Low temperature spectrum of cytochrome *c*

KEILIN AND HARTREE have pioneered in visual observations of spectra of biological and other material at the temperature of liquid air[1]. Advantages of their low temperature technique are that the absorption bands are shifted in a characteristic manner and are sharpened and intensified so that identification of certain cytochromes is materially assisted. A disadvantage of the use of the microspectroscope is that no permanent record is obtained. Such spectra are distinct enough so that relatively simple spectrophotometric methods are applicable, and are especially suitable for recording by the sensitive spectrophotometers developed for the study of turbid cell suspensions by CHANCE and his coworkers[2,3,4]. The latter techniques are apparently superior to the visual microspectroscopic method used by KEILIN AND HARTREE. These recordings show clearly absorption bands of cytochrome *c* that were identified by KEILIN AND HARTREE as a "multitude of absorption bands". We report briefly our results on reduced cytochrome *c*.

Method. The usual Beckman cuvette holder is replaced with a special holder. One mm optical path lucite cuvettes are placed in an unsilvered Dewar flask through which the two light beams pass from the monochromator to the photocell. Solutions of reduced cytochrome *c* are diluted with an equal volume of glycerol and chilled in liquid air. The vitrified material is then allowed to warm to about —55° C where a heavy turbidity is formed. These samples are then rechilled to —189° C and spectra recorded.

Fig. 1 shows the visible spectra of reduced cytochrome *c* when the sample was at room temperature and at the temperature of liquid air. At room temperature three principal absorption bands can be seen. The maxima of these bands are as follows: α, 550 mμ; β, 520 mμ; and γ, 416 mμ. At liquid air temperatures the α band splits into three bands with maxima at 549, 546, and 538.5 mμ. The β band separates into three major absorption bands with maxima at 526, 519, and 508.5 mμ with indications of at least three other absorption bands with maxima at 515, 511, and 503 mμ. The γ band has a maximum at about 414 mμ at liquid air temperatures. Both commercially available cytochrome *c* (Sigma Chemical Company) and cytochrome *c* which has been purified on IRC 50 ion exchange resin[5] show the splitting of the α- and β-bands. The α-band at 546 mμ is depressed, however, if the cytochrome *c* has been incubated in 0.05 *N* NaOH prior to reduction and chilling.

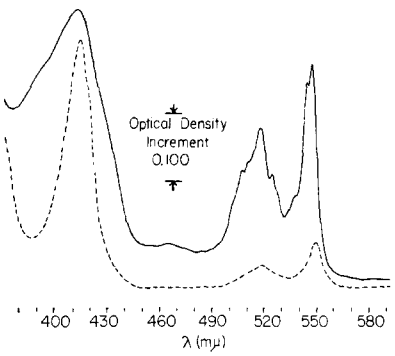

Fig. 1. Absorption spectra of reduced cytochrome *c*. The dotted curve represents the spectrum obtained when the sample is at room temperature; the solid curve is at liquid air temperatures. The sample cuvette contained 0.1 ml of $2 \cdot 10^{-4}$ *M* cytochrome *c*, 0.4 ml of phosphate buffer, 0.1 *M* of pH 7.4, a few crystals of sodium dithionite, and 0.5 ml of glycerol. Spectral interval, 0.5 to 1 mμ; optical path, 1 mm.

The spectra obtained of samples chilled in liquid air using a recording spectrophotometric technique confirms the observation of KEILIN AND HARTREE[1] that there is a sharpening of the absorption bands, a slight shifting of the maxima to shorter wavelengths, and an intensification of the light absorption by the bands.

The use of low temperature spectrophotometry illustrated here is presently being applied to the study of the spectra of a variety of biological materials. It is hoped that these results may be reported in the near future.

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