Pure & Appl. Chem., Vol. 60, No. 4, pp. 581–586, 1988.
Printed in Great Britain.

Artificial allosteric systems

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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, O_2, 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 O_2 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 relacing 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 O_2 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 1 Proposed mechanisms in cooperativity

	cooperativity and allosteric system	ref.
Hill (1910)	single step with K_1	2a
Adair (1925)	four steps with different K's	2b
Pauling (1935)	$K_1 < K_2 < K_3 < K_4$ four monotonically increasing K's, subunit interaction	2c
Monod, Wymand and Changeux (1965)	T ⇌ R concerted	2d
Koshland, Nemethy and Filmer (1966)	T ⇌ R sequential	2e
Conway and Koshland (1968)	modified sequential	2f

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

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).

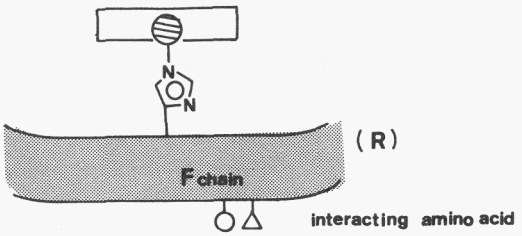


Fig. 1. Ferrous porphyrin reaction center in a monomeric O_2 binding protein in its deoxy form. Compare this schematic structure to that of deoxy-Hb in Fig. 2.

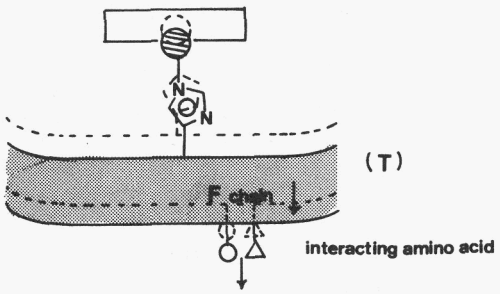


Fig. 2. Ferrous porphyrin reaction center in deoxy Hb.

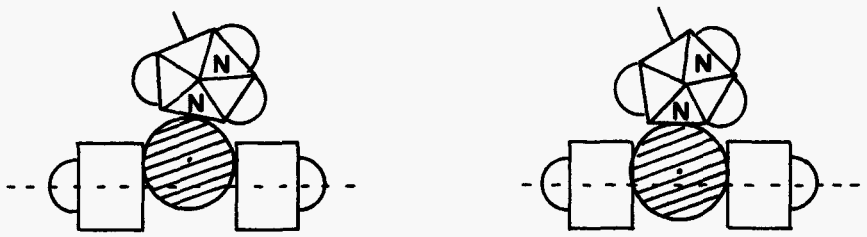
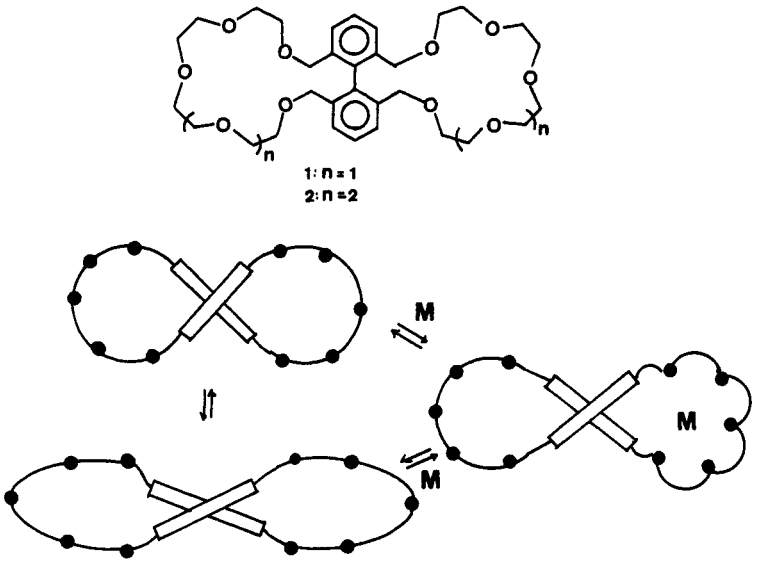


Fig. 3. Schematic representation of the results of X-ray crystallographies of (a) deoxy Hb and (b) deoxy Mb, from ref. 3b with modification; CH_4, CH_2 mean meso positions of porphyrin ring.

ARTIFICIAL ALLOSTERIC MOLECULAR SYSTEM

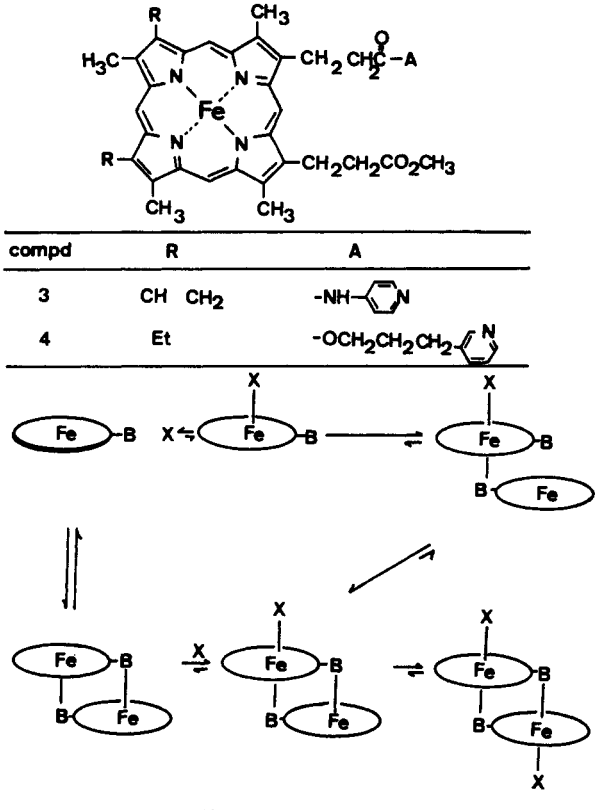
An allosteric system must be provided i) smooth conformarional change of a subunit from the T- to R-state, (ii) consederably lower O_2 affinity of the T- than the R-state, (iii) "information transfer" from subunit 1 to subunit 2, (etc.), (iv) "local configuration activity change" by information transferred. 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, intramolecularly (ref. 4, 5, 6).



Rebek's cooperative metal binding system.

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.



compd	R	A
3	CH CH_2	-NH-
4	Et	$-OCH_2CH_2CH_2-$

X = CN^-, CO

Dimeric cooperative binding system (Traylor's model)

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 mimick the Hb/Mb allosteric system. In Fig. 4, a sketchy desctiption 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 T states of very similar structure and function → first O_2 binding → Fe-O bond formation → (2) inderect induced configuration change T → R (Fe-L distance) → (3) total conformation change in bound subunit (T → R) →

(1') slightly different T_1, T_2*

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

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

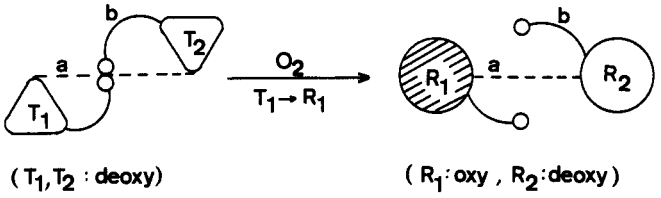


Fig 5 Possible artificial allosteric system of Hb type

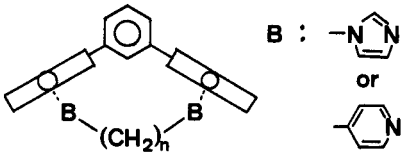
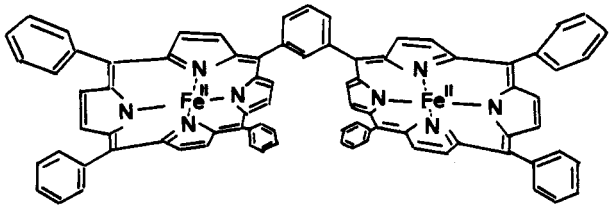


Fig 6 Allosteric molecules of Tabushi.

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-diimidazolylmethane, 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 O_2, CO, or coordinating bases strongly and reversibly. The reversible O_2 adsorptions to the complex 1 (CO, base) was ascertained by repeated appearance and disappearance of the electronic absorptions characteristic of P·Fe(II)·O_2·B (oxy) and P·Fe(II)·B (deoxy) complexes on repeated O_2 application and appevacuation.



Fe_2^{II}·Gable porphyrin

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

		CO	1MI	O_2	ref.
deoxy Hb	K_1	10		0.007	8a
	K_4	149		0.67	8a
monomer Hb		625		2.94	8b
deoxy Mb		83.3		1.42	8b
Gable·M_2·DIM	K_1	0.8[a]	300[b]	0.002[c]	8c
	K_2	18 [a]	11000[b]	0.018[c]	8c
Gable·Fe^{II}_2·DIP		330 [a]			12
TPP·M·1MI			45000[b]	0.025[c]	8c

a: $M=Fe^{II}$, DMF, 25°C, $mmHg^{-1}$; b: $M=Zn^{II}$, benzene, 24°C, M^{-1};
c: $M=Co^{II}$, DMF, -20°C, $mmHg^{-1}$

The T-complex showed much lower affinities toward O_2, 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 ($B(CH_2)_nB$) 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, α,ω-N,N-diimidazolylpropane, for example, was used as an intersubunit bridge. The R-complex thus formed showed "normal" affinity toward O_2.

In the rather wide concentration range of bisimidazolylmethane remarkable positive cooperativity in the CO (ref. 9), base (ref. 10) or 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 characterisitics (Fig 7).

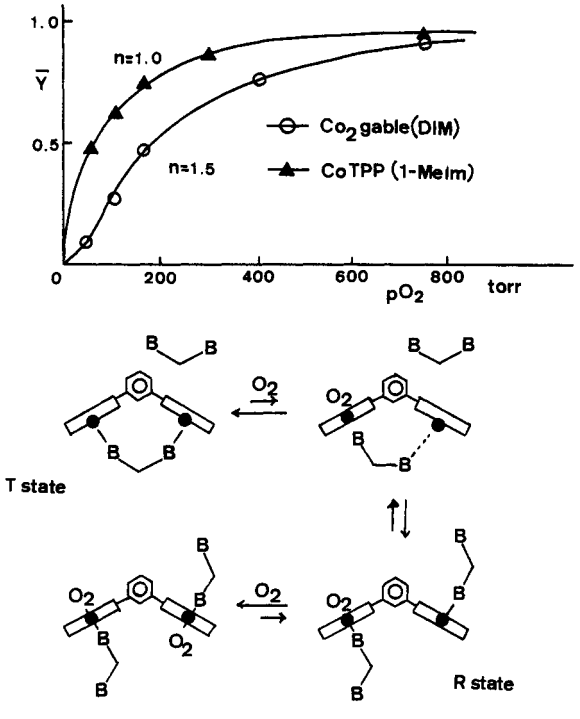


Fig. 7. O_2 adsorption isotherms in the artificial system and mechanism of allosteric O_2 binding (Tabushi's model)

Hill's coefficient estimated was 1.9, 1.7 and 1.5, for CO, N-MeIm, and O_2 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 K_{n+1}/K_n is much better indices than Hill's coefficient to define allosteric effect in a quantitative fashion.

REFERENCES

1. Abbreviation in this review: Hb, hemoglobin; Mb, myoglobin; aa, amino acid; Im, imidazolyl; R, relaxed; T, tense; TPP, tetraphenylporphyrin; B, base; Gable, gable porphyrin; P, porphyrin ligand; HIm, imidazole; DMI, 1,2-dimethylimidazole; 1MI, 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., 63, 529 (1925)
   c) L. Pauling, Proc. Natl. Acad. Sci. USA, 21, 186 (1935)
   d) J. Monod, J. Wymand, J.-P. Changeux, J. Mol. Biol., 12, 88 (1935)
   e) D.E. Koshland, Jr., G. Nemethy, D. Filmer, Biochemistry, 5, 365 (1966)
   f) A. Conway, D.E. Koshland, Jr., Ibid., 7, 4011 (1968)
3 a) M.F. Perutz, Nature (London), 228, 726 (1970)
   b) J. Baldwin, C. Chothia, J. Mol. Biol. 129, 175 (1979)
   c) A. Brzozowski, Z. Derewenda, E. Dodson, G. Dodson, M. Grabowski, R. Liddington, T. Sharzynski, D. Vallely, Ibid, 305, 74 (1984)
   d) G.L. Woolery, M.A. Waters, K.S. Suslick, L.S. Powers, T.G. Spiro, J. Am. Chem. Soc., 107, 2370 (1985)
4 J. Rebek, Jr., R.V. Wattley, T. Costello, R. Gadwood, L. Marshall, J. Am. Chem. Soc. 93, 584 (1981)
5 K. Onan, J. Rebek, Jr., T. Constello, L. Marshall, J. Am. Chem. Soc., 105, 6759 (1983)
6 J. Rebek, Jr., T. Costello, L. Marshall, R. Wattley, R.C. Gadwood, K. Onan, J. Am. Chem. Soc., 107, 7481 (1985)
7 T.G. Traylor, M.J. Mitchell, J.P. Ciccone, S. Nelson, J. Am. Chem. Soc., 104, 4986 (1982)
8 a) for CO binding: W. Huestis, M.A. Raftery, J. Biochem. 14, 1886, (1975); for O_2 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 O_2 binding: see ref. 11
9 I. Tabushi, S. Kugimiya, M.G. Kinnaird, T. Sasaki, J. Am. Chem. Soc., 107, 4192 (1985)
10 I. Tabushi, S. Kugimiya, T. Sasaki, J. Am. Chem. Soc., 107, 5159 (1985)
11 I. Tabushi, T. Sasaki, J. Am. Chem. Soc., 105, 2901 (1983)
12 I. Tabushi, S. Kugimiya, J. Am. Chem. Soc. 108, 6926 (1986)
13 I. Tabushi, T. Nishiya, Israel J. of Chem. in press