily large for a two-subunit system which has a theoretically maximal Hill's coefficient of 2 (ref. 13). However, we should mention here that Kn/K₀ is much better indices than Hill's coefficient to define allosteric effect in a quantitative fashion.

REFERENCES

1. Abbreviation in this review: Hb, hemoglobin; Md, myoglobin; aa, amino acid; Im, imidazoly; R, relaxed; T, tense; TPP, tetraphenylporphyrin; B, base; Gable, gable porphyrin; P, porphyrin ligand; HIM, imidazole; DMI, 1,2-dimethylimidazole; IMI, 1-methylimidazole; 2MI, 2-methylimidazole; DIM, N,N-diimidazolylmethane; DIP, α,ω-di-N-imidazolylpropane

2 a) A.V. Hill, J. Physiol (London), 40, VI-VIII (1910)
   b) G.S. Adair, J. Biol. Chem., 53, 529 (1925)
   e) D.E. Koshland, Jr., G. Nemethy, D. Filmer, Biochemistry, 5, 365 (1966)

3 a) M.F. Perutz, Nature (London), 228, 726 (1970)


5 a) for CO binding: W. Huestis, M.A. Raftery, J. Biochem. 14, 1886, (1975); for O₂ binding: K. Imai, Biochem., 12, 798 (1972)
   b) E. Antonini, M. Brunori, "Hemoglobin and Myoglobin in their Reactions with Ligands", New York, 1971, p.225
   c) for CO binding: see ref. 10; for 1-MeIm binding: see ref. 9, for O₂ binding: see ref. 11

   c) I. Tabushi, T. Nishiya, Israel J. of Chem. in press