SHORT COMMUNICATION

Perturbation of Respiration in *Candida utilis*:
Induction of Metabolic Oscillations

By DAVID LLOYD AND JUDITH BALL

Department of Microbiology, University College, Newport Road, Cardiff CF2 1TA

(Received 20 February 1979)

The effects of potentially perturbing influences on the respiration of glucose-grown *Candida utilis* were studied using an open oxygen electrode system. Periods of anaerobiosis as short as 2 min produced an oscillation in respiration after the air supply was restored. Longer exposure to anoxia was followed by an overshoot in dissolved oxygen after switching back to a gas phase of air. Centrifugation, cold shock or nutrient starvation caused less disturbance to respiration rates than did anaerobiosis. The high frequency oscillations (period about 5 min) resulting from anaerobic-aerobic transitions are contrasted with the slow cell cycle-dependent oscillations previously observed in synchronous cultures.

INTRODUCTION

Oscillations in pool sizes and redox states of metabolic intermediates have provided insights into the mechanisms of metabolic control via feedback circuits (Higgins, 1967; Degn, 1972); the oscillatory kinetics of glycolytic reactions in cell-free extracts and in whole cells can be explained in terms of feedback through stoichiometric reactions involving NADH and ATP and non-stoichiometric interactions at allosteric control sites (Pye & Chance, 1966). Any disturbance of steady-state conditions in a microbial culture may lead to an astable state in which oscillatory metabolic behaviour is manifest; thus, lowering of the dissolved O$_2$ tension in a chemostat culture of glucose-grown *Klebsiella aerogenes* to a critical value induced oscillations of respiration (Harrison & Pirt, 1967). Anaerobic shock of these cultures produced oscillation of the redox state of NADH and in pool levels of ATP after aerobiosis was restored (Harrison, 1976).

In this paper we describe studies of the effects on respiration of several potentially perturbing influences in the yeast *Candida utilis*. Centrifugation, cold shock, nutrient starvation and periods of anaerobiosis are all conditions which may occur while manipulating cultures during the establishment of synchronous growth, even during selection synchrony; it is important to determine the effects of perturbation in order to distinguish them from events of the cell cycle. Anaerobiosis, even for periods of a few minutes, has the most marked effect of the conditions studied; the resulting respiratory oscillations differ fundamentally from the cell cycle-dependent respiratory oscillations previously described (Poole *et al.*, 1973; Edwards *et al.*, 1975; Lloyd *et al.*, 1978; Edwards & Lloyd, 1978; Kader & Lloyd, 1979).
Short communication

Fig. 1 (a, b, c). Effects of anaerobiosis on the steady-state levels of dissolved \(O_2\) in suspensions of *C. utilis*. Washed cell suspensions in 50 mM-potassium phosphate buffer (pH 7.2) containing \(10^8\) cells ml\(^{-1}\) were maintained under a gas phase of \(N_2\) for 15 min with stirring, and then air was switched on as indicated: (a) no added substrate; (b) 50 mM-glycerol present; (c) 50 mM-glucose present. (d) Effect of anaerobiosis on the steady-state level of dissolved \(O_2\) in a stationary-phase culture of *C. utilis* after growth with glucose. The culture contained \(1.9 \times 10^8\) cells ml\(^{-1}\), and air was switched on after 25 min of \(N_2\) flow.

METHODS

Organism and growth conditions. *Candida utilis* NCYC 193 was maintained and grown on defined media as described by Kader & Lloyd (1979). Organisms were counted in a Thoma haemocytometer slide (Hawksley, Lancing, Sussex).

Measurement of steady-state levels of dissolved \(O_2\). The apparatus used for measurement of levels of dissolved \(O_2\) was similar to that described by Degn & Wohlrab (1971). It consisted of a stainless steel reaction vessel of 4.5 ml working volume, with a membrane-covered \(O_2\) electrode immersed in the liquid which was stirred at a constant rate by a synchronous motor. Air or \(N_2\) entered the reaction vessel through an inlet in the lid and flowed continuously over the surface of the stirred liquid. All measurements were made at 30 °C. Values for the \(O_2\) transfer constant were about 0.23 min\(^{-1}\).

RESULTS

Effects of anaerobiosis

We monitored the effects of 15 min periods of anaerobiosis on the re-establishment of steady-state levels of respiration of washed glucose-grown cells suspended in potassium
phosphate buffer (pH 7.2) under a gas phase of air. In the absence of added substrate (Fig. 1a), restoration of the air supply led to a monotonic increase in the dissolved $O_2$ level and the steady-state value ($214 \mu M-O_2$) was established after 30 min [$Q_{O_2} = 6.0 \mu M-O_2$ min$^{-1}$ ($10^8$ organisms)$^{-1}$]. In the presence of 50 mM-glycerol (Fig. 1b), the increasing level of dissolved $O_2$ following the anaerobic-aerobic transition showed discontinuities, presumably due to small fluctuations in the respiration rate, which eventually attained a $Q_{O_2}$ of $20.8 \mu M-O_2$ min$^{-1}$ ($10^8$ organisms)$^{-1}$. Recovery of respiration after anaerobiosis in the presence of 50 mM-glucose (Fig. 1c) differed in two respects from the pattern observed with the non-fermentable substrate. On admitting air, the level of dissolved $O_2$ showed a marked overshoot (i.e. the respiratory system was not initially working at full capacity) and only after about 20 min was the quasi-steady-state level of respiration attained [$Q_{O_2} = 46 \mu M-O_2$ min$^{-1}$ ($10^8$ organisms)$^{-1}$]. In this state irregular fluctuations in the level of dissolved $O_2$ were observed. Similar changes were observed in a stationary-phase culture of organisms which had been grown with glucose (Fig. 1d). After 25 min anaerobiosis, the anaerobic-aerobic transition again showed the overshoot in level of dissolved $O_2$, and the attainment of the steady-state level of respiration proceeded through a phase of highly-damped respiratory oscillations (period about 5 min) which lasted for 50 min. The effects of varying periods of anaerobiosis on organisms growing in the presence of glucose were also studied. Switching from air to $N_2$ for 1 min resulted in a transient decrease in the level of dissolved $O_2$ from 165 to $82 \mu M-O_2$, and within 15 min the unperturbed steady-state level of dissolved $O_2$ was re-established. Switching to $N_2$ for 2 min was just adequate to decrease dissolved $O_2$ to an undetectable level; in this case, restoration of aerobiosis gave an oscillation of respiration but no overshoot in dissolved $O_2$ tension. After $N_2$ had replaced air in the gas phase for 10 min, anaerobiosis lasted for 7 min, and the anaerobic-aerobic transition showed first the overshoot and then the oscillation of dissolved $O_2$. Both the overshoot and the oscillatory phases were also evident under similar conditions in the presence of $3 \mu M$-carbonyl cyanide $m$-chlorophenylhydrazone, an uncoupler of mitochondrial energy conservation.

Effects of centrifugation, cold shock and starvation on respiration of glucose-grown organisms

A culture of glucose-grown organisms which had attained the stationary phase was centrifuged at 30°C, immediately resuspended and transferred to the reaction vessel of the open system. The attainment of the steady-state level of respiration under an atmosphere of air was essentially monotonic, although over the first 30 min a small-amplitude oscillation (period about 5 min) was detectable at high amplification. Recovery from 5 or 30 min aerobic exposure to 0°C of cultures growing in the presence of glucose resulted in little detectable perturbation, even at high amplification. Starvation of glucose-grown cells for 3-5 h followed by re-inoculation into conditioned growth medium also caused little disturbance of respiratory activity.

DISCUSSION

Provided that anaerobiosis is avoided, cultures of C. utilis can be centrifuged, exposed to 0°C or starved of nutrients with little or no lasting signs of disturbance to respiratory metabolism after return to optimal growth conditions. It should be stressed, however, that the method of measurement employed here, although one which presents a continuous readout, is insensitive to fluctuations which may occur more rapidly than the rather slow response time (t4 about 2 min). However, this time constant is adequate for the study of many fluctuations typical of the metabolic time domain.

A much more pronounced perturbation of respiration can be demonstrated after periods of anaerobiosis in cultures grown on glucose. These responses show a time-dependent variation; thus, whereas a period of anaerobiosis of 2 min gave rise to respiratory oscillation
on restoration of aerobiosis, longer periods without O₂ gave, in addition, an overshoot in dissolved O₂ on switching back to a gas phase of air. Similar overshoots have been observed with several organisms, and the phenomenon has been ascribed to a retardation of respiration at the dehydrogenase end of the respiratory chain by an uncharacterized control mechanism (H. Degn, personal communication). It is likely that the respiratory oscillations arise from interaction between mitochondrial products and early allosteric control sites of the glycolytic or pentose phosphate pathways; organisms growing with glycerol show much less of a tendency to the oscillatory state.

These experiments suggest that perturbation of respiratory metabolism, at least on the time scale of hours, need not be a serious problem as long as anaerobiosis (particularly in cultures containing glucose) is avoided. Thus, in the study of the development of respiration in organisms growing in synchronous cultures, the effects of perturbation (intentional or unintentional) can be distinguished from cell cycle events as follows: (i) discontinuities produced by perturbation typically have periods of the order of minutes, whereas those involving cell cycle events have longer periods (of the order of hours); (ii) perturbation often results in highly damped oscillations, whereas cell cycle-dependent oscillations of respiration are attenuated only as synchrony decays.

We are indebted to Dr H. Degn for his invaluable advice and to Mr N. Williams for construction of the open O₂ electrode system.

REFERENCES