Glycolysis and respiration in yeasts

The Pasteur effect studied by mass spectrometry

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1. Simultaneous and continuous measurements of changes in CO_2 and O_2 concentrations in glucose-metabolizing yeast suspensions by mass spectrometry enabled a study of the Pasteur effect (aerobic inhibition of glycolysis) in *Saccharomyces uvarum* and *Schizosaccharomyces pombe*. 2. A different control mechanism operates in *Candida utilis* to give a damped oscillation after the anaerobic–aerobic transition. 3. The apparent K_m values for respiration of the three yeasts were in the range $1.3\text{--}1.8\ \mu\text{M-O}_2$. 4. The apparent K_m values for O_2 of the Pasteur effect were 5 and $13\ \mu\text{M}$ for catabolite-repressed and derepressed *S. uvarum* respectively and $7\ \mu\text{M}$ for *Sch. pombe*. 5. These results are discussed with respect to currently accepted mechanisms for the control of glycolysis.

The inhibition of carbohydrate utilization by O_2 has been observed in many cell-types (Krebs, 1972), and since the original discovery of this effect by Pasteur (1861) different mechanisms have been proposed. Energy demand in many yeasts may be satisfied either by highly efficient mitochondrial energy conservation, or by the much less efficient glycolytic generation of ATP. Transition from the anaerobic to the aerobic state leads to a suppression of glycolytic flux, observable as a decreased rate of CO_2 evolution; the more efficient ATP-generating system suppresses the less efficient one. The effect is reversible, so that an increased rate of production of CO_2 is observed going from aerobiosis to the anaerobic state. The Pasteur effect in yeast involves the integration of a series of feedback mechanisms (Sols, 1967), including allosteric activation of NAD^+ -dependent isocitrate dehydrogenase by AMP (Hathaway & Atkinson, 1963), inactivation of phosphofructokinase by ATP and citrate (or activation by AMP or P_i) (Ramaiah *et al.*, 1964; Salas *et al.*, 1965), and inactivation of glucose transport (Sols, 1967).

Despite an extensive literature, there is just one previous report of the O_2 -concentration dependency of the Pasteur effect (Laser, 1937). In the present

Abbreviation used: CCCP, *m*-chlorocarbonylcyanide phenylhydrazone.

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work we have taken advantage of a novel method to do this; simultaneous continuous measurements of O_2 and CO_2 concentrations in a suspension of yeasts using a quadrupole mass spectrometer with a membrane-covered inlet. We show that the Pasteur effect is more easily seen in catabolite-derepressed (but highly fermentative) yeasts, e.g. *Saccharomyces uvarum* and *Schizosaccharomyces pombe*, than in the more aerobic species *Candida utilis*, and also that the apparent K_m for O_2 of the Pasteur effect is higher than that for respiration.

Experimental

Maintenance, growth and harvesting of the organisms

C. utilis N.C.Y.C. 193 was maintained and grown on defined medium containing 1% (w/v) glucose as described previously (Kader & Lloyd, 1979). *S. uvarum* (formerly *S. carlsbergensis*) N.C.Y.C. 74S and *Sch. pombe* 972h[−] were maintained on 10% (w/v) glucose/malt extract/agar slopes and grown on defined medium containing 1% (w/v) glucose, as described by Mitchison (1970), but with 0.5% (w/v) $(\text{NH}_4)_2\text{SO}_4$ as nitrogen source. All three yeasts were grown at 30°C with rotary shaking (100 rev./min) in 200 ml cultures in 500 ml conical flasks. Organisms were counted in a Thoma haemocytometer slide (Hawkesley, Lancing, Sussex, U.K.) after suitable dilution. They were harvested by centrifugation at 2000 g for 2 min at 4°C, washed twice in 20 vol. of

15 mM-NaCl and finally resuspended in 5 vol. of this solution.

Measurements of O_2 and CO_2 by mass spectrometry

Concentrations of O_2 and CO_2 were measured in yeast suspension (4.5 ml in 10 mM-sodium citrate buffer, pH 4.0); other buffers gave similar results. Measurements were in a thermostatted open reaction vessel (30°C) of 7 ml total volume using a quadrupole mass spectrometer type Q8 from VG Micromass Ltd., Winsford, Cheshire, U.K., fitted with a turbo-pump type TPH100 from A. Pfeiffer Vacuum-technik (D-6334 Asslar 1, Germany). Stirring was by a cross-shaped stirrer fixed to a stainless-steel shaft, entering through a hole in the lid, and driven by a motor at fixed speed (1200 rev./min). The membrane-covered inlet to the mass spectrometer was sealed into a port lying below the surface of the vortex. The membrane (12 μ m Teflon) served both to separate the cell suspension from the vacuum and as a gasket between reaction vessel and mass spectrometer inlet. The response time of the system was limited by the rate of stirring; the half-times for equilibrium with O_2 and CO_2 were slightly below 2 min, corresponding to transfer constants of $7.2 \times 10^{-3} s^{-1}$ and $5.8 \times 10^{-3} s^{-1}$ respectively. The m/z numbers 32 and 44 were continuously scanned (O_2 and CO_2 respectively); the scanning cycle was 0.3 s. The surface of the reaction mixture was in contact with an O_2/N_2 gas mixture whose composition was controlled by a calibrated digital gas mixer (Lundsgaard & Degn, 1973; Degn & Lundsgaard, 1980). Steady state rates of O_2 con-

sumption and CO_2 production were calculated from steady state concentrations (Degn *et al.*, 1980). The concentration of O_2 in the buffer in equilibrium with atmospheric air was assumed to be 240 μ M. CO_2 measurement was calibrated by the injection of μ l amounts of 100 mM- $NaHCO_3$ into the buffer in the reaction vessel. Other buffers gave similar results to those with citrate buffer.

Results

Changes in levels of dissolved O_2 and CO_2 in a non-proliferating suspension of glucose-repressed *S. uvarum* (Fig. 1) indicated that addition of 11 mM-glucose had no effect on the respiration rate. When the suspension was made anaerobic, dissolved CO_2 was increased by about 40%; the Pasteur effect was also observed as an inhibition of glycolytically generated CO_2 , when O_2 was readmitted. Addition of 6.6 μ M-CCCP, an uncoupler of mitochondrial energy conservation, stimulated CO_2 production anaerobically (Galeotti *et al.*, 1968) and abolished the Pasteur effect; transition to aerobiosis was then accompanied by further increase in CO_2 evolution.

Continuous and simultaneous measurements of changes in O_2 and CO_2 concentrations in *Sch. pombe* gave similar results (Fig. 2), except that the attainment of anaerobiosis was accompanied by a transient decrease in CO_2 production: a delay of about 2 min preceded the enhanced CO_2 evolution characteristic of the Pasteur effect. Again, CCCP added under anaerobic conditions stimulated CO_2 evolution and abolished the Pasteur effect.

Fig. 1. O_2 and CO_2 concentrations in a washed cell suspension of *S. uvarum* in an open system, measured by mass spectrometry

Cell concentration in the culture was 1.6×10^8 organisms $\cdot$ ml $^{-1}$ and in the reaction vessel 1.4×10^8 organisms $\cdot$ ml $^{-1}$. At the arrows the composition of the gas stream entering the open system was altered or additions were made as shown. ---, Dissolved O_2 concentration; —, dissolved CO_2 concentration.

Fig. 2. O_2 and CO_2 concentrations in a washed cell suspension of *Sch. pombe* in an open system, measured by mass spectrometry

Cell concentration in the culture was 7×10^7 organisms $\cdot$ ml $^{-1}$ and in the reaction vessel 1.4×10^8 organisms $\cdot$ ml $^{-1}$. At the arrows the composition of the gas stream entering the open system was altered or additions were made as shown. ---, Dissolved O_2 concentration; —, dissolved CO_2 concentration.

Fig. 3. O_2 and CO_2 concentrations in a washed cell suspension of *C. utilis* in an open system, measured by mass spectrometry

Cell concentration in the culture was 2×10^8 organisms $\cdot$ ml $^{-1}$, and in the reaction vessel 5×10^7 organisms $\cdot$ ml $^{-1}$. At the arrows the composition of the gas stream entering the open system was altered or additions were made as shown. ---, Dissolved O_2 concentration; —, dissolved CO_2 concentration.

For *C. utilis*, quite different results were obtained (Fig. 3); CO_2 evolution anaerobically was always less than that observed under aerobic conditions.

Restoration of aerobiosis was characterized by an overshoot in O_2 concentration followed by a damped oscillation. Addition of 0.5 mM-potassium phosphate

Fig. 4. Reciprocal plots of respiration rate against $[O_2]$ for washed cell suspensions of yeasts (a) *S. uvarum* with 3.2×10^8 cells $\cdot$ ml $^{-1}$ (●) and 6.8×10^8 cells $\cdot$ ml $^{-1}$ (○) harvested from a culture containing 1.6×10^8 cells $\cdot$ ml $^{-1}$. (b) *Sch. pombe* with 3.5×10^7 cells $\cdot$ ml $^{-1}$ (●) and 1.4×10^8 cells $\cdot$ ml $^{-1}$ (○) harvested from a culture containing 7×10^7 cells $\cdot$ ml $^{-1}$. (c) *C. utilis* with 1.8×10^7 cells $\cdot$ ml $^{-1}$ (●) and 2×10^7 cells $\cdot$ ml $^{-1}$ (○) harvested from a culture containing 2×10^8 cells $\cdot$ ml $^{-1}$.

did not measurably effect the rate of respiration, but $3 \mu\text{M}$ -CCCP gave a two-fold stimulation of CO_2 production.

Steady state rates of O_2 consumption and CO_2 production were measured at different O_2 concentrations by setting the O_2 content of the gas phase at different fixed values and observing the resulting steady state concentrations of O_2 and CO_2 . Lineweaver-Burk plots for respiration rates, obtained from steady state O_2 concentrations (Fig. 4), indicate K_m values of 1.8, 1.7 and $1.3 \mu\text{M}$ - O_2 respectively for *S. uvarum*, *Sch. pombe* and *C. utilis*. In *S. uvarum*, growth with glucose gives a diauxic population increase; glucose is first converted by 'aerobic fermentation' to ethanol and then catabolite derepression results in organisms capable of ethanol oxidation (for review see Lloyd, 1974). The Pasteur effect was slight in glucose-repressed organisms (Fig. 5); at $19 \mu\text{M}$ - O_2 CO_2 evolution was decreased by only 14%. The rate of O_2 consumption subtracted from that of CO_2 evolution gives the glycolytic CO_2 production rate.

Reciprocals of glycolytic CO_2 evolution rates were plotted as a function of O_2 concentrations. Assuming non-competitive inhibition of glycolysis by O_2 , a value of $K_i = 13 \mu\text{M}$ - O_2 was obtained. With glucose-derepressed *S. uvarum*, the Pasteur effect was much more marked; 30% inhibition of CO_2 evolution was observed at $20 \mu\text{M}$ - O_2 , and the corresponding value for K_i for glycolysis was

$5 \mu\text{M}$ - O_2 . *Sch. pombe* behaved like glucose-derepressed *S. uvarum* (Fig. 6), and the K_i for the inhibition of glycolysis was $7 \mu\text{M}$ - O_2 .

Discussion

S. uvarum is a highly fermentative yeast (Moss *et al.*, 1971) that shows catabolite repression of the mitochondrial system when grown aerobically with glucose at concentrations greater than about $100 \mu\text{M}$ (Cartledge & Lloyd, 1972; Wales *et al.*, 1980). Catabolite-derepressed organisms show a pronounced Pasteur effect which is abolished by an uncoupler of mitochondrial energy conservation. *Sch. pombe* is another highly fermentative catabolite-repressible yeast (De Deken, 1966) which differs from *S. uvarum* in that it is capable of only limited anaerobic growth (Heslot *et al.*, 1970). Again the rate of glycolytic metabolism in catabolite-derepressed cells as measured by CO_2 production rates is determined by the presence of O_2 . The highly aerophilic *C. utilis* grows only very slowly under anaerobic conditions, and shows maximum mitochondrial development when grown at O_2 concentrations less than $0.1 \mu\text{M}$ (Moss *et al.*, 1969). As much as 50% of glucose oxidation proceeds by the hexose monophosphate pathway in this organism (Blumenthal *et al.*, 1954; Dawson *et al.*, 1976). Under the conditions employed here the Pasteur effect was not observed; a different control pheno-

Fig. 5. Rates of O_2 consumption, total CO_2 production and glycolytic CO_2 production as a function of O_2 concentrations in washed cell suspensions of *S. uvarum*
(a) Respiration rates (●) and total CO_2 production (○) at 6.4×10^8 cells $\cdot ml^{-1}$ (broken lines) and 4.8×10^8 cells $\cdot ml^{-1}$ (solid lines). (b) Reciprocal rates of glycolytic CO_2 production plotted against O_2 concentration. The intercept at the abscissa determines the inhibitor constant K_i .

Fig. 6. Rates of O_2 consumption, total CO_2 production and glycolytic CO_2 production as a function of O_2 concentration in washed cell suspensions of *Sch. pombe*
(a) Respiration rates (●) and total CO_2 production (○) at 1.6×10^8 cells $\cdot ml^{-1}$ (broken lines) and 4×10^7 cells $\cdot ml^{-1}$ (solid lines). (b) Reciprocal rates of glycolytic CO_2 production plotted against O_2 concentration. The intercept at the abscissa determines the inhibitor constant K_i .

menon was evident. Thus anaerobiosis produced a transient inactivation of the respiratory system, which was observed as an overshoot in dissolved O_2

when aerobiosis was restored, followed by an oscillation which became attenuated over the course of about 1 h (Lloyd & Ball, 1979). The control

mechanisms underlying this respiratory overshoot have not been determined, although it is of widespread occurrence in obligately-aerobic lower eukaryotes (H. Degn, unpublished work).

The respiration of all three yeasts showed high apparent K_m values for O_2 , within the range 1–2 μM - O_2 . In both *S. uvarum* and *Sch. pombe*, the Pasteur effect was characterized by a much lower affinity for O_2 (K_m values within the range 6–14 μM , depending on the organism and the stage of growth). It is difficult to account for these observations in terms of the accepted explanations for the Pasteur effect. If changes in pool sizes of intermediates (adenine nucleotides, P_i , citrate) or in redox states of intracellular compartments produced as a consequence of respiratory activity are entirely responsible for the Pasteur effect, then it would be anticipated that the affinities for O_2 of both systems would be similar. Further work is suggested by this paradox.

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