Short Communications and Preliminary Notes

Cytochrome c_3, a bifunctional haematohaematin

The cytochrome of the anaerobic sulphate-reducing bacteria[1,2] will be termed cytochrome c_3, since its systematic name—*Desulphovibrio desulphuricans* cytochrome 553—is cumbersome. The protein has been extracted and purified by a procedure, to be described in detail elsewhere, involving acid-precipitation from extracts of acetone-dried cells followed by chromatography on cellulose and Amberlite IRC 50. Evidence for carrier activity in the reduction of sulphate and related ions has been reported[3].

The product was a deep red autoxidizable water-soluble powder, homogeneous in the ultracentrifuge and by paper electrophoresis, containing less than 5% of impurity absorbing at 280 mμ when chromatographed on IRC 50 columns as described by BOARDMAN AND PARTRIDGE[4]. The following properties have been examined:

(i) *Iso-electric point:* paper electrophoresis in phosphate buffers with pure cytochrome *c* as control indicated an iso-electric point between pH 10.3 and 10.6.

(ii) *Redox potential:* potentiometric titration with sodium dithionite in the presence of anthracene-2-sulphonic acid as a poising agent gave a value of $E_0' = -205 \pm 4$ mV at 30°.

(iii) *Spectrum:* the oxidized material had peaks at $\alpha = 535$, $\gamma = 410$, $\sigma = 350$ mμ, with an inflection at ~568 mμ. The reduced material had peaks at $\alpha = 553.4$, $\beta = 525.0$, $\gamma = 419$ mμ; the further ultra-violet region of the reduced form was not investigated, since, owing to the low E_0' of c_3, only dithionite is a suitable reducing agent and this is opaque below ~380 mμ.

(iv) *Specific extinction coefficient:* the pure reduced material had an ε_{sp} of 4.2 at its α-peak. This is roughly twice that of cytochrome *c*, and indicates that c_3 has either half the molecular weight of *c*, or twice as many haemine groups per molecule. In the latter case a molecular weight of ~12,500 would be expected.

(v) *Sedimentation coefficient:* a test in the ultra-centrifuge carried out by Dr. A. G. OGSTON at Oxford indicated a value $S_{20,w} = 1.93 \cdot 10^{-13}$. This indicates a minimum molecular weight of 10,200, consistent with a double-haemin molecule similar in size to that of cytochrome *c*.

(vi) *Iron content:* 8.1 mg cytochrome c_3 was wet-ashed and analysed for iron by the *o*-phenanthroline procedure. It contained 0.92% Fe, roughly twice that of pure cytochrome *c*, again consistent with a double-haemin structure.

(vii) *Chemical stability:* cytochrome c_3 was stable to boiling for five minutes, and no haemin was released with mineral acid—or acetic-acetone. Ether-insoluble porphyrin was released from the reduced form by HCl (3.3 *N*); its α-band lay at 554 mμ (*cf*: 553 mμ for "porphyrin *c*"). Hence the pigment probably has thio-ether links between the protein and haemin similar to those of cytochrome *c*.

(viii) *Prosthetic group:* treatment with silver sulphate and acetic acid[5] yielded a haemin (Soret peak 390 mμ in KH_2PO_4, pH 7; *cf*: 391 mμ for a control preparation of haematohaemin from cytochrome *c*) which after extraction formed a pyridine haemochrome spectroscopically similar to pyridine haematohaemochrome (408, 517.2, 546.0 mμ). Reductive fission of c_3 with Na/Hg[6] yielded a porphyrin having the spectrum of mesoporphyrin and a chlorin, both products being spectroscopically like those derived from cytochrome *c*.

These studies lead to the conclusion that cytochrome c_3 is a bifunctional haematohaematin with thio-ether haemin-protein links. They will be reported in detail elsewhere.

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