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METABOLIC OSCILLATIONS OF *ESCHERICHIA COLI* RECORDED BY MICROCALORIMETRY*

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SUMMARY

Microcalorimetric experiments on the periodic heat production of a culture of *Escherichia coli* are described. The behaviour is detected in a hermetically closed environment long after nutrients and oxygen are exhausted in the medium. The calorimetric technique is reported for a plausible cooperative behaviour of cells induced in the case by the final mixed-acid fermentation metabolism.

INTRODUCTION

Oscillating reactions have been widely described in recent times (ref. 1) as an important hint concerning the regulating effects in cell organisms. Nevertheless, most available experimental evidence (ref. 2) corresponds to the well- known glycolysis. We report here the non-glycolytic periodic behaviour of a cell population of *Escherichia coli*, and we provide some indirect arguments in order to identify this effect as a self-mantained mixed-acid fermentation.

Glycolisis pathway, a chain of at least eleven enzyme-catalysed steps, belongs to a kind of phenomena ocurring far from thermodynamic equilibrium. This is the case of the well known Belousov-Zhabotinski reaction, the Briggs-Rauscher reaction or the ABA reactions (ref. 3). Numerous contributions in this field revealed that this so called "order through fluctuations" occurs only in a critical domain of parameter values outside of which the reactions proceed in a monotonous manner.

In this paper we show how a similar behaviour (with similar biological consequences) could be performed simply by coupling two steps of a reaction chain, i.e. the mixed-acid fermentation. The characteristic "window" would be controled in this case by the concentration of present CO_2.

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METHODS AND MATERIALS

The experiments were performed in a modified isoperibolic batch microcalorimeter with vessels of 7 ml and a sensitivity of 112 mV/W. The calorimetric vessel is supplied with a stirrer and an injection tube in order to proceed, if necessary, with both mechanical and chemical perturbations.

All experiments were run at 30 ^{o}C in either minimal medium (2 g/l of glucose) or lactate medium (2 g/l of lactate) with an innoculum prepared in the same manner (ref. 4).

RESULTS

As a central result we present in Fig. 1 the recording of the calorimetric signal. The calorimetric base line is not considered here in order to display the long time evolution of the bacterial culture. The first part of the P-t curve shows the typical result corresponding to an Escherichia coli growing in a hermetically closed minimal medium, i.e. an almost imperceptible aerobic phase (corresponding to the initial available oxygen) and a second anaerobic phase corresponding to glycolysis.

After the first day (and therefore long after available glucose and oxygen are exhausted in the medium) a temporal organization becomes visible. This phenomenon culminates between the third and fourth day in a structure of a rather constant period (1 h), slowly decays and definitely ends on the sixth day.

At this point one already suspects a relationship between the recorded periodic behaviour and the formation of end products from pyruvic acid (in its turn resulting from the earlier glycolysis).

In order to draw a kind of "identikit picture" of the phenomenon under study, we proceed with some further experiments, namely:

E_2, the same as experiment E_1 displayed in Fig. 1, but with a lactate medium. Result: the energetic peaks appear now from the very begining.

E_3, the experiment starts like E_1 but 0.1 ml of glucose (2 g/l) are added during the temporal organization. Result: the peaks are inhibited.

E_4, the experiment starts like E_1 but the vessel is opened to the atmosphere during the temporal organization. Result: peaks are inhibited.

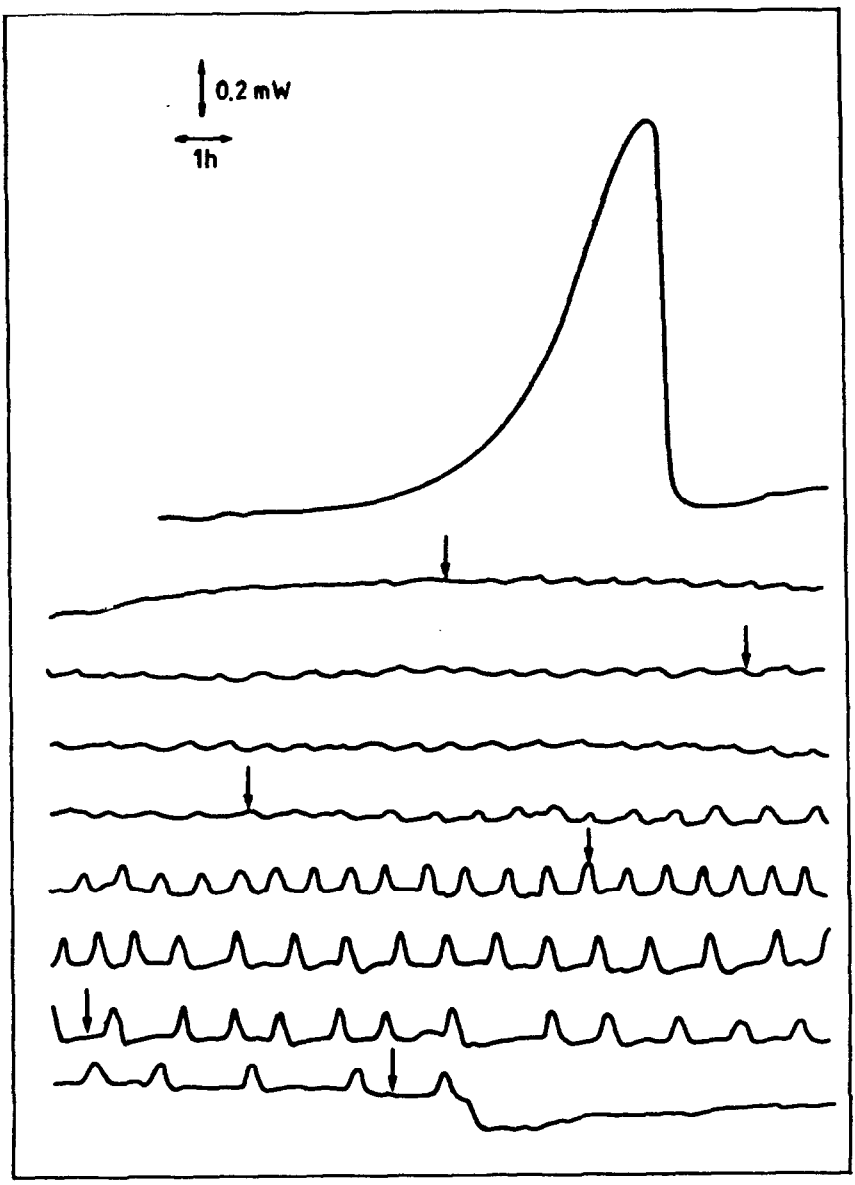

Fig. 1. P-t curve of Escherichia coli growing in minimal medium at 30 °C. Arrows indicate whole days.

E_5, experiment E_1 is perturbed by mechanical actions (i.e. stirring or addition of Ringer) during the temporal organization. Result: the peaks are not inhibited and the temporal behaviour follows with its "regular" evolution.

E_6, experiment E_1 is perturbed by the addition of 0.1 ml of Sodium Iodoacetic (0.02 g/l) during the temporal organization. Result: the peaks are not inhibited and the temporal behaviour follows with its "regular" evolution.

E_7, E_6 is perturbed by addition of 0.1 ml of glucose (2g/l). Result: the peaks are not inhibited and the temporal behaviour follows with its "regular" evolution.

DISCUSSION AND CONCLUDING REMARKS

Fig. 2 shows the well known scheme of pathways in the formation of the characteristic end products from the pyruvic acid, in particular, the mixed-acid fermentation of Escherichia coli.

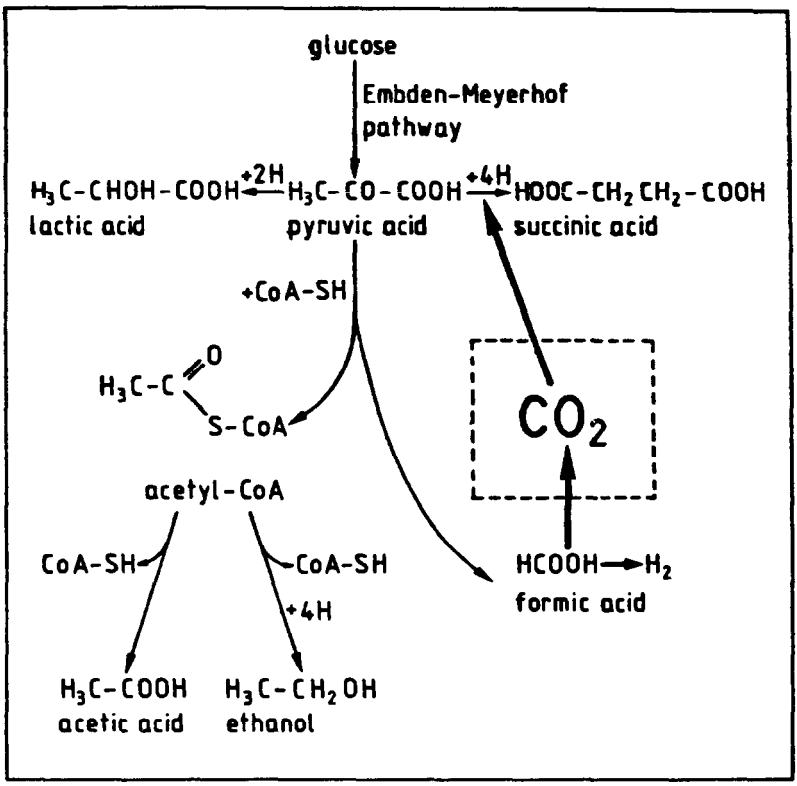

Fig. 2. The mixed-acid fermentation pathways

Let us try an explanation that matches with the identikit picture of the preceeding section :

Once the glycolysis is virtually concluded, the Escherichia coli bacteria are able to perform the transition from pyruvate (the product of glycolysis) to formate since the synthesis of pyruvate formate lyase is induced in anaerobic conditions. Moreover, pyruvate formate lyase is irreversible and rapidly inactivated under air. Formate is then cleaved into CO_2 and H_2 by means of formate hydrogen lyase (Ref. 5). Escherichia coli contains this activity under anaerobic conditions (Nitrate is not present in the medium but a formate dehydrogenase (FDH_1) could also contribute to this step if enough fumarate is available). Now, CO_2 is released as a consequence of this step so that a certain partial pressure is attained in the hermetically closed environment. This parameter is essential for the understanding of the cooperative effect. A minimum critical value is probably needed in order to activate the formation of succinate (an end product of mixed-acid fermentation) from the pyruvate, whereas a maximum critical value inhibits, logically, the elimination of formate (another end product). The former reaction consumes CO_2 and the latter produces it. This is the central coupling that produces the temporal organization detected by the calorimeter. Note that this interpretation perfectly matches with all the experiments listed in the preceeding section. Nevertheless, some positive proof is still needed in order to ensure it definitely. The continous recording of some of the end products or of CO_2 concentration is in fact a rather difficult technical question. A good idea is, we believe, to proceed to the simultaneous recording of heat production and pressure. We hope to report these results very soon.

Note also that the energetic translation of the periodic phenomenon clearly means that a cooperative effect is happening, which involves a large part of the population. This is also compatible with our suggestion since the CO_2 pressure is obviously an external and common controller from the point of view of a single bacteria, but a self controller for the whole population. Two lines of investigation remain open: the study of the proposed mechanism in the context of the general "dissipative structures" and its significance in the context of the biological hierarchies.

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