

## Pattern Formation and Marangoni Convection during Oscillating Glycolysis

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Spatial pattern formation during oscillating glycolysis in a thin layer of extract from yeast cells is investigated by two-dimensional spectrophotometry using video techniques and by dark-field detection of refractive index gradients. The time intervals of repetitively reappearing transmission patterns coincide with the phases of maximum metabolic turnover during each glycolytic cycle. The spatial arrangement of the patterns is strongly correlated with a network of convection cells which permanently exists in the liquid layer. The patterns are generated by the coupling of reaction with Marangoni type convection due to gradients in surface tension which are mainly caused by evaporative cooling and influenced by chemical composition and reactivity.

### Introduction

Symmetry-breaking transitions from spatially uniform to macroscopically inhomogeneous distributions of reactants may occur in chemical or biochemical solutions under suitable far from equilibrium conditions, as predicted and experimentally verified by many authors [1–5]. At wavelengths specific for the absorption of reduced nicotinamide adenine dinucleotide (NADH) formation of regular patterns (length scale 1 mm) has been observed during oscillating glycolysis in thin layers of extract from yeast [6]. More insight into the coupling of biochemical metabolism and transport processes has been gained by two-dimensional spectrophotometry using UV sensitive digital video techniques [7] combined with a method for visualizing spatial distributions of refractive index gradients [8]. The photometric technique allows for temporal correlation between oscillations and formation of patterns which are found to appear twice during each glycolytic cycle and which are detectable at NADH specific wavelengths. Near simul-

taneous detection of refractive index gradients proves that the pattern morphology is determined by a polygonal network of convection cells which, after an induction period of several minutes, remains detectable in the sample. Our experiments show the importance of hydrodynamic flow in a biochemical solution layer due to unbalanced forces at a liquid/gas interface (Marangoni convection) as already discussed for chemically reactive systems of related nature [9–13].

### Materials and Methods

The cytoplasmic medium used for the experiments was extracted from yeast cells (*Saccharomyces Carlsbergensis*) grown under aerobic conditions according to a published procedure [14]. The protein content (*i.e.* 49 mg/ml) corresponds to roughly one third of the concentration in the cytoplasm of an intact yeast cell. In this medium the metabolic degradation of glycolytic substrates such as glycogen is performed in an oscillatory manner with periods of the order of several minutes.

Experiments were started after placing 1.5 ml of filtered extract (0.44  $\mu\text{m}$  Millipore filter) supplied with glycogen in an open petri dish of 3.2 cm diameter. Transmission measurements were carried out with a spectrophotometer suitable for two-dimensional quantitative analysis of spatial patterns in the NADH absorption region [7]. Distributions of refractive index gradients in the same sample were recorded by fast switching to a dark-field type technique.

Digital images could be collected at a frequency up to 30 per minute with a UV sensitive video camera (Hamamatsu C-1000, vidicon tube N983) having a raster resolution of  $512 \times 512$  picture elements (pixels) and an intensity resolution of 256 digital units (greylevels). After storage on magnetic disk the transmission data were corrected for temporal fluctuations of the light source as well as for static inhomogeneities in the illuminating light field and the target sensitivity. Pixel noise was reduced by calculating moving averages of pixel frames which are small compared to the length scale of the structures [7].

### Results and Discussion

A temporal sequence of digital transmission data obtained at 380 nm is presented in Fig. 1. Glycolysis

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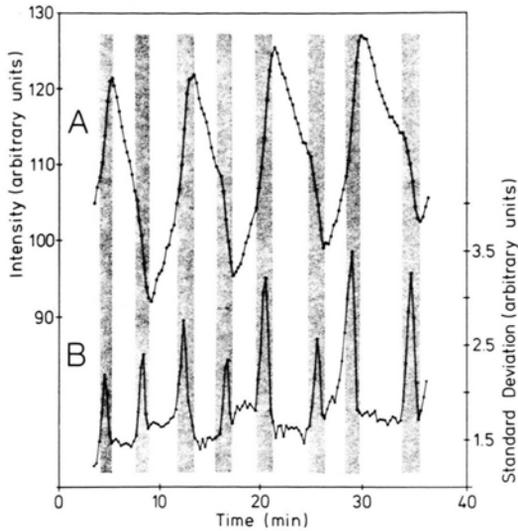


Fig. 1. Temporal relation between oscillations and spatial pattern formation at 380 nm in a 1.8 mm layer of yeast extract supplied with glycogen. The average intensity of transmitted light (A) and the standard deviation of the spatial intensity distribution (B) are evaluated from  $400 \times 200$  pixel sections of corrected digital images and plotted at a frequency of 4 per min. The shaded stripes indicate the time intervals of pattern appearance determined by visual inspection of the images. Their extent corresponds to the width of the peaks in graph B.

in the bulk solution oscillates with periods of 9 minutes as can be seen from the spatially averaged light intensities in graph A. Evaluation of the intensity distribution of each image in terms of standard deviation shows a series of distinct peaks (graph B) which precisely coincide with those time intervals during which transmission patterns as shown below (Fig. 2)

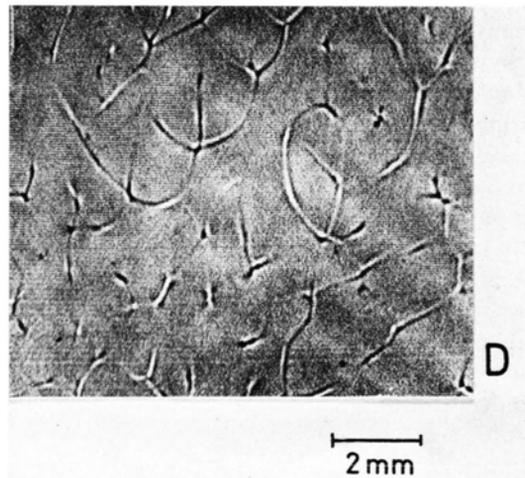
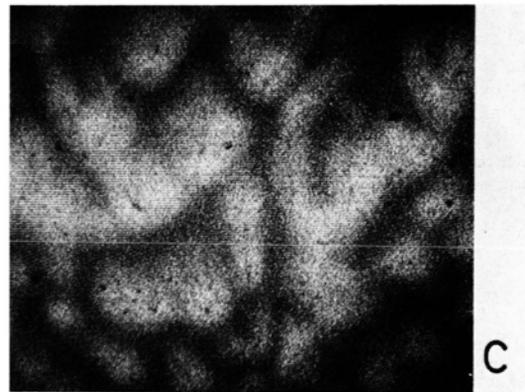
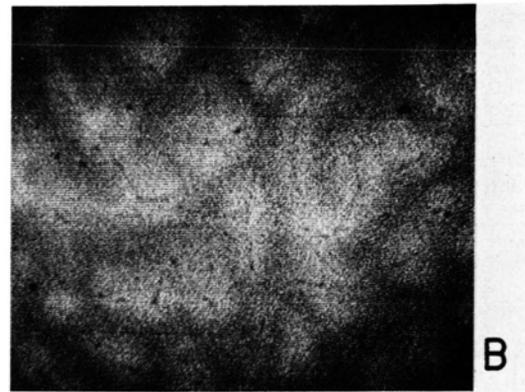
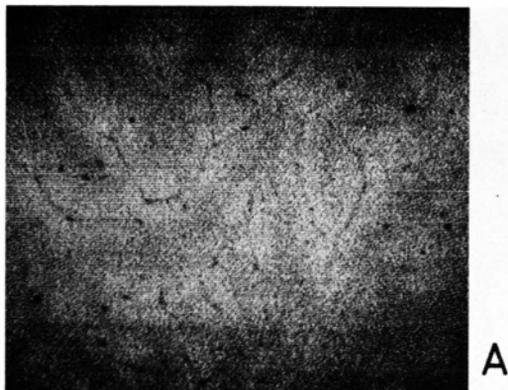


Fig. 2. Images of spatial pattern formation in a 1.8 mm layer of oscillating yeast extract. The pictures were taken 7.5 min (A), 8.0 min (B), 8.5 min (C), and 9.0 min (D) after the start of the experiment (compare Fig. 1). Patterns A to C were detected with directly transmitted light at 380 nm, pattern D with a modified dark-field method at 548 nm.

are detected by visual inspection of the images on a TV screen (vertical shaded stripes in Fig. 1). The standard deviation thus provides a heuristic means for quantitative detection of this type of transmission patterns. Extension of the shaded stripes to Fig. 1A reveals that patterning occurs twice during each glycolytic cycle just before the bulk NADH absorption reaches its minimum or maximum value. Within these time intervals changes in the NADH absorption are especially fast indicating high metabolic turnover.

A more detailed look at the pattern morphology and its temporal evolution is provided by the photographs of Fig. 2 taken during a representative period of pattern occurrence. Picture A for the initial stage mainly consists of a network of thin dark lines with several branching points. These lines have been found to be permanently present (after an induction period of a few minutes) in layers of yeast extract even without any oscillatory glycolytic reaction [8]. They result from shadows cast by pronounced local gradients in refractive index which are directly related to the distribution of temperature and/or chemical composition in the liquid [15] and do not specifically indicate changes in NADH absorption. All lines are surrounded by barely visible bright patches of approximately 1 mm width. Half a minute later these NADH specific areas and their correlation with the faint lines along their central axis are more pronounced (Fig. 2B). After another 30 seconds, when maximum contrast is reached (Fig. 2C), the faint lines are somewhat blurred but can still be clearly identified as lines of refractive index gradients on switching to the dark-field type method as demonstrated in Fig. 2D. The geometrical characteristics of structures C and D are closely related to each other. Note, for instance, the ellipsoidal segment in the right half of Fig. 2D which coincides with the similarly shaped, more diffuse bright area in the corresponding section of Fig. 2C. Another example is the fork-like threefold branching point to the left of the ellipse in picture D which is easily recognized to form

the backbone of a bright area in picture C. While the patchy transmission pattern C vanishes within the next seconds, the line pattern D persists. After being subject to slow and minor changes [8] it constitutes the morphology of the next transmission pattern to appear.

Furthermore we observe the following: (1) The typical length scale of the structures increases when the depth of the layer is increased. (2) Macroscopic dust particles which may be present in the sample tend to move towards the lines of refractive index gradients. (3) Pattern formation is coupled with the existence of horizontal temperature gradients of up to 0.5 °C/cm from the center towards the boundaries of the dish, while vertical temperature gradients are not detectable within 0.05 °C. (4) Covering the dish or placing a glass plate directly on the surface of the solution leads to disappearance of any inhomogeneities in temperature. Under these conditions both refractive index gradients and transmission patterns fail to develop.

From our experimental findings we conclude that unbalanced forces at a liquid/gas interface (Marangoni convection) are a prerequisite for patterning in an open layer of oscillating yeast extract, analogous to recent results for layers of NADH solutions [13] and a non-reactive cytoplasmic medium [8]. In this instance gradients in surface tension are set up by inhomogeneities in temperature due to evaporation. The observed gradients in refractive index indicate subsequent formation of convection cells coupled with undulation of the surface [15]. Their spatial arrangement determines the periodically reappearing transmission patterns which, according to Lambert-Beer's law, reflect transient local variations in the concentration of biochemical compounds and/or the depth of the liquid layer [13]. The glycolytic system thus generates chemical reaction-convection patterns, a class of patterns in which symmetry is broken by interfacial instabilities due to inhomogeneities in temperature or in chemical composition [9–13].

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