THE ACTION SPECTRUM FOR RELIEF OF CO-INHIBITION IN GASTRIC CELLS FROM RAJA ERINACEA: EVIDENCE FOR AN ALTERNATIVE CYTOCHROME OXIDASE

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Acid secretion by chambered skate gastric mucosa drops to near zero in the absence of oxygen. The terminal oxidase inhibitor carbon monoxide (CO) at a high CO/O_2 ratio does not fully block acid secretion (MDIBL Bull. 26:43-46, 1986), nor does it greatly inhibit oxygen uptake by cells from this tissue (MDIBL Bull. 28:14-15, 1989). This small inhibition is partially reversible by light; therefore, the spectrum for the relief of inhibition as a function of wavelength should determine the absorption spectrum of the CO-complex of the functional terminal oxidase. In particular, it should be possible to determine whether the oxidase is the conventional cytochrome oxidase (a_3) or some other pigment. Previous studies (MDIBL Bull. 30:25-26, 1991) suggested a different oxidase, but the spectra were not definitive. Improvements to the apparatus were required for convincing spectra.

Cells were removed from the gastric mucosa by pronase digestion as previously described (ibid.), digesting for 3 hours in 75 ml of Forster's solution containing (mM) NaCl, 200; $NaHCO_3$, 30; KCl, 10; $CaCl_2$, 5; $MgCl_2$, 2; Na_2HPO_4, 1; glucose, 25; urea, 350; plus 140 units/ml pronase (Streptomyces griseus) at 30°C which were washed once in Forster's solution, and stored at 5°C until used. Normally, a preparation made one morning could be used that afternoon and the following day.

Commercial baker's yeast was used as a cytochrome a_3-containing control, using the above solution, with NaCl reduced to 110 mM and no urea, glucose or pronase. A suspension of 10 mg/ml proved satisfactory, with 1% EtOH as respira- substrate.

The photochemical action spectrum (PCAS) apparatus has previously been described (ibid.). Briefly, we adjusted the intensity of light at variable wavelengths (VW) until it had the same effect on oxygen uptake (oxygen electrode) as a standard intensity of light at a standard wavelength (SW) of 500 nm, and measured the two intensities. The ratio of these intensities is the relative efficiency of light at the VW to that at the SW. The light-measuring system was modified by the addition of a chopper disk which interrups the light beam once per second, and the use of an analog-to-digital converter and an Apple II computer (Comp. Appl. in Biol. Sci. 4:331-335, 1988) to collect, display, average and record the intensity data. Sixteen peak-peak differences were measured, with the mean taken as the intensity; the relative standard error of this determination was about 0.5% at full intensity. The oxygen electrode output was also collected and displayed by the computer, which allowed signal averaging to minimize noise at high sensitivities. With this method, the Teflon membrane could be removed from the Instech electrode, which gave a more rapid response and allowed the use of 2 μl of cell suspension per run. The resulting noise in the oxygen electrode output corresponded to about 0.1% O_2. The gas mixture around the drop was 5% CO_2, 20% O_2 and 75% CO, and the concentration of cells and the position of the electrode was adjusted to give between 1 and 10% O_2 at the electrode tip when illuminated with light at the 500 nm reference wavelength. For each run, a measurement was made with VW = SW = 500 nm. Instrumental differences may make

this value not equal unity; therefore, the observed values at other VW's were divided by this correction factor to remove this source of error.

Yeast respiration is sensitive to CO, and this suspension was very responsive in the apparatus, giving the spectrum shown as figure 1, top, which is a good representation of the absorption spectrum of the cytochrome oxidase-CO complex, which agrees with information previously available for cytochrome a_3. Skate cells were less responsive to light, with some preparations being completely insensitive, which introduces errors into the determination and results in a noisy spectrum, as shown in figure 1, bottom. The most effective wavelength is in the vicinity of 580 nm in skate, while it is clearly at 590 nm in yeast. The ratio of spectral height at 590 to that at 580 was 0.75 in skate cells, while it was 1.57 in yeast cells.

While another peak is expected near 430 nm, the performance of the apparatus in this wavelength range did not give confidence in the results. Due to the characteristics of the lamp and monochromators, the maximum intensity which could be generated at 400 nm is only 1/10 that available at 500 nm, which seems to be too little for accurate results.

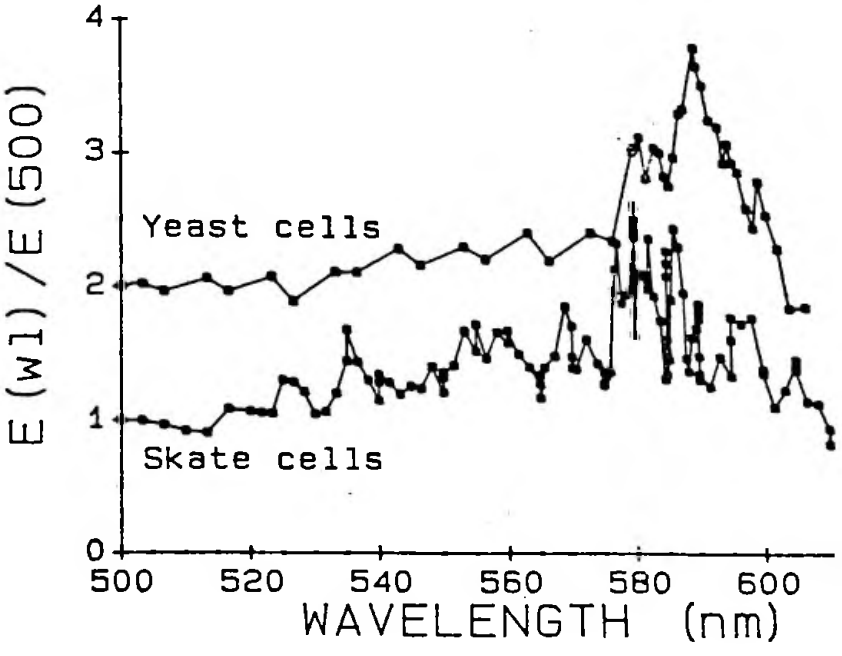

Figure 1:
Action spectra for the relief of CO-inhibition in yeast and skate cells. Data (yeast N=57, skate N=148) smoothed by a 3-point formula, with connecting lines. Yeast spectrum displaced +1 unit.

The ability of this tissue to continue to secrete in the presence of high $CO-O_2$ ratios, the insensitivity of its oxygen uptake to CO, N_3^- and CN^-, the appearance of a reduced cytochrome peak at 590 nm in the presence of N_3^-, and now the identification of a ca. 580 peak of the CO-complex of the functional oxidase all suggest that a component of the oxygen uptake in this tissue is mediated by an oxidase which is not the conventional mitochondrial cytochrome $a+_3$. While this oxidase remains to be isolated and characterized, it may well be localized to the plama membrane and play a role in the secretion of H^+ by this tissue, as previously suggested (Ann. N.Y. Acad. Sci. 574:219, 1989).

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