Periodic Signal Transmission through Metabolic Pathways with Michaelian Kinetics

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The propagation of oscillations in metabolite concentrations mediated by simple Michaelis−Menten enzymes is studied from a theoretical viewpoint. Sinusoidal input waves are investigated, and the resulting output velocities are analyzed. As a first approach, both irreversible and reversible reactions with linear kinetics are examined. Analytical expressions result for the ratios of output to input amplitudes (damping factors), as well as for phase shifts. It is shown that transmission of sinusoidal oscillations by Michaelis enzymes is approximately linear around the mean input flux even for high amplitudes of the velocity. Accordingly, contributions of superior harmonics to the overall waveform can be neglected. The predicted maximum phase shift for these systems is a quarter of a cycle per reaction step, which occurs at high frequencies. Effective rate constants are introduced that are needed for the accurate prediction of output amplitudes. By using them, calculations are presented suggesting that complete transmission can be expected for low- or medium-saturated glycolytic enzymes.

Introduction

Oscillations have been observed in metabolic systems for over thirty years.[1] The glycolytic pathway is one of the most extensively studied in a wide variety of cells,[2] including yeast strains, such as *Saccharomyces cerivisiae*[3,4] and *S. calsbergensis*,[5] muscle[6] and liver cells,[7] etc. Both intact cells[4] and extract preparations[8,9] have been used for experimentally eliciting oscillations in the concentrations of metabolites. Periodic behavior is easier to find under stress, like starvation or anaerobic conditions.[4,10] For this reason, it has been suggested that glycolytic oscillations may have a biological function in stringent circumstances. Particularly, they may have thermodynamic advantages[11,12] and contribute to the optimization of the ATP/ADP ratio.[13]

The endogenous origin of self-oscillations in glycolysis has been investigated.[14] Several theoretical models have been put forward since the early attempts of Higgins[15] and Sel'kov.[16] Most of them involve phosphofructokinase (PFK) as the enzyme from which oscillations originate (oscillophore),[17,18] although other candidates have been considered.[19] In general, allosteric effects are essential in order to justify experimental observations. Specifically, inhibitory effects of ATP (substrate) on PFK,[16] as well as activation by fructose 1,6-bisphosphate (product),[15,17] seem to be important. As for substrate inhibition, it can participate in the generating mechanism of oscillations if several substrates are present.[20] The influence of the growth phase on oscillations has also been taken into account.[4] Other experimental observations, like synchronization of oscillations mediated by intercellular acetaldehyde after mixing of asynchronous yeast populations, have recently been modeled.[21,22]

The realization that all glycolytic metabolites, including cofactors such as NADH, oscillate with the same frequency is consistent with the assumption that oscillations originate at a single point in the pathway and helps develop simplified models. Nevertheless, wave phases and amplitudes are different at every step of the pathway,[9,23] because of transmission effects. Two controlling steps apart from PFK have been identified in glycolysis, namely, glyceraldehyde 3-phosphate dehydrogenase and pyruvate kinase reactions. They involve NADH and ATP synthesis, respectively, indicating a role for cosubstrates in oscillation spread. Furthermore, the maintenance of oscillations far beyond the step where they are generated can greatly be justified by wave propagation through these cosubstrates.[5]

However, other steps of glycolysis are one-molecular transformations, which involve neither ATP nor NADH. Significant examples are the reactions catalyzed by glucose 6-phosphate isomerase, triose phosphate isomerase, phosphoglycerate isomerase, and enolase. The fact that they also oscillate needs further explanation. As far as we know, though, analysis of transmission through these reactions has not been looked into carefully. The purpose of this paper is to establish the conditions under which it is reasonable to expect oscillations to be transmitted through biochemical reactions not entailing wave-generating mechanisms, with particular reference to Michaelis−Menten kinetics.

Systems and Methods

The transmission of sinusoidal waves of the form $J_{in}(t) = \langle J_{in} \rangle + A_{in} \cos(\omega_0 t)$ through chemical reactions was studied. The parameter $\langle J_{in} \rangle$ represents the mean input flux, A_{in} is the semiamplitude of the wave, and ω_0 is the frequency of oscillation. The effects of reactions with linear kinetics were considered first. Both irreversible and reversible reactions with first-order product removal were modeled by means of linear differential equations. Laplace transform techniques were used to solve the equations in order to obtain the impulse response of the system (cf. Appendix). Damping factors for the amplitude, as well as phase shifts, were determined by Fourier transformation.

Numerical simulations of enzyme-catalyzed reactions were conducted in order to compare their transmission properties with

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the corresponding linear systems. The standard Michaelis rate equations were used, but the detailed mechanism was also simulated to check for the adequacy of the quasi-steady-state hypothesis. With the aim of analyzing the harmonic composition of the transmitted oscillation, the Fourier transform of selected waves was calculated.

Results

Transmission through Noncatalyzed Reactions with Linear Kinetics. An irreversible reaction with linear kinetics and a rate constant of k responds following an exponential decay, $r(t) = ke^{-kt}h(t)$, where $h(t)$ is the Heaviside step function. Its Fourier transform is $R(\omega) = k/(k + i\omega)$. Expressing it in polar coordinates, it is easy to obtain the damping factor of the reaction

$$\alpha_r^{irr} = (1 + \theta^2)^{-1/2} \quad (1)$$

where the nondimensional constant θ is defined as the ratio between the frequency, ω_0, and the kinetic constant, k. This damping factor is always less than unity, preventing amplification of the signal. In a similar way, the phase of the transform is

$$\phi_r^{irr} = -\text{arctg}(\theta) \quad (2)$$

From eq 2 it is clear that the maximum phase shift that can be introduced by the reaction is a retardation of a quarter of a cycle ($\pi/2$ rad).

The irreversible reactions of a sequential pathway are insensitive to the dynamics of succeeding reactions.[24] Moreover, the damping factors of a series of linear systems multiply, and the phase shifts add (cf. Appendix). Hence, the order of fast and slow reactions in a metabolic pathway is unimportant. Conversely, the dynamics of a reversible reaction depends on other reactions in the pathway. Thus, for linear kinetics with irreversible extraction of the product, the following damping factor was found:

$$\alpha_r^{rev} = \left(\frac{\theta_{out}^2 + 1}{\theta_1^2\theta_2^2 + \theta_1^2 + \theta_2^2 + 1}\right)^{1/2} \quad (3)$$

The three nondimensional parameters are $\theta_{1,2} = \omega/|\lambda_{1,2}|$ and $\theta_{out} = \omega/k_{out}$, where $\lambda_{1,2} = [-(k_+ + k_- + k_{out}) \pm ((k_+ + k_- + k_{out})^2 - 4k_+k_{out})^{1/2}]/2$ are the eigenvalues of the system and k_{out} is the kinetic constant of the velocity of removal. The damping factor of this velocity results from multiplying eq 1 for θ_{out} and eq 3. The phase of the reversible velocity is

$$\phi_r^{rev} = \text{arctg}\left(\frac{\theta_1 + \theta_2 - \theta_{out} + \theta_1\theta_2\theta_{out}}{\theta_1\theta_2 - \theta_{out}(\theta_1 + \theta_2) - 1}\right) \quad (4)$$

Again, the maximum phase shift per step is a quarter of a cycle. The damping factor of a reversible velocity is always lower than its irreversible counterpart. On the contrary, it is possible to obtain a higher phase shift for the reversible reaction, by operating at high frequencies (Figure 1). The phase shift at the output can be obtained by summing the phase angle for the irreversible (eq 2) and the reversible (eq 4) reactions. A simple calculation yields

$$\phi_r^{out} = \text{arctg}\left(\frac{\theta_1 + \theta_2}{\theta_1\theta_2 - 1}\right) \quad (5)$$

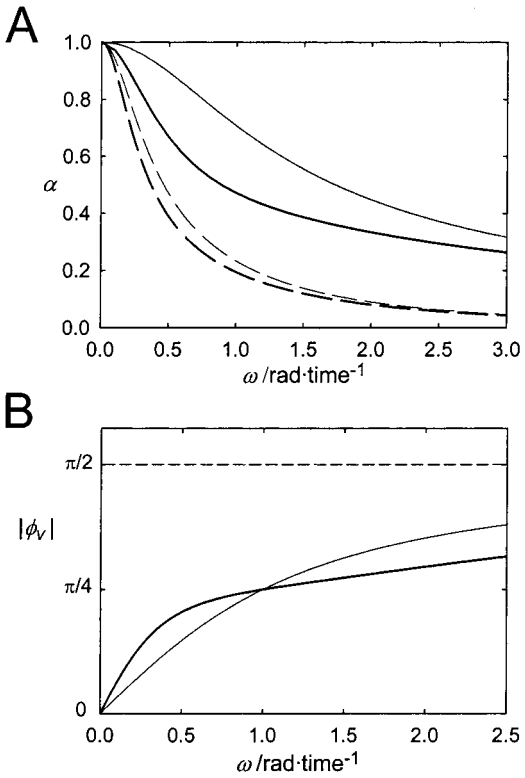

Figure 1. Transmission of sinusoidal oscillations through reactions with linear kinetics. Results are shown for rate constants equal to one unless otherwise stated. Reaction schemes are described in the text. (A) Damping factors as functions of the input frequency. Irreversible reactions (light solid line) transmit oscillations more efficiently than reversible reactions with identical direct rate constant (heavy solid line). Dashed lines correspond to damping factors at the irreversible removal of product after two consecutive reversible steps. One of them is 10 times faster. Its two possible locations were modeled, at the first (heavy line) and second (light line) reactions. The damping factor is greater in the former case. (B) Phase shift (retardation) of the velocity oscillation for an irreversible (light line) and reversible (heavy line) reaction. The intersection is noteworthy, as it does not occur on damping factors. The dashed line indicates the maximum possible shift.

The order of fast and slow reversible reactions also affects the magnitude of the damping factors. Analytical expressions cannot be derived for two or more consecutive reactions, because of the high degree of the characteristic polynomial. Nevertheless, numerical simulations show that the damping factor of the velocity of removal is lower when the fast steps precede the slow ones (Figure 1A). We observe that a general conclusion for reactions with linear kinetics is that both damping factors and phase shifts are independent of the input amplitude and mean flux.

Transmission through Irreversible Michaelis−Menten Enzymes. Enzymes behave linearly at low substrate concentrations or fluxes.[25,26] In such cases, irreversible Michaelis−Menten enzymes display first-order kinetics with a rate constant of V_m/K_M, V_m and K_M being the maximum velocity and the Michaelis constant of the enzyme, respectively. As a consequence, the amplitude of the velocity is a linear function of the input amplitude. This is not expected for general nonlinear systems. However, and rather surprisingly, numerical simulations revealed that such a relationship holds for any mean flux, no matter how close it is to the maximum velocity. Furthermore, although it is generally observed that the linear damping factor (eq 1) represents an upper limit, if the frequency of the input oscillation is sufficiently low, the amplitude of the velocity is fairly insensitive to variations of the mean flux (Figure 2). Hence,

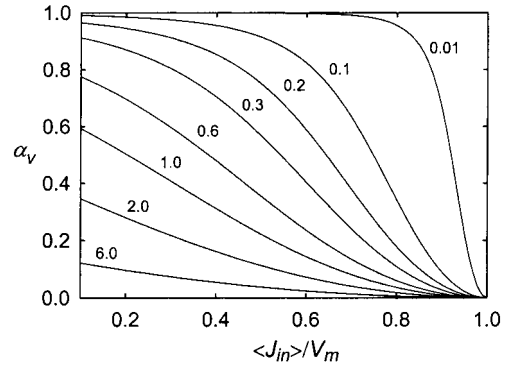

Figure 2. Dependence of velocity damping factors on the mean input flux for irreversible Michaelis−Menten enzymes ($V_m = 10$; $K_M = 11$; product removal rate: $k_{out} = 1.0$). Oscillation frequencies are referred to over each curve. Normalization of input fluxes and frequencies is commented on in the discussion.

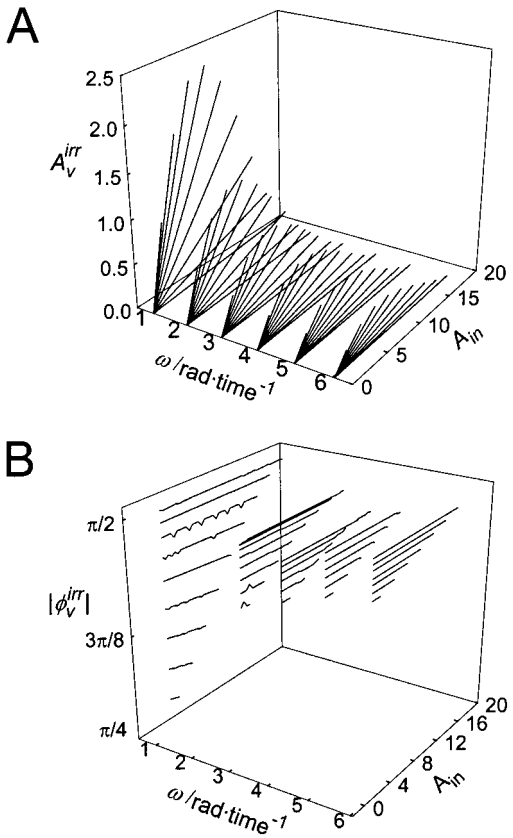

Figure 3. Transmission of sinusoidal oscillations through an irreversible Michaelis−Menten enzyme ($V_m = 10$; $K_M = 11$; product removal rate: $k_{out} = 1.0$). Input amplitudes are limited to the mean input fluxes. (A) Velocity amplitudes are linear functions of the input: straight lines are obtained for any given pair of frequency and mean flux (integer values of the latter are shown; $\langle J_{in}\rangle = 1, 2, ..., 9$). Lower slopes result as the latter approaches the maximum velocity. (B) Phase shifts of the velocity with respect to the input are nearly independent of the input amplitude. Each line corresponds to one different mean input flux. As its value increases, higher shifts result. The numerical errors of phase shifts are more significant. Some frequencies have been omitted for the sake of clarity.

although the enzyme damps the amplitude of a sinusoidal oscillation more efficiently than the corresponding noncatalyzed reaction, as expected, some linear features are still present in this kind of system.

Plots of the amplitude of the velocity vs the input amplitude, for different frequencies and mean input fluxes (Figure 3A), can be fitted by linear regression with excellent regression

TABLE 1: Amplitudes of the Velocities of Irreversible Michaelis−Menten Enzymes Functioning under Sinusoidal Input Flux

ω / rad time^{-1}	$\langle J_{in}\rangle$	r^a	ordinate^b	slope^b	$A_r{}^c$	ratio^d
0.1	1.0	1.0000000	0.0004	0.9902	0.9909	0.9993
	3.0	0.9999573	0.0301	0.9544	0.9757	0.9544
	5.0	0.9991192	0.2094	0.8182	0.9153	0.8939
	7.0	0.9991425	0.2046	0.5623	0.6332	0.8880
	9.0	0.9999999	0.0004	0.0904	0.0905	0.9989
1.0	1.0	1.0000000	(+)	0.5929	0.5929	0.9999
	3.0	0.9999999	0.0006	0.4066	0.4069	0.9991
	5.0	1.0000000	0.0002	0.2215	0.2216	0.9996
	7.0	1.0000000	(+)	0.0815	0.0815	0.9999
	9.0	0.9999997	(+)	0.0091	0.0091	0.9999
5.0	1.0	1.0000000	(−)	0.1455	0.1454	0.9996
	3.0	1.0000000	(−)	0.0886	0.0886	0.9998
	5.0	1.0000000	(+)	0.0453	0.0453	0.9999
	7.0	0.9999999	(+)	0.0163	0.0163	0.9999
	9.0	0.9999963	(−)	0.0018	0.0018	0.9995

[a] Linear fit regression coefficient; $r = 1$ for best fit. [b] Numerically calculated velocity amplitudes corresponding to input amplitudes ranging from 0 to 90% $\langle J_{in}\rangle$ and enzyme parameters as in Figures 2 and 3 were fitted to the straight line A_{out} = ordinate + slope A_{in}. Slopes can be interpreted as damping factors. (+) and (−) denote origin ordinates lower than 10^{-4} and their signs. [c] Calculated amplitudes (eqs 1 and 7). [d] Ratio of lower amplitude to higher amplitude for the numerical and calculated values. The worst ratios are obtained for medium-high mean input fluxes (obvious at low frequency).

coefficients (Table 1). Such linear functions indicate that the Fourier transform of the apparent transfer (response) function does not depend on the input amplitude of the sinusoidal input wave. Thus, the behavior is *locally linear*, even though the enzyme dynamics is nonlinear. Results concerning phase shift further support this conclusion, because plots of the phase of the velocity vs input amplitude are nearly horizontal (Figure 3B). Input dependence of the response, a characteristic of nonlinear systems, is maintained only in that the slopes of input vs velocity amplitudes vary as the mean input flux does.

These findings suggest that, for any given mean input flux and frequency, the frequency response of an irreversible Michaelis−Menten enzyme can be described by first-order kinetics with an effective rate constant. From the Taylor expansion of the enzyme velocity around the steady-state concentration of substrate

$$\frac{V_m S}{K_M + S} = \langle J_{in}\rangle + \sum_{i=1}^{\infty} \frac{(V_m - \langle J_{in}\rangle)^{i+1}}{(K_M V_m)^i} [S - \langle S\rangle]^i \qquad (6)$$

where S is the concentration of substrate and $\langle S\rangle = \langle J_{in}\rangle K_M/(V_m - \langle J_{in}\rangle)$ is the mean substrate concentration, and taking into account that $\langle J_{in}\rangle / V_m < 1$, it results that the effective rate constant is

$$k^{eff} = \frac{(V_m - \langle J_{in}\rangle)^2}{K_M V_m} \qquad (7)$$

Equation 7 is the first derivative of the Michaelis rate equation with respect to the substrate concentration, evaluated at $\langle S\rangle$. Substituting eq 7 in eqs 1 and 2, good approximations of the damping factor and the phase shift for the irreversible enzyme under a sinusoidal input flux are obtained (Table 1).

Because the Michaelis rate equation is nonlinear, the velocity is not exactly sinusoidal but contains contributions of superior harmonics. However, harmonics must be negligible with respect

to the fundamental frequency. Otherwise, the curve velocity vs input amplitude would be nonlinear. Numerical calculations of the Fourier transform of the asymptotic oscillations are consistent with this observation. No more than three or four harmonics are appreciable, and their amplitudes are small as compared to that of the fundamental frequency, with the greater corresponding to the first harmonic (Figure 4). Additionally, harmonics become more important as the semiamplitude of the input increases toward its limiting value, i.e., the mean flux. These results can be rationalized by assuming that both the substrate concentration and the enzyme velocity are sinusoidal. An estimation suggests that the amplitude of the first harmonic is usually much less than 15% that of the fundamental frequency (cf. Appendix).

Transmission through Reversible Michaelis−Menten Enzymes. All enzymes are reversible to some extent. Reversibility is important in vivo, because concentrations of reaction products are appreciable, as opposed to in vitro experiments, where initial velocities are measured in the absence of product. For this reason, it is desirable to extend the preceding analysis to reversible Michaelis enzymes. As in the irreversible case, linear functions of the velocity vs input amplitudes were found in numerical simulations of reversible Michaelis enzymes with first-order removal of product.

The reversible Michaelis velocity

$$v = \frac{\frac{V_m^S}{K_M^S}S - \frac{V_m^P}{K_M^P}P}{1 + \frac{S}{K_M^S} + \frac{P}{K_M^P}} \tag{8}$$

where S and P are substrate and product concentrations and K_M and V_m are Michaelis constants and maximum velocities, respectively, can be expanded around the mean concentrations of substrate and product:

$$\langle S\rangle = \frac{\langle J_{in}\rangle K_M^S(k_{out}K_M^P + V_m^P + \langle J_{in}\rangle)}{k_{out}K_M^P(V_m^S - \langle J_{in}\rangle)} \tag{9}$$

$$\langle P\rangle = \frac{\langle J_{in}\rangle}{k_{out}} \tag{10}$$

The resulting effective first-order direct and inverse rate constants (k_+^{eff} and k_-^{eff}, respectively) are

$$k_+^{eff} = \left(\frac{\partial \nu_{rev}}{\partial S}\right)_{\langle S\rangle,\langle P\rangle} = \frac{K_M^S K_M^P[V_m^S K_M^P + \langle P\rangle(V_m^S + V_m^P)]}{(K_M^S K_M^P + K_M^P\langle S\rangle + K_M^S\langle P\rangle)^2} \tag{11}$$

$$k_-^{eff} = \left|\left(\frac{\partial \nu_{rev}}{\partial P}\right)_{\langle S\rangle,\langle P\rangle}\right| = \frac{K_M^S K_M^P[V_m^P K_M^S + \langle S\rangle(V_m^S + V_m^P)]}{(K_M^S K_M^P + K_M^P\langle S\rangle + K_M^S\langle P\rangle)^2} \tag{12}$$

These rate coefficients have to be substituted in θ_1 and θ_2, and then in eqs 3 and 4, to approximate the damping factor and the phase shift of the enzyme. Examples of the comparison between numerical and estimated values for the characteristics of transmission are shown in Table 2.

Discussion

Transmission of oscillations along metabolic pathways is an open question. Most of the effort devoted to the study of periodic behavior in metabolism has been directed to explaining the mechanisms responsible for the existence of oscillations.[1,2] On the contrary, transmission of the wave toward distant locations

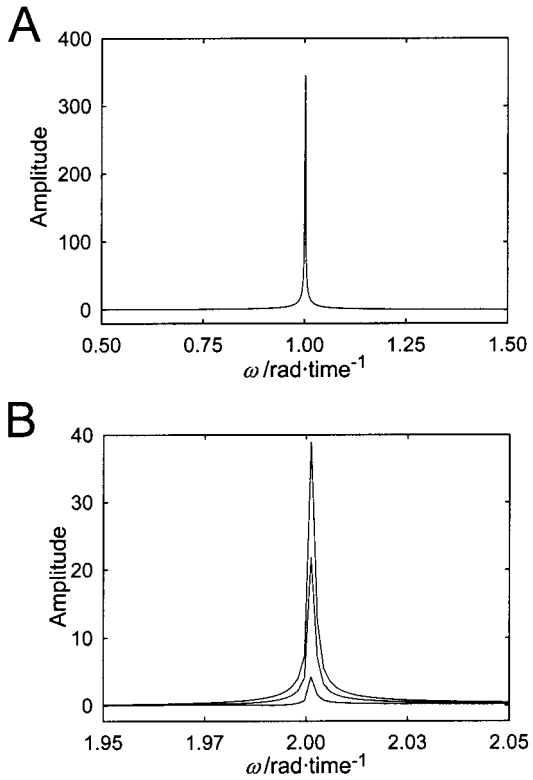

Figure 4. Harmonic decomposition of irreversible enzyme velocities under sinusoidal input fluxes. The kinetic parameters were $V_m = 10$, $K_M = 11$, and $k_{out} = 1.0$, with $\omega = 1$ and $\langle J_{in}\rangle = 5.0$ (where superior harmonics are predicted to be maximum). Fourier analysis was applied to asymptotic oscillations. (A) Amplitude of the fundamental frequency contribution normalized to the input amplitude. (B) Normalized amplitudes of the first harmonic contribution increase, according to predictions, as the input amplitude does, but are always negligible with respect to the fundamental amplitude. The curves displayed have been obtained, from lowest to highest, for semiamplitude values of 0.5, 2.5, and 4.5.

TABLE 2: Amplitudes of the Velocities of Reversible Michaelis−Menten Enzymes Functioning under Sinusoidal Input Flux

ω/ rad time^{-1}	$\langle J_{in}\rangle$	r[a]	ordinate[b]	slope[b]	A_r[c]	ratio[d]
0.1	1.0	0.9999996	0.0013	0.9725	0.9751	0.9973
	3.0	0.9999539	0.0293	0.8748	0.8959	0.9765
	5.0	0.9999984	0.0078	0.6560	0.6598	0.9943
	7.0	0.9999834	−0.0118	0.2801	0.2764	0.9869
	9.0	1.0000000	(−)	0.0305	0.0305	0.9995
1.0	1.0	0.9999999	−0.0004	0.4515	0.4507	0.9983
	3.0	0.9999993	−0.0009	0.2254	0.2247	0.9970
	5.0	0.9999998	−0.0003	0.0978	0.0977	0.9984
	7.0	1.0000000	(−)	0.0310	0.0310	0.9996
	9.0	0.9999966	(+)	0.0031	0.0031	0.9994
5.0	1.0	1.0000000	(−)	0.1195	0.1194	0.9996
	3.0	1.0000000	(−)	0.0546	0.0545	0.9997
	5.0	1.0000000	(+)	0.0224	0.0223	0.9999
	7.0	0.9999996	(−)	0.0067	0.0067	0.9999
	9.0	0.9999143	(−)	0.0006	0.0006	0.9997

[a] Linear fit regression coefficient; $r = 1$ for best fit. [b] Numerically calculated velocity amplitudes corresponding to input amplitudes ranging from 0% to 90% $\langle J_{in}\rangle$ were fitted to the straight line A_{out} = ordinate + slope(A_{in}). The kinetic parameters of the enzyme were $V_m^S = 10$, $V_m^P = 1$, $K_M^S = 11$, and $K_M^P = 5.5$, with a product removal rate of $k_{out} = 1.0$. (+) and (−) denote origin ordinates lower than 10^{-4} and their signs. [c] Calculated amplitudes (eqs 3, 11, and 12). [d] Ratio of lower amplitude to higher amplitude for the numerical and calculated values.

in the pathway has only been discussed as a secondary aspect of kinetic models. It must be recognized that the main contribution to the presence of oscillations far away from oscillophores seems to be the interchange of ATP and NADH between different enzymes. However, reactions not involving these intermediates may significantly transmit oscillations. Such a possibility may be important for giving complete and accurate descriptions of interference and resonance effects. Glycolysis, probably the most studied pathway with respect to metabolic oscillations, may present this kind of complexity, because wave spread is mediated by ATP, but transmission down the chain of reactions cannot be ruled out.

In this work, linear kinetics were considered first. It was shown that both reversible and irreversible one-molecular reactions act as low pass filters, in the sense that low frequencies are transmitted whereas high ones are damped. Nondimensional parameters, denoted by θ, can be defined according to the particular kinetics of the system, which are ratios of frequencies and eigenvalues (i.e., kinetic constants in irreversible systems). If the parameter θ is less than one, wave propagation is guaranteed, whereas for high values, oscillations become damped and phase shifted. Remarkably, there is a correlation between phase shifts and damping of amplitudes. Damping factors are lower and somewhat more complex for reversible reactions. Curiously enough, phase shifts exceed that of the irreversible reaction only under certain critical frequency. For instance, for the particular case that all kinetic constants are the same, say k, $\phi_r^{rev}(\omega) = \text{arctg}[-(\theta^3 + 2\theta)/(2\theta^2 + 1)]$, $\theta = \omega/k$, which is greater (in absolute value) than the irreversible phase for $\omega_0 < 1$ and lower otherwise (Figure 1B).

A general conclusion for linear reactions is that phase shifts are limited to a quarter of a cycle per reaction step. This is an important fact that may be useful to distinguish whether an enzyme acts as an oscillophore or not. Actually, it is a common observation that those glycolytic reactions involved in the generation or distant transmission of oscillations present phase shifts of half a cycle between substrates and products. ATP and ADP are metabolites of this kind, which participate in the PFK reaction. Because other reactions seem to be nearly in phase, such features have been used for distinguishing controlling steps in wave spread. Importantly, according to our results, the low phase shifts measured for many metabolites suggest a nearly complete transmission through most of the glycolytic reactions and an important role of propagation along the chain in the overall amplitudes and phases.

Although linear kinetics ensures transmission of low frequencies, enzymes are nonlinear, and other effects affect wave propagation. Michaelis enzymes were investigated to ascertain the influence of frequency and saturation on wave amplitudes and phases. Numerical simulations demonstrate that the mean input flux is a fundamental parameter determining damping factors and phase shifts, whereas amplitudes are unimportant. We have shown that the slope of the Michaelis hyperbola (velocity vs concentration) at the mean input flux can be used, to a very good approximation, as the effective kinetic constant of the locally linearized system (eq 7). Because such a slope decreases at high fluxes, enzymes may attenuate frequencies that would be wholly transmitted in a linear system. Particularly, the degree of saturation has to be considered.

It results from eq 7 that rescaling the maximum velocity by some factor produces a scaling of the effective constant, provided that the input flux is also scaled by the same factor. To keep θ constant, and thus the damping factor and the phase shift, the frequency of oscillation has to be rescaled too. As a result, it is possible to represent damping factors as functions of the degree of saturation if a standard frequency is defined as ω/V_m (cf. Figure 2).

Many glycolytic enzymes are known to function at low saturation (<20%, except for 60% saturation for triose-phosphate isomerase).[27] This favors oscillation transmission. However, propagation depends not only on the degree of saturation but on the frequency as well. To illustrate this fact, an approximate calculation can be made for the reaction catalyzed by enolase (EC 4.2.1.11), i.e., 2-phospho-D-glycerate (2PG) dehydration to yield phosphoenolpyruvate (PEP). This is a reaction of the lower part of glycolysis. Reported Michaelis constants are 0.10 mM, both for 2PG[28] and PEP.[23] The enzyme catalytic constant is 4320 min^{-1},[27] from which a maximum forward velocity of 408 mM min^{-1} is obtained, assuming an enzyme concentration of 0.09 mM.[27] By using an equilibrium constant of 6.3,[28] a reverse catalytic constant of 686 min^{-1} and a reverse maximum velocity of 62 mM min^{-1} result. Piruvate kinase follows enolase in the pathway and catalyzes an approximately irreversible reaction. Therefore, PEP can be assumed to be extracted at a rate of 362 min^{-1} by taking a concentration of 0.15 mM[29] in eq 10, with an average saturation of 13.3%.[27] The reported concentration of 2PG is 0.1 mM,[29] consistent with all the parameters, as can be checked by recalculating this concentration from eq 9 (0.065 mM). The forward and backward effective constants, as calculated from eqs 11 and 12, are 908 and 434 min^{-1}, respectively. These are significantly lower than the maximum effective constants, V_m/K_M, i.e., 4080 and 620 min^{-1}, correspondingly. However, for an oscillation frequency of 1 min,[1,3,30] the predicted damping factor for the enolase velocity is 0.99975 (eq 3) and the phase shift is a delay of 0.013 rad (eq 4). This agrees well with the observation that 2PG and PEP are in phase with 3PG, the preceding metabolite in the pathway.[5]

Whereas in linear systems damping factors are multiplicative and frequencies are independent, transmission through enzymes is only locally linear. It was mentioned in the results that the velocity of an enzyme operating under a sinusoidal input flux of substrate is approximately sinusoidal, but presents all superior harmonics. It is shown in the appendix that all harmonic amplitudes are negligible with respect to that of the fundamental frequency. This supports linear approximations. However, harmonics should be taken into account when analyzing sequences of enzymes. It is not clear whether frequencies are independent of each other, at least approximately, in Michaelis enzymes. Further work needs to be done in this direction. It would be desirable that reaction sequences could be decomposed in successive steps. Because enzymes produce more damping on high frequencies than similar linear systems (those with a kinetic constant of V_m/K_M), harmonics should be greatly attenuated. Thus, we anticipate that they can be disregarded in general.

We conclude that enzyme-to-enzyme wave propagation down the glycolytic pathway is significant. Although saturated enzymes are expected to attenuate amplitudes almost completely, most enzymes function at medium or even low saturation. Additionally, catalytic constants usually exceed the frequency of metabolite oscillations, thus aiding to transmit the wave. However, published reports are not sufficient to assess every aspect of the discussed theory. To verify it, in vitro assays are required that allow the experimenter to manipulate the input frequency. An easier alternative would be to vary enzyme concentrations so as to achieve increased saturations. In vivo studies, although more complex to control, would be feasible

by using enzyme defective mutants. These would also serve to confirm the postulated thermodynamic advantages of oscillations.

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Appendix

Contribution of Harmonics to Oscillation Transmission by Michaelian Enzymes. The response of a linear system is the convolution of the input with the impulse response, $r(t)$.[31] The Fourier transform of $r(t)$, $\mathbf{R}(\omega) = \int_{-\infty}^{+\infty} r(t)e^{i\omega t}\,dt$, characterizes the response in the frequency domain.[32] Because the transform of a convolution is the product of transforms, we have, in polar coordinates

$$\alpha_{out}(\omega)e^{i\phi_{out}(\omega)} = \alpha_{in}(\omega)\alpha_r(\omega)e^{i[\phi_{in}(\omega) + \phi_r(\omega)]} \qquad (A1)$$

where α and ϕ are amplitudes and phases of the Fourier transform and the subscripts in and out refer to the input and output of the system, respectively. Hence, the amplitude of $\mathbf{R}(\omega)$ is a damping factor, $\phi_r(\omega)$ is a phase shift, and, in general, amplitudes multiply and phases add.

The Fourier transform of the sinusoidal input with phase zero is $J_{in}(\omega) = 2\pi\langle J_{in}\rangle\delta(\omega) + \pi A_{in}[\delta(\omega - \omega_0) + \delta(\omega + \omega_0)]$, where $\delta(\omega)$ is Dirac's delta. The output $J_{out}(t) = \langle J_{in}\rangle + A_{in}\alpha_r(\omega_0)\cos(\omega_0 t + \phi_r(\omega_0))$ results from substituting $J_{in}(\omega)$ in eq A1 and transforming back to the time domain. Therefore, the frequency of the output oscillation is that of the input, ω_0. Thus, linear systems do not introduce new frequencies.

On the contrary, nonlinear systems contribute frequencies that were not present in the input spectrum. These can be fundamental frequencies introduced by the system and harmonics of the fundamental frequencies of the input. The former are produced by intrinsic oscillophores, like PFK. The latter are introduced by any nonlinear system in different degrees. To assess the magnitude of superior harmonics in the response of an irreversible Michaelis−Menten enzyme, we assume that both the substrate concentration and the enzyme velocity are sinusoidal and check the accuracy of this hypothesis by inserting the concentration in the velocity. First, we note that the substrate concentration is in phase with the velocity. Thus, the concentration is, in exponential notation

$$S \approx \langle S\rangle + A_S[(e^{i\omega_0 t} + e^{-i\omega_0 t})/2] \qquad (A2)$$

where A_S is the semiamplitude of the oscillation of substrate. Alternatively, S can be implicitly expressed from the rate equation as

$$S \approx \frac{K_M + S}{V_m}[B_0 + B_1(e^{i\omega_0 t} + e^{-i\omega_0 t})] \qquad (A3)$$

B_0 and B_1 being coefficients of a truncated Fourier series of the velocity, representing the mean value and half the semiamplitude of the oscillation, respectively. Notice that the assumption of sinusoidal velocity implies that higher harmonics of the velocity can be neglected. Substituting eq A2 into eq A3 yields

$$\langle S\rangle + A_S[(e^{i\omega_0 t} + e^{-i\omega_0 t})/2] \approx V_m^{-1}[(\langle S\rangle + K_M)B_0 + A_S B_1 + (A_S B_0/2 + (\langle S\rangle + K_M)B_1)(e^{i\omega_0 t} + e^{-i\omega_0 t}) + A_S(B_1/2)(e^{i2\omega_0 t} + e^{-i2\omega_0 t})] \qquad (A4)$$

Because of nonlinearities, the first harmonic ($\omega = 2\omega_0$) appears in the right member of eq A4. By identifying coefficients, we observe that

$$B_0 = \frac{\langle S\rangle V_m - A_S B_1}{\langle S\rangle + K_M}$$

$$B_1 = \frac{A_S V_m K_M}{2(\langle S\rangle + K_M)^2 - A_S^2} \qquad (A5)$$

The assumption (eq A2) holds if the first harmonic is negligible, that is, if the ratio between the amplitudes of the first harmonic and of the fundamental frequency ($A_S B_1/V_M$ and A_S, respectively) satisfy the condition

$$\frac{B_1}{V_m} = \frac{A_S K_M (V_m - \langle J_{in}\rangle)^2}{2V_m^2 K_M^2 - A_S^2 (V_m - \langle J_{in}\rangle)^2} \ll 1 \qquad (A6)$$

The derivative of eq A6 with respect to A_S is always positive. Thus, the approximation will improve as the substrate amplitude drops, i.e., as the input amplitude does. This is in agreement with numerical calculations. Furthermore, the approximation is expected to be quite good in general, as demonstrated by taking $A_S = \langle S\rangle$ in eq A6. This yields the maximum hypothetical ratio between harmonic amplitudes, $\langle J_{in}\rangle(V_m - \langle J_{in}\rangle)/(2V_m^2 - \langle J_{in}\rangle^2)$, which vanishes at low and high mean input fluxes. Its maximum occurs at $\langle J_{in}\rangle = (2 - \sqrt{2})V_m$, where $(B_1/V_m)_{max} = (3\sqrt{2} - 4)/(4\sqrt{2} - 4) \approx 0.15$, and it is independent of the maximum velocity. Such a maximum is observed in numerical calculations (cf. Table 1). However, because the oscillation of substrate is usually damped ($A_S < \langle S\rangle$), the first harmonic is typically much less than 15%.

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