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THE BIOLOGICAL FUNCTIONS OF LOW-FREQUENCY VIBRATIONS (PHONONS) 4. RESONANCE EFFECTS AND ALLOSTERIC TRANSITION *

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Based on the internal structure of oligoprotein as well as the basic physical characterics of vibrations, it is deduced that the low-frequency vibrations possess some exceptional functions in transmitting biological information at the molecular level. In particular, according to the viewpoint of energy exchange and intramolecular displacement, it is demonstrated that the low-frequency resonance plays a very significant role during the dynamic process of allosterism of an oligomeric protein molecule. Furthermore, the cooperative reaction between hemoglobins and ligands is taken as an example, through which it is seen that some observed phenomena, whose dynamic principle has thus far been unclear, can be explicitly interpreted in terms of the concept of low-frequency resonance.

1. Introduction

During the last decade more and more evidence from Raman spectra [1–4] and far-infrared spectra [5] has indicated that there obviously exist low-frequency modes in some proteins and DNA. Many efforts have been made in attempting to reveal the origin of this kind of low-frequency vibration [6–10]. Meanwhile, much speculation concerning their biological functions has been put forward, which can be classified as follows:

(1) Green [11], Ji [12], and Fröhlich [13] – presuming the catalytic function of enzyme is closely associated with the low-frequency vibrations.

(2) Careri et al. [14] and Englander [15] – intending to determine the relation between this internal motion and the hydrogen-exchange properties occurring in proteins and nucleic acids.

(3) Chou and Chen [16,17] – demonstrating that the associations between insulin and insulin receptor as well as insulin and antibody would concomitantly excite low-frequency phonons (whose wave numbers are 10–100 cm^{-1}), otherwise a thermodynamic 'deficit' could not be compensated for. Based on this, a physical picture was conceived in which this kind of low-frequency phonon might play an important role in transmitting dynamic information in biomacromolecules.

(4) Chou et al. [18] – analysing the relationship between the low-frequency motion in biomacromolecules and their conformational change, and further speculating such a microscopic mechanism that activation and deactivation of a protein molecule might be directly related to the excitation and annihilation of some special low-frequency phonons, the so-called activating low-frequency phonons.

(5) Sobell et al. [19,20], and Zhou [21] – inferring that the low-frequency (acoustic) phonons are crucial from the viewpoint of dynamics to

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DNA breathing and drug intercalation.

(6) Chou [7–9] – investigating the relation of the low-frequency vibrations with the microenvironment involved in order to obtain insight into the internal relation between the activity of a protein molecule and its activating low frequency.

As is well known, in nature vibrations play a unique role in transmitting information. This kind of uniqueness is closely dependent on the intrinsic physical characters of vibrations. In molecular biology, however, there are many different information-transmission phenomena. Therefore, it is undoubtedly interesting to discuss the biological functions of low-frequency vibrations in terms of their basic physical character. This paper is devoted to determining the relationship between the resonance effect of low-frequency vibrations and the allosteric transmission in oligomeric proteins.

2. Illumination from a basic natural phenomenon

A basic and intriguing subject today in molecular biology is how to elucidate the allosterism of oligomeric proteins from the viewpoint of dynamics [20]. Generally, the cooperative effects in oligoproteins are roughly attributed to the interactions between subunits. However, through what kind of mode do these subunits interact with each other? What is the dynamic process of this interaction? To answer these questions, investigations at a deeper level are needed. Below, let us first consider a fundamental phenomenon in nature.

Assume several pairs of pendulums are supported from a string as in fig. 1, and are adjusted in length so as to make the periods of the members of a pair be identical. Thus, on displacing any one pendulum, the second member of its pair will start to swing, but the others remain almost undisturbed. This is a simple example of resonance, from which we see that a vibrating body will cause a second body to move in sympathy, subject to the two coupling bodies possessing the same intrinsic frequencies. When two oscillators are in resonance, the energy transfer between them is by way of feedback, and therefore has the following two remarkable characteristics: (a) high efficiency, viz., nearly 100% of the energy can be transferred in such a way; (b) rapidity, which means the speed of resonant exchange in energy is very fast.

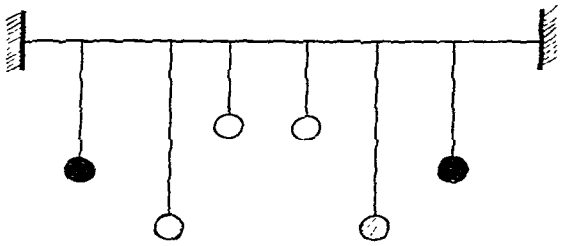

Fig. 1. Three pairs of pendulums supported from a string.

On the other hand, oligoproteins generally contain some subunits whose primary structures are identical or basically the same, e.g., a hemoglobin molecule consists of a two α-subunits and two β-subunits (see section 3 for further description), so that there must exist some identical intrinsic frequencies between two like subunits. As a consequence of this, such an oligoprotein molecule will possess a strong potential for resonance, and therefore the energy transfer between its two like subunits is very efficient. Besides, the allosteric transition generally involves a considerable intramolecular displacement. However, as is well known, when a portion of a biomacromolecule is excited to vibrate with a given energy, the low-frequency vibrations will possess much bigger amplitudes and result in larger displacements of atoms than any high-frequency vibrations. Therefore, from the viewpoints of both energy transfer and internal movement, the low-frequency resonance is very likely to play a distinctive role during the allosteric process of an oligoprotein molecule.

3. Low-frequency vibration spectra

Suppose the low-frequency vibrational spectrum of the ith subunit in an oligoprotein molecule is expressed by

$$\Gamma_i = \{\tilde{\nu}_1', \tilde{\nu}_2', \dots\} \quad (1)$$

where $\tilde{\nu}_1'$, $\tilde{\nu}_2'$, ... are the corresponding individual wave numbers, among which the largest one should be less than 100 cm^{-1} according to the implication

of low-frequency modes in biomacromolecules as given by Chou and Chen [16]. For instance, the low-frequency vibrational spectra for the subunits of hemoglobin can thus be written as

$$\begin{aligned} \Gamma_{\alpha 1} &= \{\tilde{\nu}_1^{\alpha 1}, \tilde{\nu}_2^{\alpha 1}, \ldots\} \\ \Gamma_{\alpha 2} &= \{\tilde{\nu}_1^{\alpha 2}, \tilde{\nu}_2^{\alpha 2}, \ldots\} \\ \Gamma_{\beta 1} &= \{\tilde{\nu}_1^{\beta 1}, \tilde{\nu}_2^{\beta 1}, \ldots\} \\ \Gamma_{\beta 2} &= \{\tilde{\nu}_1^{\beta 2}, \tilde{\nu}_2^{\beta 2}, \ldots\} \end{aligned} \tag{2}$$

Now, the point is how to determine the individual low-frequency wave numbers for a given subunit.

Recognizing the extreme complexity and inherent flexibility of protein molecules, rather than the discrete model that is suitable for the normal mode calculation method, here we would prefer to adopt the continuity model to determine the low-frequency spectra. In principle, the normal mode calculation method can be applied to calculate vibrational movements in any molecules, but in practice this is unfortunately computationally impossible owing to lack of symmetry in biomacromolecules and limitations on computer size and speed. When studying the high-frequency vibrations of a molecule, which involve very small relative displacements and very strong molecular forces, such as covalent bonds, between neighbor individual atoms, one has no choice but to adopt a discrete model. However, for the low-frequency motions in a biological macromolecule, which involve much larger effective masses and much weaker force constants [16,17], and whose modes can be compared to an accordion-like motion [1], heartbeat pulsation [11,12] or similar types of motions that somehow involve many atoms and actually span a much larger dimension than the length of a covalent bond, it is not only more convenient but also physically reasonable to adopt the continuity model. Employing the continuity model, one will of course lose the information about the high-frequency motions in a biomacromolecule. Nevertheless, in investigating the action mechanism of biomacromolecules, the low-frequency motion displays much more significant dynamic functions than the high-frequency motion, as was mentioned above and will be further demonstrated later. Besides, in terms of the continuity model, we can easily acquire an intuitive and explicit physical picture of the internal low-frequency motion in a biomacromolecule, which is no doubt very helpful for study in this field.

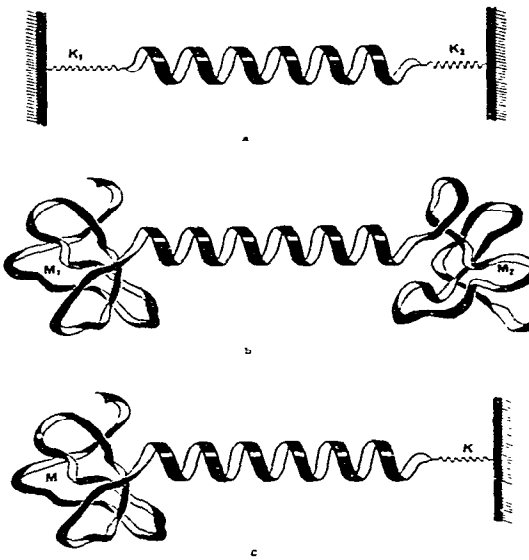

Fig. 2. The vibration system of the helical structure in which the two ends of a helix are linked to (a) two mass-negligible springs with force constants K_1 and K_2, respectively; (b) two sequential fragments with masses M_1 and M_2; (c) a mass-negligible spring with force constant K and a sequential fragment with mass M, respectively.

According to the continuity model, an α-helix can be likened to a spring whose mass, however, is not negligible, viz., to a spring with distributed mass [7,8]. Based on this, the fundamental frequency (lowest frequency) can be, according to different terminal conditions, expressed respectively as follows:

(1) If the two ends of a helix are linked to two mass-negligible springs (e.g., chemical bonds) as illustrated in fig. 2a, we then have [7]

$$\tilde{\nu} = \frac{\nu}{c} = \frac{1}{2\pi c}\left[\frac{k + K^*}{(\alpha_1^3 + \alpha_2^3)\rho L/3}\right]^{1/2} \tag{3}$$

with

$$\alpha = K_2/(K_1 + K_2),\ \alpha_2 = K_1/(K_1 + K_2) \tag{4}$$

$$K^* = K_1 K_2/(K_1 + K_2) \tag{5}$$

where ν is the fundamental frequency of the system concerned, $\bar{\nu}$ the corresponding wave number, c the speed of light, k the stretching force constant of the helix, ρ the mass per unit length along the axis of the helix, L the of helical axis, and K_1 and K_2 the force constants of the two attached mass-negligible springs, respectively.

(2) If the two ends of a helix are linked to two fragments with masses M_1 and M_2, respectively (fig. 2b), we then have [8]

$$\bar{\nu} = \frac{\nu}{c} = \frac{1}{2\pi c}\left[\frac{k}{M^* + (\beta_1^3 + \beta_2^3)\rho L/3}\right]^{1/2} \tag{6}$$

with

$$\beta_1 = M_2/(M_1 + M_2) \quad \beta_2 = M_1/(M_1 + M_2) \tag{7}$$

$$M^* = M_1 M_2/(M_1 + M_2) \tag{8}$$

(3) If one end of a helix is linked to a mass-negligible spring, and the other to a peptide fragment with mass M (fig. 2c), then it follows (see the appendix for the derivation) that

$$\bar{\nu} = \frac{\nu}{c} = \frac{1}{2\pi c}\left[\frac{K^\dagger}{M + (1 + \gamma + \gamma^2)\rho L/3}\right]^{1/2} \tag{9}$$

with

$$\gamma = k/(k + K) \tag{10}$$

$$K^\dagger = kK/(k + K) \tag{11}$$

A more detailed justification concerning the above models as well as the formulae derived therefrom was presented in refs. 7–9, where a number of concrete examples were also given which indicate an excellent agreement between observations and the results calculated in terms of eqs. 3–8.

4. Dynamic approach to allosteric process

Now let us apply the concept of low-frequency resonance and its basic physical properties to probe into the allosteric phenomenon of oligoproteins. As is well known, hemoglobin is a typical oligoprotein for studying the allosteric transition and cooperative effect. Hemoglobin is formed by the noncovalent association of four subunits, each of which contains a polypeptide chain. The chains are of two types, denoted α and β, which differ primarily in the number and sequence of amino acid residues [22]. Each subunit contains a bound heme group, and eight helical segments labeled A–H (fig. 3), separated by extended or nonhelical regions labeled according to the helices at either end. Thus, CD is a nonhelical segment between helices C and D, EF is the one between helices E and F, and so forth. In this way the whole single polypeptide chain is folded into a complicated but highly specific tertiary structure which is approximately spherical. For clearness, an isolated subunit is depicted in fig. 4. The same general description applies to [23] both α- and β-subunits, whether or not oxygenated, for α- and β-subunits themselves have quite similar tertiary structures. However, a more detailed description will indicate that α- and β-chains do not have identical tertiary structures, and that each type of chain can exist in one of two possible conformations, which virtually reflects an apparent change of tertiary conformation due to binding a ligand.

Suppose the aforementioned eight helices in the α- and β-chains are denoted by A_α, B_α,..., H_α,

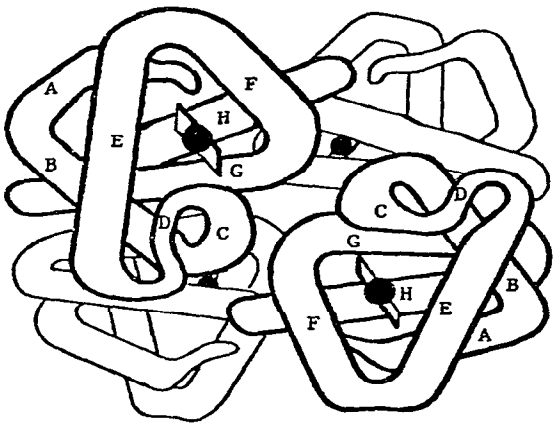

Fig. 3. An illustration of the hemoglobin molecule, which is formed by the noncovalent association of two α-subunits and two β-subunits. Each subunit contains a bound heme group, and eight helical segments labeled A–H (see text).

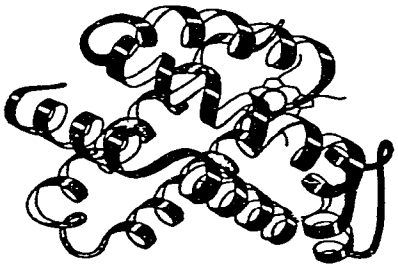

Fig. 4. A general illustration for an isolated subunit of hemoglobin, which applies to both α- and β-subunits since, roughly speaking, α- and β-subunits have quite similar tertiary structure although a more detailed description will indicate they are not identical (see text).

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A_α: Ser-Ala-Ala-Asp-Lys-Thr-Asn-Val-Lys-Ala-Ala-Try-Ser-Lys-Val-Gly
     (3)                                                        (18)
A_β: Ser-Gly-Glu-Glu-Lys-Ala-Ala-Val-Leu-Ala-Leu-Try-Asp-Lys-Val
     (4)                                                    (18)

B_α: His-Ala-Gly-Glu-Tyr-Gly-Ala-Glu-Ala-Leu-Glu-Arg-Met-Phe-Leu-Gly
     (20)                                                          (35)
B_β: Asn-Glu-Glu-Glu-Val-Gly-Gly-Glu-Ala-Leu-Gly-Arg-Leu-Leu-Val-Val
     (19)                                                          (34)

C_α: Phe-Pro-Thr-Thr-Lys-Thr-Thr
     (36)                   (42)
C_β: Tyr-Pro-Try-Thr-Gln-Arg-Phe
     (35)                   (41)

D_α: His-Phe-Asp-Leu-Ser-His-Gly
     (45)                   (51)
D_β: Asp-Pro-Gly-Ala-Val-Met-Gly
     (50)                   (56)

E_α: Ser-Ala-Gln-Val-Lys-Ala-His-Gly-Lys-Lys-Val-Ala-Asp-Gly-Leu-Thr-Leu
     (52)
                                                          -Ala-Val-Gly
                                                                  (71)

E_β: Asn-Pro-Lys-Val-Lys-Ala-His-Gly-Lys-Lys-Val-Leu-His-Ser-Phe-Gly-Glu
     (57)
                                                          -Gly-Val-His
                                                                  (76)

F_α: Leu-Ser-Asp-Leu-Ser-Asn-Leu-His-Ala
     (80)                          (88)
F_β: Phe-Ala-Ala-Leu-Ser-Glu-Leu-His-Cys
     (85)                          (93)
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G_α: Asp-Pro-Val-Asn-Phe-Lys-Leu-Leu-Ser-His-Cys-Leu-Leu-Ser-Thr-Leu-Ala
(94)
-Val-His
(112)

G_β: Asp-Pro-Glu-Asn-Phe-Arg-Leu-Leu-Gly-Asn-Val-Leu-Ala-Leu-Val-Val-Ala
(99)
-Arg-His
(117)

H_α: Thr-Pro-Ala-Val-His-Ala-Ser-Leu-Asp-Lys-Phe-Leu-Ser-Ser-Val-Ser-Thr
(118)
-Val-Leu-Thr-Ser
(138)

H_β: Thr-Pro-Glu-Leu-Gln-Ala-Ser-Tyr-Gln-Lys-Val-Val-Ala-Gly-Val-Ala-Asn
(123)
-Ala-Leu-Ala-His
(143)

and A_β, B_β,..., H_β, respectively. For horse hemoglobin, their amino acid sequences are known as [22,24]
where the subscripts indicate the orders of the corresponding amino acid residues in a polypeptide chain, counting from the amino end (N terminal).

Based on the above general description, the fundamental frequencies of helices B and E–G can be calculated according to the model illustrated in fig. 2a [7], while the fundamental frequencies of helices A and H, which are located near the initial and terminal ends of the polypeptide chain, respectively, can be calculated by the model of fig. 2c. (Helix C is a 3_{10} helix, besides helices C and D contain less than eight residues. They are too short to generate low-frequency modes [8], and therefore will not be considered further.) In calculations, K_1 and K_2 in eqns. 4 and 5 and K in eqns. 10 and 11 should be substituted by $H(\mathrm{NC'C_\alpha}) = 0.19 \times 10^5$ dyn/cm [25], the bending force constant [7,8] at the folding point, for all the helices along the polypeptide chain are folded with each other (figs. 3 and 4). However,the mass M in eq. 9 should be substituted by the mass of the end sequential fragment Val-Leu, Val-Gln-Leu, Lys-Tyr-Arg, and Lys-Tyr-His for A_α, A_β, H_α, and H_β, respectively [22].

The value of k, the stretching force constant of the helix itself, is related to the constituent hydrogen bonds (fig. 5), and can be calculated by the following formula [8]:

$$k = \begin{cases} \dfrac{6k^*}{5i} & \text{if } j = 0 \\ \dfrac{12k^*}{10i + 12/j} & \text{if } 1 \le j \le 4 \\ \dfrac{12k^*}{10i + 3 + 12/(j-4)} & \text{if } 5 \le j \le 8 \\ \dfrac{12k^*}{10i + 6 + 12/(j-8)} & \text{if } 9 \le j \le 10 \end{cases} \tag{12}$$

where

$$\left.\begin{aligned} i &= \left[\!\left[\frac{n-4}{11}\right]\!\right] \\ j &= (n-4) - 11i \end{aligned}\right\} \tag{13}$$

where n (≥ 5) is the number of the constituent amino acid residues in an α-helix, and the truncation operator $[\![\ ]\!]$ means taking the integral part of the number therein; e.g., $[\![7/2]\!] = 3$, $[\![13/3]\!] = [\![12/3]\!] = 4$, $[\![3/4]\!] = 0$, and so forth, and

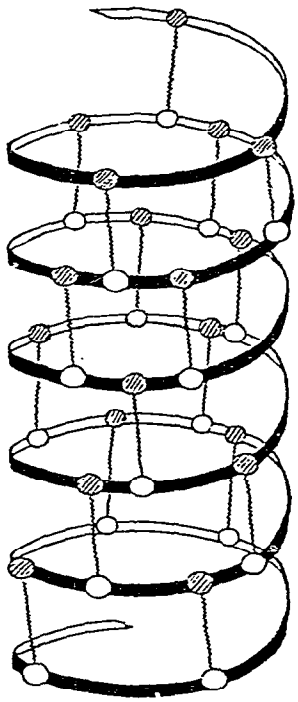

Fig. 5. The α-helix and its hydrogen bonds. (Unfilled circle) Peptide oxygen. (hatched circle) peptide nitrogen. (zigzag line) hydrogen bond. It is obviously seen from this picture that the force constant of an α-helix is related to the force constants of its hydrogen bonds. See refs. 7 and 8 for a detailed description.

$$k^* = \sqrt{\left(k_H^S \cos\theta\right)^2 + k_H^B \sin\theta)^2} = 0.12 \times 10^5 \text{ dyn/cm} \quad (14)$$

in which $\theta \cong 26°$ is the deviation angle between the helix axis and the constituent hydrogen bonds, and $k_H^S = 0.13 \times 10^5$ dyn/cm and $k_H^B = 0.03 \times 10^5$ dyn/cm are the stretching and bending force constants of the hydrogen bond [25], respectively. For the helix set $\{A_\alpha,B_\alpha,E_\alpha,F_\alpha,G_\alpha,H_\alpha\}$ in the α-subunit, and the helix set $\{A_\beta,B_\beta,E_\beta,F_\beta,G_\beta,H_\beta\}$ in the β-subunit, the values of the corresponding stretching force constants k are found in terms of eqs. 12–14 to be {6/11, 6/11, 12/25, 4/5, 12/3, 12/19}k *, and {6/5, 6/11, 12/25, 4/5, 12/13, 12/19}k *, respectively.

To a reasonable approximation, ρ, the mass per unit length of a helix, may be supposed to be even [7,9]; therefore, in the equations concerned, $\rho L = m$ is the mass of a helix, which can be easily figured out according to its amino acid constituents. For example, for the helix sets $\{A_\alpha,B_\alpha,E_\alpha,F_\alpha,G_\alpha,H_\alpha\}$ and $\{A_\beta,B_\beta,E_\beta,F_\beta,G_\beta,H_\beta\}$, the corresponding masses are {1616, 1733, 1934, 951, 2091, 2143} Da and {1598, 1665, 2157, 972, 2117, 2150} Da, respectively.

Applying all these data, as well as eqs. 3–5 and 9–11, we find that the low-frequency spectra for subunits α_1, α_2, β_1 and β_2 are

$$\Gamma_{\alpha_1} = \Gamma_{\alpha_2} = \{9.4, 43.4, 40.2, 64.0, 44.7, 7.9\} \text{ cm}^{-1} \quad (15)$$

$$\Gamma_{\beta_1} = \Gamma_{\beta_2} = \{10.7, 44.2, 38.0, 63.2, 44.4, 8.0\} \text{ cm}^{-1} \quad (16)$$

wherein the values of wave numbers are arranged according to the order of helices A, B, E, F, G, H of the polypeptide chains.

From eqs. 15 and 16 we see that the low-frequency spectra of α_1- and α_2-subunits are identical, and so are the low-frequency spectra of β_1- and β_2-subunits. The low-frequency spectrum of α-subunit and that of β-subunit are different, and the differences in wave number between two corresponding helices fall within the range of 2.2 cm^{-1}. As is well known, the relation of the resonance amplitude with the intrinsic frequency difference is generally described by some sort of steep functions, such as a delta-type function; i.e., a slight difference in intrinsic frequency between two resonant bodies will cause the resonance amplitude to decrease very rapidly. Consequently, the energy transfer through resonance between subunits α_1 and α_2, or between subunits β_1 and β_2, is much more efficient than that between α- and β-subunits.

The above analysis can be well used to explain the following cooperative phenomenon occurring in the association between hemoglobins and

oxygens:

$$\begin{aligned} &Hb + O_2 \overset{K_1}{\rightleftharpoons} HbO_2 \\ &HbO_2 + O_2 \overset{K_2}{\rightleftharpoons} Hb(O_2)_2 \\ &Hb(O_2)_2 + O_2 \overset{K_3}{\rightleftharpoons} Hb(O_2)_3 \\ &Hb(O_2)_3 + O_2 \overset{K_4}{\rightleftharpoons} Hb(O_2)_4 \end{aligned} \tag{17}$$

According to the general viewpoint of positive cooperativity, the prior subunit of an oligoprotein after binding a ligand will exert an influence on the active site of the next subunit through the interaction between subunits, so as to increase its association constant in binding the next ligand. However, the experimental results [26,27] show that, in the reactions described by eq. 17, rather than $K_1 < K_2 < K_3 < K_4$, we have [18]

$$K_1 < K_3 < K_2 < K_4 \tag{18}$$

which obviously cannot be explained satisfactorily by simple attribution to the general interaction between subunits, especially since K_3 is virtually one order of magnitude less than K_2. However, such a phenomenon can be very naturally elucidated from the viewpoint of low-frequency resonance between subunits. For example, when the α_1-subunit binds an oxygen molecule, the energy released is easily transferred to the α_2-subunit by way of resonance exchange as described in section 2, resulting in a conformational change of the α_2-subunit from a 'tense state' to a 'relaxed state' according to Perutz [24], or a transition from an 'inactivated state' to 'activated state' according to Chou et al. [18]. As a result of such an allosteric transition, the α_2-subunit can very easily bind a ligand. An analogous phenomenon would also take place between β_1- and β_2-subunits. However, such an induced activation process [18] via resonance does not occur as easily, or at least not as efficiently, between α- and β-subunits because their low-frequency spectra are not identical. That is why we have $K_3 < K_2$.

Furthermore, the concept of an allosteric process driven by low-frequency resonance between subunits can also be used to predict the transition sequence during the associations between hemoglobins and ligands; i.e., according to the higher efficiency of the induced activation via resonance between two like subunits, the sequence of a hemoglobin binding ligands should always occur such that the α_1-subunit is followed by the α_2-subunit, or the β_1-subunit by the β_2-subunit, as described by

$$\begin{aligned} &[{}^*\alpha_1 \alpha_2 \beta_1 \beta_2] \rightarrow [{}^*\alpha_1 {}^*\alpha_2 \beta_1 \beta_2] \rightarrow [{}^*\alpha_1 {}^*\alpha_2 {}^*\beta_1 \beta_2] \\ &\quad \rightarrow [{}^*\alpha_1 {}^*\alpha_2 {}^*\beta_1 {}^*\beta_2] \end{aligned}$$

or

$$\begin{aligned} &[\alpha_1 \alpha_2 {}^*\beta_1 \beta_2] \rightarrow [\alpha_1 \alpha_2 {}^*\beta_1 {}^*\beta_2] \rightarrow [{}^*\alpha_1 \alpha_2 {}^*\beta_1 {}^*\beta_2] \\ &\quad \rightarrow [{}^*\alpha_1 {}^*\alpha_2 {}^*\beta_1 {}^*\beta_2] \end{aligned}$$

but almost never happens like

$$[{}^*\alpha_1 \alpha_2 \beta_1 \beta_2] \rightarrow [{}^*\alpha_1 \alpha_2 {}^*\beta_1 \beta_2] \rightarrow \cdots$$

or

$$[\alpha_1 \alpha_2 {}^*\beta_1 \beta_2] \rightarrow [{}^*\alpha_1 \alpha_2 {}^*\beta_1 \beta_2] \rightarrow \cdots$$

where the left superior asterisk indicates the subunit having bound a ligand. (Note that α_1 and α_2 are identical, as are β_1 and β_2. Therefore, the sequence order between α_1 and α_2, or between β_1 and β_2, does not make any difference.) So far observations have confirmed such an inference [22,24,28,29].

Furthermore, let us discuss the allosteric transmission from the viewpoint of internal movement of a molecule. As is already known, the key point during the allosteric process of hemoglobin is in the position of the iron atom and the proximal histidine related to the porphyrin plane [22]. Since the porphyrin is in contact with about 60 atoms of the globin chain, any change in such a relative position will act as a very effective trigger to start a sequence of conformational changes [22,24]. Observations indicate that after binding a ligand O_2, the iron atom will almost simultaneously move about 0.75 Å into the porphyrin plane [22,24]. Such a considerable internal displacement, which plays a key role in the allosteric process, is obviously beyond the reach of any high-frequency amplitudes thus incurred in a biomacromolecule.

Now let us see what is expected for the low-frequency amplitude.

As is well known, an oscillator with frequency ν will excite phonons with energy $h\nu$ for each phonon, where h is Planck's constant. At first sight, one is liable to be under the illusion that the low-frequency vibration seems hardly important in energy transfer via a couple-oscillator mechanism for the energy $h\nu$ of a phonon with wave number 10–100 cm^{-1} is much less than k_BT (k_B, Boltzmanns constant; T, absolute temperature). Careful consideration will tell us, however, that it is just due to $h\nu \ll k_BT$ that more phonons with this kind of low frequency can be excited for a given system under the action of the same energy so as to generate a more significant phonon entropy, which is crucially important in solving the thermodynamic paradox occurring in the associations between insulin and insulin receptor as well as insulin and insulin antibody [16,17]. Furthermore, it is also due to $h\nu \ll k_BT$ that a relatively much larger amplitude will be reached, which is a basic requirement in internal displacement for triggering the allosteric transition of a protein molecule as mentioned above. This last point will be further demonstrated as follows.

Because phonons are bosons [16,17], according to Bose-Einstein statistics, the mean number of phonons at energy level $h\nu$ is determined at thermal equilibrium by (it should be realized that the following derivation just serves to illustrate that, under the condition as excited by the same energy, the low-frequency phonons correspond to a much larger amplitude of vibration than the high-frequency ones. Such a conclusion is generally valid, irrespective of a system being in thermal equilibrium; it is just for the convenience of formulation to assume the condition of thermal equilibrium):

$$\langle n\rangle_T = \frac{1}{e^{h\nu/k_BT}-1} = \begin{cases} 0, & \text{if } h\nu \gg k_BT \\ k_BT/h\nu, & \text{if } h\nu \ll k_BT \end{cases} \quad (19)$$

The above expression concerning the number of phonons once again indicates that only the low-frequency phonons can be considerably excited under the general condition, and hence possess much more significant biological functions than high-frequency phonons from both thermodynamic [16] and dynamic [7,8] viewpoints. According to eq. 19 the thermal vibration energy of the low-frequency oscillator is

$$\langle E\rangle_T = \langle n\rangle_T h\nu = \left.\frac{h\nu}{e^{h\nu/k_BT}-1}\right|_{h\nu \ll k_BT} \simeq k_BT \quad (20)$$

recalling that for low-frequency mode with $\tilde{\nu} < 50$ cm^{-1}, $h\nu \ll k_BT$ holds at room temperature. On the other hand, according to energy conservation, $\langle E\rangle_T$ should be equal to the maximum potential energy thus reached. Therefore, for the vibration system in fig. 2a, the thermal low-frequency amplitude is [7]

$$\sigma_T = \sqrt{\frac{2\langle E\rangle_T}{k+K^*}} \simeq \sqrt{\frac{2k_BT}{k+K^*}} \quad (21)$$

for the vibration system in fig. 2b, we have [8]

$$\sigma_T \simeq \sqrt{\frac{2k_BT}{k}} \quad (22)$$

and for the system shown in fig. 2c (see eq. A7)

$$\sigma_T \simeq \sqrt{\frac{2k_BT}{K^\dagger}} \quad (23)$$

For the helices A, B and E–H, the thermal low-frequency amplitudes calculated at $T = 300$ K according to eqs. 21 and 23 fall in the region of 0.2–0.4 Å. These magnitudes are much closer to 0.75 Å in comparison with high-frequency amplitudes in biomacromolecules, but are still much less than 0.75 Å. This is rational since, as expected, the mere thermal movement cannot trigger the allosteric transition without some additional energy. The additional energy is none other than E^*, the energy released when a ligand is bound to the oligoprotein molecule. Since this kind of association is not covalent binding but something like coordination binding, the energy E^* thus excited may not be very large but is at least greater than 10 k_BT. If we take this additional energy into consideration, and replace the thermal energy $\langle E\rangle_T \simeq k_BT$ in eqs. 21–23 by $(k_BT + \eta E^*)$ where $0 < \eta < 1$, the low-frequency amplitudes thus obtained can certainly attain the magnitude of 0.75

Å. The introduction of the factor η is because generally only a fraction of the binding energy may be used to excite the relevant low-frequency vibration.

5. Conclusion

In the macroscopic world vibrations play a unique role in transmitting various information. This is naturally associated with the question: Are the vibrations also important in transmitting biological information at the molecular level? The discussion based on the basic physical characteristics of low-frequency vibrations and the internal structures of biomacromolecules indicates that, from the viewpoints of energy transfer and intramolecular displacement, the low-frequency resonance plays a very significant role in the allosteric process of oligoproteins. In particular, some observed phenomena in the cooperative reactions between hemoglobins and ligands, whose dynamic mechanism has thus far remained a puzzle, can be naturally deduced from the concept of low-frequency resonance.

Appendix

To derive the fundamental frequency of the vibrational system as illustrated in fig. 2c, let us suppose that σ is the maximum extent of stretching of the system, and that σ_k and σ_K are the maximum extents of stretching of the springs k and K, respectively. Obviously, we have

$$\sigma = \sigma_k + \sigma_K \tag{A1}$$

According to force equilibrium, we have

$$k\sigma_k = K\sigma_K \tag{A2}$$

Combination of eqs. A1 and A2 will give

$$\left.\begin{aligned}\sigma_K &= \gamma\sigma \\ \sigma_k &= (1-\gamma)\sigma\end{aligned}\right\} \tag{A3}$$

where γ is defined by eq. 10. The displacements of any points $(0 \le x \le L)$ on the helix [7,8] at any time can be described by

$$u(x, t) = \sigma_K \sin \omega t + \frac{x}{L}\sigma_k \sin \omega t \tag{A4}$$

where ω is the rotational frequency. Then the maximum kinetic energy of a mass element $\rho\Delta x$ of the helix, at a distance x_i from the linking point of the helix to the spring K, is

$$\begin{aligned}\max(\Delta T') &= \frac{\rho\Delta x}{2}\max\left(\frac{\mathrm{d}u}{\mathrm{d}t}\right)^2_{x_i} \\ &= \frac{\rho\Delta x}{2}\left(\sigma_K + \frac{x_i}{L}\sigma_k\right)^2\omega^2\end{aligned} \tag{A5}$$

The total maximum kinetic energy of the vibrational system is

$$\begin{aligned}\max T &= \frac{M}{2}\max\left(\frac{\mathrm{d}u}{\mathrm{d}t}\right)^2_{x=L} + \lim_{\Delta x\to 0}\left[\sum_i \max(\Delta T^i)\right] \\ &= \frac{M}{2}\omega^2\sigma^2 + \frac{\rho}{2}\omega^2\int_0^L\left(\sigma_K + \frac{x}{L}\sigma_k\right)^2\mathrm{d}x \\ &= \frac{\omega^2}{2}\left\{M + \frac{\rho L}{3}(1+\gamma+\gamma^2)\right\}\sigma^2\end{aligned} \tag{A6}$$

On the other hand, the maximum potential of the system is

$$\max U = \frac{K}{2}\sigma_K^2 + \frac{k}{2}\sigma_k^2 = \tfrac{1}{2}K^\dagger\sigma^2 \tag{A7}$$

where $K^\dagger$ is defined by eq. 11. According to energy conservation, viz., $\max T = \max U$, we have

$$\omega^2\left\{M + \frac{\rho L}{3}(1+\gamma+\gamma^2)\right\} = K^\dagger$$

which gives

$$\omega = \left[\frac{K^\dagger}{M + (1+\gamma+\gamma^2)L/3}\right]^{1/2}$$

Therefore, the wave number $\bar{\nu} = \omega/2\pi c$ is obtained as given by eq. 9.

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