

Glycolysis and Respiration in Yeasts: the Effect of Ammonium Ions Studied by Mass Spectrometry

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Addition of NH_4^+ in the presence of glucose to washed suspensions of *Saccharomyces uvarum*, *Schizosaccharomyces pombe* or *Candida utilis* greatly increased glycolytic CO_2 production and slightly stimulated respiration. In all three organisms the ammonium ion effect was distinguishable from the effect of an uncoupler of aerobic energy conservation (carbonyl cyanide *m*-chlorophenylhydrazone; CCCP). The Pasteur effect (aerobic inhibition of glycolysis) in the fermentative yeasts also proceeded independently of the ammonium ion effect. Possible control mechanisms are discussed.

INTRODUCTION

The Pasteur effect (inhibition by O_2 of the utilization of carbohydrates: Krebs, 1972) involves the integration of several feedback mechanisms; especially important are the allosteric effectors of phosphofructokinase. This enzyme is inhibited by ATP and citrate, and activated by AMP, P_i , NH_4^+ and K^+ (Ramaiah *et al.*, 1964; Salas *et al.*, 1965; Mavis & Stellwagen, 1970). In this report we show that the addition of NH_4^+ to washed non-proliferating cell suspensions of yeasts stimulates glycolysis; this 'ammonium ion effect' is quite distinct from the stimulatory effect of CCCP and from the Pasteur effect.

METHODS

Organisms and culture conditions. *Candida utilis* NCYC 193 was maintained and grown on defined medium containing 1% (w/v) glucose as described previously (Kader & Lloyd, 1979). *Saccharomyces uvarum* (formerly *S. carlsbergensis*) NCYC 745 and *Schizosaccharomyces pombe* 972 h⁻ were maintained on 10% glucose/malt extract/agar slopes and grown on defined medium containing 1% glucose as described by Mitchison (1970), but with 0.5% $(\text{NH}_4)_2\text{SO}_4$ as nitrogen source. All three yeasts were grown at 30 °C with rotary shaking (100 r.p.m.) as 200 ml cultures in 500 ml conical flasks. Organisms were counted in a Thoma haemocytometer slide (Hawkesly, 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.

Measurement of O_2 and CO_2 by mass spectrometry. Concentrations of O_2 and CO_2 were measured in yeast suspensions (4.5 ml in 10 mM-sodium citrate buffer, pH 4.0) in a thermostated open reaction vessel of 7 ml total volume using a quadrupole mass spectrometer type Q8 (VG Micromass, Winsford, Cheshire, U.K.) fitted with a turbopump type TPN 100 (A. Pfeiffer Vacuum Technic, D-6334 Asslar, F.R.G.). Similar results were obtained with other buffers, suggesting that the cells are not permeable to citrate. Details of the equipment have been described previously (Lloyd *et al.*, 1981). Mass per charge numbers 32 and 44 were scanned for O_2 and CO_2 respectively; half times for equilibration correspond to transfer constants of $7.2 \times 10^{-3} \text{ s}^{-1}$ and $5.8 \times 10^{-3} \text{ s}^{-1}$ respectively and the scanning cycle was 0.3 s. The gas phase was controlled by a calibrated digital gas mixer (Lundsgaard & Degn, 1973). Steady-state rates of O_2 consumption and CO_2 evolution 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 μM . CO_2 measurement was calibrated by the injection of small amounts of 100 mM- NaHCO_3 into the buffer in the reaction vessel.

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Abbreviation: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

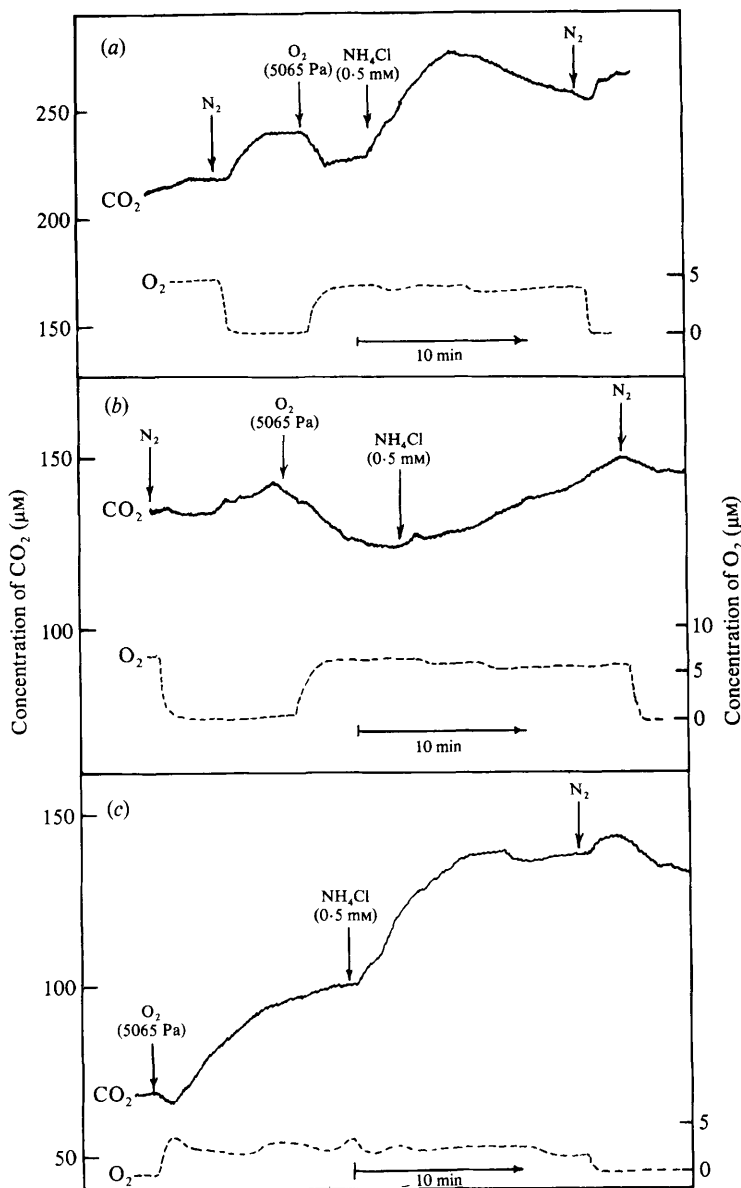


Fig. 1. O₂ and CO₂ concentrations in washed cell suspensions of yeast: effect of ammonium ions. (a) *Saccharomyces uvarum*, (b) *Schizosaccharomyces pombe*, (c) *Candida utilis*. Cell concentrations in the cultures at harvesting were 2×10^8 , 8×10^7 , 2×10^8 and in the reaction vessel 1.6×10^8 , 8×10^7 , 5×10^7 in (a), (b) and (c) respectively; 11 mM-glucose was present throughout. At arrows additions were made or the composition of the gas stream entering the open system was altered as indicated. ----, O₂ (mass 32); —, CO₂ (mass 44).

RESULTS AND DISCUSSION

Figure 1 demonstrates the ammonium ion effect in the three yeasts. In the presence of 11 mM-glucose, switching the gas phase from 5% O₂ to N₂ stimulated CO₂ evolution, and the effect (Pasteur effect) was reversible. In *Saccharomyces uvarum* (Fig. 1a) addition of 0.5 mM-NH₄Cl (final concn) to the aerobic suspension gave a more pronounced stimulation of CO₂ evolution, which reached a maximum after about 5 min. Respiration was also slightly stimulated (<5%).

Before CO₂ concentration had returned to its initial value the Pasteur effect was still demonstrable as a stimulation of CO₂ production on making the suspension anaerobic. Addition of NH₄Cl anaerobically gave a burst of CO₂ evolution (not shown). Stimulation of glycolysis and respiration by an uncoupler of aerobic energy conservation (CCCP) was observed before or after addition of NH₄Cl (not shown). The ammonium ion effect was also observed in *Schizosaccharomyces pombe* (Fig. 1 b), and in *Candida utilis* (Fig. 1 c), although the Pasteur effect was not evident in the latter. Control experiments indicated the specificity of stimulation by NH₄⁺; thus addition of 0.5 mM-NaCl produced no response, whereas 0.5 mM-NH₄OH in potassium phosphate buffer gave similar effects to those observed for NH₄Cl.

A possible explanation for the ammonium ion effect lies in the activation of phosphofructokinase (Mavis & Stellwagen, 1970); consumption of ammonium ions to produce amino acids and proteins may explain the transient nature of the effect. That the effects of anaerobiosis, uncoupler and ammonium ions are additive suggests that maximal activation of phosphofructokinase requires a combination of circumstances. Increased respiration in the presence of NH₄⁺ may reflect increased energy demand for transport or biosynthetic reactions. These observations may be related to those of Devine & Slaughter (1980) who reported that ethanol production was accelerated in media containing high concentrations of ammonium ions.

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