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The calculation of the Interaction Energy Between Subunits of Hemoglobin molecule

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THE CALCULATION OF THE INTERACTION ENERGY BETWEEN SUBUNITS OF HEMOGLOBIN MOLECULE

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SUMMARY

The interaction energies between subunits are calculated for oxy- and deoxyhemoglobin by taking into account the van der Waals interaction, the electrostatic interaction and the hydrogen bonds.

In the transition from deoxy to oxy form, the van der Waals interaction becomes attractive only by 8 kcal/mole as a whole for hemoglobin, although the interaction in each pair of subunits except for the pair of subunits $\alpha_1 \alpha_2$ is much varied by the order of 15 kcal/mole. Moreover, the electrostatic interaction becomes more repulsive by 5 kcal/mole in the pair of $\alpha_1\beta_2$ (or $\alpha_2\beta_1$) without taking into account the salt bridges. Therefore the energy difference between deoxy and oxy quaternary structure is mainly to be attributed to the twelve salt bridges in which the C-terminals of four subunits and DPG participate.

The calculated result leads to a conclusion that the entropy factor due to the rotation of C-terminal is essential in the transition from deoxy quaternary structure to oxy quaternary structure. This substantiates Perutz' model energetically, and give some quide to Monod-Wyman-Changeux's model.

INTRODUCTION

During the past decades, a great deal of work has been done to clarify the mechanism of heme-heme interaction in the oxygenation process of hemoglobin. But the mechanism has been tried to be explained only abstractly in terms of Monod-Wyman-Changeux's model ¹⁰ or Koshland-Némethy-Filmer's.² model.

The Monod-Wyman-Changeux's model postulates the existence of only two stable states (the R-state and the T-state) that are accessible to hemoglobin. Namely, all the subunits in the quaternary T-state are unreactive and those in the quaternary R-state are reactive. This postulate leads naturally to the relation $K_1 \leq K_2 \leq K_3 \leq K_4$ among the equilibrium constants K_1 , K_2 , K_3 and K_4 in Adair's ³ theory.

Koshland-Nemethy-Filmer's sequential model implies that the change in tertiary structure of each subunit directly affects the ligand affinity of its neighbours. Hence, according to this conception, the energy which is used to change each subunit from an unreactive form to a reactive one is different at each step of the oxygenation process.

However, recently, extensive X-ray diffraction analyses have been made by Perutz *et al.* ⁴⁻⁸⁾ to determine the atomic coordinates of oxy- and deoxyhemoglobin. Moreover, Perutz ⁹⁾ proposed the stereochemical mechanisms of both heme-heme interaction and Bohr effect based on the comparison of the atomic coordinates of oxy- and deoxyhemoglobin. But, his model does not seem to be well substantiated on the

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basis of energetics.

In the present paper, the energies of van der Waals interaction, electrostatic interaction, and hydrogen bonds between subunits are calculated for deoxy- and oxyhemoglobin with the atomic coordinates of hemoglobins.⁸⁻¹⁰⁾ The calculated result will provide some guide to the models mentioned above.

The sequential and structural notations for hemoglobin conform to those of Perutz et al..4.6

ATOMIC COORDINATES OF HEMOGLOBIN USED IN THE PRESENT CALCULATION

I used the atomic coordinates determined from the 3.0 Å resolution electron density map of horse decoxyhemoglobin and those determined from the 2.8 Å resolution map of horse oxyhemoglobin dy Perutz *et al.*,^{8, 9)} There are a few pairs of atoms within too short a distance in the atomic coordinates. However, they can be separated by slight rotation about a single bond with only a small energy change of tertiary structure. The treatments of these atom pairs are described in later section.



Fig. 1. Bond angles used in determination of the atomic coordinates of hydrogen atoms. All the nomenclature conforms to that of Edsall et al.¹² (a), data are taken from Pauling,¹³
(b), data are taken from Marsh.¹⁴ (c) ~ (f), all bond angles are taken to be the tetrahedral angle of 109.5°. (g), X-H bond belonging to the ring of phenylalanine, histidine, tryptophan and tyrosine is placed both in the plane and on the bisectors of the external angles of the ring.

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The coordinates of hydrogen atoms, which had not been listed in their table, were determined with the use of the bond lengths estimated as the sums of covalent radii ¹¹⁾ of the participant atoms and of the bond angles shown in Fig. 1. More rigorous selection of bond lengths and bond angles may not be Yasuhide Arata : The calculation of the interaction energy between subunits of hemoglobin molecule

needed because the atomic coordinates of hydrogen atoms are restricted by the coordinates of the atoms other than hydrogen which were already determined and because our purpose is not to calculate the conformational energy of tertiary structure but to estimate the energy difference between the quaternary structures of deoxy- and oxyhemoglobin.

There are found eight salt bridges between subunits in deoxyhemoglobin, namely, Val NA1(1) NH $_3^+ \cdots$ COO - Arg HC3 (141), Lys H10 (127) NH $_3^+ \cdots$ COO - Arg HC3 (141) and Asp H9 (126) COO - ... Gua + Arg HC3 (141) between α_1 (α_2) and α_2 (α_1) subunits and Lys C5 (40) NH $_3^+ \cdots$ COO - His HC3 (146) between α_1 (α_2) and β_2 (β_1) subunits, but these salt bridges are ruptured in oxyhemoglobin.

METHOD OF ENERGY CALCULATION

The calculations of the interaction energies between subunits are carried out by taking into account electrostatic interaction, van der Waals interaction and hydrogen bonds. These three types of interactions are assumed to be superposed in the evaluation of the interaction energies between subunits.

(A) Electrostatic Interaction

In the estimation of the electrostatic interaction between subunits, first must be considered how to treat the proton distribution on cationic and anionic sites in hemoglobin. For this purpose, the intrinsic pK of sites placed deeply in protein, the effective dielectric constant between a pair of charges in protein and *etc.* are examined with Tanford and Kirkwood's formula¹⁵⁾ for the work done in charging the sites on a globular protein. This formula is

$$W(\xi_{1}, \xi_{2}, \dots, \xi_{m}) = \frac{e^{2}}{2} \sum_{k=1}^{m} \sum_{l=1}^{m} \xi_{k} \xi_{l} A_{kl} - \frac{e^{2}}{2b} \sum_{k=1}^{m} \sum_{l=1}^{m} \xi_{k} \xi_{l} B_{kl} - \frac{e^{2}}{2a} \sum_{k=1}^{m} \sum_{l=1}^{m} \xi_{k} \xi_{l} C_{kl}, \qquad (1)$$

where

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$$A_{kl} = \frac{1}{D_i r_{kl}}$$
(2)

$$B_{kl} = \frac{1}{D_{i}} \sum_{n=0}^{\infty} \frac{(n+1)(D-D_{i})}{(n+1)D+nD_{i}} \left(\frac{\tau_{k}\tau_{l}}{b^{2}}\right)^{n} P_{n} (\cos\theta_{kl}).$$
(3)

The full expression for C_{kl} is not written out here because C_{kl} is a complicated function and because the third term is small with respect to the usual ionic strength. In these equations, $P_n (\cos \theta_{kl})$ represents ordinary Legendre polynomials, and r_k , r_l , r_{kl} and θ_{kl} are identified by Fig. 2; D_i and D are the dielectric constants of protein and solvent, respectively; b is the radius of the protein; a is the radial distance to which the salt ions in the solvent are excluded; ξ_k is the charge on the k-th sites, therefore ξ_k takes the values 0 or +1 for cationic sites, 0 or -1 for anionic ones. The first term (A term) is the direct electrostic interaction between the charges on the protein, the second term(B term) is the energy arising from the fact that the protein molecule is in a bounded cavity within a medium of higher dielectric constant D, and the third term(C term) is the interaction with the ions in solvent. In the following calculation, the values of a and b are assumed to be 31.0 Å ¹⁶ and 33.5 Å ¹⁷ respectively, for both oxy- and deoxyhemoglobin, and the dielectric constants $D_i = 4$ and D=81 are used.



2. Model of protein molecule. The points k and / represent the locations of the two charges. The dielectric constant is D_i within the radius b, and D outside it. Salt ions in the solvent cannot penetrate within the radius a.

(from Tanford and Kirkwood¹⁵⁾)

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According to Tanford and Kirkwood,¹⁵⁾ an intrinsic standard free energy change, $(\Delta F_{int}^*)_k$, for the k-th site on the protein, which is characterized by the intrinsic dissociation constant $(K_{int})_k$ with the relation $(\Delta F_{int})_k = -2.303RT (pK_{int})_k$, is given as follows. For a cationic site

$$(\Delta F_{\text{int}})_{k} = \Delta F_{k}^{\bullet} + \frac{e^{2}}{2b} (A_{kk} - B_{kk}) - \mu_{\text{H}}^{\bullet} +$$
(4)

and for an anionic site

$$(\Delta F_{\text{int}})_{k} = \Delta F_{k}^{*} - \frac{\mathbf{e}^{2}}{2b} (A_{kk} - B_{kk}) - \mu_{\text{H}}^{*} +$$
(5)

where $\mu_{\rm H}$ is the standard chemical potential of an hydrogen ion in the solution, ΔF_k^* is the difference between the free energy of a protein molecule having a proton on site k, in its hypothetical discharged form, and of a similarly discharged molecule not having a proton on site k. The ΔF_k^* and A_{kk} in equation 4 or 5 have the same value for all sites of a particular chemical kind, but B_{kk} depends on the depth of the site k within the molecule, as given by $(r_k/b)^2$ from equation 3 with $\cos\theta_{kk} = 1$.

It is also indicated by Tanford ¹⁸⁾ that the charged sites of the small organic molecule should be located at the depth of about 1 Å from the surface, when the Kirkwood's model is applied. Therefore, let us consider a "reference *j*-th site" which is located at the depth of 1 Å from the effective surface of protein and is of the same chemical kind as the *k*-th site. Then, the same relation as the equation 4 or 5 is obtained, for a cationic site

$$(\Delta F_{int}^{*})_{j} = \Delta F_{j}^{*} + \frac{e^{2}}{2b} (A_{jj} - B_{jj}) - \mu_{H}^{*} +$$
(6)

and for an anionic site

$$(\Delta F_{int}^{\bullet})_{j} = \Delta F_{j}^{\bullet} - \frac{e^{2}}{2b} (A_{jj} - B_{jj}) - \mu_{H}^{\bullet} +$$
⁽⁷⁾

By combination with equations 4 and 6, or 5 and 7, and by recalling the relations $\Delta F_k^* = \Delta F_i^*$ and $A_{kk} = A_{jj}$,



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we get, for a cationic site

$$(pK_{int})_{k} = (pK_{int})_{j} - \frac{1}{2.303RT} \frac{e^{2}}{2b} (B_{jj} - B_{kk}), \qquad (8)$$

and for an anionic site

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$$(pK_{int})_{k} = (pK_{int})_{j} + \frac{1}{2.303RT} \frac{e^{2}}{2b} (B_{jj} - B_{kk}).$$
(9)

Finally, the pK value of the k-th site immersed in protein is obtained approximately by replacing $(pK_{int})_j$ in equation 8 or 9 with the intrinsic pK of the corresponding free amino acid. The value of $e^2 B_{kk}/2b$ is shown in Fig. 3 as a function of the distance from the center of the molecule for three values of b.

It may be easily seen from Fig. 3 that the pK value of the sites placed within 20 Å from the center of protein varies by about 16 unit from that of free amino acid for $29 \sim 33$ Å of b. The used values of $(pK_{int})_j$ in equation 8 or 9 for arginine (quanidinium), Lysine $(\epsilon \cdot NH_3^+)$, value $(a \cdot NH_3^+)$, histidine (imidazolium), aspartic acid (COOH), and glutamic acid (COOH) are 13.2, 10.28, 9.62, 5.97, 3.65 and 2.18¹⁹ respectively.





[Proton Distribution]

I investigated, with the atomic model, the distribution of the proton binding and releasing sites of arginine, histidine, lysine, aspartic acid, glutamic acid, N-terminal, and C-terminal residues. Most of these sites are distributed over the surface of a hemoglobin molecule and are in contact with solvent. However, only 28 sites of these residues are buried interior to the protein, within 22 Å from the center of the protein, in the case of both oxy- and deoxyhemoglobin. The residues to which these 28 sites belong are common for oxy- and deoxyhemoglobin. They are as follows.

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α chain; Arg B12(31), Asp G1(94), Lys G6(99), His G10(103), His H5(122), Asp H9(126),
Lys H10(127), Arg HC3(141)
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β chain; Val NA1(1), Arg B12(30), Asp G1(99), Glu G3(101), Arg G6(104), His HC3(146)

Although the sites distributed over the surface may be charged by binding or releasing a proton, they may have little effect on the interaction energy between subunits because of the water shielding. This is ascertained by estimating the effective dielectric constant $D_{\rm E}$ between a pair of charges with the equations 1-3. The value of $D_{\rm E}$ depends primarily on the location of the charges with respect to the interface between the protein and solvent.

The value of D_E is calculated between a pair of charges, one on the surface and another in the interior of protein, for the case of $\theta_{kl} = 0$, and is shown in Fig. 4 as a function of distance from the center of protein. Fig. 4 shows that the charges over the surface have little effect on the interior within 25 Å from the center. As a matter of course, D_E is about 80 for a pair of charges on the surface. Therefore these residues over the surface are treated as neutral ones on the determination of the proton distribution. This treatment may be permitted also in estimating the energy difference between the quaternary structures of oxy- and deoxyhemoglobin.



Fig. 4. The effective dielectric constant
$$D_E$$

between a pair of charges, one on the
surface and another in the interior of
the protein. D_E is calculated by using
equations 1-3 with $\theta_{kl} = 0$ and $d = r_l =$
31.0 Å and is shown as a function of .
distance r_k of the interior placed charge
from the center of the molecule.

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Now, the proton distribution on the 28 sites deeply immersed in the protein must be determined by using the interaction energy between the charges on sites and the intrinsic free energy change arising from addition of a proton on a site. When W denotes a W from which the self energy term is omitted, the energy $E(\xi_1, \xi_2, \dots, \xi_{28})$ corresponding to a set proton distribution is given as

$$E(\xi_1, \xi_2, \cdots, \xi_{28}) = W'(\xi_1, \xi_2, \cdots, \xi_{28}) + 2.303 RT \sum_{k=1}^{\infty} \xi_k |pH - (pK_{int})_k|.$$
(10)

The self energy term in equation 1 is included in the pK_{int} in equation 10. The values of $E(\xi_1, \xi_2, \cdots, \xi_{28})$ are calculated for all the possible configurations under the usual conditions (ionic strength of 0.1M, pH value of 7.0, and at the temperature of 25°C). The used values of pK_{int} are obtained with the method mentioned in the preceding section. The calculated values of $E(\xi_1, \xi_2, \cdots, \xi_{28})$ are shown in

Fig. 5 for both oxy- and deoxyhemoglobin.

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The energy difference between the second lowest level and the lowest is about 16 kcal/mole for both oxy- and deoxyhemoglobin, and is much greater than the value of RT at room temperature (about 0.6 kcal/mole). Therefore, the proton distribution is determined to coincide with the lowest configuration $\xi_1 = \xi_2 = \dots = \xi_{28} = 0$. Namely, the 28 residues taken into account are also treated as neutral ones in calculation. Such a tendency is suggested by Tanford.²⁰



The electrostatic interaction energies between subunits are calculated with the same formula as the first term of equation 1, taking into account the partial charges on all constituent atoms of hemoglobin, namely,

$$U_{\rm E} = \sum_{k} \sum_{l} q_{k} q_{l} / D_{\rm i} r_{kl}, \qquad (11)$$

where r_{kl} is the inter-atomic distance between atom k in one subunit and atom l in another; q_k and q_l are partial charges on these atoms; D_i is the effective dielectric constant and $D_i = 4$ is used for all atom pairs. The partial charges for neutral amino acids have been determined by Poland and Scheraga²¹⁾ for homopolymers of the important amino acids. Their results may also be used as a good approximation for heteropolymers, because the backbone charges for the different homopolymers are all about the same.

Their partial charges are used in the present calculation. For the pairs of atoms within too short a distance, the sum of the van der Waals radii of the two atoms in the pair is assigned as the distance between them.

The energies of the salt bridges between subunits in deoxyhemoglobin are not included in the calculation mentioned above. It is an overestimation to calculate the interaction energy between subunits, including the salt bridges, by equation 11 directly, because the salt bridges are made over the surface and are in contact with solvent. Thus the energies of the salt bridges must not be evaluated with equation 11. The energy of a salt bridge is estimated as the interaction energy between a pair of charges separated by 3.5Å over the surface of the molecule by equation 1. The energy value thus estimated is -1.5 kcal/mole for a salt bridge.

(B) Van der Waals Interaction

A Lennard-Jones "6-12" potential function is used for the interaction of a pair of atom i in one subunit and atom j in another:

$$U_{ij} = d_{ij} / r_{ij}^{2} - e_{ij} / r_{ij}^{6},$$
(12)

where r_{ij} is the inter-atomic distance. The used values of d_{ij} and e_{ij} for all pairs between C, N, O and H are taken from Scott and Scheraga's paper.^{22;} The calculations are carried out for the following pairs of atoms; pairs of non-hydrogen atoms (C, N and O) within 6 Å, pairs of non-hydrogen atoms and hydrogen atoms within 5 Å, and pairs of hydrogen atoms within 4 Å. The van der Waals interaction between subunits is evaluated by the superposition of the interactions of all the pairs of atoms mentioned above. For the pairs of atoms within too short a distance mentioned in the previous section, the energy value of the van der Waals interaction for each of these pairs is assumed to be zero.

(C) Hydrogen Bond

The energies of hydrogen bonds are calculated with the use of the potential function proposed by Scott and Scheraga²²⁾ This is an extension of the potential function proposed by Lippincott and Schroeder²³⁾ to include the dependency upon the direction of a hydrogen bond. Namely,

$$U_{\rm HB} = -D^{*} (\cos^{2} \theta_{1} + \cos^{2} \theta_{2}) \exp\left[\frac{-n^{*} (R - r - r_{0}^{*})^{2}}{2 (R - r)}\right] -A \exp(-bR) - \frac{1}{2}A \left(\frac{R_{0}}{R}\right)^{6} \exp(-bR_{0}).$$
(13)

0

The values for the adjustable parameters A, b, $D_i^* n_i^* r_0^*$ and R_0 are obtained from Schroeder and Lippincott's paper ²⁴ for various types of hydrogen bonds. The variable R is the distance of N-O or O-O in angstroms; θ_1 and θ_2 are the angles formed by the intersections of the direction of N-H(or O-H) and those of the two lone-pair orbitals of the 0 atom.

RESULT

The calculated values of the electrostatic interaction energies between subunits except for salt bridges are listed in Tables I-IV and those of the van der Waals interactions are listed in Table V for the four pairs of subunits, a_1a_2 , $\beta_1\beta_2$, $a_1\beta_1$ and $a_1\beta_2$. In these tables, the energy values are shown in kcal/ mole for every pair of segments. For each pair of segments, the upper values show the energy in oxyhemoglobin and the lower values in deoxyhemoglobin. The values in Table VI show the sums of the energy values in Tables I-IV and those in Table V for each pair of subunits. In this table, the numbers in parentheses show the energy values obtained taking into account the salt bridges mentioned in the previous section. First the main features of these interactions are taken up briefly by pairs of subunits. Yasuhide Arata: The ealculation of the interaction energy between subunits of hemoglobin molecule

Table I. The calculated energy values of the electrostatic interaction between a_1 and a_2 subunits for oxy and deoxy forms. The upper and the lower values for each pair correspond to the ones for oxy and deoxy forms, respectively. All the residues are treated as neutral. The dielectric constant $D_i = 4$ is used for all atom pairs. The energy values are given in units of kcal/mole.

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α ₂ α ₁	N A	A	A B	В	С	C D	E	EF	F	FG	G	GH	Н	НC
N A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.3
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-0.1	-0.2
А		-0.1	0.0	0.1	-0.1	0.1	-0.1	-0.1	0.2	0.3	0.0	0.0	-1.2	-0.9
		-0.1	0.0	0.1	-0.1	0.0	-0.1	-0.2	0.1	0.5	0.5	0.0	-0.5	-0.4
A B			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0
			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
в				0.2	-0.1	0.0	-0.1	0.0	. 0.1	0.1	-0.1	0.0	-0.1	-0.2
				0.2	-0.1	0.0	-0.1	0.0	0.0	0.0	-0.1	0.0	-0.1	0.0
с		2			0.1	0.0	0.0	0.0	-0.1	0.0	0.2	0.0	-0.1	0.0
					0.1	0.0	0.1	0.0	-0.1	0.0	0.2	0.0	-0.1	0.0
СД						0.0	0.0	0.0	0.0	0.0	-0.1	0.0	0.1	0.0
						0.0	0.0	0.0	0.0	0.0	0.0	0.0	0 1	Q 0
Е							0.1	0.0	-0.1	-0.1	-0.2	0.0	0.4	0.2
							0.1	0.0	0.0	0.0	-0.2	0.0	0.5	0.0
EF								0.1	0.0	0.0	0.2	0.0	-0.3	-0.2
								0.0	0.0	0.0	0.1	0.0	-0.1	0.0
F									-0.3	0.3	0.1	0.1	-0.4	0.0
									0.0	0.0	0.1	0.1	-0.4	0.0
FG										0.0	0.1	0.0	-0.1	0.0
										0.0	0.1	0.0	-0.1	0.0
G											10	-0.1	0.0	-0.1
											0.1	-0.1	0.0	-0.1
СΗ												0.0	0.1	0.1
												0.0	0.1	0.0
н													0.2	0.8
													-0.1	-0.1
нс														-0.2
														0.0

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Table II. The calculated energy values of the electrostatic interaction between β_1 and β_2 subunits for oxy and deoxy forms. The upper and the lower values for each pair correspond to the ones for oxy and deoxy forms, respectively. All the residues are treated as neutral. The dielectric constant $D_i = 4$ is used for all atom pairs. The energy values are given in units of kcal/mole.

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β_1 β_2	N A	A	В	С	C D	D	Е	EF	F	FG	G	GH	Н	НC	_
N A	0.0	0.0	0.0	0 .1	0.0	0.0	0.0	0.1	0.0	-0.2	0.0	0.0	0.2	-0.3	
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
A		0.0	0.0	-0.1	0.0	0.0	0.0	-0.1	-0.1	01	0.1	0.0	-0.4	-0.2	
		0.0	0.0	-0.1	0.0	0.0	-0.1	-0.1	0.1	0.1	0.1	0.0	-0.5	0.1	
В			0.3	-0.2	0.0	0.0	0.0	0.2	0.1	0.0	-0.2	0.0	0.0	0.0	
			0.1	-0.1	0.0	0.0	-0.1	0.0	0.1	0.0	-0.1	0.0	-0.1	0.0	
С				0.1	Q. 0	0.0	0.1	0.0	-0.1	0.0	0.4	0.0	-0.3	0.0	
				0.0	0.0	0.0	0.0	0.0	-0.1	0.0	0.2	0.0	-0.1	0.0	
CD					0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
					0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
D						0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	
						0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Е							0.2	0.1	-0.1	-0.1	-0.2	0.0	0.8	0.2	
							0.2	0.0	-0.1	-0.1	-0.1	0.0	0.3	0.0	
EF								0.0	0.0	0.0	0.0	0.0	-0.1	-0.1	
								0.0	0.0	0.0	0.1	0.0	-0.2	0.0	
F									L 0	0.0	0.1	0.0	-0.6	-0.1	
									0.1	0.0	0.0	0.0	-0.3	0.0	
FG										0.0	0.0	0.0	-0.2	0.0	
										0.0	0.0	0.0	0.0	0.0	
G											-0.7	-0.1	0.2	0.2	
											0.7	0.0	0.1	0.0	
GH												0.0	0.1	0.0	
												0.0	0.1	0.0	
H													0.6	-0.4	
													-0.6	-0.2	
НC														0.0	
														0.0	

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Table III. The calculated energy values of the electrostatic interaction between a_1 and β_1 subunits for oxy and deoxy forms. The upper and the lower values for each pair correspond to the ones for oxy and deoxy forms, respectively. All the residues are treated as neutral. The dielectric constant $D_i = 4$ is used for all atom pirs. The energy values are given in units of kcal/mole.

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β_1	N A	A	В	с	C D	D	E	EF	F	FG	G	GH	H	нс
NA	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Α	0.0	0.0	-0.9	0.4	-0.1	0.5	0.3	-0.1	-0.1	-0.1	1.1	-0.1	-0.6	0.0
	0.0	0.0	-0.6	0.1	-0.2	0.4	0.4	-0.1	-0.1	0.0	0.3	0.0	-0.4	0.0
A B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	Q 0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
В	0.0	-0.5	0.0	-0.1	0.0	0.2	0.6	-0.1	-0.1	-0.1	-0.3	0.4	0.0	-0.1
	0.0	-0.5	0.1	0.0	0.0	0.1	0.4	-0.1	-0.1	0.0	-0.3	0.7	-0.8	0.0
с	0.0	0.1	-0.1	0.1	0.0	0.0	-0.2	0.0	0.0	0.0	0.0	-0.2	0.0	0.0
	0.1	0.1	0.0	0.0	0.0	0.0	-0.2	0.1	0.1	0.0	0.1	-0.1	0.4	0.0
CD	0.0	-0.2	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.0	-0.3	0.0
	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0 .0	0.0	0.2	0.0	-0.3	0.0
Е	0.0	0.2	0.4	-0.2	0.0	-0.2	-0.3	0.1	0.1	0.0	-0.2	-0.2	0.6	0.0
	0.0	0.2	0.3	-0.1	0.0	-0.2	-0.3	0.1	0.1	0.0	-0.1	-0.1	0.4	0.0
EF	0.0	0.0	-0.2	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	-0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F	0.0	-0.1	-0.1	0.0	0.0	0.1	0.2	0.0	0.0	0.0	-0.1	0.1	0.1	0.0
	0.0	-0.1	-0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	-0.2	0.0	0.0	0.0
FG	0.0	-0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	-0.1	0.0	0.1	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
G	-0.1	0.4	-0.5	-0.1	0.0	0.0	-0.2	0.0	-0.1	0.0	3.1	-0.9	-0.6	0.0
	0.0	0.5	-0.3	0.2	0.0	0.0	-0.2	-0.1	-0.1	0.0	3.3	-0.9	-0.5	0.0
GН	0.0	0.1	0.6	-0.1	0.0	-0.1	-0.3	0.0	0.0	0.0	-1.0	0.2	0.2	0.0
	0.0	0.0	1.7	-0.1	0.0	-0.3	-0.4	0.0	0.1	0.0	-1.0	0.3	0.2	0.0
н	0.0	-0.4	1.1	-0.3	-0.1	1.0	0.7	0.0	0.0	0.0	-1.6	0.3	0.4	0.0
	0.0	-0.4	-0.1	0.0	0.0	1.1	0.8	0.0	0.0	0.1	-1.2	0.2	0.2	0.0
нс	0.0	0.0	-0.1	0.1	0.0	0.0	-0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

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Table IV. The calculated energy values of the electrostatic interaction between α_1 and β_2 subunits for oxy and deoxy forms. The upper and the lower values for each pair correspond to the ones for oxy and deoxy forms, respectively. All the residues are treated as neutral. The dielectric constant $D_i = 4$ is used for all atom pairs. The energy values are given in units of kcal/mole.

β_2	N A	А	В	С	C D	D	E	EF	F	FG	G	GH	н	НC
N A	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A	0.0	0.0	-0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	-0.1	0.0	0.2	0.0
	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	-0.2	0.0	0.2	0.0
A B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
В	0.0	0.0	0.1	0.2	-0.1	0.1	0.2	0.1	0.6	0.0	-0.7	0.0	1.1	0.3
	0.0	0.0	0.0	0.0	-0.1	0.0	0.2	0.1	0.3	-0.2	-0.6	0.0	1.0	0.8
с	0.0	0.0	0.0	1.6	0.0	0.0	-0.3	0.0	-0.1	1.1	0.7	0.0	-0.7	-0.1
	0.0	0.1	0.1	1.0	-0.1	0.0	-0.2	0.1	1.1	-0.1	-0.9	0.0	-0.8	-0.9
C D	0.0	0.0	-0.1	-0.3	0.0	0.0	0.2	0.0	0.1	-0.2	-0.1	0.0	0.2	0.1
	Q. O	0.0	-0.1	-0.5	0.1	0.0	0.1	0.0	-0.5	-0.6	0.3	0.0	-0.1	0.0
Е	0.0	0.0	0.1	-0.3	0.0	-0.1	0.0	0.0	-0.1	-0.1	0.1	0.0	-0.2	0.0
	0.0	0.0	0.1	-0.2	0.0	-0.1	0.0	-0.1	-0.2	-0.1	0.1	0.0	-0.3	-0.1
EF	0.0	0.0	0.0	0.0	0.0	0.0	-0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F	0.0	0.1	1.1	-1.3	0.0	-0.1	-0.3	0.1	0.3	0.2	-0.5	0.0	0.2	0.0
	0.0	0.1	0.6	-0.1	-0.1	-0.1	-0.3	0.0	0.3	0.1	-0.5	0.0	0.2	0.0
FG	0.0	0.1	0.2	1.5	-0.4	0.1	-0.2	0.1	0.3	0.2	-0.2	0.0	0.1	0.0
	0.0	0.0	0.0	0.0	0.6	0.1	-0.2	0.0	0.1	0.1	0.0	0.0	0.0	0.0
G	0.1	-0.1	-1.2	1.6	0.1	0.0	0.1	-0.1	-0.4	-0.1	2.2	0.0	-0.4	0.1
	0.0	-0.1	-0.8	1.4	0.1	0.1	0.1	0.0	-0.3	0.0	2.1	0.0	-0.2	0.0
СН	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Н	0.0	0.0	1.3	-1.0	0.0	-0.2	-0.3	0.1	0.2	0.1	-0.4	0.0	0.0	0.0
	0.0	0.1	1.0	-0.4	0.0	-0.2	-0.3	0.0	0.2	0.1	-0.4	0.0	0.0	0.0
НC	0.0	0.0	0.3	0.2	0.1	-0.2	-0.1	0.0	-0.1	-0.1	0.7	0.0	-0.3	0.0
	0.0	0.0	-0.3	0.2	0.1	-0.1	0.0	0.0	0.0	0.0	0.2	0.0	-0.1	0.0

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Table V. The calculated energy values of the van der Waals interaction. The upper and the lower values for each pair correspond to the ones for oxy and deoxy forms, respectively. The energy values are given in units of kcal/mole.

(a) $\alpha_1 \alpha_2$ contact

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(b) $\alpha_1\beta_1$ contact

α ₁ α ₂	G	н	НC	_	β_1	В	с	D	G	GH	Н
					В				-0.1	-1.6	-22.1
NA		-0.2	-3.3						0.0	-0.9	-16.2
		0.0	-2.6		с				-1.0		-3.8
A			-0 J						0.0		-1.4
			-0.1		G				-16.8	-5.8	-6.3
G	-0.1		-0.3						-15.1	-5.2	-4.0
	-0.9		0.0		GН	-2.2			-5.1		
н			-8.3			-1.0			-5.6		
			-9.9		н	-8.7 -10.3	-1.9 -1.6	-1.5 -2.1	-3.2 -2.3		

(c) $\beta_1\beta_2$ contact

(d) $a_1\beta_2$ contact

β_1 β_2	G	Н	НC	_	β_2	В	С	C D	F	FG	G	НС
N A		-0.1	-6.3		с		-6.7		0.0	-3.7	-0.4	-0.4
		0.0	0.0				0.0		-0.3	-4.0	-5.3	-6.9
G	- 0.3				CD					0.0		
	0.0									-1.4		
					FG		-11.7	0.0				
							-10.6	-0.8				
					G		-5.1				-6.2	
							-5.8				-9.1	
					нс	-0.1	-4.7					
						-2.1	-9.0					

(1) between a_1 and a_2 subunits

The value of the van der Waals interaction energy is almost the same for both deoxy- and oxyhemoglobin. Although the electrostatic interaction energy has the same value for both deoxy- and oxyhemoglobin except for the salt bridges in deoxyhemoglobin, the deoxy form is lower in energy by about 10 kcal/mole due to the six salt bridges on C-terminals mentioned in the previous section.

(2) between β_1 and β_2 subunits

In deoxyhemoglobin, there is no van der Waals interaction, but in oxyhemoglobin, there arise a small number of van der Waals contacts and the energy difference of the van der Waals interaction between them is fairly large. The electrostatic interaction is somewhat attractive in both deoxy- and oxyhemoglobin. If the effect of DPG which forms four salt bridges between β chains in the deoxy form is taken into account, the electrostatic interaction energy may become lower by 7 kcal/mole in deoxyhemoglobin.

(3) between α_1 and β_1 subunits

[Deoxyhemoglobin]

The energy value of the van der Waals interaction is calculated to be -66 kcal/mole. The energy of the electrostatic interaction is small and is repulsive. The energy of the three hydrogen bonds which are found in this $a_1\beta_1$ contact on the basis of atomic coordinates is calculated to be -3 kcal/mole. [Oxyhemoglobin]

The van der Waals contact is the most extensive among all pairs of subunits and its value energy amounts to -80 kcal/mole which is more attractive than that of deoxyhemoglobin by 14 kcal/mole. This large energy change is mainly contributed by the pair of segments $B(\alpha) - H(\beta)$. The electrostatic interaction is also somewhat repulsive. No energy difference between deoxy and oxyhemoglobin is found in the There are three hydrogen bonds of type N·H…O and one of type O·H…O. electrostatic interaction. The energy value of the three hydrogen bonds may be expected to be -13 kcal/mole at most, because the -4 maximum strength of the hydrogen bond N-H…O is about -3 kcal/mole and that of O-H…O is about kcal/mole. However calculated value for the four hydrogen bonds is only -4 kcal/mole with the atomic coordinates of oxyhemoglobin.

(4) between α_1 and β_2 subunits

[Deoxyhemoglobin]

The energy of the van der Waals interaction amounts to -55 kcal/mole. The energy value of electrostatic interaction is small although there is a salt bridge in which the C-terminal of the β chain participates. The energy of the four hydrogen bonds which are found in this contact is calculated to be -3 kcal/mole.

[oxyhemoglobin]

Although the pair of segments $C(a)-C(\beta)$ has strong van der Waals interaction, the total value of the van der Waals interaction is reduced to -39 kcal/mole between these subunits. This large energy change is mainly contributed by the reduction of van der Waals interaction in the pairs of segments C(a)-HC (β) and $C(a)-G(\beta)$. The electrostatic interaction is fairly repulsive. Two hydrogen bonds N-H…O appear and their energy value may be expected to be -6 kcal/mole at most. But, its value is only -1 kcal/mole in my calculation.

At the first sight of these results, it seems to us that the energy changes here and there in the

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hemoglobin molecule in the transition from the deoxy to the oxy form and a simple rule can not be derived from them at the present time. But, if we look at the total interaction energy of a whole hemoglobin molecule, we find an important character in the transition.

Table VI.The calculated interaction energies between subunits.The energy values of oxy- anddeoxyhemoglobin are sums of those in Tables I—IV and the sums of those in Table V, respectively.In parentheses, the energy values including the energy of the twelve salt bridges between subunits areshown.The energy values are given in units of kcal/mole.

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		Оху		Deoxy						
	Van der Waals	Electrostatic	Hydrogen bond	Van der Waals	Electrostatic	Hydrogen bond				
α1 α2	- 25	- 1	0	- 26	-2(-11)	0				
$\beta_1 \beta_2$	- 13	- 1	0	0	-2(-8)	0				
$\alpha_1 \beta_1$	- 80	2	- 4	- 66	3	- 3				
$\alpha_1 \beta_2$	-39	7	- 1	- 55	2(1)	- 3				

As is clearly seen from Table VI, the van der Waals interaction contributes much more to the stabilization of the quaternary structure than the electrostatic interaction and the hydrogen bonds in both deoxy- and oxyhemoglobin. Although, in every pair of subunits except for the $\alpha_1 \alpha_2$ pair, a large energy change of 13~15 kcal/mole in the van der Waals interaction occurs in the transition from the deoxy to the oxy form, this interaction energy is lower in the oxy form than in the deoxy form by only 8 kcal/mole for the whole hemoglobin molecule. Moreover, the electrostatic interaction is sufficiently more repulsive in the oxy form than in the deoxy form to cancel the energy difference in the van der Waals interaction.

Table VII. The total values of the interaction energies between the subunits. The energy values of oxy- and deoxyhemoglobin are the sums of those in Table VI. In parentheses, the energy values including the energy of the twelve salt bridges between subunits are shown. The energy values are given in units of kcal/mole.

		Inter subunits	· · · · · · · · · · · · · · · · · · ·	Total
	Van der Waals	Electrostatic	Hydrogen bond	
Оху	- 276	16	- 10	- 270
Deoxy	- 268	6 (-18)	-12	-274(-292)

Thus, the total energy difference between the oxy and deoxy forms becomes only 4 kcal/mole without the salt bridges. This feature is shown in Table VII, where the interaction energies for each pair of subunits in Table VI are summed up. Taking into account the twelve salt bridges in the deoxyhemoglobin, the six salt bridges between the α subunits, the two salt bridges between the α and β subunits and the four salt bridges which are made between the two β subunits through a DPG molecule, the deoxy form becomes lower in energy than the oxy form by 22 kcal/mole, where the energy value of one salt bridge is estimated to be -1.5 kcal/mole as mentioned in the previous section. The energy values including the

energy of these salt bridges are shown in parentheses in Table VII. This result leads to a following picture. The deoxy quaternary structure is comparable with, or a little lower than the oxy quaternary structure in internal energy without the salt bridges and it is the salt bridges that stabilize the deoxy form. This may support the Perutz' model and his pointing out the significance of the salt bridges.

The calculations were carried out on the FACOM 230-60 at the Computer Center, kyoto University and on the NEAC 2200-700 at the Computer Center, Osaka University.

DISCUSSION

Since the hydrophobic interaction is not considered in the present calculation, the total values of the interaction energies among the subunits in Table VII will not necessarily be the absolute energies of the quaternary structures of deoxy- and oxyhemoglobin in the solvent, respectively. However, the conformational change in the transition from deoxy- to oxyhemoglobin is small in the sense that the proportion of the non-polar residues to the polar residues over the molecular surface is almost invarient in both deoxyand oxyhemoglobin outside the regions where the C-terminal residues and a DPG molecule make salt bridges in the deoxy form. The enthalpy change due to the volume change is also negligibly small. This is ascertained by the following estimation. For example, let us assume such a large volume change that deoxyand oxyhemoglobin are globular molecules with the radii of 30 Å and 35 Å, respectively. Then, oxyhemoglobin has a larger volume than deoxyhemoglobin by about 6×10^{-20} cm³. Under the usual condition of I atm, the enthalpy change is evaluated to be only about I kcal/mole even for this volume change. Therefore the calculated result in the present paper may give one important guide to the difference between deoxy- and oxyhemoglobin in energy of the quaternary structure.

But, it may be asked whether the difference of the total energies in Table VII may be regarded asthe energy difference of the quaternary structures of deoxy- and oxyhemoglobin. One questionable point is the treatment of the salt bridges in which the C-terminal residues participate. In the transition from deoxy to oxy form, the charges, even if they may be regarded as making salt bridges on the surface of a globular protein in the deoxy form may jut out into water. Also a hydrophobic part in the region may make more contact with water. The former lowers the energy and the latter raises the energy. The same situation may be more plausible in the contact between β subunits. In the deoxy form, a DPG molecule make bridges. between these subunits and there appears no van der Waals contact pair. In the oxy form, on the other hand, the DPG molecule is released from the β subunits, and there arise van der Waals contacts between the segment pairs, NA-HC, G-G and NA-H as shown in Table V. It should be noted that the DPG molecule is situated in the central cavity and its charged groups are within hydrogen-bonding distance of valine NA1and histidine H21 in the deoxy form, according to the atomic model proposed bv Perutz.9) Therefore, the water molecules in the central cavity may be partly expelled from there in the transition. But the central cavity is lined with polar residues. Hence, even if the water molecules are expelled from the central cavity, this calls to mind the two effects on the energy which are complementary, i.e. one lowers the energy by non-polar residues taking less contact with water and the other raises the energy by polar residues taking less contact with water. As is well known, when a hydrophobic molecule is put into water, both enthalpy and entropy decrease due to the change of the structure of the water around it, and the increase of the free energy amounts to the order of kcal/mole at room temperature for a simple alkyl group. Thus, we would have to make a close examination of the changes in

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contact with water, if it were possible. At present, we expect optimistically that the effects of the polar part and the non-polar part on the water may cancel each other energetically, and that the resultant change in energy may be attributed only to the salt bridges mentioned above. In the following, it will be shown that the value of the allosteric parameter in Monod-Wyman-Changeux's model can be obtained with this optimism.

The result of the present calculation shows that deoxy quaternary structure is lower in energy by 22 kcal/mole including the eight salt bridges in the C-terminal residues and the four of the DPG molecule (see Table VII). Even if all these salt bridges are broken due to the binding of four oxygen molecules, it seems that deoxy quaternary structure is still lower in energy by 4 kcal/mole than the oxy quaternary structure. Where is the energy to make the transformation from deoxy to oxy quaternary structure in the oxygenation?

Perutz⁹⁾ suggests the rotation of the last and the penultimate residues of four chains on the basis of his X-ray analyses. Thus, if it is assumed that the freedom of rotation about a single bond is three and there are four such rotatable bonds in the C-terminal of each subunit, the free energy of rotation in the C-terminals of the four subunits is estimated to be $4 \times 4 \times RT \ln 3$. At 25°C, the value of this energy amounts to -10 kcal/mole and the free energy of oxy quaternary structure is lowered by 6 kcal/mole below that of deoxy quaternary structure in which the salt bridges are broken. Taking into account the salt bridges, deoxy quaternary structure is lower in energy by 12 kcal/mole than oxy quaternary structure. The energy values of these structures are shown in Fig. 6, where V is the difference of the van der Waals contacts, electrostatic interaction and hydrogen bonds, E is the energy of salt bridges and $4 \times RT \ln W$ is the energy due the rotation of C-terminals.



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Fig. 6. Energy levels of various structures. Energy difference V is due to the van der Waals contacts, electrostatic interaction and hydrogen bonds between subunits and is calculated to be 4 kcal/mole. Energy E is due to the twelve salt bridges and its value is assigned to be 18 kcal/mole. Energy difference 4RTlnW is due to the rotation of C-terminals in four subunits and its value is assigned to be 10 kcal/ mole at 25°C.

If our deoxy and oxy quaternary structures are assumed to correspond respectively to the T and R states in Monod-Wyman-Changeux's model, then the allosteric parameter L, which is defined as the proportion of T state to R state, is evaluated as exp $\{-4lnW+(E+V)/RT\}$. With the use of the cstimated values for W, E and V, the allosteric parameter L is evaluated to be 3.6×10^8 at 25° . Moreover, if it is assumed that the difference between the oxygen equilibrium constants K_T in T state and K_R in R state is due to only the strain energy which is nearly equal to, or a little less than, the energy of three salt bridges per one subunit, the proportion of K_T to K_R which is usually denoted by c, is evaluated

ated to be 5×10^{-4} or more at 25°C. These values for L and c are of the same order of magnitude as $(2.56 \pm 1.98) \times 10^{8}$ and 1.48×10^{-3} which were obtained from the analysis of human adult hemoglobin in the presence of 2 mM DPG at pH 7.4 and 25°C by Imai.²⁵) Szabo and Karplus ²⁶) has proposed one model in which he tries to represent quantitatively the diagram proposed by Perutz ⁹ in Monod-Wyman-Changeux's scheme. In his model, however, the allosteric parameter L is still an adjustable parameter. On the other hand, my result shows that both L and c may be evaluated semi-quantitatively from the present calculation.

Further examination of the mechanism of heme-heme interaction will be reported in a succeeding paper.

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