Biophysical Chemistry 8 (1978) 71–80

FIELD-INDUCED INSTABILITIES IN TWO-COMPONENT SYSTEMS: THE CELL-FREE GLYCOLYTIC SYSTEM

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Received 27 December 1977

The effects of weak static electric field on a cell-free glycolytic system, which is known to exhibit oscillatory behavior, have been studied using an allosteric model (due to Goldbeter and Lefever). Linear stability analysis is used to determine the change in the nature of stability and the consequent appearance of dissipative structures, due to the electric field. The results show that for this system all the necessary conditions for a field induced instability are satisfied. An order of magnitude calculation of the field strength shows that field strength in the range 10–100 V/cm is required to produce observable change in the system's behavior.

1. Introduction

Open chemical systems far from equilibrium are known to exhibit stable states with spatio-temporal organization. Such structures whose formation depends on the dissipative processes in the system are termed dissipative structures [1, 2, 3]. Often and especially in biochemical systems, the reacting chemicals are in a solution, and when in a solution some of the species of molecules acquire a net charge and become ions.

In such systems it is possible to introduce an additional transport mechanism, viz. ionic drift, by subjecting the system to an electric field. The possible influences of this transport on the stability of the system can be understood by considering its influence on the growth of concentration fluctuations that occur locally. The system is unstable if the overall effect of the chemical reaction and the transport mechanisms on the fluctuations is to amplify them. Considering a small volume ΔV in which a fluctuation occurs, we see that diffusional transport in general tends to damp this fluctuation and if the mobilities of the different ionic species are almost equal, the presence of an electric field simply introduces an overall drift motion on the fluctuation without having any influence on its growth or decay. However, if the mobilities are unequal then the difference in the drift velocities would amount to selectively removing the faster ions from the volume ΔV, the same being true if the diffusion constants are unequal. Depending on the type of chemical reaction, this selective removal could result in the growth of the fluctuation and have a destabilizing influence on the system. Recently the general effects of static electric fields on the symmetry lowering instabilities have been studied by one of the authors [4]. Here we present a detailed analysis of a two component system and apply the results to a well-characterized system viz. the cell-free glycolytic system.

Glycolytic systems that exhibit oscillatory behavior have been extensively studied [6–15]. These studies indicate that the oscillations originate at the reaction step involving the enzyme phospho-fructokinase (PFK). Goldbeter and Lefever have developed an allosteric model [5] for this reaction step, which accounts for the

observed behavior with remarkable accuracy. For our purpose we use this model to study the effects of static electric fields on the dissipative structures of glycolytic systems.

2. The Goldbeter–Lefever model

The overall reaction step that involves PFK is

ATP + fructose-6-phosphate

$$\xrightarrow{\text{PFK}}$$ ADP + fructose-1, 6-diphosphate

Observed oscillations in the densities of the metabolites originate at this step due to the interaction between ATP, PFK and ADP. Experiments on phase shifts show that fructose-6-phosphate and fructose-1, 6-diphosphate do not play any role in the mechanism of the oscillations [11, 15, 17] and hence it is sufficient to consider only ATP as the substrate and only ADP as the product.

The Goldbeter–Lefever model (referred to hereafter as the G–L model) is based on the following assumptions:

(i) The enzyme PFK is an allosteric dimer which has two conformations, an active conformation which is denoted by R and an inactive conformation which is denoted by T [‡].

(ii-a) The substrate (ATP) is supplied at a constant rate and it binds to both R and T forms, however, with different affinities (i.e. in the terminology of Monod et al. [16] we have a K-system).

(ii-b) The substrate-R complex then decomposes irreversibility to yield the product (ADP) and the enzyme. The substrate-T complex does not yield the product, i.e. the R and T forms have differing catalytic activity (in the terminology of Monod et al. we have here a V-system).

(iii-a) The product which is a positive effector binds only to the R-form.

(iii-b) The product is removed from the system at a rate proportional to its concentration.

The validity of these assumptions and their physiological significance are discussed in detail in an article by Goldbeter and Nicolis [18]. However, we have the following comment to make regarding assumption (ii-b). Here we have assumed that the substrate-T complex is totally inactive and does not decompose to give the product. This assumption is not crucial to the system's oscillatory behavior and in fact G–L model was later generalized [18] to take into account the substrate-T complex decomposing to give the product. Such an analysis shows that the essential features, and in particular the nature of the symmetry breaking instabilities, are unaltered. In the present work, since we are interested in the effects of external field on the instabilities, we adopt this simplifying assumption.

Assumptions (ii-a, b) and (iii-b) drive the system far from equilibrium while the differential binding and catalytic activity give rise to cooperative nonlinear effects that are essential for the formation of dissipative structures.

On the basis of this model and some further assumptions, the kinetic equations of the system can be written as (see [5] and [18] for details)

$$\frac{\partial \alpha}{\partial t} = a\left\{\sigma_1 - \frac{[2D_0\epsilon/(\epsilon+1)]\,\alpha(1+\gamma)^2[1+\alpha/(\epsilon+1)]}{L(1+\alpha c)^2+(1+\gamma)^2[1+\alpha/(\epsilon+1)]^2}\right\} \quad (1a)$$

$$\frac{\partial \gamma}{\partial t} = a\left\{\frac{[2D_0\epsilon/(\epsilon+1)]\,\alpha(1+\gamma)^2[1+\alpha/(\epsilon+1)]}{L(1+\alpha c)^2+(1+\gamma)^2[1+\alpha/(\epsilon+1)]^2} - \sigma_2\gamma\right\} \quad (1b)$$

where α and γ are normalized concentrations [5, 16] of the substrate and product respectively; σ_1 is proportional to the rate at which the substrate is supplied to the system; σ_2 is proportional to the rate of product removal; L is the allosteric constant equal to ratio of T form concentration to the R form concentration in the absence of ligands; c is the non-exclusive binding coefficient which is a measure of the degree of differential affinity of the substrate to the two forms of the enzyme; D_0 is the total concentration of the enzyme and ϵ and a are quantities depending on the kinetic constants.

Further, in aqueous solution ATP and ADP acquire a net charge due to the partial ionization of the phosphate groups, and as a result, in the presence of an electric field the local densities will be altered due to their mobility. Considering diffusion also then, the change in the local densities of the metabolites is due to (i) the chemical reaction, (ii) diffusion. and (iii) in

[‡] At pH near 7 it may very well be that the active form of PFK exists as a tetramer, but it can be shown that the results are not changed in any radical way if we continue to regard it as dimeric [18].

the presence of an electric field, the ionic mobility. Considering these processes the equations of the system become [4]

$$\frac{\partial \alpha}{\partial t} = a[\sigma_1 - \Sigma] - \nabla\cdot(M_\alpha \alpha E) + \nabla\cdot(D_\alpha \nabla\alpha) \qquad (2a)$$

$$\frac{\partial \gamma}{\partial t} = a[\Sigma - \sigma_2\gamma] - \nabla\cdot(M_\gamma \gamma E) + \nabla\cdot(D_\gamma \nabla\gamma) \qquad (2b)$$

where

$$\Sigma = \frac{[2D_0\epsilon/(\epsilon+1)]\,\alpha(1+\gamma)^2[1+\alpha/(\epsilon+1)]}{L(1+\alpha c)^2 + (1+\gamma)^2[1+\alpha/(\epsilon+1)]^2}\,.$$

D_α, D_γ are the diffusion tensors for α and γ respectively. M_α and M_γ are mobility tensors. Since the ionization of ATP and ADP is not always complete, we assume that the proper factor of multiplication is included in M_α and M_γ. Note that due to the directionality introduced by the electric field the tensorial nature of the diffusion should be taken into consideration.

As to the electrolytic nature of the system, we make the following simplifying assumptions:

(a) There are other ions in the system (keeping in mind all the other species of molecules that are involved in the glycolytic pathways) such that the divergence of the electric field in the solution is vanishingly small when the system is in a homogeneous state, and the density variations of the different ionic species in the reaction give rise to local fields that are very small compared to the external electric field. We note here that in the cell-free glycolytic system, at concentrations for which it is capable of giving rise to oscillatory instabilities ATP is not a significant negative effector of PFK, although the complex Mg-ATP could be. This has been observed in muscle extracts [22, 23]. We therefore assume that no Mg^{++} is present in solution and that the supporting electrolyte consists of other ions.

(b) The external electric field is static and in the z-direction. Also, the field intensities under consideration are low so that the effects of joule heating could be neglected since it is known that the glycolytic system is sensitive to temperature [19]. We also assume that the second Wien effect can be neglected.

(c) The mobilities M_α and M_γ are scalars.

Denoting the diffusion constants parallel to the field by $D_\parallel$ and perpendicular to the field by $D_\perp$, with the above assumptions we can write equations (2a) and (2b) as

$$\frac{\partial \alpha}{\partial t} = a[\sigma_1 - \Sigma] - M_\alpha E\frac{\partial \alpha}{\partial z} + D_{\perp\alpha}\left(\frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2}\right)\alpha + D_{\parallel\alpha}\frac{\partial^2 \alpha}{\partial z^2}, \qquad (3a)$$

$$\frac{\partial \gamma}{\partial t} = a[\Sigma - \sigma_2\gamma] - M_\gamma E\frac{\partial \gamma}{\partial z} + D_{\perp\gamma}\left(\frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2}\right)\gamma + D_{\parallel\gamma}\frac{\partial^2 \gamma}{\partial z^2}. \qquad (3b)$$

This system has two homogeneous steady states, only one of them being physically acceptable. This state is given by

$$\gamma_0 = \sigma_1/\sigma_2 \qquad (4a)$$

$$\alpha_0 = \frac{\{[D_0\epsilon/(\epsilon+1)]\,\Gamma^2 - \sigma_1[Lc + \Gamma^2/(\epsilon+1)]\} - \Gamma\delta^{1/2}}{\{\sigma_1[Lc^2 + \Gamma^2/(\epsilon+1)^2] - 2D_0\epsilon\Gamma^2/(\epsilon+1)^2\}}\,. \qquad (4b)$$

where

$$\Gamma = (1 + \sigma_1/\sigma_2) \qquad (5)$$

and

$$\delta = 2\sigma_1 L[D_0\epsilon/(\epsilon+1)]\,[1/(\epsilon+1) - c] + [D_0\epsilon\Gamma/(\epsilon+1)]^2 - \sigma_1^2 L[1/(\epsilon+1) - c]^2\,. \qquad (6)$$

3. Field induced instability

To investigate the nature of stability of this steady state, we linearize the system of equations (3a), (3b) about this steady state, and taking the Fourier transform of the perturbations from the steady state $\delta\alpha$ and $\delta\gamma$ we obtain

$$\frac{\partial}{\partial t}\begin{pmatrix}\delta\alpha\\ \delta\gamma\end{pmatrix} = F'(\alpha_0, \gamma_0)\begin{pmatrix}\delta\alpha\\ \delta\gamma\end{pmatrix} + \begin{pmatrix}-(D_{\parallel\alpha}k_z^2 + D_{\perp\alpha}k^2 + iEM_\alpha k_z) & 0\\ 0 & -(D_{\parallel\gamma}k_z^2 + D_{\perp\gamma}k^2 + iEM_\gamma k_z)\end{pmatrix}\begin{pmatrix}\delta\alpha\\ \delta\gamma\end{pmatrix} \qquad (7)$$

where $F'(\alpha_0, \gamma_0)$ is the Jacobian of the non-linear kinetic part evaluated at (α_0, γ_0) and $k_z^2 + k_y^2$ is the transverse component of the wave vector.

If the diffusion coefficients and the mobilities for

both the components are the same, i.e., if $D_{\parallel\alpha} = D_{\parallel\gamma} = D_{\perp\alpha} = D_{\perp\gamma} = D$ and $M_\alpha = M_\gamma = M$, then the eigenvalues λ_i of this system are of the form

$$\lambda_i = \lambda_{F'i} - iEMk_z - D(k^2 + k_z^2), \tag{8}$$

where $\lambda_{F'i}$ is the eigenvalue of $F'(\alpha_0, \gamma_0)$. In this case we see that diffusion makes the system more stable while the ionic mobility alters only the frequency of oscillation. For the nature of stability to be altered by the ionic mobility, it is necessary that the mobility of the components be unequal. In the glycolytic system this condition is satisfied because the ionic mobility of ATP and ADP are unequal. Since it is the difference in the transport coefficients that changes the behavior of the system, it is convenient to define

$$D_{\parallel\gamma} \equiv D_{\parallel\alpha} + \delta_1\,, \qquad D_{\perp\gamma} \equiv D_{\perp\alpha} + \delta_2\,, \tag{9a,b}$$

$$M_\gamma \equiv M_\alpha + \mu\,, \qquad \Delta \equiv -(\delta_1 k_z^2 + \delta_2 k^2 + iE\mu) \tag{9c,d}$$

and rewrite eq. (7) as

$$\frac{\partial}{\partial t}\begin{pmatrix}\delta\alpha\\ \delta\gamma\end{pmatrix} = \left[\begin{pmatrix}C & 0\\ 0 & C\end{pmatrix} + \begin{pmatrix}a_{11} & a_{12}\\ a_{21} & a_{22}+\Delta\end{pmatrix}\right]\begin{pmatrix}\delta\alpha\\ \delta\gamma\end{pmatrix} \tag{10}$$

where $C \equiv -(D_{\parallel\alpha}k_z^2 + D_{\perp\alpha}k^2 + iEM_\alpha)$ and $a_{ij}, i,j = 1,2$ are the matrix elements of $F'(\alpha_0, \gamma_0)$. The eigenvalues of this system will be of the form

$$\lambda = C + \lambda'\,, \tag{11}$$

where λ' is the eigenvalue of the second matrix. The characteristic equation that determines λ' is

$$\lambda'^2 - \lambda'(a_{11} + a_{22} + \Delta) + a_{11}a_{22} + a_{11}\Delta - a_{21}a_{12} = 0$$

the roots of which are

$$\lambda'_\pm = \frac{a_{11} + a_{22} + \Delta}{2} \pm \left\{\frac{(a_{11} + a_{22} + \Delta)^2 - 4(a_{11}a_{22} + a_{11}\Delta - a_{21}a_{12})}{2}\right\}^{1/2}.$$

With

$$d \equiv (a_{11} + a_{22})^2 - 4(a_{11}a_{22} - a_{12}a_{21}) \tag{12}$$

$$\alpha' \equiv (k^2\delta_1^2 + k_z^2\delta_2)^2 - k_z^2\mu^2E^2 - 2(k^2\delta_1 + k_z^2\delta_2)(a_{22} - a_{11}) \tag{13}$$

and

$$\beta \equiv -2k\mu E[(a_{22} - a_{11}) - (k^2\delta_1 + k_z^2\delta_2)] \tag{14}$$

these roots can be written as

$$\lambda_\pm = R_\pm + iI_\pm\,, \tag{15}$$

where

$$R_\pm = \mathrm{Re}\left[C + \frac{a_{11} + a_{22} + \Delta}{2}\right] \pm \tfrac{1}{2}\left\{\frac{(d + \alpha') + \sqrt{(d+\alpha')^2 + \beta^2}}{2}\right\}^{1/2}, \tag{16}$$

$$I_\pm = \mathrm{Im}\left[C + \frac{a_{11} + a_{22} + \Delta}{2}\right] \pm \tfrac{1}{2}\left\{\frac{\sqrt{(d+\alpha')^2 + \beta^2} - (d + \alpha')}{2}\right\}^{1/2}. \tag{17}$$

The system becomes unstable when $R_\pm > 0$ with a linear frequency of oscillation $I_\pm/2\pi$.

We are interested in the possibility of inducing an instability through an electric field. This will be possible if the largest real part R_+, is negative for E less than a critical value E_c, and becomes positive when E exceeds E_c. If this condition is satisfied, then as the field strength is increased the system will become unstable when E reaches the critical value E_c, and this induced instability will result in the formation of a spatio-temporal dissipative structure.

From the above expressions the stability of the system in three dimensions can be inferred, and in general it will not be identical to the one dimensional case due to the dependence of α and β on the transverse component of the wave vector. However, for the sake of simplicity and in order to compare our results with those of Goldbeter and Lefever's analysis, which is in one dimension, we consider the special case of one dimension. In this case we see that R_+ is a function of k_z^2 and E^2 and we consider it as a family of functions of k_z^2 parametrized by E^2. With $\eta \equiv k_z^2$ and $\gamma \equiv E^2\mu^2$ we write

$$R_\gamma(\eta) = (a_{11} + a_{22})/2 - \eta(D + \delta/2) + \tfrac{1}{2}\sqrt{g_\gamma(\eta)}/2 \tag{18}$$

where

$$g(\eta) = d + \eta^2\delta^2 - \eta(\gamma + 2(a_{22} - a_{11})\delta) + \{[d + \eta^2\xi^2 - \eta(\gamma + 2(a_{22} - a_{11})\delta)]^2 + 4\eta\gamma[(a_{22} - a_{11}) - \eta\delta]^2\}^{1/2} \tag{19}$$

Here we have dropped the subscripts for D and δ, since we are considering a one dimensional system.

From these equations it follows that $R_y(\eta)$ has the property

$$R_y(0) = \begin{cases} (a_{11} + a_{22})/2 & \text{if } d \leqslant 0 \\ (a_{11} + a_{22})/2 + \sqrt{d}/2 & \text{if } d > 0 \end{cases} \quad (20)$$

The case of $d < 0$ corresponds to kinetics that give rise to an oscillatory instability, and since the glycolytic system has an oscillatory instability, we consider this case.

To determine the points of instability, we need to determine the conditions under which $R_y(\eta)$ crosses zero, the critical points being the zeros of $R_y(\eta)$. The qualitative dependence of $R_y(\eta)$ on the electric field can be understood by noting the following properties of $g_y(\eta)$, which are proved in the appendix

(i) $g_y(\eta) \geqslant 0$ and is monotonic in y if $a_{12}a_{21} < 0$

(ii) $\underset{y\to\infty}{\text{Lt}} \sqrt{g_y(\eta)}/2 = |(a_{22} - a_{11}) - \eta\delta| \quad (\eta \geqslant 0)$

(iii) For large η, $g_y(\eta) \sim |(a_{22} - a_{11}) - \eta\delta|$

(iv) $dg_y(0)/d\eta = 2y(a_{22} - a_{11})^2/|d|$

From these properties we know qualitatively the behavior of the family of functions $\sqrt{g_y(\eta)}/2$ and the zeros of $R_y(\eta)$ are the points of intersections (if any) of the straight line

$$\eta(D + \delta/2) - (a_{11} + a_{22})/2 \quad \text{(A)}$$

and the curve

$$\tfrac{1}{2}\sqrt{g_y(\eta)}/2. \quad \text{(B)}$$

The requirement that the system be stable in the absence of the electric field is satisfied if $(a_{11} + a_{22}) < 0$. This together with the asymptotic behavior of $g_y(\eta)$ expressed by property (iii) implies that a necessary condition for (A) and (B) to intersect is

$$-(a_{11} + a_{22}) < |a_{22} - a_{11}|. \quad (21)$$

Fig. 1 indicates the case when $d < 0$ and $(a_{22} - a_{11})$ and $\delta (= D_2 - D_1)$ having opposite signs. By property (i), we see that the curve (B) moves upwards as the field strength is increased. The condition $a_{12}a_{21} < 0$ means that the interaction between the two components of the system is such that the destruction of one component leads to the creation of the other, and clearly this condition is satisfied for almost all two component systems. Properties (ii) and (iv) then imply

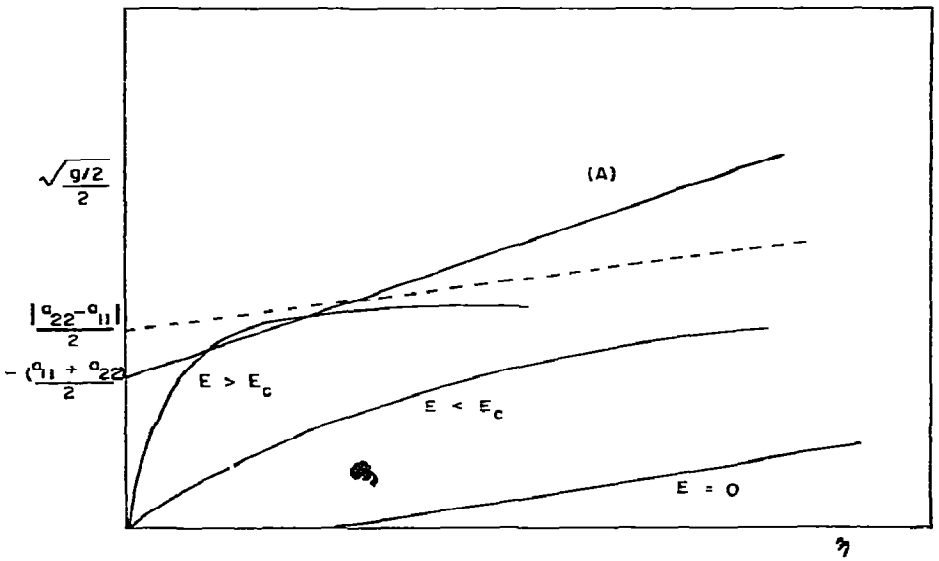

Fig. 1. The family of curves (B) for varying values of the electric field. The critical points are the points of intersection with curve (A). Curve (B) tends to the dashed line as the field strength tends to infinity.

that for sufficiently large y, curve (B) will intersect the straight line (A). This in turn implies that $R_y(\eta)$ will cross zero and that the system will become unstable for sufficiently large y (i.e. field strength). The case of δ and $(a_{22} - a_{11})$ having the same sign, leaves the behavior of $g_y(\eta)$ unchanged for small and large η, and only the behavior for intermediate values of η is different from that shown in fig. 1.

Hence we see when $d < 0$ i.e. when the system has an oscillatory instability, condition (21) is necessary and sufficient for the field to induce an instability.

Also it is easy to see that condition (21) is a reflection of the general result that the real parts of the eigenvalues of $A + iB$, where A and B are real matrices, are bounded by the largest and the smallest eigenvalues of $(A + A^T)/2 + i(B - B^T)/2$. If B is diagonal, as is the case with the mobility matrix, any change in the nature of stability should occur within the bounds of the eigenvalues of $(A + A^T)/2$. If the largest eigenvalue of $(A + A^T)/2$ is negative, then the real parts of the eigenvalues of $A + iB$ are negative regardless of B.

4. Numerical study

The results derived above being applicable in general to any two component system with an oscillatory instability, we now return our attention to the glycolytic system. The G–L model of the glycolytic system gives [5, 18]

$$a_{11} = -aAC, \quad a_{22} = aBC - \sigma_2 a, \quad a_{12} = aBC,$$

$$a_{21} = -aAC, \tag{22a–d}$$

where

$$A = L(1+\gamma_0)\{\alpha_0^2 c[2/(\epsilon+1) - c] + 2\alpha_0/(\epsilon+1) + 1\}$$

$$+ (1+\gamma_0)^3[1 + \alpha_0/(\epsilon+1)]^2, \tag{23}$$

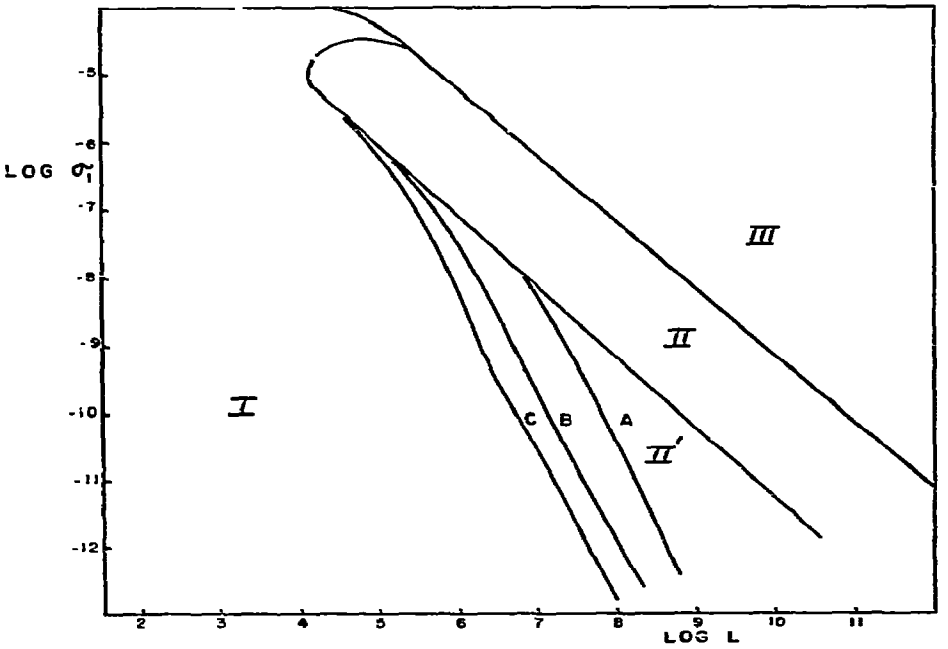

Fig. 2. Stability diagrams in the $L-\sigma_1$ plane for various values of E in V/cm. (A) $E = 10$ V/cm; (B) $E = 50$ V/cm; (C) $E = 100$ V/cm. The values of the other parameters are $\sigma_2 = \sigma_1/2$, $C = 10^{-2}$, $\epsilon = 10^{-1}$, $D_0 = 5 \times 10^{-4}$, $a = 10$/mM s, $D_\alpha = D_\gamma = 10^{-6}$ cm^2/s, $M = 10^{-5}$ cm^2 volt^{-1} s^{-1}, $\mu = 10^{-5}$ cm^2 volt^{-1} ϵ^{-1}. In region I the steady states are stable. Region II' has field induced instability leading to spatio-temporal oscillations. Region II has homogeneous oscillations. Region III has no physically admissible steady states.

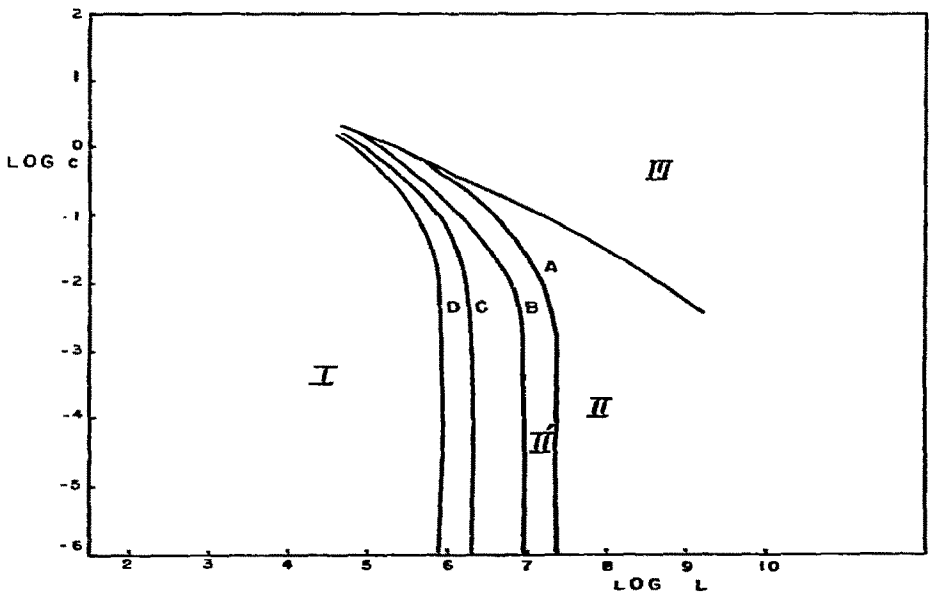

Fig. 3. Stability diagram in the $L-c$ plane for various values of E in V/cm. (A) $E = 0$ V/cm; (B) $E = 10$ V/cm; (C) $E = 50$ V/cm; (D) $E = 100$ V/cm. The values of the other parameters are $\epsilon = 10^{-1}$, $\sigma_1 = 10^{-8}\ s^{-1}$, $\sigma_2 = 5 \times 10^{-9}\ s^{-1}$, $D_0 = 5 \times 10^{-4}$, $a = 10$/mM s, $D_\alpha = D_\gamma = 10^{-6}\ cm^2/s$, $M = 10^{-5}\ cm^2\ volt^{-1}\ s^{-1}$, $\mu = 10^{-5}\ cm^2\ volt^{-1}\ s^{-1}$. Regions I, II, II' and III are as in fig. 2.

$$B = 2\alpha_0 L(1+\alpha_0/(\epsilon+1))(1+\alpha_0 c)^2\,, \tag{24}$$

$$C = \frac{2[D\epsilon/(\epsilon+1)]\,[1+\gamma_0]}{L(1+\alpha_0 c)^2 + (1+\gamma_0)^2(1+\alpha_0/(\epsilon+1))} \tag{25}$$

Numerical calculation shows that for empirically meaningful values of L the allosteric constant, c, the nonexclusive binding coefficient, σ_1, the substrate input and σ_2 the product removal rate, condition (21) is satisfied and the system can undergo an instability induced by the electric field. (Note that $a_{12}a_{21} < 0$ since A, B, C and a are positive by definition.)

In order to obtain the order of magnitude of the field strengths necessary to induce an instability, a numerical calculation was performed and the stability diagrams in the L-σ_1 and L-c planes are shown in figs. 2 and 3 for values of E between 10 and 100 V/cm. The values of the parameters are chosen according to the data obtained by Blangy et al. for E. coli [21]; also see [5] and [8].

The diffusion coefficients were given the value $10^{-6}\ cm^2/s$. With this value for D the mobilities were estimated using the relation $M/D = Ze/kT$ and an order of magnitude value of $\mu = 10^{-5}\ cm^{-1}\ vol^{-1}$ was used in the computation.

The linear period of oscillation, i.e. the period of oscillation close to the point of instability, for various values of the electric field are shown in table 1.

5. Concluding remarks

Some of the general effects of electric fields on dissipative structures emerge from this analysis. The induced instabilities are in general spatio-temporal. Further, we have an additional degree of freedom in controlling the stability of the system and we have shown that these instabilities arise for low enough values of the electric fields. Due to this additional control we now have on the system, it should be possible to measure some physiological quantities in biological systems by knowing the critical points of field induced in-

Table 1
Linear period with corresponding values of σ_1. $c = 10^{-2}$, $\epsilon = 10^{-1}$

Log L	$E = 10$	$E = 50$	$E = 100$
5.5	×	9 s	10 s
		(log σ_1 = 6.76)	(log σ_1 = 7.1)
6	×	31 s	55 s
		(log σ_1 = 7.5)	(log σ_1 = 8.4)
7	295 s	75 s	74 s
	(log σ_1 = 8.25)	(log σ_1 = 10.1)	(log σ_1 = 10.7)

× denotes no field induced instability.

stabilities. For example, if we know L and c in the above system and if the system is in the stable region I, by noting the value of the field that induces an instability one could obtain the value of σ_1. Clearly one could expect this to be a general feature in that the electric field can be used as a probe to obtain information about the system.

Also an immediate implication of these results comes to mind on considering that the glycolytic pathway in its full generality (involving more than the two variables we have considered here) is still, at the present time, a likely candidate for the role of master oscillator in circadian and infradian periodicities. It seems easier to understand from these results why electric field-induced shifts are in fact observed in circadian rhythm.

We hope to publish in the future a note on the application of the present results to the modulation of infradian periodicities in pacemaker neurons of invertebrate ganglia.

Acknowledgements

We would like to thank Prof. I. Prigogine for his continued interest in the present work and encouragement. We would also like to thank Dr. A. Goldbeter for the valuable discussions we have had with him. This work was done with the support of a grant from the Robert A. Welch Foundation and of the Solvay Institutes (Brussels).

Appendix

$$g_y(\eta) = d + \eta^2\delta^2 - \eta(y + 2(a_{22} - a_{11})\delta) + \{[d + \eta^2\delta^2 - \eta(y + 2(a_{22} - a_{11})\delta)]^2 + 4\eta y [(a_{22} - a_{11}) - \eta\delta]^2\}^{1/2}$$

(i) Since $y \geq 0$ by definition $g_y(\eta) \geq 0$.

Using the definition of d given in eq. (12), we can write $g_y(\eta)$ as

$$g_y(\eta) = [(a_{22}-a_{11}) - \eta\delta]^2 + 4a_{12}a_{21} - \eta y \{[((a_{22}-a_{11})-\eta\delta)^2 + 4a_{12}a_{21} - \eta y]^2 + 4\eta y[(a_{22}-a_{11})-\eta\delta]^2\}^{1/2} \tag{A.1}$$

which can be written as

$$g_y(\eta) = (a + 4a_{12}a_{21} - \eta y) + \{(a + 4a_{12}a_{21} - \eta y)^2 + 4\eta y a\}^{1/2} \tag{A.2}$$

where

$$a \equiv [(a_{22} - a_{11}) - \eta\delta]^2 \tag{A.3}$$

Taking the derivative with respect to y, we obtain

$$\frac{\mathrm{d}}{\mathrm{d}y} g_y(\eta) = -\eta \left\{ 1 + \frac{(a + 4a_{12}a_{21} - \eta y) - 2a}{\{(a + 4a_{12}a_{21} - \eta y)^2 + 4\eta y a\}^{1/2}} \right\} \tag{A.4}$$

which can be rewritten as

$$\frac{\mathrm{d}}{\mathrm{d}y} g_y(\eta) = -\eta \left\{ 1 + \frac{4a_{12}a_{21} - \eta y - a}{\{(4a_{12}a_{21} - \eta y - a)^2 + 16a\, a_{12}a_{21}\}^{1/2}} \right\} \tag{A.5}$$

The rhs is positive if $\eta y > -a + 4a_{12}a_{21}$ and $a_{12}a_{21} < 0$. Since a and ηy are positive by definition, $a_{12}a_{21} < 0$ is a

sufficient condition for $g_y(\eta)$ to be monotonic in y.

(ii) Eq. (A.2) can be rewritten as

$$g_y(\eta) = (a + 4a_{12}a_{21} - \eta y) + \{(4a_{12}a_{21} - \eta y - a)^2 + 16a\,a_{12}a_{21}\}^{1/2}.$$

For $\eta \neq 0$ and large y we get

$$g_y(\eta) \approx (a + 4a_{12}a_{21} - \eta y) + |4a_{12}a_{21} - \eta y - a| \left\{1 + \frac{8a\,a_{12}a_{21}}{(4a_{12}a_{21} - \eta y - a)^2}\right\} \tag{A.6}$$

when $\eta y > a + 4a_{12}a_{21}$ we get

$$g_y(\eta) \approx 2a + \frac{8a\,a_{12}a_{21}}{|4a_{12}a_{21} - \eta y - a|}$$

Hence

$$\lim_{y\to\infty} \sqrt{g_y(\eta)/2} = \sqrt{a} = |a_{22} - a_{11} - \eta\delta| \tag{A.7}$$

(iii) Computing the derivative of g with respect to η, we get

$$\frac{\mathrm{d}}{\mathrm{d}\eta} g_y(\eta) = \left\{1 + \frac{d + \eta^2\delta^2 - \eta s}{\sqrt{[d + \eta^2\delta^2 - \eta s]^2 + 4\eta y\,[a - \eta\delta]^2}}\right\}(2\delta^2\eta - s) + \frac{2y(a - 3\eta\delta)(a - \eta\delta)}{\sqrt{[d + \eta^2\delta^2 - \eta s]^2 + 4\eta y\,[a - \eta\delta]^2}} \tag{A.8}$$

where

$$s \equiv y + 2(a_{22} - a_{11})\delta\,, \qquad a \equiv (a_{22} - a_{11})\,.$$

When $\eta = 0$, since $d < 0$, we get

$$\mathrm{d}g_y(0)/\mathrm{d}y = 2y\,a^2/|d|\,, \tag{A.9}$$

which could be made arbitrarily large by increasing y (i.e., by increasing the field strength).

(iv) To determine the asymptotic behavior of $\sqrt{g_y(\eta)/2}$, we write $g_y(\eta)$ in the form

$$g_y(\eta) = (a + b - c)\{(a + b - c)^2 + 4ac\}^{1/2} \tag{A.10}$$

where

$$a \equiv (a_{22} - a_{11} - \eta\delta)^2\,, \qquad b \equiv 4a_{12}a_{21}\,, \qquad c \equiv \eta y\,,$$

which could be rewritten as

$$g_y(\eta) = (a + b - c) + \{(a + b + c)^2 - 4bc\}^{1/2}.$$

For large η, $|a + b + c| > |bc|$, and making a binomial expansion and neglecting the small terms, we get

$$g_y(\eta) \approx (a + b - c) + (a + b + c) \approx 2(a + b).$$

Thus for large η we get

$$\sqrt{g_y(\eta)/2} \sim \sqrt{a + b} \sim |a_{22} - a_{11} - \eta\delta|\,. \tag{A.11}$$

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