ELSEVIER

Biophysical Chemistry 125 (2007) 112–116

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

# Simple and complex spatiotemporal structures in a glycolytic allosteric enzyme model

Lu Zhang, Qingyu Gao *, Qun Wang, Xing Zhang

*College of Chemical Technology, China University of Mining and Technology, Xuzhou 221008, PR China*

Received 29 December 2005; received in revised form 9 July 2006; accepted 10 July 2006
Available online 4 August 2006

**Abstract**

Pattern formation in glycolysis is studied with a classical reaction-diffusion allosteric enzyme model. It is found that, similar to recent experimental reports in the yeast extracts, a small magnitude local perturbation can induce transient target waves in a two dimensional oscillatory medium. An above threshold stimulation generates target waves which eventually evolve into spatiotemporal chaos upon collisions with the boundary or other wave activities. Detailed simulation studies show that the studied simple glycolytic reaction-diffusion model can support three types of spatiotemporal behaviors which are independent of the boundary conditions: (1) a spatially uniform stable steady state, (2) periodic global oscillations and (3) spatiotemporal chaos.


*Keywords:* Glycolysis; Pattern formation; Spatiotemporal chaos

## 1. Introduction

Glycolysis, a sequence of 10 enzyme-catalyzed reactions that convert sugars into pyruvate, plays an important role in the metabolism. Under certain conditions, the degradation of glucose to pyruvate via glycolysis exhibits temporal oscillations in stirred systems or is accompanied by pattern formation in spatially extended medium. Temporal oscillations in glycolysis have been observed in many kinds of biosystems, including yeast cell-free extracts [1–7], yeast cell suspension [8], ascites tumor cells [9], cardiac muscle extracts [10], skeletal muscle extracts [11], rat liver extracts [12], blowflies [13], pancreatic β-cells [14], heart cells [15] and the reconstituted enzyme systems [16]. In early studies, only simple oscillations were observed [1,2]. Later, with the stochastic or periodic input of the substrate, quasi-periodicity, chaos, as well as entrainment were found [3–5]. Recently, the employment of a CSTR permits the achievement of sustained complex oscillations in the autonomous glycolytic system, which include period-doubled, mixed-mode and quasi-periodic oscillations and chaos [6,7].

When the oscillatory glycolysis is coupled with mass transport processes such as diffusion, the self-organized spatial patterns can be developed. For example, glycolytic waves have been experimentally found in single cells and yeast extracts [17–22]. Due to its maneuverability, studies in the glycolytic yeast extracts have become a primary experimental approach to gain insight into glycolytic pattern formation. In the glycolytic yeast extracts, waves can be induced by a local perturbation on the activity of the allosteric enzyme phosphofructokinase (PFK), which is usually accomplished through the local injection of an activator of PFK, fructose-2,6-bisphosphate (F-2,6-$P_2$). Most of the existing investigations on glycolytic pattern formation were performed when the yeast extracts are in an excitable state, where circular and spiral shaped excitation waves can be observed after a local perturbation. In contrast, there are few studies on glycolytic pattern formation under the dynamics that the extracts undergo global oscillations. So far, only transient phase waves were reported in the oscillatory glycolytic medium [20].

Several models have been developed to characterize the self-organized glycolytic pattern formation [23–25]. Despite the differences among these models, they all attribute the nonlinear kinetic source of glycolysis to PFK, which is activated by one of

* Corresponding author. Fax: +86 516 399 5758.
*E-mail address:* gaoqy@cumt.edu.cn (Q. Gao).

0301-4622/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2006.07.004

the products of the enzymatic reaction. Based on the allosteric characteristic of PFK, Goldbeter and Lefever developed a two-variable model and reproduced the limit cycle behaviors observed in experiments [26]. Taking diffusion into account, Goldbeter altered it into a reaction-diffusion model [23]. With Dirichlet boundary conditions, various spatiotemporal patterns (depending on the boundary conditions and the size of the space) have been observed in the one dimensional (1D) system in the simulations. These patterns include stationary spatial structures, standing and propagating concentration waves [24]. Introducing the function of subcellular structures on the glycolytic dynamics *in vivo*, Marmillot et al. observed pulses in their simulations in the heterogeneous glycolytic system with the model [24].

Since existing theoretical studies with the allosteric enzyme model did not mimic the experimental configurations in a way that the glycolytic waves were produced by locally perturbing the excitable or global oscillatory yeast extracts in a sealed petri dish, little is known regarding whether the allosteric property of PFK plays a critical role in the waves formation in the experiments *in vitro* from earlier numerical simulations. In addition, in comparison with the research activity in the glycolytic temporal dynamics and pattern formation in other biosystems, e.g. $Ca^{2+}$ waves, spatiotemporal dynamics in glycolysis have been rarely investigated, especially in the theoretical aspect. Yet, considering the complexity of dynamics in glycolysis, rich glycolytic patterns shall be expected. It thus motivates us to carry out numerical simulations with the classical two-variable reaction-diffusion allosteric enzyme model of glycolysis in this study. To mimic the experiments in yeast extracts, we employed no-flux boundary conditions and initiated waves by a local perturbation on the spatially uniform state which allows us to examine the effect of the allosteric property of PFK on the pattern formation.

## 2. Model

The reaction-diffusion model employed here was developed by Goldbeter [23], which is based on the allosteric enzyme model proposed in 1972 [26]. The model describes the kinetic processes of ATP and ADP and focuses on the significance of PFK in the nonlinear kinetics of glycolysis. According to the mechanism, PFK catalyzes the reaction: Fructose-6-phosphate + ATP → fructose-1,6-bisphosphate + ADP, which is the only rate limiting reaction step in the glycolytic process. The PFK is assumed to exist under two conformations: "active" (R) and "inactive" (T); transitions between the two forms are fully concerted. The substrate (ATP) binds to R and T with different affinities, but only the complexes with R decompose to yield the product (ADP); the product-ligand binds exclusively to the active form R and therefore acts as a positive effector of the enzyme. The substrate is supplied to the system at a constant rate, while the product is removed by a first-order reaction.

Applying a quasi-steady-state hypothesis to the enzymatic forms, the evolution of the matabolite concentrations in time and space is given by the following reaction-diffusion equations:

$$\frac{\mathrm{d}\alpha}{\mathrm{d}t} = a\left[\sigma_1 - \frac{\left(\frac{2E_0\varepsilon}{\varepsilon+1}\right)\alpha\left(1+\frac{\alpha}{\varepsilon+1}\right)(1+\beta)^2}{L(1+\alpha c)^2+(1+\beta)^2\left(1+\frac{\alpha}{\varepsilon+1}\right)^2}\right] + D_\alpha\frac{\partial^2\alpha}{\partial r^2} \tag{1a}$$

$$\frac{\mathrm{d}\beta}{\mathrm{d}t} = a\left[\frac{\left(\frac{2E_0\varepsilon}{\varepsilon+1}\right)\alpha\left(1+\frac{\alpha}{\varepsilon+1}\right)(1+\beta)^2}{L(1+\alpha c)^2+(1+\beta)^2\left(1+\frac{\alpha}{\varepsilon+1}\right)^2} - \sigma_2\beta\right] + D_\beta\frac{\partial^2\beta}{\partial r^2} \tag{1b}$$

where $\alpha$ and $\beta$ denote, respectively, the concentrations of substrate (ATP) and product (ADP); $a$ is the unitary kinetic constant for the binding of substrate and product to the enzyme; $E_0$ is the total enzyme concentration; $L$ is the allosteric constant; $c$ is the nonexclusive binding coefficient for the substrate; $\varepsilon$ is related to the irreversible decomposition of the active (R form) enzyme–substrate complex; $\sigma_1$ is the reduced injection rate of the substrate; $\sigma_2$ is related to the outflow of the product; and $D_\alpha$ and $D_\beta$ are the diffusion coefficients of the substrate (ATP) and the product (ADP), respectively. More detailed description of the model can be found elsewhere [23,26].

Kinetics of the above model in the absence of diffusion have been intensely studied [23,24,26], and the results agreed very well with the experimental observations in many aspects, such as the oscillatory amplitude, oscillatory period, dependence of enzymic activity on substrate injection rate, etc. So it is qualified to be used to explore glycolytic pattern formation, which is less known both in experimental and theoretical aspects. Euler integration method is used in simulation. Space and time steps used in the following simulations are 0.01 cm and 0.02 s, respectively. Qualitative the same results were obtained when smaller space and time steps were employed. Preliminary studies indicate that the above reaction-diffusion model does not support excitability, but exhibits global limit cycle oscillations.

## 3. Results

In experimental studies, glycolytic waves are initiated by a temporary local concentration increase of an activator of PFK, which is performed by injecting F-2,6-$P_2$ or improving the concentration of ADP through the addition of ATPase. To mimic the experiments on wave formation in the global oscillatory glycolytic extracts (see Fig. 6 of Ref. [20]), in our simulation, we locally increase the concentration of ADP when the reaction-diffusion model exhibits global oscillations in a two dimensional (2D) system and observe almost the same phenomena (see Fig. 1). In the experiments, after a local injection of F-2,6-$P_2$ to the uniformly oscillatory state, the

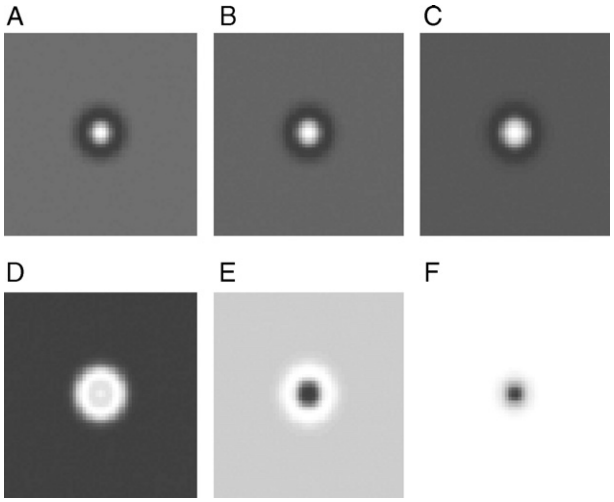


Fig. 1. Transient ADP waves induced by a small magnitude perturbation (ADP increased by five times of the initial global concentration) in the centre (9×9 pixels) of the global oscillatory state. A–C show the generation of a traveling ADP wave. D–E show the annihilation of the wave by a global ADP oscillation, leaving a small area of the system out of phase (F). The darker patterns signify a higher concentration. Time intervals are 70 s (A, B), 60 s (B, C), 80 s (C, D), 40 s (D, E) and 60 s (E, F). All images are 101×101 pixels. Numerical values of the parameters are as follows: $E_0=5\times10^{-3}$ mM, $a=10^5$ mM$^{-1}$ s$^{-1}$, $\varepsilon=0.1$, $c=0.01$, $L=5\times10^6$, $\sigma_1=1.1\times10^{-6}$ mM and $\sigma_2=10^{-6}$ mM. $D_\alpha=D_\beta=10^{-6}$ cm$^2$·s$^{-1}$.

wave is initiated and propagates through part of the extract for the overall NADH concentration increase due to the global glycolytic oscillations. In our simulation, after a local small increase of ADP concentration, the scenario of wave initiation and subsequent decay could also be observed, which indicates that the allosteric property of PFK indeed plays a crucial role in the glycolytic pattern formation in the corresponding experiment.

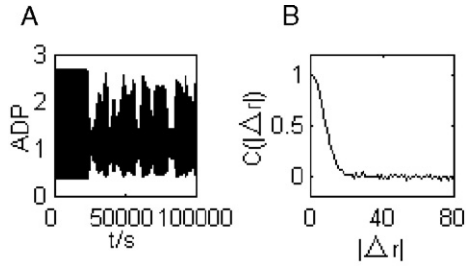


Fig. 3. Characterization of the spatiotemporal chaotic waves in Fig. 2. (A) Time sequence of ADP obtained at the point (101,101) marked by a black square in Fig. 2(A). (B) Spatiotemporal correlation function obtained in the area bordered by the black triangle shown in Fig. 2E.

Significantly, Fig. 2 shows that continuous target waves can be produced by a large magnitude local perturbation in the 2D glycolytic medium. The target waves are geometrically regular, but their amplitudes are indeed fluctuating in time. Such a scene can be easily discerned from Fig. 3A, in which the time series of local oscillations are plotted. The time series is collected at the point noted by a filled square in Fig. 2. Fig. 3A illustrates that before the target wave reaches the point, the local dynamics is simple periodic oscillations, but the oscillation become irregular after the first circular wave passes through the sampling point.

Upon collisions with the boundary or other wave activities, these target waves break to form defect structure. The symmetric appearance of the defect structure arises from the initial configuration of the medium as well as the initial perturbation. Although the defect structure looks symmetric as a whole, it is turbulent in any one of the eight equal parts. It is confirmed from the exponential decay of its correlation function (see Fig. 3B). The number of defects $n$ in the symmetric defect structure

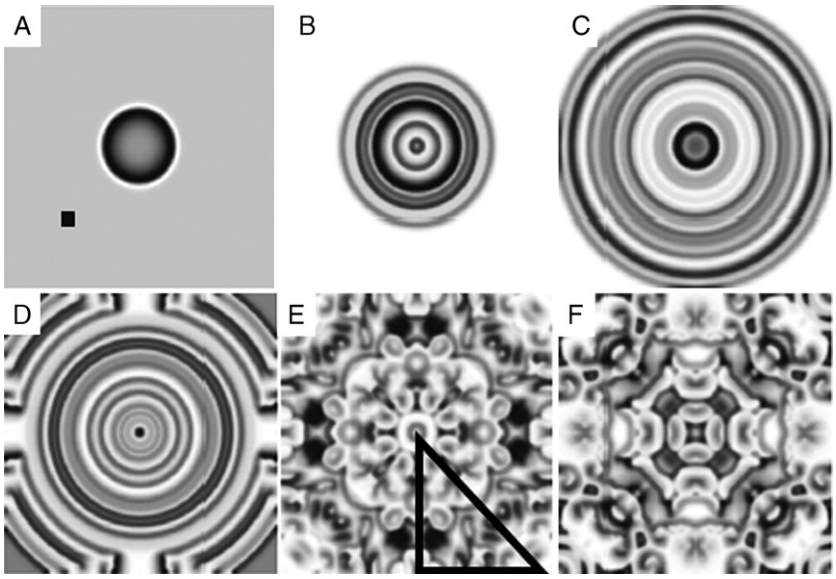


Fig. 2. Evolution of irregular ADP waves as a function of time, which are induced by a large magnitude perturbation (ADP increased by 10 times of the initial global concentration) in the centre (9×9 pixels) of the global oscillatory state: (A) 10,000 s, (B) 20,000 s, (C) 35,000 s, (D) 40,000 s, (E) 80,000 s and (F) 100,000 s. A–B show the generation of the irregular circular ADP waves. C–D show the scenes a short time after the waves reach the boundaries. E–F show the symmetric defect structures. All images are 401×401 pixels. The darker patterns signify a higher concentration. All parameters are the same as in Fig. 1.

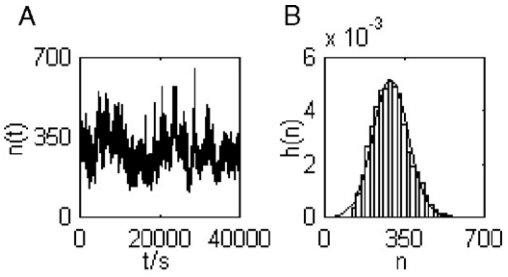


Fig. 4. Statistical properties of the symmetric defect structure in Fig. 2. (A) Number $n$ of defects in the system as a function of time. (B) Histogram. The solid curve is the corresponding normal distribution.

has been plotted as a function of time in Fig. 4A, where the number of defects $n$ increases rapidly until a relatively constant level is reached. The corresponding distribution of $n$ is shown in the form of normalized histogram (Fig. 4B), which agrees very well with a normal distribution with expectation and deviation being obtained from Fig. 4A.

Figs. 1 and 2 show that perturbations of different magnitudes will lead to different spatiotemporal behaviors. However, one should note that, despite that the initiation of the continuous waves depends on the magnitude of the local concentration perturbation on ADP, the initiation of target waves exhibiting irregular amplitudes is independent of boundary conditions and is solely arising from the coupling of diffusion transportation and the oscillatory reaction dynamics.

Detailed simulations have also been carried out in one-dimensional media to investigate the possible spatiotemporal states induced by a local perturbation on ADP concentrations. As control parameters concerning the injection rate of substrate and outflow rate of product, $\sigma_1$ and $\sigma_2$ are the most accessible ones in experiments, the following simulations are performed by systematically adjusting these two parameters. Fig. 5 shows the spatiotemporal dynamics supported by the reaction-diffusion model in the $\sigma_1/\sigma_2$–$\sigma_2$ phase plane. In the absence of diffusion process, the system has two distinct dynamic states in the $\sigma_1/\sigma_2$–$\sigma_2$ phase plane, namely, stable steady state and limit cycles. Correspondingly, the reaction-diffusion model can

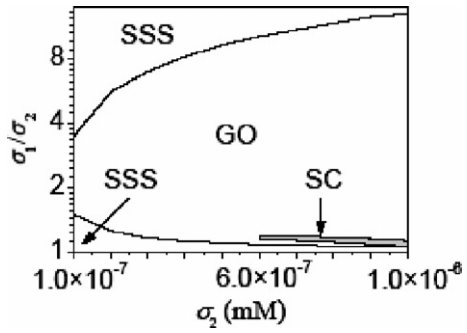


Fig. 5. Bifurcation diagram in the $\sigma_1/\sigma_2$–$\sigma_2$ phase plane. SSS, GO and SC denote the parameter regions which support spatially uniform stable steady state, global oscillations and spatiotemporal chaos, respectively. Other parameters are the same as in Fig. 1.

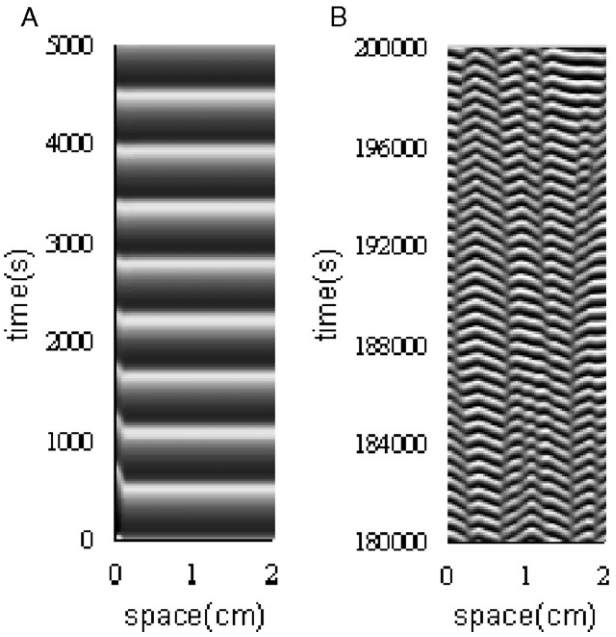


Fig. 6. Time–space plots of ADP pattern formation process after a local perturbation in the center (9 pixels) of a 4 cm long 1D system at the conditions $\sigma_1=8.88\times10^{-7}$ mM and $\sigma_2=8\times10^{-7}$ mM. In the perturbation area, ADP concentration is increased by 1.0 mM for A and 8.68333 mM for B. The presented space is the right half of the whole system (including the middle point). The darker patterns signify a lower concentration. Other parameters are the same as in Fig. 1.

exhibit spatially uniform stable steady state (SSS) and global oscillations (GO). Two solid lines in Fig. 5 indicate where a transition from stable to global oscillatory state takes place. Our simulation illustrates that the transition from SSS to GO occurs through supercritical Hopf-bifurcations. Despite our search, no wave activity could be initiated in the SSS regimes, implicating these conditions are not excitable. Under global oscillatory conditions (GO), our simulations show that there exists a small parameter region in which spatiotemporal chaos (SC) could be induced by big perturbation and such an area is very close to low Hopf-bifurcation point. Again, within the parameter region of SC whether the reaction-diffusion medium exhibit global oscillations or spatiotemporal irregular behavior depends on the magnitude of the initial perturbation, similar to the results seen in the 2D medium. However, outside of SC region, the small and big perturbation to global oscillations can only induce transient waves.

Fig. 6 presents the responses of the system to the perturbations of different magnitudes at the conditions $\sigma_2=8\times10^{-7}$ mM and $\sigma_1=1.11\sigma_2$. For a very small magnitude perturbation, the system recovers to the global oscillation state immediately (see Fig. 6A). As the amplitude of the local perturbation increases, the system takes a longer time to recover to the uniform global oscillations. Eventually, when the amplitude of the local oscillation is above a threshold level (see Fig. 6B), spatiotemporal chaos develop in the otherwise uniform medium. We employed both non-flux and periodic boundary conditions in this 1D simulation and got the same results.

## 4. Conclusions

Pattern formation in the reaction-diffusion system has been intensively studied to reveal their functions in various biological processes, such as metabolism, signal transduction and organism development [27–29]. Glycolytic waves are generated in the metabolism, behave as a biological signal and might influence the organism development. Studies in this paper show that in the presence of diffusion the allosteric property of PFK is capable of inducing spatiotemporal chaotic behavior in glycolytic metabolites. Spatiotemporal chaos in glycolysis have not been observed in experiments of glycolytic pattern formation and thus results obtained in this study shall provide useful guidance for future experimental exploration on such interesting nonlinear behavior as well as their biological functions.

Studies on the effect of a local perturbation on the global oscillatory state show that when the system locates in the parameter region far from the supercritical Hopf-bifurcation point, a local perturbation will always decline quickly regardless how large its amplitude is. Consequently, the reaction-diffusion medium will return to the uniform global oscillations. On the other hand, within the parameter region near low supercritical Hopf-bifurcation point, the global oscillations state becomes sensitive to the local perturbation, where perturbations of different magnitudes induce quite different dynamic states. The system in such a parameter region is similar to real living organisms which are sensitive to external disturbance.

In conclusion, we made detailed numerical studies on the reaction-diffusion allosteric enzyme model of glycolysis with a focus on the pattern formation processes after a local perturbation. The configuration of our simulation is tended to mimic recent experiments performed in yeast extracts. Earlier experimental phenomena are qualitatively reproduced in the simulation and new phenomena which have not been obtained in experiments, more specifically spatiotemporal chaos, are also obtained here.

## Acknowledgements

This work was supported by NSFC (20573134), RFDP (20050290512) and NCET (05-0477). We thank Dr. Jichang Wang for fruitful discussions.

## References

[1] B. Chance, B. Hess, A. Betz, DPNH oscillation in a cell-free extract of *S. carlshergensis*, Biochem. Biophys. Res. Commun. 16 (1964) 182–187.
[2] B. Hess, K. Brand, Continuous oscillations in a cell-free extract of *S. carlsbergensis*, Biochem. Biophys. Res. Commun. 23 (1966) 102–108.
[3] A. Boiteux, A. Goldbeter, B. Hess, Control of oscillating glycolysis of yeast by stochastic, periodic and steady source of substrate: a model and experimental study, Proc. Natl. Acad. Sci. U. S. A. 72 (1975) 3829–3833.
[4] M. Markus, D. Kuschmitz, B. Hess, Chaotic dynamics in yeast glycolysis under periodic substrate input flux, FEBS Lett. 172 (1984) 235–238.
[5] M. Markus, S.C. Muller, B. Hess, Observation of entrainment, quasiperiodicity and chaos in glycolyzing yeast extracts under periodic glucose input, Ber. Bunsenges. Phys. Chem. 89 (1985) 651–654.
[6] K. Nielsen, P.G. Sorensen, F. Hynne, Chaos in glycolysis, J. Theor. Biol. 186 (1997) 303–306.
[7] K. Nielsen, P.G. Sorensen, F. Hynne, H.-G. Busse, Sustained oscillations in glycolysis: an experimental and theoretical study of chaotic and complex periodic behavior and of quenching of simple oscillations, Biophys. Chemist. 72 (1998) 49–62.
[8] S. Dano, P.G. Sorensen, F. Hynne, Sustained oscillations in living cells, Nature 402 (1999) 320–322.
[9] K.H. Ibsen, K.W. Schiller, Oscillations of nucleotides and glycolytic intermediates in aerobic suspensions of Ehrlich ascites tumor cells, Biochim. Biophys. Acta 131 (1967) 405–407.
[10] R. Frenkel, Control of reduced diphosphopyridine nucleotide oscillations in beef heart extracts: I. Effect of modifiers of phosphofructokinase activity, Arch. Biochem. Biophys. 125 (1968) 151–156.
[11] K. Tornheim, J.M. Lowenstein, The purine nucleotide cycle: V. Control of phosphofructokinase and glycolytic oscillations in muscle extracts, J. Biol. Chem. 250 (1975) 6304–6314.
[12] J. Frenzel, W. Schellenberger, K. Eschrich, Bistability and damped oscillations in the fructose 6-phosphate/fructose 1,6-bisphosphate cycle in cell-free extracts from rat liver, Biol. Chem. Hoppe-Seyler 376 (1995) 17–24.
[13] K.G. Collatz, M. Horning, Age dependent changes of a biochemical rhythm—the glycolytic oscillator of the blowfly *Phormia terraenovae*, Comp. Biochem. Physiol. 96B (1990) 771–774.
[14] H.-F. Chou, N. Berman, E. Ipp, Oscillations of lactate released from islets of Langerhans: evidence for oscillatory glycolysis in beta-cells, Am. J. Physiol. 262 (1992) E800–E805.
[15] B. O'Rourke, B.M. Ramza, E. Marban, Oscillations of membrane current and excitability driven by metabolic oscillations in heart cells, Science 265 (1994) 962–966.
[16] K. Eschrich, W. Schellenberger, E. Hofmann, Sustained oscillations in a reconstituted enzyme system containing phosphofructokinase and fruotose 1,6-bisphosphatase, Arch. Biochem. Biophys. 222 (1983) 657–660.
[17] H.R. Petty, R.G. Worth, A.L. Kindzelskii, Imaging sustained dissipative patterns in the metabolism of individual living cells, Phys. Rev. Lett. 84 (2000) 2754–2757.
[18] T. Mair, S.C. Muller, Traveling NADH and proton waves during oscillatory glycolysis in vitro, J. Biol. Chem. 271 (1996) 627–630.
[19] S.C. Muller, T. Mair, O. Steinbock, Traveling waves in yeast extract and in cultures of *Dictyostelium discoideum*, Biophys. Chemist. 72 (1998) 37–47.
[20] T. Mair, C. Warnke, S.C. Muller, Spatio-temporal dynamics in glycolysis, Faraday Discuss. 120 (2001) 249–259.
[21] S. Bagyan, T. Mair, E. Dulos, J. Boissonade, P.D. Kepper, S.C. Muller, Glycolytic oscillations and waves in an open spatial reactor: impact of feedback regulation of phosphofructokinase, Biophys. Chemist. 116 (2005) 67–76.
[22] T. Shinjyo, Y. Nakagawa, T. Ueda, Hierarchic spatio-temporal dynamics in glycolysis, Physica, D 84 (1995) 212–219.
[23] A. Goldbeter, Patterns of spatiotemporal organization in an allosteric enzyme model, Proc. Natl. Acad. Sci. U.S.A. 70 (1973) 3255–3259.
[24] P. Marmillot, J.-F. Hervagault, G.R. Welch, Patterns of spatiotemporal organization in an "ambiquitous" enzyme model, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 12103–12107.
[25] G. Baier, M. Muller, H. Orsnes, Excitable spatio-temporal chaos in a model of glycolysis, J. Phys. Chem., B 106 (2002) 3275–3282.
[26] A. Goldbeter, R. Lefever, Dissipative structures for an allosteric model. Application to glycolytic oscillations, Biophys. J. 12 (1972) 1302–1315.
[27] D.E. Bentil, J.D. Murray, On the mechanical theory of biological pattern formation, Physica, D 63 (1992) 161–190.
[28] H. Meinhardt, Growth and patterning—dynamics of stripe formation, Nature 376 (1995) 722–723.
[29] M.P. Harris, S. Williamson, J.F. Fallon, H. Meinhardt, O. Prum, Molecular evidence for an activator-inhibitor mechanism in development of embryonic feather branching, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 11734–11739.