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On the absolute meaning of the energy scale $\sim kT$ in the 'thermal interference' involved in enzyme coupled reactions

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Summary

We propose a novel mechanism involved in enzyme-catalyzed coupled reactions in biological systems. It is assumed that an enzyme sets a constraint on the coupling ratio between the degrees of freedom coupled in the reaction. We show that this assumption leads to a mixing of free energy values between the degrees of freedom coupled in the reaction. This is an interference effect of thermal origin, which shares some properties with the quantum interference effect. We discuss the possibility that the energy scale $\sim kT$ has an absolute meaning in determining the magnitude of the thermal interference effect.

1. Introduction

Coupling is a ubiquitous and important mechanism employed in many crucial aspects of the biological processes. The exact nature of this very important biological process, however, is not yet known. Recently, through the observation of the properties of mechanochemical coupling in muscle contraction (Yanagida *et al.* 1985, Harada *et al.* 1990, Higuchi & Goldman 1991, Ohno & Kodama 1991, Yasunaga & Wakabayashi 1991, Lombardi *et al.* 1992, Irving *et al.* 1992), a new question as to the nature of coupling in biological systems has been brought up. Through the analysis of this question, we present, in this paper, a novel mechanism of coupling in biological systems. We postulate that a 'thermal interference effect', namely the interference between mutually inconsistent events coupled through the action of the enzyme, is involved in the coupled reactions. This is an effect similar to the quantum interference effect (see for example, Dirac 1958 or Bohm 1951), but of different physical origin. We discuss the possibility that, as in the quantum interference effect, there is an absolute meaning to the scale of energy $\sim kT$ (where k is Boltzmann's constant, and T is the absolute temperature) in the mechanism leading to the thermal interference effect.

The new question about coupling that has been brought up through the studies of muscle contraction can be summarized as follows.

Under constant temperature and constant pressure conditions of biological systems, the Gibbs free energy determines the direction in which reaction proceeds. Namely, a reaction must involve a negative free energy change in order to proceed spontaneously. In coupling, enzyme E drives a reaction step involving an unfavorable (positive) free energy change by coupling it to another step involving a favorable (negative) free energy change. Let us denote the two coupled reaction steps as $A_1 \rightarrow A_2$ (energetically unfavorable reaction) and $B_1 \rightarrow B_2$ (energetically favorable reaction).

Through the action of enzyme E, these two degrees of freedom become interrelated. Coupling ratio C is defined as the ratio of the net occurrence of these two reaction steps (Mogi 1993a).

$$C = \frac{\text{occurrence of } A_1 \rightarrow A_2}{\text{occurrence of } B_1 \rightarrow B_2}$$

In the conventional understanding of the coupled reaction (see, for example, Stryer 1988), it has been assumed that coupling ratio C is one. In this situation, it was assumed that when one unit of reaction $A_1 \rightarrow A_2$ occurs, one unit of reaction $B_1 \rightarrow B_2$ also occurs. Namely, the conventional scheme is

In coupling, $EA_1B_1 \rightarrow EA_2B_2$ always occurs (A)

However, it has been suggested in the study of muscle contraction and other biological systems (*e.g.*, Shimizu *et al.* 1991) that the coupling ratio is not necessarily one. In particular, in the study of muscle contraction, coupling ratio C has been shown to be much larger than one under certain conditions. This means that the two reactions $A_1 \rightarrow A_2$ and $B_1 \rightarrow B_2$ do not always occur simultaneously. Namely, in view of the recent experimental evidence coupling should rather be expressed in the scheme

In coupling, sometimes $EA_1B_1 \rightarrow EA_1B_2$ occurs
and sometimes $EA_1B_1 \rightarrow EA_2B_1$ occurs . (B)

In the new scheme, the conventional coupling scheme (A) can be considered as a special case in which the two reactions in scheme (B) occur simultaneously.

To illustrate the theoretical problems that

arise from the above picture of coupling, let us assume, for example, that the coupling ratio is N, where N is some integer larger than 1. The energetically favorable reaction $B_1 \rightarrow B_2$ occurs, on average, only in one case out of N occurrences of the energetically unfavorable reaction $A_1 \rightarrow A_2$. Namely, in $N-1$ out of N cases, the energetically unfavorable reaction $A_1 \rightarrow A_2$ should occur without the accompanying occurrence of the driving reaction $B_1 \rightarrow B_2$. We then ask ourselves how the unfavorable reaction $A_1 \rightarrow A_2$ is driven in the absence of the favorable reaction $B_1 \rightarrow B_2$. To understand such a mode of coupled reactions, we need a coupling mechanism in which the energetically unfavorable reaction can be driven on the 'credit' that the energetically favorable reaction would occur in one out of N cases.

Many hypotheses have been proposed to explain a 'credit' mechanism such as formulated above. Storage of the free energy liberated by the driving reaction $B_1 \rightarrow B_2$ in some mode of the protein structure (thermal ratchet model) has been suggested as a possible solution to this question (Feynman *et al.* 1963, Oosawa 1989, Vale & Oosawa 1990, Cordova *et al.* 1992). However, although this scheme can reproduce the basic properties of the experimental data, in view of the rapid decay time of any fluctuation in protein structure (*e.g.,* Brooks et al. 1988) it is very difficult to incorporate this model in the realistic dynamics of protein in water. It may appear that inertia can drive the unfavorable reaction, especially when the driven reaction $A_1 \rightarrow A_2$ is the translocation of macromolecules. However, inertia cannot function as an energy-storing mechanism because of the low value of Reynolds number for macromolecules in water (Purcell 1977, Shapere & Wilczek 1987). The existence of multiple intermediate states is also unlikely to explain the observed large values of the coupling ratio in muscle contraction, as it is unlikely that the multiple intermediate states can sequentially assume the alternating conformations of the motor molecules.

To conclude, there is a very severe conceptual difficulty when we try to understand a coupling mechanism in which the coupling ratio is not one. In view of the recent experimental evidence, we must abandon the conventional scheme, and endeavor to find a coupling principle which can incorporate the newly revealed nature of coupling. In particular, we need a 'credit' mechanism in which the energetically unfavorable reaction is driven on the 'credit' that the energetically favorable reaction would occur with a certain probability.

In the next section, we propose a coupling principle which can solve the problem formulated above.

2. Coupling ratio constraint mechanism

In this section, we propose a novel coupling mechanism (the 'coupling ratio constraint' mechanism).

Let us denote the two coupled degrees of freedom as x ($A_1 \rightarrow A_2$, the energetically unfavorable reaction) and y ($B_1 \rightarrow B_2$, the energetically favorable reaction). We formulate the coupled reaction system as a random walk in a two-dimensional plane (x, y). First we consider the situation in the absence of coupling enzyme E. At each transition time, transition can occur in one of the 4 directions $(1,0)$, $(-1,0)$, $(0,1)$, $(0,-1)$, with probabilities v_1, v_2, u_1, u_2, respectively.

The normalization condition is

$$v_1 + v_2 + u_1 + u_2 = 1. \qquad (1)$$

In the absence of coupling enzyme E, there is no correlation between the two degrees of freedom. Let us assume that transition $A_1 \rightarrow A_2$ involves a free energy change of $\Delta E_x > 0$, and

transition $B_1 \rightarrow B_2$ involves a free energy change of $\Delta E_y < 0$.

The transition probabilities v_1, v_2, u_1, u_2 and free energy gradients ΔE_x, ΔE_y characterize the uncoupled system. We have the Arrhenius relations

$$\frac{v_1}{v_2} = \exp\left(-\frac{\Delta E_x}{kT}\right)$$
$$\frac{u_1}{u_2} = \exp\left(-\frac{\Delta E_y}{kT}\right). \quad (2)$$

If we take an appropriate origin, the state of the system represented by the coordinate (x,y) has the free energy value of $E(x,y)$, where

$$E(x,y) = x\Delta E_x + y\Delta E_y. \quad (3)$$

When we introduce enzyme E, the two degrees of freedom are correlated through the action of the enzyme. As a result, it is expected that the transition probabilities v_1, v_2, u_1, u_2 and the free energy gradients ΔE_x, ΔE_y are transformed. Let us write the transformed transition rate constants and free energy gradients as v_1', v_2', u_1', u_2' and $\Delta E_x'$, $\Delta E_y'$. The essence of the coupling mechanism is reflected in the transformation

$$(v_1, v_2, u_1, u_2, \Delta E_x, \Delta E_y)_{uncoupled} \rightarrow (v_1', v_2', u_1', u_2', \Delta E_x', \Delta E_y')_{coupled} \quad (4)$$

We will refer to the transformed energy gradients $\Delta E_x'$ and $\Delta E_y'$ as the *effective* energy gradients. The *effective* energy gradients are expected to be relevant only as far as the dynamical behavior of the system is concerned (*e.g.*, in determining the kinetic rate constants). Since an enzyme cannot change the free energy balance of a system, the *actual* free energy gradients ΔE_x and ΔE_y are expected to be invariant. Therefore, there would be, in general, a discrepancy between the *effective* and *actual* energy gradients.

Note that for reasons that will be clarified later, the arguments developed in this section are considered to be applicable only to the cases where the absolute values of the energy gradients ΔE_x and ΔE_y are smaller or comparable to $\sim kT$ (see sections 3 & 4). When the absolute values of ΔE_x and ΔE_y are larger than $\sim kT$, a modification of the scheme is needed, as discussed in section 4.

The novel coupling mechanism that we propose in this paper is based on the following two fundamental assumptions.

(1) Through the action of the enzyme, the ratios of the forward and backward transition probabilities in the coupled degrees of freedom are fixed at a constant value. **As a result, the coupling reaction is effectively constrained to proceed in a single (*average*) degree of freedom** (the coupling ratio constraint hypothesis, Fig.1).
(2) Although there is, in general, a discrepancy between the *effective* and *actual* free energy values, they take the same value along the *average* degree of freedom specified by the coupling ratio constraint.

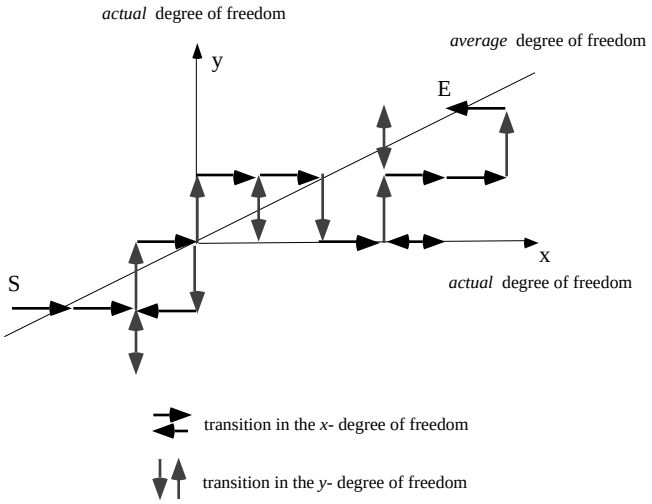

Fig.1 The coupling ratio constraint mechanism

In the coupling ratio constraint mechanism, it is assumed that the coupling ratio (the ratio of the transition rate constants in the coupled degrees of freedom) is fixed at a constant value by the enzyme. The actual transitions, however, continue to occur in two dimensions. In this figure, the average degree of freedom is shown by the bold line. The transitions of the system are restricted, *on average*, to this single degree of freedom. An example of a sequence of the actual transitions is represented by the directed edges. When an edge has two arrowheads, the occurrence of a reversible transition is suggested.

Now let us obtain the transformed transition probabilities and energy gradients based on the above assumptions.

The normalization condition is again

$$v_1' + v_2' + u_1' + u_2' = 1 \quad . \tag{5}$$

The coupling ratio C defined in the previous section can be written as

$$C = \frac{v_1' - v_2'}{u_1' - u_2'} \quad .$$

We express the *average* degree of freedom specified by the coupling ratio constraint by the 'coupling ratio vector' *(p,q)*, where

$$p^2 + q^2 = 1 \quad .$$

In the following discussion, we assume, without a loss of generality, that $p, q > 0$.

The coupling ratio constraint hypothesis can be written as

$$\frac{v_1'}{u_1'} = \frac{v_2'}{u_2'} = \frac{p}{q} \quad . \tag{6-A}$$

Under condition (6-A), the coupling ratio takes a constant value of

$$C = \frac{v_1' - v_2'}{u_1' - u_2'} = \frac{p}{q} \quad . \tag{6-B}$$

Note that condition (6-A) is a stronger requirement than condition (6-B). This stronger requirement is necessary and appropriate for two reasons. The first reason is that if we require only condition (6-B), we would need one more equation to determine the transformed rate constants and free energy gradients. In other words, we are left with one free parameter, and there is no natural physical or biological requirement that would provide the necessary equation. The second reason is that requirement (6-B) is a generalization of the principle of detailed balancing (Bridgman 1928) in that both the forward and backward reactions proceed in the same *average* degree of freedom.

The random walk is now *on average* restricted in the direction of the coupling ratio vector *(p,q)*. In other words, there is effectively only one degree of freedom. The requirement that

the effective free energy and the actual free energy take the same value along the average degree of freedom can be written as

$$p\Delta E_x + q\Delta E_y = p\Delta E_x' + q\Delta E_y' \quad . \tag{7}$$

The Arrhenius relations for the transformed transition probabilities and free energy gradients are

$$\frac{v_1'}{v_2'} = \exp\left(-\frac{\Delta E_x'}{kT}\right)$$

$$\frac{u_1'}{u_2'} = \exp\left(-\frac{\Delta E_y'}{kT}\right) \quad . \tag{8}$$

From relations (5), (6-A) and (7)-(8), the effective free energy gradients are obtained as a single value $\Delta E'$.

$$\Delta E_x' = \Delta E_y' = \Delta E' = \frac{p\Delta E_x + q\Delta E_y}{p+q} \tag{9}$$

The transformed transition probabilities are obtained as

$$v_1' = \frac{pe^{-\frac{\Delta E'}{kT}}}{(p+q)\left(1+e^{-\frac{\Delta E'}{kT}}\right)}$$

$$v_2' = \frac{p}{(p+q)\left(1+e^{-\frac{\Delta E'}{kT}}\right)}$$

$$u_1' = \frac{qe^{-\frac{\Delta E'}{kT}}}{(p+q)\left(1+e^{-\frac{\Delta E'}{kT}}\right)}$$

$$u_2' = \frac{q}{(p+q)\left(1+e^{-\frac{\Delta E'}{kT}}\right)} \quad . \tag{10}$$

Equations (9) and (10) give transformation (4), realized through the coupling ratio constraint mechanism.

It is interesting to consider the implications of formula (9). Recall that we have assumed that $\Delta E_x > 0$ and $\Delta E_y < 0$. The transition in the x degree of freedom cannot proceed by itself. Through the 'coupling ratio constraint' of (6-A), the free energy gradients in the two coupled degrees of freedom are 'mixed' into a single value of $\Delta E'$. As long as the condition

$$\Delta E' = \frac{p\Delta E_x + q\Delta E_y}{p+q} < 0$$

is satisfied (*i.e.*, if the coupling ratio is not very large), the transition in the x- degree of freedom would be driven by an effective free energy gradient of $\Delta E'$. In this way, the negative free energy change ΔE_y accompanying the favorable transition $B_1 \rightarrow B_2$ is used to drive the unfavorable reaction $A_1 \rightarrow A_2$. This is a 'free energy mixing' effect, which can reproduce the essential features of a coupling reaction for which coupling ratio C is not one.

The effective energy value of the state *(x,y)* is now given as

$$E'(x,y) = x\Delta E_x' + y\Delta E_y'$$
$$= (x+y)\frac{p\Delta E_x + q\Delta E_y}{p+q} \quad . \qquad (11)$$

We see that the question formulated in the previous section of how the enzyme can establish a 'credit' mechanism is now answered. Namely, in the above scheme, the unfavorable reaction $A_1 \rightarrow A_2$ is driven on the 'credit' that the favorable reaction $B_1 \rightarrow B_2$ would, *on average*, occur in one out of C cases. We also see that there is a 'thermal interference effect' involved in our scheme. The 'thermal interference effect' is formulated as the interference between two mutually inconsistent events that are coupled through the action of enzyme E. In our scheme, the two events

$$EA_1B_1 \rightarrow EA_1B_2$$

and

$$EA_1B_1 \rightarrow EA_2B_1$$

are mutually inconsistent. However, through the action of the enzyme, these two inconsistent events are coupled, and the free energy gradients accompanying these events 'interfere' with each other. As a result, we obtain a single effective free energy gradient (11) which is common for these two mutually inconsistent events. This is a novel feature of the 'coupling ratio constraint' mechanism.

As a concrete example, let us consider the sliding movement of actin filament driven by the hydrolysis of ATP observed in muscle contraction. It has been experimentally demonstrated that several elementary 'working strokes' are conducted during the hydrolysis of one ATP molecule (Lombardi *et al.* 1992). The free energy liberated by the hydrolysis of ATP under physiological conditions is about 12 *kcal/mol* (Shriver 1984) or 20 kT per molecule at room temperature (~300K). If we assume, for example, that the 'load' ΔE_x is 3 kT, and the coupling ratio is 4, the effective free energy gradient $\Delta E'$ will be -1.6 kT. Therefore, each 'working stroke' would be driven by a constant effective free energy gradient, *regardless of the particular time at which the hydrolysis of ATP is completed*. This picture is compatible with the smooth sliding movement of actin filament observed in *in vitro* motility assays (Ishijima *et al.* 1991, Harada *et al.* 1990).

Let us verify that the energy balance (i.e., the change in the Gibbs free energy) is always negative, as is required by the second law of thermodynamics. We assume that after n steps of random walks, the system has undergone a transition of $(x(n), y(n))$. The energy balance $U(n)$ (change in the Gibbs free energy) for the coupled reaction is defined as

$$U(n) = x(n)\Delta E_x + y(n)\Delta E_y \quad . \qquad (12)$$

The average value of the energy balance is then calculated as

$$\langle U(n)\rangle = \langle x(n)\rangle \Delta E_x + \langle y(n)\rangle \Delta E_y$$
$$= n(u_1' - u_2')\left(\frac{p}{q}\Delta E_x + \Delta E_y\right)$$
$$= -n\frac{1 - e^{-\frac{\Delta E'}{kT}}}{1 + e^{-\frac{\Delta E'}{kT}}}\Delta E' \quad . \qquad (13)$$

We see that the average value of the energy balance is always zero or negative, regardless of the value of the effective free energy gradient $\Delta E'$. Therefore, in our scheme, the second law of thermodynamics is not violated, and there is no Maxwell's demon (Maxwell 1871) at work.

3. Statistical properties of difference between effective and actual free energy values

In the previous section, we presented the 'coupling ratio constraint' mechanism which can explain the basic properties of a coupled reaction where the coupling ratio is not one. We formulated the model in terms of two different concepts of free energy values, namely, the effective and actual free energy values. We postulated that the effective energy values are relevant when we consider the dynamical behavior (the kinetic rate constants, etc.) of the system. The actual free energy values, on the other hand, are assumed to be relevant when we consider the energy balance. In the 'coupling ratio constraint' mechanism, there is, in general, a discrepancy between the effective and actual free energy values. The difference between the effective and actual free energy values is a novel feature of the 'coupling ratio constraint' mechanism. In the conventional scheme of coupled reactions, there is no discrepancy between the effective and actual free energy values (see, for example, Mogi 1993a).

The question then arises as to the origin of the difference between the effective and actual free energy values. Since the difference between the two free energy values can ultimately be only accounted for by the heat bath, it is reasonable to assume that the difference between the effective and actual free energy values is provided *temporarily* by the thermal fluctuation. Namely, we assume that the coupling ratio constraint mechanism works by utilizing a temporal energy difference (i.e., the difference between the effective and actual free energy gradients) provided *on loan* from the heat bath in the form of the thermal fluctuation.

In consolidating the physical validity of the 'coupling ratio constraint' mechanism, the statistical properties of the scheme are expected to be important. In particular, if the difference between the 'effective' and 'actual' free energy values is to be provided by the thermal fluctuation, then it would have to satisfy certain conditions in accordance with the statistical properties of the thermal fluctuation. Let us therefore check if the statistical behavior of the difference between the 'effective' and 'actual' free energy values is compatible with the assumption that its origin is the thermal fluctuation.

Let us again begin with the assumption that after n steps of random walks, the system has undergone a transition of $(x(n), y(n))$. The transition of the system $(x(n), y(n))$ can be decomposed into a superposition of the coupling ratio vector (p,q) and a unit vector $(-q,p)$ perpendicular to it.

$$\big(x(n), y(n)\big) = a(n)(p,q) + b(n)(-q, p) \tag{14}$$

We have

$$\begin{aligned} a(n) &= px(n) + qy(n) \\ b(n) &= -qx(n) + py(n) \end{aligned} \tag{15}$$

The average values of $a(n)$ and $b(n)$ are obtained as

$$\begin{aligned} \langle a(n)\rangle &= p\big(v_1' - v_2'\big)n + q\big(u_1' - u_2'\big)n = \frac{1}{p}\big(v_1' - v_2'\big)n \\ \langle b(n)\rangle &= 0 \end{aligned} \tag{16}$$

The standard deviation of $b(n)$ is related to the standard deviations of $x(n)$ and $y(n)$ as

$$\sigma_{b(n)}^2 = q^2\sigma_{x(n)}^2 + p^2\sigma_{y(n)}^2 \tag{17}$$

where the standard deviations on the right side are given as

$$\sigma^2_{x(n)} = n\left(v_1' + v_2'\right)\left(u_1' + u_2'\right) + 4nv_1'v_2'$$
$$\sigma^2_{y(n)} = n\left(u_1' + u_2'\right)\left(v_1' + v_2'\right) + 4nu_1'u_2' \quad . \tag{18}$$

Using these relations, and making use of (10), the standard deviation of *b(n)* is obtained as

$$\sigma_{b(n)} = \sqrt{n}\,\frac{\sqrt{pq}\sqrt{\left(1 + e^{-\frac{\Delta E'}{kT}}\right)^2 + 8pqe^{-\frac{\Delta E'}{kT}}}}{(p+q)\left(1 + e^{-\frac{\Delta E'}{kT}}\right)} \quad . \tag{19}$$

Let us now examine the statistical properties of the difference between the effective energy value E' (11) and the actual energy value E (3). If the system has undergone a transition of *(x(n), y(n)),* the difference between the effective energy value $E'(x(n), y(n))$ and the actual energy value $E(x(n),y(n))$ is given as

$$\begin{aligned}\delta E(n) &= E'(x(n),y(n)) - E(x(n),y(n)) \\ &= x(n)\Delta E_x{}' + y(n)\Delta E_y{}' - \left(x(n)\Delta E_x + y(n)\Delta E_y\right) \\ &= \frac{(py(n) - qx(n))}{p+q}\left(\Delta E_x - \Delta E_y\right) \\ &= \frac{b(n)}{p+q}\left(\Delta E_x - \Delta E_y\right)\end{aligned} \tag{20}$$

The average value of $\delta E(n)$ is therefore

$$\begin{aligned}\langle \delta E(n) \rangle &= \frac{\langle b(n) \rangle}{p+q}\left(\Delta E_x - \Delta E_y\right) \\ &= 0\end{aligned} \tag{21}$$

Namely, there is, on average, no discrepancy between the effective and actual free energy values. This is in accordance with the assumption that the origin of the difference between the effective and actual free energy gradients is the thermal fluctuation. Note also that this result is guaranteed by the condition of (7).

Using (19), we obtain the standard deviation for $\delta E(n)$ as

$$\sigma_{\delta E(n)} = \sqrt{n}(\Delta E_x - \Delta E_y)\,\frac{\sqrt{pq}\sqrt{\left(1 + e^{-\frac{\Delta E'}{kT}}\right)^2 + 8pqe^{-\frac{\Delta E'}{kT}}}}{(p+q)^2\left(1 + e^{-\frac{\Delta E'}{kT}}\right)} \tag{22}$$

A problem arises when we examine the property of formula (22). From the nature of thermal fluctuation, the probability of the occurrence of a fluctuation of the scale $\sim\Delta E$ is expected to scale as

$$\sim e^{-\frac{\Delta E}{kT}} \tag{23}$$

However, the fluctuation in the difference between the effective and actual energy values $\delta E(n)$ (22) for the coupled reaction does not scale as in condition (23). It is seen, for example, that the fluctuation given in (22) increases linearly with the absolute values of ΔE_x and ΔE_y when the effective energy gradient $\Delta E'$ is *~0*. This situation is clearly incompatible with the assumption that the origin of the difference between the actual and effective energy values is the thermal fluctuation. This is the 'fluctuation problem'.

We therefore postulate that there is some mechanism that restricts the 'thermal interference' effect so that the 'fluctuation problem' disappears. One possibility would be that the coupling constraint mechanism as developed in the previous section is valid only when the conditions

$$\left|\Delta E_x\right| <\sim kT \quad and \quad \left|\Delta E_y\right| <\sim kT \tag{24}$$

are satisfied. In this picture, the 'thermal

interference effect' would be prominent only when the conditions in (24) are satisfied.

We conclude from the argument developed in this section that there is a mechanism which restricts the occurrence of 'thermal interference' in accordance with the free energy change involved. It is to be noted that the situation is similar to the case of quantum interference, where the interference is restricted within the energy scale of $\sim h$. However, in the case of 'thermal interference' the role of scale $\sim kT$ is expected to be more complicated, as will be discussed later.

4. Absolute meaning of scale $\sim kT$

In the previous section, we saw that the coupling ratio constraint mechanism introduced in this paper does not *on average* violate the second law of thermodynamics. However, we have seen that the energy fluctuation is expected to become too large when the energy gradients ΔE_x and ΔE_y become large compared to the energy scale $\sim kT$.

In this section, we show that if we introduce an absolute meaning to scale $\sim kT$ and restrict the 'thermal interference' within this energy scale, we can solve the 'fluctuation problem'.

Let us write down again the equations that determine the transformed rate constants and free energy gradients

$$(v_1', v_2', u_1', u_2', \Delta E_x', \Delta E_y')_{coupled}$$

in the 'coupling ratio constraint' mechanism.

Namely,

$$\begin{aligned}
&v_1' + v_2' + u_1' + u_2' = 1 \\
&(v_1' - v_2')(\Delta E_x' - \Delta E_x) + (u_1' - u_2')(\Delta E_y' - \Delta E_y) = 0 \\
&\frac{v_1'}{v_2'} = e^{-\frac{\Delta E_x'}{kT}} \\
&\frac{u_1'}{u_2'} = e^{-\frac{\Delta E_y'}{kT}}
\end{aligned} \qquad (25)$$

We solve equations (25) taking the effective free energy gradients $\Delta E_x', \Delta E_y'$ as given.

We then obtain

$$\begin{aligned}
v_1' &= \frac{e^{-\frac{\Delta E_x'}{kT}}\left(1 - e^{-\frac{\Delta E_y'}{kT}}\right)(\Delta E_y - \Delta E_y')}{W} \\
v_2' &= \frac{\left(1 - e^{-\frac{\Delta E_y'}{kT}}\right)(\Delta E_y - \Delta E_y')}{W} \\
u_1' &= \frac{e^{-\frac{\Delta E_y'}{kT}}\left(1 - e^{-\frac{\Delta E_x'}{kT}}\right)(\Delta E_x' - \Delta E_x)}{W} \\
u_2' &= \frac{\left(1 - e^{-\frac{\Delta E_x'}{kT}}\right)(\Delta E_x' - \Delta E_x)}{W}
\end{aligned}, \qquad (26)$$

where

$$W = \left(1 + e^{-\frac{\Delta E_x'}{kT}}\right)\left(1 - e^{-\frac{\Delta E_y'}{kT}}\right)(\Delta E_y - \Delta E_y') + \left(1 - e^{-\frac{\Delta E_x'}{kT}}\right)\left(1 + e^{-\frac{\Delta E_y'}{kT}}\right)(\Delta E_x' - \Delta E_x)$$

.

We now introduce an absolute meaning to scale $\sim kT$ and restrict the 'thermal interference' within this energy scale. In order to achieve this, we define *scale function F*, which is assumed to satisfy the following conditions.

(1) F is a monotonously decreasing function of the absolute values of the free energy gradients ΔE_x and ΔE_y.

$$\frac{\partial F}{\partial|\Delta E_x|} < 0$$

$$\frac{\partial F}{\partial|\Delta E_y|} < 0$$

(2) F tends to 1 as both $|\Delta E_x|$ and $|\Delta E_y|$ approach zero.

$$\lim_{\substack{|\Delta E_x|\to 0\\ |\Delta E_y|\to 0}} F = 1$$

(3) F tends to zero as $|\Delta E_x|$ and $|\Delta E_y|$ become large.

$$\lim_{|\Delta E_x|\to\infty} F = \lim_{|\Delta E_y|\to\infty} F = 0$$

(4) The characteristic energy scale of function F is $\sim kT$.

An example of scale function F that satisfies the above four conditions would be

$$F = e^{-\frac{\Delta E_x^{\,2}+\Delta E_y^{\,2}}{4(kT)^2}}. \qquad (27)$$

We assume that the effective free energy gradients $\Delta E_x'$ and $\Delta E_y'$ depend linearly on scale function F,

$$\Delta E_x' = \Delta E_x + F\left(\Delta E' - \Delta E_x\right)$$
$$\Delta E_y' = \Delta E_y + F\left(\Delta E' - \Delta E_y\right), \qquad (28)$$

where $\Delta E'$ is the effective energy gradient (9) derived in the coupling ratio constraint mechanism of section 2.

In (28), scale function F can be considered to be the measure of the extent to which the coupling ratio constraint (and the thermal interference effect) (6-A) holds. When $F=1$, the effective energy gradients in the x- and y- degrees of freedom are given by the single value of E', and there is maximum thermal interference effect. When scale function $F=0$, there is no change in the energy gradients, and there is no thermal interference effect. In addition, the thermal interference effect is prominent only in the energy scale of $<\sim kT$. Namely, scale function F introduces an absolute meaning to scale $\sim kT$, and restricts the thermal interference effect within this energy scale.

Under the assumption of (28), the standard deviation for $\delta E(n)$ is obtained as

$$\sigma_{\delta E(n)} = \sqrt{nF}\,\frac{\left(\Delta E_x - \Delta E_y\right)\sqrt{pq\left(1-e^{-\frac{2\Delta E_x'}{kT}}\right)\left(1-e^{-\frac{2\Delta E_y'}{kT}}\right)+4p^2q^2\left(e^{-\frac{\Delta E_x'}{kT}}-e^{-\frac{\Delta E_y'}{kT}}\right)^2+e^{-\frac{\Delta E_x'}{kT}}\left(1-e^{-\frac{\Delta E_y'}{kT}}\right)}}{(p+q)\left|p\left(1+e^{-\frac{\Delta E_x'}{kT}}\right)\left(1-e^{-\frac{\Delta E_y'}{kT}}\right)+q\left(1-e^{-\frac{\Delta E_x'}{kT}}\right)\left(1+e^{-\frac{\Delta E_y'}{kT}}\right)\right|} \qquad (29)$$

Note that $\sigma_{\delta E(n)}$ is proportional to scale function F. We see that $\sigma_{\delta E(n)}$ now tends to zero when the absolute values of the energy gradients $|\Delta E_x|$ and $|\Delta E_y|$ become large. This is in accordance with the assumption that the difference between the effective and actual free energy values is provided by the thermal fluctuation.

In conclusion, we have seen that if we introduce an absolute meaning to scale $\sim kT$, we can make the coupling ratio constraint mechanism reasonable in its fluctuation behavior. The thermal interference effect would then be prominent only in the energy scale of $<\sim kT$.

Let us note, however, that at the present stage, it is premature to discuss the nature of scale function F in a more exact manner. It is expected that there are some complications, as discussed in the next section.

5. Discussions

In this paper, we proposed a coupling mechanism which can explain the basic features of coupling observed in biological systems.

We argued that in considering the mechanism of enzyme coupled reactions, it is important to take into consideration the experimental observation that the coupling ratio is not necessarily one. The importance of this kind of coupling mode (loose coupling) has already been pointed out (Oosawa & Masai 1982, Oosawa & Hayashi 1983).

We have assumed that the action of the enzyme can be stated in the condition that the coupling ratio is fixed at a constant value (the coupling ratio constraint mechanism). We have shown that this assumption successfully explains the transduction of free energy in a coupled reaction where the coupling ratio is not one. It is interesting to consider the enzymatic mechanisms that may underlie such a constraint on the coupling ratio. In particular, condition (6-A) should reflect some symmetry in the reaction space of the coupled reactions imposed by the enzyme, and it is of much interest to consider the molecular mechanisms which may be involved.

From the analysis of the scheme that we presented in this paper, we can make the following predictions about the nature of mechanochemical coupling in muscle contraction (Mogi 1993b).

(1) ATP is not necessarily hydrolyzed when one working stroke occurs.
(2) When ATP is not hydrolyzed, the chemical state of myosin is the same before and after the working stroke.
(3) The sliding of actin filament is uniform regardless of the particular time at which the hydrolysis of ATP is completed.
(4) The multiple working strokes during the hydrolysis of ATP is driven by exactly the same amount of free energy change.
(5) The number of intermediate states involved in coupling can be much smaller than the number of working strokes conducted per ATP hydrolyzed.

These predictions can be tested in future experiments to verify the validity of the scheme presented in this paper.

The coupling mechanism we introduced in this paper shows a 'thermal interference effect', in analogy with the quantum interference effect. The basic features of our model that lead to the 'thermal interference effect' can be summarized as follows.

(1) A stochastic process in two degrees of freedom is restricted,*on average,* to one degree of freedom, through the 'coupling ratio constraint' imposed by the enzyme.
(2) The *actual* transitions, however, continue to occur in two degrees of freedom.
(3) The 'discrepancy' between the *average* single degree of freedom and the *actual* two degrees of freedom leads to an interference between the two coupled degrees of freedom.

It is to be noted that the logical structure leading to the 'thermal interference effect' is similar to the logical structure leading to the quantum interference effect. Several authors have pointed out the possible connection between the quantum and stochastic processes (*e.g.* Barnes & Silverman 1934, Nelson 1966, Nelson 1967).

However, it should be stressed that the 'thermal interference effect' that we proposed here is independent of the quantum interference effect. Limited cases of quantum mechanical effect are observed in enzyme kinetics (*e. g.*, electron tunneling (de Vault 1984), and tunneling of hydrogen atoms (Cha *et al.* 1989)). However, since the degrees of freedom in our model are actually the 'degenerate' expressions for many internal degrees of freedom, our system is too 'macroscopic' for any quantum processes to take effect. Therefore, the 'thermal interference effect' that we proposed in this paper is of purely stochastic character.

In quantum mechanics, an absolute meaning is given to the size of the system (Dirac 1958). Namely, the quantum interference effect occurs only when the scale of the system involved is of the order of Planck's constant *h.* We have suggested that there is a similar absolute meaning to energy scale $\sim kT$ in the thermal interference effect. Namely, if we assume that the thermal interference effect is prominent only when the energy scale involved in coupling is of the order of

~kT, we can solve the 'fluctuation problem'.

It should be noted, however, that it is, at present, not clear how scale function F is exactly given as a function of the energy gradients involved in the coupled reaction. In muscle contraction, the free energy change accompanying the phosphate release step (the step considered to be essential in mechanochemical coupling) is about 7 *kcal/mol* (Shriver 1984) or 12 kT per molecule at room temperature (~300K). If scale function F decreases rapidly in energy scale >~kT, the thermal interference effect involved in mechanochemical coupling under physiological conditions would be small. However, experimental evidence *in vivo* (Higuchi & Goldman 1991, Lombardi *et al.* 1992) demonstrates that the coupling ratio is large under physiological conditions, suggesting the existence of a substantial thermal interference effect. One possibility which we did not discuss in detail in this paper is that scale function F may depend critically only on the positive (unfavorable) free energy gradient ΔE_x . Another possibility is that scale function F may depend on coupling ratio C. It would be at the present stage premature, however, to discuss the exact nature of scale function F.

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