NEAR-INFRARED SPECTROPHOTOMETRIC MONITORING OF HAEMOGLOBIN AND CYTOCHROME a,a₃ IN SITU

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INTRODUCTION

In the near-infrared region, two chromophores of biological interest, haemoglobin and cytochrome a,a₃, have specific absorption bands, which respond to the oxygenation state of living tissues. Several authors have applied near-infrared spectroscopy to their analysis in situ (e.g. Jobsis, 1977; Gianni et al., 1982; Kariman and Burkhart, 1985a), but quantitative analyses in tissue have encountered difficulties from the overlap of changes in the amount and oxygenation state of haemoglobin and the redox state of cytochrome a,a₃.

We have developed a method to measure changes in the content of oxy- and total (oxy- plus deoxy-) haemoglobin in rat head (Hazeki and Tamura, unpublished) and skeletal muscle (Seiyama, Hazeki and Tamura, 1987). In these studies we illuminated rat tissues with near-infrared light and analysed the light transmitted through them. The quantification was made on the basis of the linear relationship between the changes of optical absorbance and haemoglobin content. In this paper we present a method to monitor the redox changes of cytochrome a,a₃ based on our previous work.

METHODS

Optical techniques

The centre of the rat head was illuminated by light from an air-cooled tungsten-iodine lamp through a light-guide of 4 mm diameter. The light transmitted through skin, cranial bone, mandible and brain tissue was collected by a three-branched light-guide with optical filters of 700, 730 and 830 nm, and led to photomultipliers. The light intensities at each wavelength were recorded and the absorbance changes calculated.

Perfused rat head preparation

Male Wistar rats weighing 180-280 g were anaesthetized with urethane (0.8 g/kg I.P.). The left and right external carotid arteries were ligated at their origins. The common carotid arteries were cannulated with polyethylene tubes and Krebs Ringer bicarbonate buffer
was infused slowly. The ascending aorta was occluded quickly at its origin and again at the point where it turned downward. The perfusate was then changed to saline containing 10 mM Na₂S₂O₄ or 1 mM NaN₃, and the infusion rate raised to 3 ml/min.

RESULTS AND DISCUSSION

Figure 1A shows the difference absorption spectrum of isolated haemoglobin solution. An isosbestic point for oxy and deoxy species was found at 805 nm. Figure 1B shows the difference absorption spectrum of rat head induced by anoxia. The spectrum in vivo was similar to that of haemoglobin solution, but 805 nm was no longer the isosbestic point. This could be attributed either to the decrease in amount of haemoglobin or to the decrease in absorption of other tissue component(s). In order to estimate the contribution of haemoglobin to in vivo spectra, we measured the absorption spectrum of haemoglobin-free rat head (Fig. 1C), in which blood was replaced with Fluosol-43 emulsion.

![Figure 1](image)

Figure 1. Difference absorption spectra of haemoglobin solution (A), normal rat head (B), and haemoglobin-free rat head (C). The spectra at oxygenated states were used as baselines (solid lines), and the anoxic spectra (broken lines) were recorded.

In this rat head the absorbance change in the region of 700–780 nm disappeared, showing that the change in this region of normal rat was mostly due to the change in haemoglobin. In contrast, the absorbance above 780 nm was decreased by anoxia even in the haemoglobin-free rat. The change was attributable to the reduction of cytochrome aₐ₄₃ in mitochondria (Griffith and Wharton, 1961; Chance, 1966 and Ferrari et al., 1983).