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CYTOCHROME C: REFLECTION SPECTRUM OF UNMODIFIED REDUCED HORSE HEART CYTOCHROME C

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Due to the modifying action of different compounds used in preparing cytochrome c, as well as, the influence of solvents, reported absorption spectra are often confusing (1, 2). In several respects reflection spectra give more real information.

It was found that very thin layers of pure, reduced cytochrome c on frosted quartz plates in pure N_2, saturated with H_2O-vapour, give excellent reflection spectra (Fig. 1), when measured against MgO as reference, (freshly prepared by burning of pure Mg and deposited on quartz). The reflectance, defined as $-10 \log$ reflection coefficient (%) was measured from $\bar{\nu}$ 4.61 μm^{-1} (217 nm) to $\bar{\nu}$ 1.43 μm^{-1} (700 nm) with quantities of 1×10^{-11} mole (0.125 µg) of reduced cytochrome c per mm^2 of reflecting area, with a mean error of about 2 % of value.

The symmetry of the reflection spectrum of reduced cytochrome c is marked (Fig. 1). The maxima are 547.5; 519.0; 407.3; 278.0 and 225.2 nm, respectively.

Reduced cytochrome c was prepared by two different methods, from horse heart cytochrome c, prepared for Sigma without trichloroacetic acid. Both preparations were made under N_2, completely free from oxygen. Since spectroscopically pure, "native", reduced cytochrome c was not obtained with ascorbic acid as reducing agent, glycolic acid was used in one method, and sodium formate in the other. Glycolic acid reduces oxidized cytochrome c directly at 20°. When sodium formate was used, (Formate/carbonate E'_o - 0.43 V,) a very small quantity of 99.9 % platinum black (Baker) was added: e.g. to 1.33 mg cytochrome c and 23 mg HCOO Na in 1ml redist. H_2O 0.2 mg 99.9 % Pt-black was added. Reduction proceeds slowly, but completely at 20°. In 1 h reduction of Fe^{+++} to Fe^{++} of the prosthetic group is complete without attack of double-bonds; Pt-black is filtered off, solution dialysed and evaporated at 20° and 1 torr. All operations were carried out under N_2.

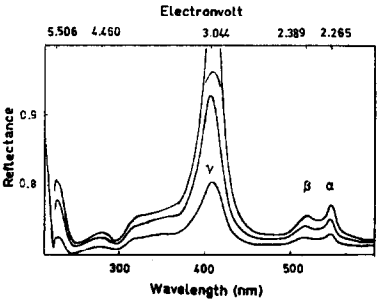

Fig. 1. Reflection spectrum of reduced cytochrome c on frosted quartz in N_2, saturated with H_2O-vapour, at 20°. Lower curve: 0.202 µg (1.63×10^{-11} mole). Middle curve: 0.404 µg (3.26×10^{-11} moles). Upper curve: 0.606 µg (4.89×10^{-11} moles) per mm^2, of reduced cytochrome c. Reference: MgO, freshly prepared by burning of pure Mg and deposited on quartz.

Even very thin layers of reduced cytochrome c of uniform thickness are easily obtained on frosted quartz, by rapid evaporation in vacuo, as above, of the appropriate volume of a solution in redist. H_2O applied on the frosted surface with a microcapillary pipette with the tip bent at a right angle. Reflection measurements were carried out as described earlier (3, 4) with a doublebeam recording spectroreflectometer and integrating sphere. Wavelength values are $\pm$ 0.3 nm, corrected for scanning and sphere errors, controlled with Holmium oxide.

The mol. wt. of reduced horse cytochrome c, prepared by both methods, was 12380, calculated from the iron content, determined as 2.2' - dipyridyl-compound (5). Reflection spectra were identical within the limit of error, given above, at the same concentration of the two preparations.

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REFERENCES

1. B.F. Van Gelder and E.C. Slater, Biochim. Biophys. Acta, 58, 593 (1962).
2. Lucile Smith, Norma Newton, and P. Scholes in "Hemes and Hemoproteins" (Ed. Britton Chance, R. Estabrook, and T. Yonetani), Academic Press, New York, London, 1966, p. 395-403.
3. H. Fredholm, Acta chem. Scand., 24, 3419 (1970).
4. H. Fredholm, Biochem. Biophys. Res. Commun., 43, 285 (1971).
5. H. Fredholm, Livsmedelsteknik, 4, 180 (1969).