Oxygen Affinities of the Hydrogenosome-containing Protozoa

*Tritrichomonas foetus* and *Dasytricha ruminantium*, and Two Aerobic Protozoa, Determined by Bacterial Bioluminescence

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Oxygen-dependent bioluminescence of *Photobacterium* (*Vibrio*) *fischeri* was used to measure oxygen affinities of four protozoa. The aerobic organisms *Acanthamoeba castellanii* and *Tetrahymena pyriformis* showed apparent $K_m$ values for $O_2$ of 0.42 and 2.43 $\mu$M respectively. The aerotolerant anaerobe *Tritrichomonas foetus*, and the more strictly anaerobic rumen ciliate *Dasytricha ruminantium*, both of which have hydrogenosomes, respired with apparent $K_m$ values of 1.08 and 1.70 $\mu$M-$O_2$. We conclude that mitochondrial respiration is not the only process conferring on organisms a high affinity for $O_2$.

INTRODUCTION

In the presence of air, some trichomonads show an active respiration, which is stable over several hours (Suzuoki & Suzuoki, 1951; Doran, 1959; Lloyd et al., 1982a, b). These aerotolerant anaerobes, typified by the cattle parasite, *Tritrichomonas foetus*, have no cytochromes (Lloyd et al., 1979a), no mitochondrial ATPase (Lloyd et al., 1979b), and no organelle bearing any morphological resemblance to mitochondria (Daniel et al., 1971; Brugerolle, 1972; Honigberg, 1978). The major organelle of their carbon metabolism is the hydrogenosome, which produces $H_2$ under anaerobic conditions (Lindmark & Müller, 1973) and acts as a respiratory organelle in the presence of $O_2$ (Čerkašov et al., 1978; Müller & Lindmark, 1978). An intensely active NADH oxidase localized in the cytosol is also present (Čerkašovová & Čerkašov, 1974). Although the growth of *T. foetus* is inhibited by $O_2$, slow growth does occur when the $O_2$ tension is kept at about 1% (v/v) (Mack & Müller, 1978).

The rumen ciliate *Dasytricha ruminantium* is usually considered to be a strict anaerobe, and produces $H_2$, as well as $CO_2$, acetate, butyrate and lactate by the fermentation of sugars (Williams & Harfoot, 1976). This holotrich remains viable only for a few minutes in the presence of air, but it respires and can survive for several hours under 1% (v/v) $O_2$ (N. Yarlett, A. G. Williams & D. Lloyd, unpublished observations). The presence of hydrogenosomes in *D. ruminantium* (Yarlett et al., 1981 a, b) suggests functional analogy with the trichomonad system; in both systems the identity of the terminal oxidases responsible for $O_2$ consumption remains to be elucidated. In this paper we show that the $O_2$ affinities of the hydrogenosome-containing protozoa *T. foetus* and *D. ruminantium* are similar to those of aerobic protozoa possessing well-developed mitochondria.

METHODS

*Growth and isolation of the organisms.* *Tritrichomonas foetus* KV<sub>1</sub> (obtained from Dr D. Müller) was cultured in Diamond’s liquid TYM medium without agar, supplemented with 10% (v/v) heat-inactivated horse serum (Diamond, 1957). Stock cultures were subcultured daily and used to inoculate 3.0 ml medium in tubes closed with

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organisms ml⁻¹. Harvesting was done by centrifuging at 1000 rev. min⁻¹ at room temperature for 2 min in an MSE bench centrifuge. Organisms were washed twice in 0.9

Acanthamoeba castellani (Neff strain) was grown axenically in shaken cultures at 30 °C as described previously (Lloyd et al., 1977). Harvesting and washing was as for T. foetus. Tetrahymena pyriformis ST was maintained and grown at 30 °C with shaking as described previously (Williams et al., 1980). Photobacterium (Vibrio) fischeri (strain MJ-1) was obtained from Dr E. G. Ruby (The Biological Laboratories, Harvard University, Cambridge, Mass., U.S.A.). Cultures (20 ml in 50 ml conical flasks) were grown with shaking for 18 h at 25 °C on Difco Photobacterium broth. The organism was maintained at 4 °C on slopes of this medium solidified with 2% (w/v) agar. In some cultures poor light emission necessitated selection of organisms from bright colonies for subsequent subculture. After harvesting by centrifugation at 10000 g for 20 min, the organisms were suspended in a buffer containing 3% (w/v) NaCl/0.1 m potassium phosphate (pH 7.0), re-centrifuged and finally resuspended in 20 ml of this buffer. Organisms were starved for 2 d in this buffer at 4 °C before use to decrease both the endogenous O₂ consumption rates and the 'residual glow' observed under argon (Schindler, 1964).

**Determination of O₂ affinities.** Steady-state respiration rates were determined as a function of O₂ concentration by measurement of the intensity of bioluminescence from a stirred suspension of _P. fischeri_ mixed with the protozoan under study. A cylindrical reaction vessel, constructed in stainless steel, with a 5:0 ml working volume and a glass bottom, was used. Stirring was by a cross-shaped stirrer fixed to a stainless steel shaft entering through a hole in the lid and driven by a synchronous motor at 1500 rev. min⁻¹. Gas entry and exit ports were via the lid (Degn et al., 1980). The vessel was jacketed and supplied with water from a thermostatically controlled water heater. Bioluminescence intensity was measured using an end-on photomultiplier (EMI-9524B) which combines high sensitivity (1000 amp lm⁻¹ at 1200 V) with low dark current (5 nA at 1200 V), Dynode potentials were obtained from a well stabilized supply (Hewlett-Packard, Harrison 6515A DN power supply). The detector circuit was that of Mayer et al. (1969). Aluminium tubes provided light-tight enclosure for the photomultiplier. Gases used were high purity argon (<3 p.p.m. O₂) and standard gas mixtures consisting of 1% or 0.05% (v/v) O₂ in N₂ (Air Products, Cardiff). Low O₂ tensions were obtained using a digital gas mixer (Lundsgaard & Degn, 1973) which provides accurate dilutions in 5% steps when supplied with argon and an O₂/N₂ mixture at pressures in a direct ratio to the dynamic viscosities of argon/N₂ (1:27-1:00). Absolute gaseous O₂ concentrations were calculated from percentage dilutions of stock gas mixtures. Humidified gas mixtures were passed over the stirred liquid vortex in the reaction vessel.

A calibration curve for light emission as a function of O₂ concentration in the liquid [0.9% (w/v) NaCl] was obtained immediately before each O₂ affinity determination. A suspension of _P. fischeri_ (5 ml; _A_mₐₓ_0.05) was stirred under argon (flow rate 200 ml min⁻¹) until all measurable O₂ was purged from the system (about 5 min). Calibration with standard mixtures then took about 10 min. Continuous recording of light intensity enabled equilibration to a new steady state after each change of gas composition to be determined. Linear response was obtained over the range 30 nM to 2 μM-O₂; residual light intensity was <0-2% of maximum emission; half-maximal luminescence was observed at 9 μM-O₂. For O₂ affinity determinations, the procedure and _P. fischeri_ suspensions were identical to those employed in the calibration procedures, except that about 10⁵ protozoa ml⁻¹ were added to the reaction mixture. The O₂ concentrations of air-saturated 0.9% (w/v) NaCl were taken as 286 μM at 19 °C, 269 μM at 22.5 °C and 258 μM at 25 °C; light emission decayed too rapidly at higher temperatures for the method to be useful. At the cell densities employed, oxygen consumption by _P. fischeri_ suspensions was negligible. Respiration rates of protozoa (Vₑ) were calculated from the relationship _Vₑ = k (Tᵣᵣ - Tₑ),_ where _Vₑ_ is the respiration rate, _k_ is the oxygen transfer constant, _Tᵣᵣ_ is the concentration of O₂ in a liquid phase containing no organisms when equilibrated with the gas phase, and _Tₑ_ is the concentration of O₂ in the liquid. The value of _k_ for the system employed was 0·35 min⁻¹ at 20 °C.

**RESULTS AND DISCUSSION**

Figure 1 shows typical responses obtained by exposing a mixed suspension of _P. fischeri_ and _D. ruminantium_ to gas mixtures of different O₂ tensions, together with bioluminescence of the bacterial suspension in the absence of the protozoan. Lineweaver–Burk plots of the dependence of endogenous respiration rates on O₂ tension in the liquid gave straight-line relationships for all four protozoa. Values obtained for the apparent _Kₘ_ for O₂ are shown in Table 1. In the case of _T. pyriformis_, as previously described (Lloyd et al., 1980), some
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Fig. 1. Light emission at 21 °C by Photobacterium fischeri exposed to gas mixtures of various compositions in (a) the presence and (b) the absence of D. ruminantium. Measurements were made in the open system on organisms suspended in 0.9 % (w/v) NaCl (10^5 organisms ml^-1). Emission intensity in the absence of protozoa gave a calibration for concentrations of dissolved O_2 (T_L); this was used to measure values for T_L at a series of fixed values of O_2 in the gas phase (T_c) in (a), and hence the respiration rate [from V_r = k (T_c - T_L)].

Table 1. Values of apparent K_m for O_2 for protozoa, determined with P. fischeri

<table>
<thead>
<tr>
<th>Organism</th>
<th>Temperature (°C)</th>
<th>Apparent K_m for O_2 (µM)</th>
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<tbody>
<tr>
<td>Tritrichomonas foetus KV_1</td>
<td>19</td>
<td>1.08 ± 0.18 (4)</td>
</tr>
<tr>
<td>Dasytricha ruminantium Schuberg</td>
<td>25</td>
<td>1.70 ± 0.30 (4)</td>
</tr>
<tr>
<td>Acanthamoeba castellanii Neff strain</td>
<td>20</td>
<td>0.42 ± 0.05 (5)</td>
</tr>
<tr>
<td>Tetrahymena pyriformis ST</td>
<td>22.5</td>
<td>2.43 ± 0.08 (4)</td>
</tr>
</tbody>
</table>

adaptation to low O_2 occurred, i.e. plots produced by decreasing O_2 with time were linear, whereas those produced by increasing O_2 were convex downwards. The K_m values for A. castellanii and T. pyriformis are in good agreement with those obtained using an O_2 electrode method (Lloyd et al., 1979c; 1980) and a membrane-covered photobacterium probe (Lloyd et al., 1981). The aerotolerant anaerobe T. foetus and the more strictly anaerobic D. ruminantium, which do not possess mitochondria, are equally as capable as the aerobic protozoa of utilizing O_2 at low tensions, as revealed by their high apparent K_m values for O_2. Whether the respiratory systems of anaerobic protozoa serve to minimize intracellular O_2 tensions as a protection for O_2-sensitive systems (Morris, 1979) remains to be investigated. A number of iron–sulphur centres and a flavoprotein with redox potential midpoints in the range -305 to -115 mV have been revealed in hydrogenosomes from T. foetus (Ohnishi et al., 1980), and the cytosol of this organism has a highly active NADH oxidase, which also gives iron–sulphur and flavin radical signals in e.p.r. spectra (Lloyd et al., 1982b). However, the identities of the terminal oxidases responsible for the high O_2 affinity of this organism and of D. ruminantium remain obscure. Although these O_2 affinities are of the same order as those of some aerobic protozoa, the oxidases responsible are evidently quite different from those found in mitochondria.

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