oxidase. Earlier measurements of breakpoints in haemoproteins may have escaped detection since the runs were rarely carried below 70°C (Eley & Spivey, 1960). Since the polarization effects for samples I and II remained after several days' pumping, the ionic conduction cannot be due to protons in adsorbed water (Eley & Leslie, 1964). The dark-current time effects in sample I disappeared at field strengths greater than 30kV/m.

The results in Fig. 1 show that serum albumin gives only u.v. photocurrents, whereas cytochrome c oxidase shows photocurrents extending from the u.v. through the visible into the i.r. Photocurrent gain is here defined as number of electrons collected at the electrode per incident photon, in our case approximately the same as per absorbed photon for the u.v. and visible. Photocurrents clearly decrease with increasing sample purity, but persist in the purest sample III, the action spectrum being similar to those found for cytochrome c and haemoglobin (Eley & Metcalfe, 1972).

The thermal activation energies $\epsilon_p$ for photocurrents $i_p$ are listed and they are much larger for the pure specimens (sample III), and again there is a breakpoint at 70°C, also observed for cytochrome c (D. D. Eley, E. Metcalfe & M. P. White, unpublished work).

Photoconduction is generally associated with electrons or holes, rather than ions, and so overall we do not associate the photoconduction with the impurities. Instead we assign the photocurrents for photon energies less than 0.56eV (3.5eV) to photon absorption by the haem, resulting in injection of electrons into the protein, where they move by narrow-band intermolecular tunnelling or hopping conduction. The reason why impurities increase the photoconduction in samples I and II is unclear at present. If similar effects arise in mixtures of phospholipid with cytochrome c oxidase it will be of interest for membrane systems.

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Alternating-Current Conduction in Dry Native and Modified Cytochrome c Oxidase

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Szent-Györgi (1941) suggested that electron transport in the respiratory chain might involve a semiconduction process via the protein molecules. Since then, the direct-current conduction has been studied in many proteins and biopolymers (see, e.g., Eley, 1962, 1968). Most proteins in the dry state have a room-temperature conductivity $\sigma$ (at 298°K) of about $10^{-18} \text{Q}^{-1} \text{cm}^{-1}$ and an activation energy $\Delta\epsilon$ of approx. 0.42eV (2.6eV) in $\sigma = \sigma_0 \exp(-\Delta\epsilon/2kT)$. This activation energy may be lowered by adsorption of water or by quinones. A narrow energy band has been assumed within protein molecules, with electron tunnelling between molecules (Eley & Spivey, 1960). Microwave Hall measurements have led to a similar tentative model for the respiratory chain (Eley et al., 1972a,b).
In recent years a mechanism of charge transport in which electrons in localized states hop over energy barriers has been considered, especially in relation to chalcogenide glasses (Mott & Davis, 1971). Measurements of alternating-current conductivity as a function of frequency serve to distinguish this process, which has been suggested for some organic materials, including proteins (Pethig, 1972). The Drude formula describes the conductivity as a function $\omega$ for electrons moving in wide bands:

$$\sigma(\omega) = \frac{\sigma(0)}{1 + \omega^2 \cdot \tau^2}$$

where $\sigma(0)$ is the steady-state (direct-current) conductivity. With typical relaxation times $\tau$ ($10^{-13}$ s) $\sigma(\omega)$ should remain constant to optical frequencies. On the other hand chalcogenide glasses show $\sigma(\omega) \propto \omega^{0.8}$ at room temperature in the MHz region of

![Fig. 1. Conductivity $\sigma$ and relative permittivity $\varepsilon$, of cytochrome c oxidase as a function of frequency $f$.](image)

Conductivity data: curve 1 (---), untreated cytochrome c oxidase, temperature 84°C; curve 2 (--), untreated cytochrome c oxidase, temperature 37.5°C; curve 1 (-----), modified cytochrome c oxidase, temperature 84°C; curve 2 (-----), modified cytochrome c oxidase, temperature 31°C. Relative-permittivity data: curve 3, untreated cytochrome c oxidase, temperature 56°C; curve 4, modified cytochrome c oxidase, temperature 56°C. Error bars shown for modified cytochrome c oxidase apply through the whole frequency range.
which Mott & Davis (1971) interpret as electron hopping between states at the Fermi level.

In these experiments we prepared cytochrome c oxidase from heavy ox heart mitochondria by Yonetani's (1967) method. Cytochrome c oxidase is complex, containing cytochrome a, cytochrome a₃, Cu and phospholipid, and it is an amorphous powder. Samples of cytochrome c oxidase and cytochrome c oxidase previously treated with N-bromosuccinimide were dialysed against 0.25 M sucrose, pH 7.0, and then sedimented by centrifugation at 100000 g, for 2 h. The N-bromosuccinimide modifies tryptophan residues and has been shown to lower cytochrome c oxidase catalytic activity and to modify the haem–protein interaction significantly (Mayer & Landon, 1972). Capacitance and conductivity measurements were carried out in a parallel-plate cell on the dry substances in a vacuum of 1 mPa as follows: (i) at 1.592 kHz by Wayne-Kerr bridge; (ii) 50 kHz to 5 MHz, Marconi Q meter; (iii) 10 MHz to 500 MHz, a transmission line with Hewlett-Packard Vector Voltmeter; (iv) 9 GHz, microwave cavity.

Fig. 2. Conductivity of cytochrome c oxidase as a function of temperature at various frequencies

○, Untreated cytochrome c oxidase; ○, modified cytochrome c oxidase.
The results in Fig. 1 when approximated by $\sigma = A \omega^s$ give values of $s = 1.11-1.14$ for cytochrome $c$ oxidase and $0.98-1.0$ for N-bromosuccinimide-modified cytochrome $c$ oxidase. This dependence is found over a frequency range for which the relative permittivity $\varepsilon_r$ is constant and at higher frequencies where $\varepsilon_r$ decreases. These values of $s$ are significantly higher than the value of 0.8 for hopping between compensated donor impurities in $n$-type silicon (Pollak & Geballe, 1961; Mott & Davis, 1971, pp. 49, 185). They are also higher than values found for cytochrome $c$ and bovine serum albumin (Pethig, 1972) and for metal organic chelates (Fendley & Jonscher, 1972). In our case the hopping process will be complex, probably involving intramolecular barriers with a distribution of heights (the preparations are amorphous) and in the presence of the associated phospholipid with dipolar relaxations in the frequency range $10^6-10^{11}\text{ s}^{-1}$, which we may infer from n.m.r. data (E. G. Finer, personal communication). This dipolar loss is here manifested in Fig. 1 by the decrease in permittivity $\varepsilon_r$ which occurs at frequencies $f (= \omega/2\pi)$ where $s$ decreases. The alternating-current conductivity of untreated cytochrome $c$ oxidase appears to be significantly lower than that of modified cytochrome $c$ oxidase, especially at low frequencies, and the permittivity values are also definitely lower. Under the conditions employed the indications are that the N-bromosuccinimide mainly modifies tryptophan residues (cf. Witkop, 1961), but we cannot rule out completely the introduction of some electron-accepting residues that could enhance the conductivity of the protein and give the observed result. There is a uniform increase in the relative permittivity of the samples with increasing temperature. Further, the alternating-current conductivity of cytochrome $c$ oxidase at 1 MHz appears to be one order of magnitude higher than that of cytochrome $c$ and two orders of magnitude higher than that of bovine serum albumin (Pethig, 1972), although further work here is necessary.

Fig. 2 shows that below 500kHz there is a marked temperature-dependence of $\sigma$ at high temperatures, which becomes temperature-independent at low temperatures. At 1.592kHz the activation energy ($\Delta\varepsilon/2$) is 0.02aJ (0.14eV) for modified cytochrome $c$ oxidase, but 0.0 for the untreated material, which is anomalous insofar as the latter has the lower conductivity and points at first sight to an even lower mobility.

In conclusion, therefore, our preliminary experiments show a frequency-dependence of alternating-current conductivity that suggests a possible hopping process of localized charge carriers (electrons) in dry cytochrome $c$ oxidase, the process being significantly affected by chemical modification of the protein. The exact nature of the hopping and its interaction with dipolar losses arising in the associated phospholipid at the higher frequencies will require further work. The higher conductivity of the biologically inactive N-bromosuccinimide-treated cytochrome $c$ oxidase suggests either that the N-bromosuccinimide is also interfering with the substrate-binding site of the cytochrome $c$ oxidase, or that its effect on the overall conductivity of the protein is opposite to that on the specific electron path for respiration.