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Temperature dependence and temperature compensation of kinetics of chemical oscillations; Belousov–Zhabotinskii reaction, glycolysis and circadian rhythms

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Abstract

Based on the distribution of activation energies around the experimental mean and averaging of rate constants we propose a theoretical scheme to examine the temperature dependence and temperature compensation of time periods of chemical oscillations. The critical finite width of the distribution is characteristic of endogeneous oscillations for compensating kinetics as observed in circadian oscillations, while the vanishing width corresponds to Arrhenius temperature dependent kinetics of non-endogeneous chemical oscillation in Belousov–Zhabotinskii reaction in a CSTR or glycolysis in cell-free yeast extracts. Our theoretical analysis is corroborated with experimental data.

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Keywords: Temperature compensation; Chemical oscillators; Circadian rhythms; BZ reaction; Oregonator model

1. Introduction

The velocity of a chemical reaction increases with temperature. Although the basic paradigm of exponential dependence of rate constant on temperature due to Arrhenius lies at the heart of chemistry over a century, the variation of temperature under diverse conditions e.g. application of homogenous temperature oscillation, stationary or varying spatial temperature gradients and temperature compensation in chemical and biological systems, have attracted wide attention in recent years (Mair et al., 2005; Hong et al., 2007; Dutt and Muller, 1993; Foerster et al., 1990; Ruoff, 1995; Rabai and Hanazaki, 1999; Ruoff and Rensing, 1996). These studies have revealed a wealth of information regarding regulatory functions of physiological processes and adaptation of bio-organisms to their environment. While complex oscillation frequencies and amplitudes in Belousov–Zhabotinskii (BZ) reaction (Zaikin and Zhabotinskii, 1970) in a stirred tank reactor vary with temperature (Dutt and Muller, 1993), application of temperature gradient (Foerster et al., 1990) initiates propagation of traveling waves in oscillatory glycolysis in yeast extracts (Mair et al., 2005). The dependence of velocity of calcium waves in frog eggs (Lechleiter and Clapham, 1992) and heart cells (Engel et al., 1995) on temperature and increased immune functionality (Rosenspire et al., 2002) due to temperature dependence of frequency of NADPH oscillations in neutrophil cells are the typical examples where temperature plays an effective means in controlling feedback in biological systems. An intriguing aspect in a related context is the temperature compensation of circadian oscillations of cells (Hastings and Sweeney, 1957) and bio-organisms which coordinate their physiological activities (Bunning, 1963; Dunlap, 1999) over a 24 hour cycle of light and dark on earth. Over a decade these studies on temperature variation and temperature compensation of experimental kinetics

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have inspired a wide ranging theoretical activities (Hong et al., 2007; Rabai and Hanazaki, 1999; Ruoff et al., 2000, 2003; Ruoff, 1992; Kovacs and Rabai, 2002; Leloup and Goldbeter, 1998; Gonze et al., 2002; Rajan and Abbott, 2007). The phenomenon that a single process (rate) can become temperature insensitive is often referred to as 'immediate temperature compensation' (Ruoff et al., 2000). Some mechanisms of temperature adaptation of enzymes have been described in Hochachka and Somero (2002).

The key point in understanding chemical oscillations is the realisation of limit cycle involving relevant reacting components of the rate-determining steps. The majority of the defining properties of the oscillations are well characterised by the limit cycle. However, kinetic rate constants being sensitive to temperature make limit cycle oscillations and the associated time periods highly susceptible to the variation of temperature. The differential behavior of a chemical oscillator like BZ or biochemical oscillator like glycolytic oscillator and a circadian oscillator is highly conspicuous by the fact that while the time period of BZ is dependent on temperature (Dutt and Muller, 1993), that of circadian oscillation is temperature compensated (Hong et al., 2007). The mechanism of temperature compensation had been analysed several years ago by Hastings and Sweeney (1957) on the basis of a hypothesis of opposing reactions in keeping with the mechanism operating in temperature compensated mechanical pendulum. More recently Ruoff et al. (2000, 2003) have suggested that a delicate balance between the positive contributions to the sum of products of control coefficients and activation energies and the corresponding sum of negative contributions may result in temperature compensation. Since the delicate balance, in general, is not robust to mutations, Hong et al. (2007) have proposed a switching mechanism for resetting the phase which forces the system to alternate between a stable steady state and a limit cycle. A mechanism of temperature compensation has also been put forward recently (Rajan and Abbott, 2007) by using a cascade of reactions forming a network which keep the ratio of rate constants fairly insensitive to temperature. The differences notwithstanding, the nature of mechanism in majority of these studies relies on the delicate adjustment and balance of control coefficients and activation energies. One of the basic requirements in such a scheme is the specificity of a set of activation energies some of which at times may have to be very large making the corresponding reaction path less probable. Secondly, the set of control coefficients are not always unique for the maintenance of the balance. In this paper we propose a generalisation of the scheme to bypass the stringent delicate balance condition involving specific activation energies or opposing reactions by using a distribution of activation energies. The critical finite width of the distribution is characteristic of the endogenous and self-regular feature of the compensating kinetics, while the vanishing width corresponds to Arrhenius temperature dependent kinetics of non-endogenous chemical oscillations. The present scheme thus allows us to understand both temperature dependence and temperature compensation of kinetics and time period on an unified footing. We illustrate the analysis in three different experimental systems: BZ reaction in a CSTR (Dutt and Muller, 1993), glycolytic oscillations in cell-free yeast extract (Mair et al., 2005) and circadian oscillations in Drosophila using standard models (Gonze and Goldbeter, 2006; Tyson et al., 1999) proposed earlier.

2. Average rate constant, temperature dependence and temperature compensation of kinetics

It is worthwhile to note that in a complex bio-organism the reacting species in the rate determining steps widely fluctuate in numbers (Gonze and Goldbeter, 2006) while crossing over the potential barriers with many local minima. Furthermore in its passage from reactant to product well a species may follow many paths corresponding to different activation energies which depend on the nature of enzymatic complexation and local heterogeneity of the reaction medium. The reduced models describing the limit cycles contain only lumped rate equations involving these species. This suggests that an average rate constant rather than a bare rate constant might be more appropriate for description of kinetics. In what follows we propose such a scheme where the averaging of rate constants is carried out over the distribution of activation energies around the experimental mean. The width of distribution can be determined by the condition of temperature compensation of kinetics in circadian oscillations in a cell signifying that the width reflects the self-regulating character of the bio-organism. For an oscillating chemical reaction like BZ on the other hand the width is not self-regulating and is determined by external condition of the reaction medium. The width in the later case tends to vanish so that one recovers the usual Arrhenius dependence of time period on temperature.

2.1. Averaging of rate constants

To realise an average rate constant based on a spread of activation energies $\langle \Delta E^2 \rangle$ and an experimental mean $\langle E \rangle$ we introduce a distribution function $P(E\langle \Delta E^2 \rangle, \langle E \rangle)$ of average activation energy E. The distribution function is normalised as

$$\int_0^\infty \mathrm{d}E\ P(E; \langle \Delta E^2 \rangle, \langle E \rangle) = 1. \tag{2.1}$$

We further assume that the spread is temperature dependent so that as $\langle \Delta E^2 \rangle = B/RT$, where R is the Universal gas constant and B is a constant to be determined. An average over Arrhenius rate constant ($k = A\mathrm{e}^{-E/RT}$) for a reaction step is then given by

$$\langle k \rangle = \int_0^\infty \mathrm{d}E A\mathrm{e}^{(-E/RT)} P(E; \langle \Delta E^2 \rangle, \langle E \rangle), \tag{2.2}$$

where A is the frequency factor. The phenomenological condition for temperature compensation requires

$$\frac{\partial\langle k\rangle}{\partial T}=0. \tag{2.3}$$

This yields the relation

$$\frac{1}{RT^2}\langle E.k\rangle+\int_0^\infty \mathrm{d}Ek\frac{\partial P}{\partial T}=0. \tag{2.4}$$

Making use of the partial derivative $\partial P/\partial T=\partial P/\partial\langle\Delta E^2\rangle\partial\langle\Delta E^2\rangle/\partial T=-B/RT^2$ in the above equation we obtain

$$B=\frac{\int_0^\infty \mathrm{d}E\ k(E)EP(E;\langle\Delta E^2\rangle,\langle E\rangle)}{\int_0^\infty \mathrm{d}E\ k(E)\partial P/\partial\langle\Delta E^2\rangle}. \tag{2.5}$$

Specializing the case for the normalised Gaussian distribution $P(E;\langle\Delta E^2\rangle,\langle E\rangle)=\sqrt{2/\pi\langle\Delta E^2\rangle}\mathrm{e}^{-(E-\langle E\rangle)^2/2\langle\Delta E^2\rangle}$ with $\langle\Delta E^2\rangle=B/RT$ it is easy to obtain

$$B=\frac{\langle k.E\rangle}{\frac{1}{2}[(RT)^2\langle k(E-\langle E\rangle)^2\rangle/B^2-RT\langle k\rangle/B]}. \tag{2.6}$$

For the given value of experimental average activation energy $\langle E\rangle$ and temperature T one can solve numerically self-consistently the above algebraic equation for B. Use of this calculated B and hence width $\langle\Delta E^2\rangle$ in Eq. (2.2) yields an average $\langle k\rangle$ which is clearly temperature compensated and the resulting time period of oscillations remains insensitive to the variation of temperature.

For the chemical reaction carried out in a homogenous medium as in a petri dish or CSTR or cell-free extract on the other hand, where the spread in distribution of activation energies tends to zero, ($\langle\Delta E^2\rangle\to 0$) the Gaussian distribution P (E; $\langle\Delta E^2\rangle$, $\langle E\rangle$) reduces to a δ-function $\delta(E-\langle E\rangle)$. Under this condition we have from Eq. (2.2)

$$\langle k\rangle=A\mathrm{e}^{(-\langle E\rangle/RT)} \tag{2.7}$$

and one recovers the Arrhenius law but in an average sense.

Eqs. (2.2) (where $\langle\Delta E^2\rangle$ is determined by Eq. (2.6)) and (2.7) are the key expressions for the average rate constant to be used in describing kinetics of chemical and biological systems that follow in the next section. It is therefore evident that the distribution of activation energies of the biological systems through mutation or otherwise has to be self-regulatory in such a way that the system adjusts its width of distribution to fulfill the condition of temperature compensation (Eq. (2.6)). This adjustment is statistical and hence more robust in contrast to the specificity of a set of activation energies as demanded by Ruoff's condition which, in general, is too stringent. Furthermore the imposition of the steps for opposing reactions and negative activation energies are avoided in this procedure. We note in passing that averaging over the distribution of activation energies is well known in the context of non-exponential kinetics and $1/f$ noise in glassy media (Weissman, 1988).

2.2. Stochastic simulation of kinetics

The stochastic simulation of kinetics can be followed by using Gillespie (1976, 1977) Algorithm and taking into consideration of each activation energy averaged rate constant $\langle k\rangle$ into the scheme. To this end we introduce the function $P(\tau,\mu)\,\mathrm{d}\tau$ as the probability that given the state $(X_1,X_2,\ldots,X_N)$ at time, the next reaction in a volume V will occur in the infinitesimal time interval $(t+\tau,t+\tau+\mathrm{d}\tau)$ and will be a specific R_μ reaction. $P(\tau,\mu)\mathrm{d}\tau$ is a joint probability density function in continuous variable $\tau(0\leqslant\tau<\infty)$ and discrete variable μ ($\mu=1,2,3,\ldots,M$). Considering h_μ as the number of distinct R_μ molecular reactant combinations available in the state space $(X_1,X_2,\ldots,X_N)$ with ($\mu=1,2,3,\ldots,M$), one has $h_\mu c_\mu\,\mathrm{d}t=a_\mu\,\mathrm{d}t$ where $a_\mu\,\mathrm{d}t$ is the probability that a R_μ will occur in V in $(t,t+\mathrm{d}t)$ given that the system is in the state $(X_1,X_2,\ldots,X_N)$ at time t. A simple consideration of master equation leads us to conclude that the reaction probability density function is given by $P(\tau,\mu)=a_\mu\exp(-a_0\tau)$ if $0\leqslant\tau<\infty$ or 0 otherwise with $a_\mu=h_\mu c_\mu$ and $a_0=\sum_{\mu=1}^{M}h_\mu c_\mu$, $\mu=1,2,\ldots,M$. In our present scheme a_μ is dependent on temperature through average $\langle k\rangle$. To generate a random pair (τ,μ) according to probability density function we choose r_1 and r_2, two random numbers from the unit interval with uniform distribution such that $\tau=1/a_0\ln(1/r_1)$ and μ to be an integer such that $\sum_{\nu=1}^{\mu-1}a_\nu<r_2a_0\leqslant\sum_{\nu=1}^{\mu}a_\nu$. Making use of τ and μ thus obtained the kinetics may be followed by increasing t by τ and adjustment of molecular population.

We now implement the above theoretical analysis in the following three sections and compare our results with experimental findings. We distinguish between two types of situations:

(I) endogenous oscillations in circadian rhythms in a cell;
(II) non-endogenous oscillations in BZ reaction in a CSTR (Dutt and Muller, 1993) or otherwise or glycolysis in cell-free extracts (Mair et al., 2005).

3. Belousov–Zhabotinskii reaction and oregonator model

Field and Noyes (1973) originally devised the oregonator model as a highly idealised minimal model for Belousov–Zhabotinskii reactions, which involves cerrium ion catalysed oscillatory Bromate oxidation of malonic acid. The model comprises of the six reacting species Y_1, Y_2, Y_3 and X_1, X_2, X_3 and the following steps.

$$X_1+Y_2\xrightarrow{k_1}Y_1,$$

$$Y_1+Y_2\xrightarrow{k_2}Z_1,$$

$$X_2+Y_1\xrightarrow{k_3}2Y_1+Y_3,$$

$$2Y_1\xrightarrow{k_4}Z_2,$$

$$X_3+Y_3\xrightarrow{k_5}Y_2.$$

Here X_1, X_2 and X_3 are in large numbers and hence they are treated as constants. k_1, k_2, k_3, k_4 and k_5 are the kinetic rate constants. Following Gillespie algorithm we specify five reaction parameters k_1X_1, k_2, k_3X_2, k_4 and k_5X_3. In order to incorporate temperature dependence we treat k_i $(i = 1, 2, \ldots, 5)$ as the corresponding activation energy averaged rate constants $\langle k_i \rangle$, according to Eq. (2.7). The reaction scheme is simulated with the following set of parameters: $k_1X_1 = 2$, $k_2 = 0.1$, $k_3X_2 = 104$, $k_4 = 0.001$ and $k_5X_3 = 26.0$ with the corresponding activation energies as 66.7, 74.1, 56.9, 85.5 and 60.4 kJ, respectively. The frequency factors ($10^{12}\,\mathrm{min}^{-1}$) are assumed to be equal.

The model admits of stable limit cycle oscillations in the specified parameter regime (Gillespie, 1977). In Fig. 1(a) we illustrate them (Y_1 vs t) for three different temperatures. It is evident that both period and amplitude are significantly affected by the variation of temperature. In Figs. 1(b) and (c) we represent the results of our numerical simulations [dark square and continuous line], by fitting with the following relations for period and amplitude:

$$Period = 3.07 \exp[-0.09 \times temperature] \tag{3.1}$$

and

$$Amplitude = -62.7 \ln[temperature - 5] + 265.9. \tag{3.2}$$

In order to correlate the simulation results with experiment, we compare the experimental results (Dutt and Muller, 1993) on the effect of temperature variation on Belousov–Zhabotinskii reaction studied by Dutt and Muller. The experiments were carried out in a thermostated

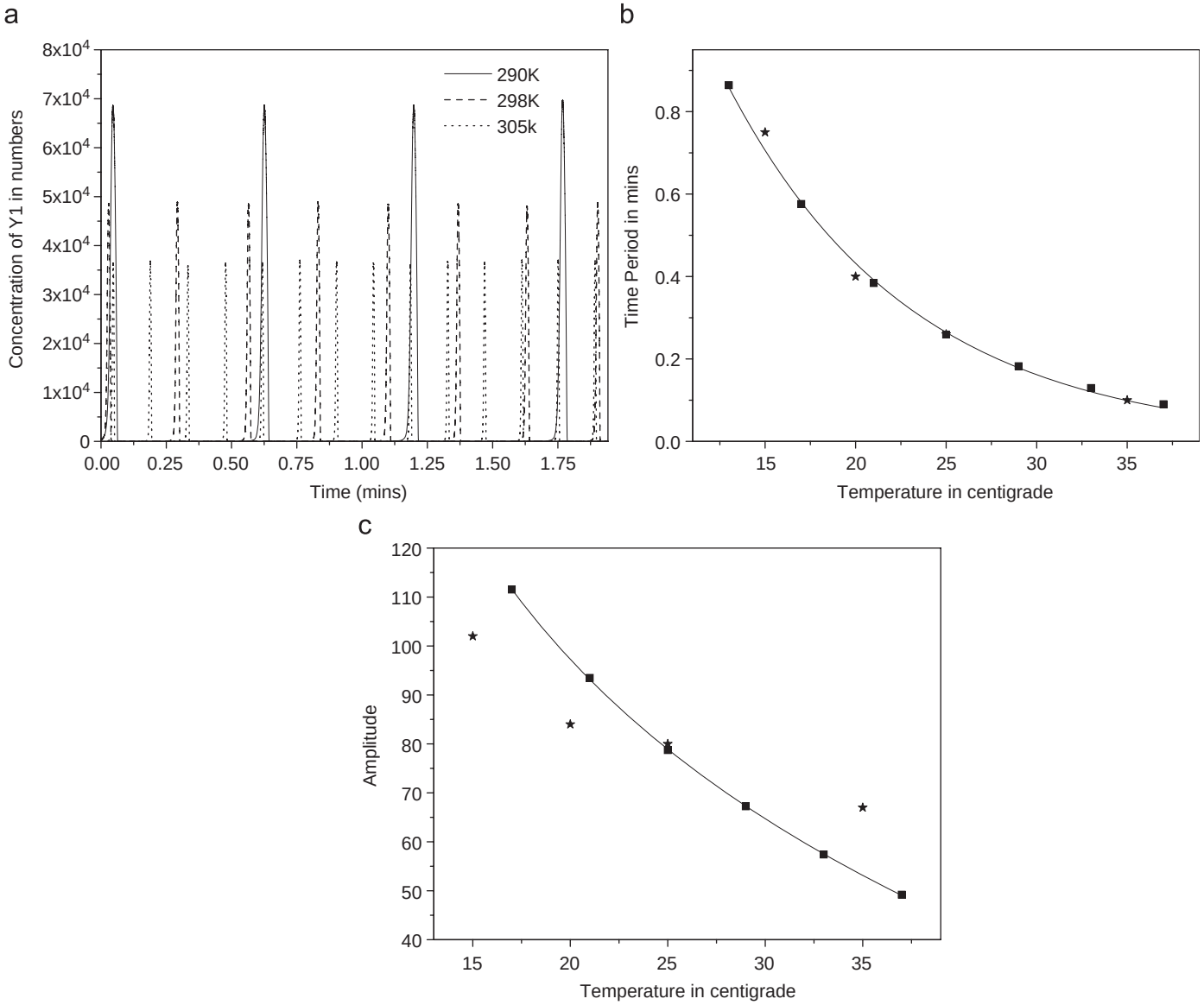

Fig. 1. (a) Oscillation of Y1 in oregonator model (numerical simulations) at three different temperatures for the parameter set given in the text. (b) Variation of time period of Y1 with temperature in oregonator model for the parameter set as mentioned in the text (Dark square and solid line-theory; Dark star-experiment). (c) Variation of amplitude of Y1 with temperature in oregonator model for the parameter set as mentioned in the text (Dark square and solid line-theory; Dark star-experiment).

CSTR with appropriate concentration of organic substrates. The experimental observation of the dependence of time period and amplitude on temperature is shown in Figs. 1(b) and (c) [star marks], respectively. The experimentally fitted empirical relations are

$$Period = 2.7\exp[-0.09 \times temperature] \tag{3.3}$$

and

$$Amplitude = -39\ln[temperature] + 205. \tag{3.4}$$

It is apparent that the experimental characteristic constant (0.09) for the variation of time period with temperature agrees fairly well with that obtained by numerical simulation. Both period and amplitude decrease with increase of temperature. Since experimentally the amplitude is a function of stirring rate, which, however, is not a component of our theoretical analysis the agreement between the simulation and the experiment in the case of amplitude-temperature relation is found to be less satisfactory as compared to period-temperature curve.

Table 1
Stochastic version of the glycolytic model of Goldbeter and Lefever (1972)

Reaction number	Reaction	Probability of the reaction
1	$\alpha \rightarrow$	γ
2	$\rightarrow \alpha$	$\sigma\phi(\alpha,\gamma)$
3	$\gamma \rightarrow$	$q\sigma\phi(\alpha,\gamma)$
4	$\rightarrow \gamma$	k_s

4. Glycolytic oscillations

Glycolysis is the source of metabolic energy in almost all living cells. Based on a scheme of autocatalytic reaction considered by Monod et al. (1965) for allosteric enzyme, Goldbeter and Lefever (1972) had proposed a minimal model (Kar and Ray, 2003, 2004) for glycolytic oscillations. The conspicuous feature of this model is captured by the allosteric enzyme, PFK, which has been found to be responsible for conversion of substrate ATP (and F6P) to ADP (and FBP), respectively. This is displayed schematically in Goldbeter (1996). The knowledge of the rate constants allows us to construct the kinetic equations for the enzyme which exists in two states R and T and the kinetic equations for the substrate (S) and the product (P). Making use of quasi-steady state approximations for the enzymatic species one arrives at the kinetic equations for the normalised substrate (α) and product (γ) concentration. In stochastic simulation of this model we attribute to each linear or nonlinear term of the kinetic equations a probability of occurrence of the corresponding reaction. Thus four reactions for the two reacting components α and γ along with the associated probabilities are given in Table 1. We use $\alpha = [S]/K_R$ and $\gamma = [P]/K_P$ which are the dimensionless concentrations of the substrate and the products, respectively. $\phi(\alpha,\gamma)$ is the complex kinetic function given by

$$\phi(\alpha,\gamma) = \frac{\alpha e(1+\alpha e)^{n-1}(1+\gamma)^n + L\theta\alpha ce'(1+\alpha ce')^{n-1}}{L(1+\alpha ce')^n + (1+\alpha e)^n(1+\gamma)^n}, \tag{4.1}$$

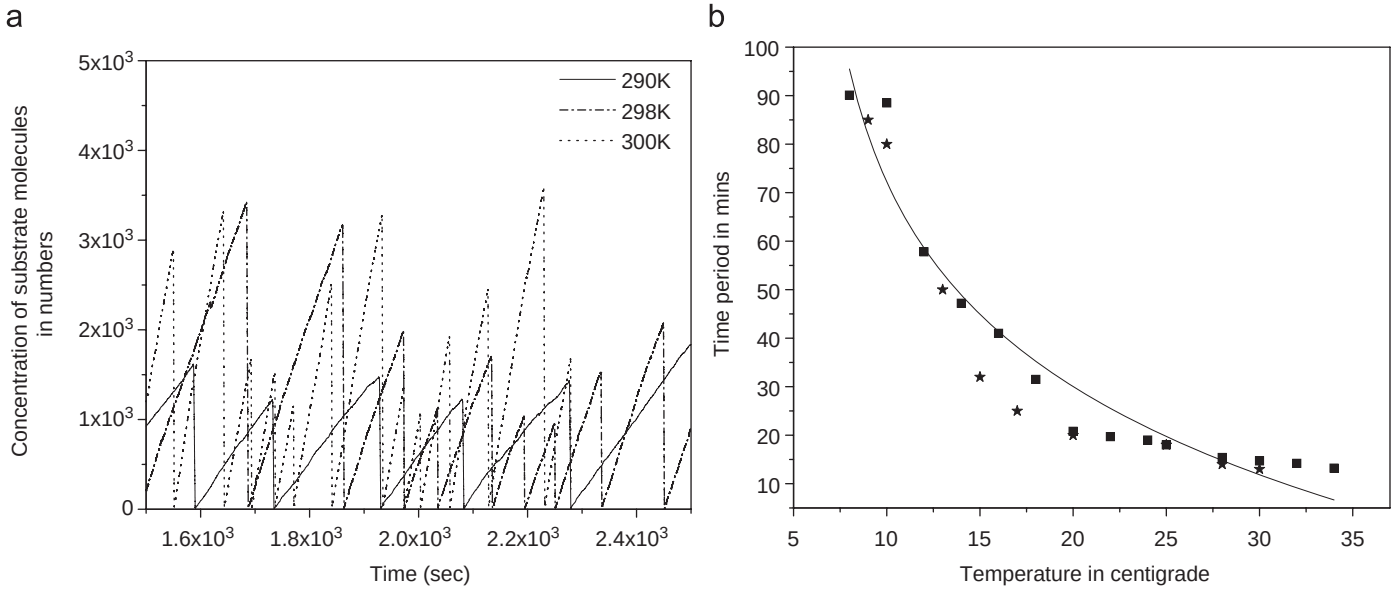

Fig. 2. (a) Sustained oscillations of the number of substrate molecules in units of K_P at three different temperatures for the parameter set given in the text (numerical simulation). (b) Variation of time period of oscillation of substrate molecules with temperature in glycolytic model for the parameter set as mentioned in the text (Dark square and solid line-theory; Dark star-experiment).

where σ is the maximun rate of enzyme reaction divided by the constant K_R. L is the allosteric constant of the enzyme. $c = K_R/K_T$ is the nonexclusive binding coefficient of the substrate. The other parameters are given as: $q = K_R/K_P$, $e = (1+\varepsilon)^{-1}$, $e' = (1+\varepsilon')^{-1}$ with $\varepsilon = k/d$, $\varepsilon' = k'/d'$ and $\theta = k'/k$. For a detailed description of the parameters and their values used in simulation we refer to earlier articles (Goldbeter, 1996; Kar and Ray, 2003). In our simulations $q = K_R/K_P$ which is assumed to be unity; the normalised dimensionless concentrations α and γ can therefore be treated as numbers, since in simulating the system with Gillespie algorithm, the required variables are the number of molecules of each species. Each time a reaction occurs the number of reacting molecules changes from one unit. Dimensionless α (and γ) should therefore be read as number of substrate molecules (and product molecules) in units of K_R ($= K_P$). The activation energies chosen for the average rate constants v, σ and k_s are 72.4, 62.1 and 74.16 kJ, respectively according to Eq. (2.7) with the frequency factor $10^{12}\,s^{-1}$. This parameter regime gives rise to limit cycle oscillations. We illustrate the oscillations of substrate concentration ([S] vs t) in number of molecules at three different temperatures as depicted in Fig. 2(a).Each curve has been obtained by averaging over a large number of trajectories ($\sim$100) and the estimated time period is the mean time period for glycolytic oscillation at each temperature. The variation of time period of oscillation with temperature is shown in Fig. 2(b) and compared to that for the experiment carried out with cell-free yeast extract by Mair et al. (2005). The agreement between theory and experiment is found to be quite satisfactory and lends to good support to Goldbeter–Lefever model for glycolysis, in the context of temperature dependence of chemical oscillations.

Table 2
Stochastic version of the three variable model of Drosophila by Gonze and Goldbeter (2006)

Reaction number	Reaction	Probability of reaction
1	$\rightarrow$ M	$\omega_1 = v_s\Omega\dfrac{(K_I\Omega)^n}{(K_I\Omega)^n + P_n^N}$
2	M $\rightarrow$	$\omega_2 = v_m\Omega\dfrac{M}{K_M\Omega + M}$
3	$\rightarrow P_C$	$\omega_3 = k_s M$
4	$P_C \rightarrow$	$\omega_4 = v_d\Omega\dfrac{P_C}{K_d\Omega + P_C}$
5	$P_C \rightarrow P_N$	$k_1 P_C$
6	$P_N \rightarrow P_C$	$k_2 P_N$

The parameter Ω ($= 1000$) is used to convert the concentration into number of molecules and controls the level of noise in the system.

5. Circadian oscillations

The seminal discovery of PER gene by Konopka and Benzer (1971) in early seventies has motivated significant advances in molecular biology of circadian rhythm in living cells ranging from fruit fly Drosophila, fungus Neurospora, Cynobacteria, plants to mammals. Ever since the proposal of a three-variable oscillator model by Goodwin (1963) in sixties a number of theoretical models have been proposed, the central theme of which lies on negative autoregulation extended by PER protein on its gene expression. To investigate temperature compensation in our scheme we consider two different models proposed by Gonze and Goldbeter (2006) and Tyson et al. (1999) (Hong et al., 2007). We have used stochastic reaction dynamics to study the circadian oscillation of these two models. The main idea of this reaction dynamics (here Gillespie algorithm) is to include the stochastically determined reaction probability density. In this approach a parameter Ω (having the unit of volume) is used to convert the concentrations into number of molecules. Since circadian oscillations are obtained in the presence of molecular noise this parameter has an added advantage of controlling the molecular noise in the system. In the numerical simulation of the two models discussed below the value of Ω is taken to be 1000.

5.1. Three variable model by Gonze and Goldbeter

The model is schematically shown in Gonze and Goldbeter (2006). This is based on the repression exerted by the nuclear form of a clock protein (P_N) on the transcription of its gene into m-RNA (M) (Goldbeter, 1996; Leloup et al., 1999). m-RNA is transported into cytosol and then translated into protein. In the cytosol the clock protein (P_C) is synthesised at a rate proportional to M. The protein enters into the nucleus in a reversible

Table 3
Description and values for the parameters used in the three-variable model by Gonze and Goldbeter (2006)

Name	Value	Unit	Description
v_s	1.6	nM h^{-1}	Max rate of transcription
v_m	0.505	nM h^{-1}	Max rate of degradation of per m-RNA
v_d	1.4	nM h^{-1}	Max rate of degradation
k_s	0.5	h^{-1}	First order rate constant for the synthesis of PER
k_1	0.5	h^{-1}	First order rate constant for the conversion of cytosolic clock protein (P_C) to nuclear clock protein(P_N)
k_2	0.6	h^{-1}	First order rate constant for the conversion of nuclear clock protein (P_N) to cytosolic clock protein (P_C)

manner. This is followed by a negative feedback loop exerted by the clock protein on gene transcription. The whole process is described by an equation of Hill type. The time evolution of the concentration of the Clock gene m-RNA (M), and cytosolic and nuclear clock protein (P_C and P_N, respectively) in a single cell can be followed by stochastic numerical simulation according to six reaction steps with associated probabilities as given in Table 2. The relevant parameters for the dynamics are described in Table 3. n is the degree of cooperativity and K_I is the threshold repression constant. The values of K_I, K_m, K_d are taken to be 1, 0.5 and 0.13 nM, respectively. The kinetic rate constants υ_s, υ_m, υ_d, k_s, k_1 and k_2 are to be replaced by activation energy averaged rate constants according to Eq. (2.2), the width being determined by Eq. (2.6). The parameter set for the mean activation energies and the scaling factors which include frequency factors are given in Table 4.In Fig. 3(a) we exhibit the representative oscillations of concentrations of m-RNA (in numbers) vs time at three different temperatures. It is apparent that the time periods are fairly independent of temperature with little phase shifts for the oscillations. Fig. 3(b) exhibits temperature compensation of the time period where the kinetic rate constants are determined by the width according to Eq. (2.6) (Dark stars). This has been compared to the case where the width of distribution tends to be vanishing implying Arrhenius dependence of period on temperature (Dark squares).

Table 4
Mean activation energies and scaling factors for the three-variable model by Gonze and Goldbeter (2006)

Rate constant	Average activation energy (kJ)	Scaling factor
υ_s nM h^{-1}	67.29	88.39
υ_m nM h^{-1}	70.15	28.69
υ_d nM h^{-1}	67.62	76.5
k_s (h^{-1})	70.17	28.4
k_1 (h^{-1})	70.17	28.4
k_2 (h^{-1})	69.72	33.7

5.2. Simplified two variable model by Tyson et al. (1999)

Tyson et al. (1999) have developed a two-variable model for circadian oscillation in Drosophila. They assumed that the PER monomers are rapidly phosphorylated and degraded whereas PER/TIM dimers are less susceptible to proteolysis. The model of Tyson et al. (1999) is similar in spirit to that of Leloup and Goldbeter (1998) with the exception that in the Leloup–Goldbeter model the role of PER phosphorylation is to introduce a time delay into the negative feedback loop whereas in case of the latter model the role of PER phosphorylation is to introduce a positive feedback in the PER accumulation. With increase of PER concentration greater proportion of protein is dimerised and protected from DBT. Therefore as the total concentration of PER (monomer + dimer) increases, the rate of total PER degradation does not increases proportionally. This nonlinearity is the essential ingredient for the mathematical model of Tyson et al. (1999). The stochastic version of the reduced model of Tyson et al. comprises of the steps of reactions with the associated probabilities as given in Table 5. We take the concentration variables $M = [\text{m-RNA}]$, $P_t = [\text{total protein}] = P_1 + 2P_2$ where $P_1 = [\text{monomer}]$ and $P_2 = [\text{dimer}]$. The stochastic

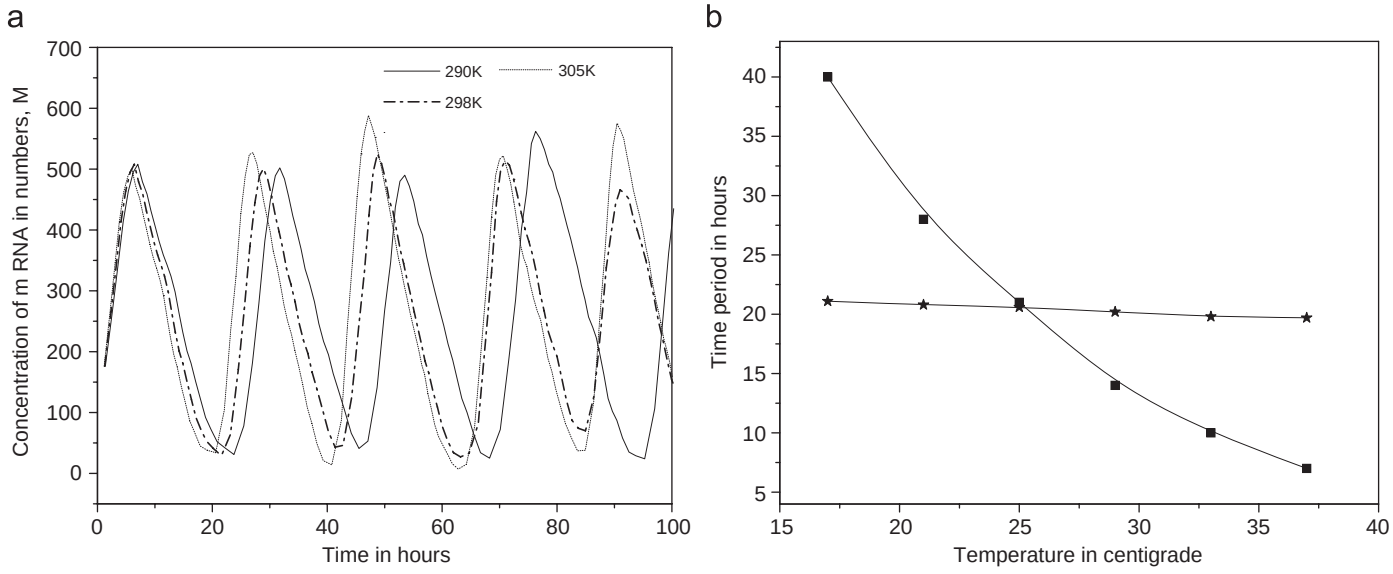

Fig. 3. (a) Oscillations of m-RNA obtained by stochastic simulation of the model for circadian rhythm by Gonze–Goldbeter with temperature compensated rate constants using Eq. (2.2) (with Eq. (2.6)) and (2.7) at three different temperatures for the parameter set mentioned in text. (b) Temperature dependence (Dark square) and temperature compensation (Dark stars) of the time period of oscillation of m-RNA in *Drosophila* (Gonze–Goldbeter model) for the parameter set mentioned in the text.

simulations have been carried out with the parameter set given in Table 6.

C_m and C_p represent characteristic concentrations for m-RNA and protein, respectively. The parameter set for the scaling factors (containing the frequency factors) and the average activation energies are given in Table 7. The results have been plotted in Fig. 4(a). Temperature compensation of the time period is clearly evident in Fig. 4(b) where one uses the average rate constants for which the widths are determined by Eq. (2.6) (Dark stars). For vanishing width one observes the usual Arrhenius temperature dependence as shown by dark squares as in the case of Gonze–Goldbeter model.

6. Conclusion

The kinetic rate constant of a chemical reaction depends on its activation energy barrier. Based on the distribution of activation energies around the experimental mean we have examined the temperature dependence and temperature compensation of kinetics of chemical oscillations and the associated time periods in chemical and biological systems in terms of activation energy averaged rate constants. A distribution of activation energies for a reaction step may imply many paths for a reacting species corresponding to different enzyme-substrate complexation or otherwise in going from a reactant state to a product state. The scheme concentrates on a single parameter, the width of distribution of which characterises the endogenous and non-endogenous oscillations. A circadian oscillation in a cell is endogenous and the width of distribution of activation energies as determined by temperature compensating condition is self-regulatory. A chemical oscillation in a BZ reaction in a CSTR or in glycolysis in a cell-free yeast extract, on the other hand is non-endogenous for which the dynamics is set by the reaction condition, e.g. stirring rate, etc. and the width tends to vanish in such cases. We emphasise that this distinction is phenomenological and our work does not elaborate any detailed biological content. However, this lends support to the fact that a critical condition based on realisation of a set specific activation energies or opposing reactions is too severe and unlikely to withstand mutations. A condition based on the statistical distribution of activation energies around an experimental mean is a relatively flexible condition and may meet the purpose. The Arrhenius temperature dependence and temperature compensation correspond to two limiting conditions on the width of distribution. Further experimental studies on temperature dependence of chemical oscillations may reveal the intermediate ranges

Table 5
Stochastic version of the two-variable model of drosophila by Tyson et al. (1999)

Reaction number	Reaction	Probability of reaction
1	$\rightarrow$ M	$\frac{\Omega v_m}{1+(P_t(1-q)/2P_{crit})^2}$ where $q=\frac{2}{1+\sqrt{1+8K_{eq}P_t/\Omega}}$
2	M $\rightarrow$	$k_m M$
3	$\rightarrow P_t$	$v_p M$
4	$P_t \rightarrow$	$\frac{\Omega(k_{p1}P_t q + k_{p2}P_t)}{\Omega J_p + P_t}$
5	$P_t \rightarrow$	$k_{p3}P_t$

The parameter Ω (= 1000) is used to convert the concentration into number of molecules and controls the level of noise in the system.

Table 7
Average activation energies and scaling factors for the two-variable model by Tyson et al. (1999)

Rate constant	Average activation energy (kJ)	Scaling factor
v_m ($C_m h^{-1}$)	68.45	54.64
k_m (h^{-1})	74.16	6.02
v_p ($C_p C_m^{-1} h^{-1}$)	70.17	28.4
k_{p1} ($C_p h^{-1}$)	62.75	50.5
k_{p2} ($C_p h^{-1}$)	77.14	1.88
k_{p3} (h^{-1})	74.15	6.02

Table 6
Description and values for the parameters used in the two-variable model by Tyson et al. (1999)

Name	Value	Unit	Description
v_m	1	$C_m h^{-1}$	Maximum rate of synthesis of m-RNA
k_m	0.1	h^{-1}	First order rate constant of m-RNA degradation
v_p	0.5	$C_p C_m^{-1} h^{-1}$	Rate constant for translation m-RNA
k_{p1}	10	$C_p h^{-1}$	V_{max} for monomer phosphorylation
k_{p2}	0.03	$C_p h^{-1}$	V_{max} for dimer phosphorylation
k_{p3}	0.1	h^{-1}	First order rate constant for proteolysis
K_{eq}	200	C_p^{-1}	Equilibrium constant for dimerisation
P_{crit}	0.1	C_p	Dimer concentration at the half maximum transcription rate
J_p	0.05	C_p	Michaelis constant for protein kinase (DBT)

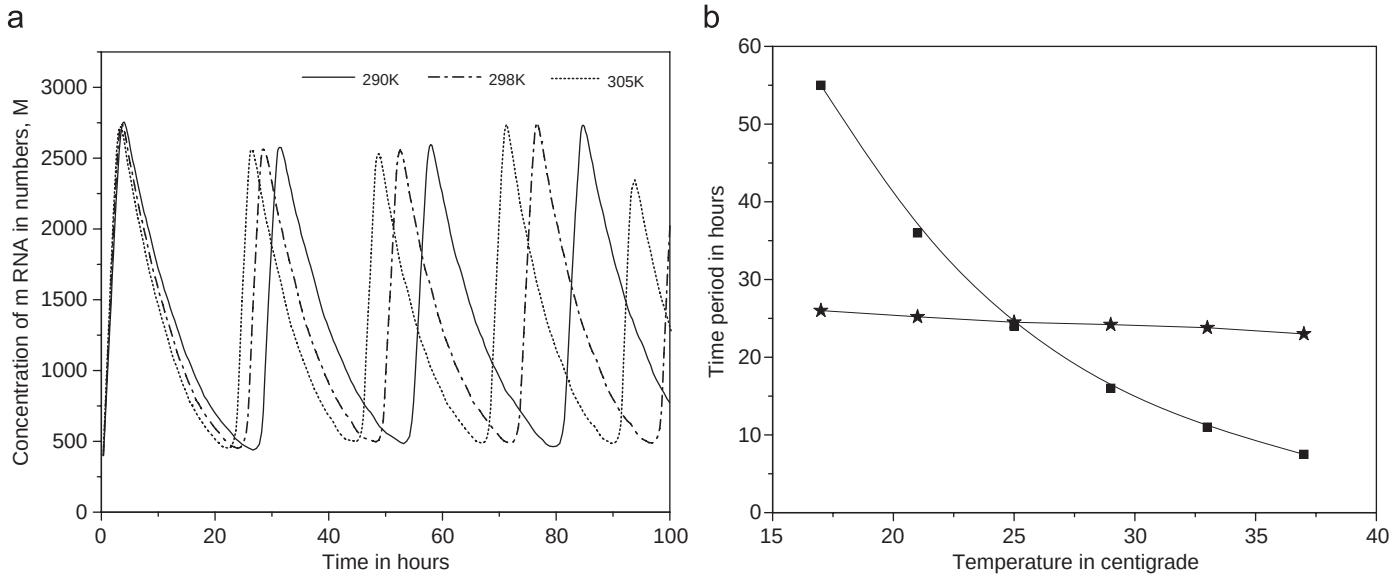

Fig. 4. (a) Oscillations of m-RNA monomer obtained by stochastic simulation of the model by Tyson et al. for circadian rhythm with temperature compensated rate constants using Eqs. (2.2) (with Eq. (2.6)) and (2.7) at three different temperatures for the parameter set mentioned in the text. (b) Temperature dependence (Dark squares) and temperature compensation (Dark stars) of time period of oscillation of m-RNA in *Drosophila* (Tyson et al. model) for the parameter set mentioned in the text.

of width for which one may observe weak, partly compensating and non-Arrhenius kinetics of oscillations.

7. Uncited references

Leloup et al. (1999); Ruoff and Rensing (1996).

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