THE ACTION SPECTRUM FOR RELIEF OF CARBON MONOXIDE INHIBITION OF RESPIRATION IN THE GASTRIC MUCOSA OF RAJA ERINACEA

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The oxygen (O_2) used by tissue respiration interacts with most tissues via cytochrome oxidase, a mitochondrial constituent. Carbon monoxide (CO) competes for O_2 at the binding site, inhibiting respiration by 50% at a CO/O_2 ratio of about 4/1. Light shifts the equilibrium for CO binding, causing dissociation of the oxidase-CO complex and thus relieving inhibition. Only photons which are absorbed can cause photodissociation, so a plot of the efficiency of light of various wavelengths in relieving CO-inhibition must correspond to the absorption spectrum of the CO-terminal oxidase complex. The sharp spectral peaks of the cytochromes facilitate identification from their spectra, with the photochemical action spectrum (PCAS) having the advantage that other light-absorbing compounds do not interfere with obtaining a spectrum of the CO-inhibited pigment.

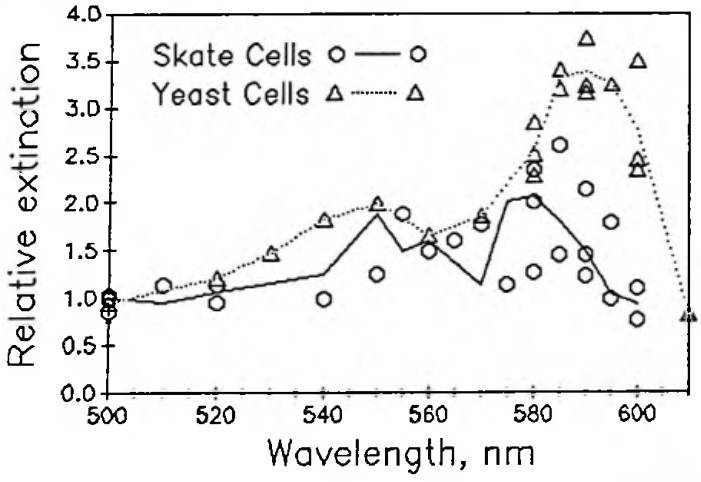
Gastric acid secretion in the frog and skate is relatively insensitive to CO, with high CO/O_2 required to achieve 20-30% inhibition. O_2 uptake by isolated gastric cells is similarly insensitive to CO, but such inhibition as occurs can be partially reversed by white light. Absorption spectra of these tissues reveal a spectral peak at 590 nm which is not suppressed by inhibitors which eliminate the peak at 605 nm due to the conventional cytochrome oxidase. Since other information suggests that the terminal oxidase in this system might not be the conventional cytochrome oxidase, it was of interest to determine the photochemical action spectrum (PCAS) for light reversal of CO-inhibition of O_2 uptake in these cells, to see whether the spectral of the CO-complex peaks occurred at 430 and 590 nm, which would indicate the presence of conventional cytochrome oxidase, or at some other wavelengths indicating a different terminal oxidase.

We used a PCAS apparatus patterned on that of Caster and Chance. A drop of fluid containing respiring cells is suspended on an oxygen electrode in an atmosphere containing both O_2 and CO. Cell respiration removes O_2 from the drop, which is replaced by diffusion from the gas space. For any respiratory rate, a steady state oxygen concentration $[O_2]$ will obtain at the O_2 electrode surface. While the complex drop geometry makes it impossible to calculate the respiratory rate from $[O_2]$, we can use this electrode as a null detector, since an increased respiratory rate decreases $[O_2]$ while decreased respiration increases $[O_2]$. The PCAS apparatus can illuminate the drop with light from either of two monochromators. One monochromator is left at a wavelength of 500 nm and at constant intensity, and serves as the reference illumination. The other monochromator is set to the test wavelength, and the intensity of illumination adjusted until switching from one monochromator to the other produces no change in oxygen concentration in the drop, which indicates that both light beams were having the same effect on the CO-oxidase complex. The intensities of the two beams were then measured with a wavelength-insensitive thermopile detector. The plot of the ratios obtained as a function of the wavelength of the variable source is the action spectrum of the cells, relative to the value at 500 nm.

The cells were removed from the gastric mucosa by pronase digestion as previously described. In brief, this required digestion of the tissue in a pronase-containing solution for 3 hours, followed by recovery of the cells by centrifugation and washing with pronase-free media. The usual yield was about 0.5 ml of a concentrated cell suspension. Since only about 5 microliters was required for the PCAS apparatus chamber, there was an excess of material. It was discovered that the cell suspension would remain active for at least 48 hours at 5°C, so it was not necessary to make a fresh preparation every day. With storage, the cells settled in the tube, resulting in differing cell densities at different depths. Advantage was taken of this to adjust the oxygen uptake in the respiring drop to a value which gave an oxygen concentration in the sensitive range, which was between about 1 and 10% oxygen.

This summer's experience has given us a good indication of the required ratio of CO/O_2 in the gas mixture and the concentration of cells in the drop, as well as some information on the form and placement of the oxygen electrode itself. The PCAS instrument itself is currently in a developmental form, and the experience of this summer suggests a number of improvements. The most important will be improvements in the light-measuring system, since it is clear this system contributes the most error to the spectrum.

The results of the use of this apparatus form an action spectrum such as that shown. In this plot, the circles represent individual readings of the efficiency of light of the indicated wavelength to that of light at the 500 nm, which is also the ratio of the extinction coefficients of the CO-complex of the terminal oxidase at these wavelengths. We believe that there is negligible error in wavelength determination, since the monochromator bandwidths were about 1.5 nm and could be reset to an accuracy of better than 0.5 nm. The greatest source of error was the light measuring system, as indicated above.



Especially at the peaks of the action spectrum, where the intensity of the variable beam was low, the error in measuring this small intensity had a large effect on the ratio and thus on the action spectrum. With these errors, it was not possible to determine whether there was any departure of the measured spectrum from the 430 nm peak, but the peak expected at 590 has been replaced by a peak at about 580 nm. Control experiments with commercial baker's yeast (a classical cytochrome oxidase system) showed the expected 590 peak, so we seem justified in believing that the gastric mucosal cells do not contain a classical cytochrome oxidase which is active under these conditions. Further work is necessary to better define this spectrum and to extend these observations to other species.

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