FEBS Letters 499 (2001) 230–234 FEBS 24978

Mathematical analysis of a mechanism for autonomous metabolic oscillations in continuous culture of *Saccharomyces cerevisiae*

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Received 5 April 2001; revised 24 May 2001; accepted 26 May 2001

First published online 6 June 2001

Edited by Judit Ovádi

Abstract Autonomous metabolic oscillations were observed in aerobic continuous culture of *Saccharomyces cerevisiae*. Experimental investigation of the underlying mechanism revealed that several pathways and regulatory couplings are involved. Here a hypothetical mechanism including the sulfate assimilation pathway, ethanol degradation and respiration is transformed into a mathematical model. Simulations confirm the ability of the model to produce limit cycle oscillations which reproduce most of the characteristic features of the system.

Key words: Metabolic oscillation; Mathematical model; Sulfate assimilation; Regulation of respiration; Hydrogen sulfide; *Saccharomyces cerevisiae*

1. Introduction

Cellular oscillations are appropriate objects for the investigation of the structure and the regulatory properties of biochemical networks. Intensively studied rhythms are the cell cycle, Ca^{2+} as well as glycolytic oscillations [1]. As a relatively new example, autonomous metabolic oscillations were described in continuous culture of yeast under aerobic conditions [2–4]. The oscillatory behavior occurs for different carbon sources in the media (glucose, ethanol or acetaldehyde) and concerns a multitude of intra- and extracellular variables. These are, for example, the oxygen uptake rate, CO_2 evolution rate, dissolved oxygen tension (DOT), the concentrations of ethanol, acetaldehyde, acetate, cysteine, glutathione, hydrogen sulfide, ATP, and NAD(P)H, and the cellular pH [2,5]. The oscillation period depends on the culture conditions and the carbon source. In ethanol media, which we focus on here, it is about 40 min. The oscillatory dynamics was found to be sustained for several weeks without any shifts in the characteristic phase relations between the metabolites (see Table 1 for examples) and in the period. It was demonstrated that the rhythm is not related to the cell cycle or glycolytic oscillations [3,4]. Therefore, a crucial question concerns the underlying mechanism of this dynamic behavior. Recently this was investigated in a series of experiments [5–7], the results of which we summarize briefly.

Obviously, oscillations can be observed in a yeast cell suspension only if the behavior of the single cells is synchronized. It was shown that synchrony is mediated by the diffusion of hydrogen sulfide, which leads to an inhibition of respiration in each cell [6,8,9]. The coupling substance is produced in the sulfate assimilation pathway [5]. This pathway includes the uptake of sulfate and its subsequent activation in an ATP-dependent reaction producing adenylyl sulfate. After several steps H_2S is formed by sulfite reductase. Sulfide can react with *O*-acetylhomoserine to cysteine. For the generation of the oscillations the regulatory properties of the uptake and the assimilation of sulfate play an important role [5,7]. The regulation of the pathway is rather complex: *O*-acetylhomoserine, methionine/*S*-adenosylmethionine and cysteine act as effectors [7]. It was demonstrated that cysteine (or one of its derivatives) has a major effect by repressing the sulfate assimilation pathway at high concentration levels [7,8,10]. In particular, the inhibition of the sulfate uptake by cysteine is considered the source of the oscillations. This hypothesis is supported by the fact that the dynamics directly depends on the availability of sulfate [5]. Additionally, ethanol metabolism, which is linked to sulfate assimilation via sulfite and redox balance, is essential for the oscillatory mechanism [6].

Apparently, the oscillation-generating mechanism concerns several biochemical pathways. This makes it difficult to come to a full understanding by a verbal discussion alone. Therefore, a hypothetical mechanism is discussed in terms of a mathematical model. It will be used to find out whether the proposed mechanism may lead to oscillatory behavior and, moreover, reproduce characteristic features of the oscillations.

2. The model

The model includes the pathways which were found to be essential for the generation of oscillations. These are, in particular, the synthesis of the amino acid cysteine by the sulfate assimilation pathway, the uptake and degradation of the carbon source ethanol, the citrate cycle and the oxidative phosphorylation. Only those regulatory couplings which were discussed to be crucial for the oscillations are taken into account. These are the inhibitory effect of cysteine on the sulfate uptake and the inhibition of the respiratory chain by sulfide. The

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0014-5793/01/$20.00
PII: S0014-5793(01)02562-5

reaction network of the model is shown in Fig. 1; the abbreviations for the intermediates are given in the legend of that figure. In Table 2 the individual processes are listed.

Because of the large number of reactions under consideration, the mathematical description is simplified as much as possible. (I) Several metabolites are lumped. This concerns for example the reduction equivalents NADH, NADPH and $FADH_2$, as well as the intermediates of the citrate cycle. (II) Because fluxes, concentrations and other parameters of the system are not ascertained, dimensionless variables (concentrations of metabolites, time) and parameters (fluxes, kinetic constants) are used. (III) The oxidative phosphorylation is described by a minimal model (see Appendix), for more extended models see [11,12].

Taking the four conserved moieties

$$A_2^c + A_3^c = A^c, \tag{1a}$$

$$A_2^m + A_3^m = A^m \tag{1b}$$

$$N_1 + N_2 = N, \tag{1c}$$

$$S_1 + S_2 = S \tag{1d}$$

into account, the model can be described by a differential equation system with 13 variables

$$\frac{d\mathit{sul}}{dt} = v_1 - v_2 \tag{2a}$$

$$\frac{d\mathit{aps}}{dt} = v_2 - v_3 \tag{2b}$$

$$\frac{d\mathit{pap}}{dt} = v_3 - v_4 \tag{2c}$$

$$\frac{d\mathit{hyd}}{dt} = v_4 - v_5 - v_{17} \tag{2d}$$

$$\frac{d\mathit{cys}}{dt} = v_5 - v_6 \tag{2e}$$

$$\frac{d\mathit{eth}}{dt} = v_{13} - v_7 \tag{2f}$$

$$\frac{d\mathit{aco}}{dt} = v_7 - v_8 - v_{15} \tag{2g}$$

$$\frac{dS_1}{dt} = v_8 - v_9 \tag{2h}$$

$$\frac{d\mathit{oxy}}{dt} = v_{10} - v_{11A} - v_{14} \tag{2i}$$

$$\frac{dA_3^m}{dt} = v_{11B} - v_{16} \tag{2j}$$

$$\frac{dN_2}{dt} = -3v_4 + 2v_7 + 4v_9 - v_{11A} \tag{2k}$$

$$\frac{dA_3^c}{dt} = -v_2 - v_3 - v_{12} + v_{16} \tag{2l}$$

$$\frac{d\mathit{oah}}{dt} = -v_5 + v_{15} - v_{18} \tag{2m}$$

where v_i denote the reaction rates according to Fig. 1. The mathematical description of these rates is given in Table 3. For most rate equations linear and bilinear functions of the substrate concentrations are used. The rate equations v_{11A} and v_{11B} for the oxidative phosphorylation are derived in the Appendix. The factors f_1 and f_2 describe the inhibition of the sulfate transport by cysteine and that of the respiratory chain by hydrogen sulfide, respectively. The parameters K_C and K_H are the inhibition constants, while n and m are cooperativity coefficients.

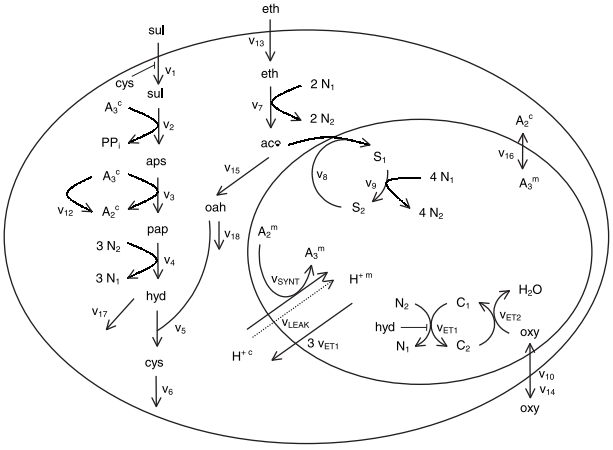

Fig. 1. Reaction scheme of the model. The following abbreviations are introduced for the metabolites: sul: sulfate ions, aps: adenylyl sulfate, pap: 3-phosphoadenylyl sulfate, hyd: hydrogen sulfide, cys: cysteine, eth: ethanol, aco: acetyl-CoA, S_1, S_2: intermediates of the citrate acid cycle, oxy: oxygen, C_1, C_2: protein complexes involved in the oxidative phosphorylation, A_3: ATP, A_2: ADP, N_1: $NAD(P)^+$, N_2: NAD(P)H, oah: *O*-acetylhomoserine. The cytosolic and mitochondrial compartments are characterized by the superscripts c and m, respectively. For an explanation of individual reactions see Table 2. The rate equations $v_{11A} = v_{ET1} = v_{ET2}$ and $v_{11B} = v_{SYNT}$ for the processes of the oxidative phosphorylation are derived in the Appendix.

3. Results and discussion

The central question to answer is whether the regulation of the sulfate uptake by cysteine can give rise to oscillatory dynamics. In a first step we therefore focus on this regulation and ignore the effect of H_2S on the respiratory chain ($K_H \to \infty$, consequently $f_2 = 1$). Simulations demonstrate the existence of parameter regions in which limit cycle oscillations occur. In Fig. 2A the dynamic behavior of several system variables is shown for a reference set of parameters (see legend of Fig. 2A,B). The periodic behavior is characterized by strong oscillations in the intermediates of the sulfur metabolism (sulfate ions, adenylyl sulfate, 3-phosphoadenylyl sulfate, hydrogen sulfide and cysteine) and only slight variations in all other variables. Obviously, the feedback inhibition within the sulfate assimilation pathway may result in oscillatory changes of all intermediates and reaction rates along this reaction chain. Therefore, also the adenine nucleotides and the redox equivalents participate as cofactors in oscillating reactions. However, the majority of these cofactor molecules are converted by ethanol degradation, including the citrate cycle and respiration (the flux through the ethanol degradation pathway is assumed to be higher than that through the sulfate assimilation pathway). These processes are not directly involved in the oscillatory mechanism. Consequently, the concentrations of the cofactors show modest oscillations and the periodicity is only slightly transduced from the oscillatory core to the rest of the cellular metabolism.

The inclusion of the inhibition of the oxidative phosphorylation by hydrogen sulfide (inhibition constant $K_H = 0.5$ and otherwise identical parameter values) leads to strong oscillations in all variables (see Fig. 2B for a selection of them). The parameters of the system are scaled in such a way that the period of the oscillation is about 40 in the reference case. Here

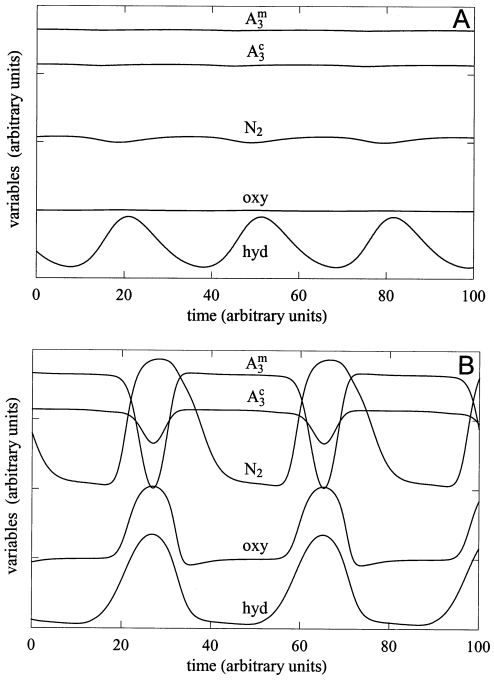

Fig. 2. Limit cycle oscillations of selected metabolites. Reference parameter values: $v_0 = 1.6$, $k_2 = 0.2$, $k_3 = 0.2$, $k_4 = 0.2$, $k_5 = 0.1$, $k_6 = 0.12$, $k_7 = 10.0$, $k_8 = 10.0$, $k_9 = 10.0$, $v_{10} = 80.0$, $k_{11} = 10.0$, $k_{12} = 5.0$, $v_{13} = 4.0$, $k_{14} = 10.0$, $k_{15} = 5.0$, $k_{16} = 10.0$, $k_{17} = 0.02$, $k_{18} = 1.0$, $n = 4.0$, $m = 4.0$, $K_A = 1.0$, $K_C = 0.1$, $\alpha = 0.1$, $A^c = 2.0$, $A^m = 2.0$, $S = 2.0$, $N = 2.0$. Individual scales for the variables: NADH 1.6–2.0, DOT 5.0–10.0, H_2S 0–2.0, ATP^c and ATP^m 0–2.0. A: Model without the inhibitory effect of sulfide on respiration ($K_H \to \infty$, $f_2 = 1$). Oscillations occur in a certain range of ethanol uptake $v_{13} = 2.9$–8.4. B: Model with inhibition of respiratory chain by hydrogen sulfide (inhibition constant $K_H = 0.5$).

the characteristic features of the experimental data and of the simulated oscillations may be compared.

Because of its central role in the oscillation mechanism and in intercellular coupling, the time course of H_2S was studied intensively [6]. It was found that sulfide, which oscillates in phase with DOT, reaches concentrations of about 1.5 μM at maximum, whereas it is below the detection limit at its minimum. DOT varies between 50 μM and 170 μM during the high and low respiration phases, respectively. The model simulations reproduce these features qualitatively. The two metabolites oscillate in phase and, moreover, the concentration of sulfide in the high respiration phase is close to zero (see Fig. 2B).

Other characteristics for the oscillatory behavior are the phase shifts of the metabolites. In addition to H_2S, ATP and NAD(P)H oscillate in phase with DOT, whereas ethanol and DOT are 90° out of phase (see Table 1). The phase shifts

Table 1
Characteristic phase relation of oscillating metabolites in experiments

Intermediate	Phase relation
ATP–DOT	in phase
NAD(P)H–DOT	nearly in phase
Ethanol–DOT	nearly 90° out of phase (DOT-max is followed by ethanol-max)
H_2S–DOT	nearly in phase (H_2S-max is followed by DOT-max)

Table 2
Processes under consideration

	Process
Sulfur metabolism and biosynthetic processes	
1	sulfate uptake into the cell
2	reaction catalyzed by sulfate adenylyltransferase
3	reaction catalyzed by adenylyl sulfate kinase
4	lumped reactions of phosphoadenylyl sulfate reductase, sulfite reductase and ferredoxin-NADPH reductase
5	lumped processes of cysteine production from H_2S and carbon metabolites
6	degradation of cysteine
15	production of *O*-acetylhomoserine
18	degradation of *O*-acetylhomoserine
19	outflow/degradation of H_2S
Ethanol degradation and citrate acid cycle	
13	ethanol influx
7	reactions of alcohol dehydrogenase, aldehyde dehydrogenase and acetyl-CoA-synthase
8, 9	lumped processes of the citrate cycle
Oxidative phosphorylation	
10	influx of oxygen
14	outflux of surplus oxygen
11A	lumped processes of respiration: electron transfer from NAD(P)H to connected with the pumping of protons from the mitochondria in the cytosol
11B	lumped processes of respiration: production of ATP, coupled to the flux of protons from the cytosol to the mitochondria
Transport processes and ATP consuming steps	
12	ATPase in the cytosol
16	translocation of the adenine nucleotides over the mitochondrial membrane
17	ATPase in the mitochondrion

of NAD(P)H and ethanol with respect to DOT are reproduced by the model (see Fig. 2B for NAD(P)H and DOT, ethanol not shown). An investigation of the phase relation of the adenine nucleotides and DOT reveals that cytosolic and mitochondrial ATP oscillate in phase, but 180° out of phase with DOT. These findings can be easily understood in terms of the model. High concentrations of hydrogen sulfide lead to an inhibition of the rate of electron transport and therefore to an accumulation of NAD(P)H and the protein complex C_1. Due to the conservation condition the concentration of the complex C_2 will decrease and consequently DOT will rise. Moreover, the inhibition of the electron transport prevents the pumping of protons out of the mitochondrion. Therefore, the ATP production rate is reduced. The out of phase oscillation of the adenine nucleotides and DOT contrasts with the situation in the experiments. Simulations show that variations of parameters change the phase relation of these metabolites only slightly. For instance, the effect of the velocity of the adenine nucleotide translocation on this phase relation is minor. This indicates that not all processes determining the oscillatory dynamics have been identified so far. Preliminary results show that a phase-dependent ATP consumption, for example by oscillating biosynthetic rates, may change the phase relation of ATP and DOT. Consequently, an experimental investigation of the processes determining the ATP balance of the system is necessary.

Another object of detailed experimental study was the dependence of the dynamic behavior on the sulfate uptake. The inorganic sulfate concentration in the culture directly controls the period of the oscillations [5]. A switch from standard medium to one with a low sulfate concentration decreases

Table 3
Rate equations of the mathematical model

$v_1 = v_0 \cdot f_1$	$f_1 = \left[1 + \left(\frac{cys}{K_C}\right)^n\right]^{-1}$
$v_2 = k_2 \cdot sul \cdot A_3^c$	$v_3 = k_3 \cdot aps \cdot A_3^c$
$v_4 = k_4 \cdot pap \cdot N_2$	$v_5 = k_5 \cdot hyd \cdot oah$
$v_6 = k_6 \cdot cys$	$v_7 = k_7 \cdot eth \cdot N_1$
$v_8 = k_8 \cdot aco \cdot S_2$	$v_9 = k_9 \cdot S_1 \cdot N_1$
$v_{10} = \text{const}$	
$v_{11A} = \frac{k_{11} \cdot N_2 \cdot oxy \cdot f_2}{\alpha \cdot N_2 + oxy}$	$f_2 = \left[1 + \left(\frac{hyd}{K_H}\right)^m\right]^{-1}$
	$v_{12} = k_{12} \cdot A_3^c$
$v_{11B} = 3v_{11A}\frac{A_2^m}{K_A + A_2^m}$	
$v_{13} = \text{const}$	$v_{14} = k_{14} \cdot oxy$
$v_{15} = k_{15} \cdot aco$	$v_{16} = k_{16} \cdot A_3^m \cdot A_2^c$
$v_{17} = k_{17} \cdot hyd$	$v_{18} = k_{18} \cdot oah$

the period of oscillation from about 40 min to 30 min [5]. In the model a lower sulfate uptake (corresponding to a lower value for v_0) also leads to shorter periods. A decrease in the sulfate uptake from the reference parameter set (with $v_0 = 1.6$, see Fig. 2B) to $v_0 = 0.13$ leads to a period of about 30. For smaller v_0 values the system shows no oscillations, but returns to a steady state.

These results can be summarized as follows.

The inhibition of the sulfate uptake by cysteine may lead to sustained oscillations. This is in accordance with the well known fact that oscillatory dynamics arises from a reaction chain with feedback inhibition if the length of the chain and the inhibition strength exceed critical values [13]. It was shown that the regulation of the respiration by sulfide has a major effect on the spreading of the oscillation in the metabolic network. The model reproduces experimental observations concerning the time course of hydrogen sulfide as one of the central metabolites of the system and the effect of the sulfate uptake on the oscillation period. Moreover, it explains the phase shifts of ethanol and NAD(P)H with respect to DOT. However, the out of phase oscillation of ATP (in cytosol and mitochondria) with DOT is in contrast to experimental data. As already discussed, the cellular ATP balance needs to be further analyzed.

Even though there are strong indications that the inhibition of the sulfate uptake is involved in the oscillatory mechanism, clear experimental evidence for that hypothesis is still lacking. Accordingly, other regulations may be discussed as a source of the oscillations. Central regulatory loops are of special interest in this context. As an example may serve the indirect negative feedback of ATP on the citrate cycle. It results from the fact that the citrate acid cycle and some biosynthetic processes have the same substrate, i.e. acetyl-CoA. An increased rate of ATP-consuming biosynthesis therefore leads to a decreased rate in the cycle and in respiration, which produces ATP. Consequently, an increase in ATP may lead to a decreased rate in the cycle. In an alternative mathematical model we have considered this mechanism the central oscillatory loop. It was shown that limit cycle oscillations may occur. However, neither the phase relation of ATP nor that of NAD(P)H with respect to DOT could be reproduced by this model.

A suitable test for the model would be the simulation of perturbation experiments. In yeast culture the influence of glutathione, acetaldehyde, the sulfide acceptor *O*-acetylhomoserine and Na_2S was investigated in this way [6,7]. For Na_2S, a donor of sulfide, the effect of the perturbation with respect to the phase of addition and strength of the perturbation pulse was quantified in phase response curves [6]. However, questions arise to what extent the response of the cell population is due to changes in the dynamics of the single cells and how far they result from synchronization effects. The experiments indicate that a damping or disappearance of the oscillatory behavior after perturbations in several cases may result from a desynchronization of the oscillations of the individual cells. Consequently, for the simulation of perturbation experiments a model of interacting cells would be more appropriate. The interaction between cells is mediated by the diffusion of hydrogen sulfide. The situation may therefore be related to glycolytic oscillations in yeast cell suspensions, where acetaldehyde acts as a coupling substance [14]. For this system the effect of interaction on the population dynamics was investigated in several mathematical studies [15–18]. It was shown that synchronization as well as antisynchronization may result from such an indirect coupling via the external solution.

Acknowledgements: H.-Y.S. was the recipient of a fellowship of the Science and Technology Agency (STA) of Japan. R.H. acknowledges the financial support by the Japan Industrial Technology Association (JITA) during two stays at the National Institute of Bioscience and Human Technology (Tsukuba).

Appendix. Mathematical description of the oxidative phosphorylation

Here a minimal description for the oxidative phosphorylation is derived. The complex processes take place in the inner membrane of mitochondria and include the electron transport from NADH to oxygen and the formation of ATP. The electrons are transported in several steps from NADH via a chain of protein complexes to oxygen. Here these proteins are assumed to be lumped into one complex (see Fig. 1). The concentrations of its two forms C_1 and C_2 are considered as a conserved moiety $C = C_1 + C_2$. The dynamics of C_1 can be described by

$$\frac{dC_1}{dt} = -v_{ET1} + v_{ET2} = -k_{ET1} \cdot N_2 \cdot C_1 + k_{ET2} \cdot (C - C_1) \cdot oxy \tag{A1}$$

Assuming a quasi steady state for C_1 determines the concentration of that metabolite and the reaction rate

$$v_{ET1} = v_{ET2} = \frac{k_{ET1} \cdot C \cdot N_2 \cdot oxy}{(k_{ET1}/k_{ET2}) \cdot N_2 + oxy}. \tag{A2}$$

With $k_{11} = k_{ET1} \cdot C$, $\alpha = k_{ET1}/k_{ET2}$ as well as the inclusion of the inhibitory effect of hydrogen sulfide by the factor f_2 this gives the rate v_{11A} (for f_2 and v_{11A} see Table 3).

While the electron transport is linked to a proton transfer out of the mitochondria, the flow of protons back through the ATP synthase is coupled to the production of ATP [19]. The concentrations of protons in the mitochondria and the cytosol are therefore determined by three fluxes: the pumping of protons due to the electron transfer γv_{ET1} ($\gamma = 3$ because of three

protons per NADH), the flux through the ATP synthase v_{SYNT} and a leak flux v_{LEAK} between both compartments. The proton concentrations fulfil mass conservation and additionally they meet a quasi steady state assumption. Therefore,

$$0 = \gamma \cdot v_{ET1} - v_{SYNT} - v_{LEAK} \quad (A3)$$

follows. The fluxes v_{SYNT} and v_{LEAK} are considered to be proportional to the concentration difference of protons in the two compartments. Moreover, v_{SYNT} is dependent on the ADP concentration inside mitochondria. Therefore, the concentration of cytosolic protons and in the end the rate equation of the ATP synthase can be calculated

$$v_{SYNT} = 3 \cdot v_{ET1} \frac{A_2^m}{(k_{LEAK}/k_{SYNT}) + A_2^m}. \quad (A4)$$

With $K_A = k_{LEAK}/k_{SYNT}$ the rate v_{11B} (see Table 3) is derived from Eq. A4.

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