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I6

Is Cytochrome aa_3 From *Thermus Thermophilus* a Single Subunit Oxidase?

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A reliable procedure has been developed for the purification of the cytochrome c_1aa_3 complex from the plasma membrane of *T. thermophilus.* The ratios heme C:heme A:Fe:Cu were found to be 1:2:3:2 confirming previous results, however, the molecular weight was found to be ~92,000 rather than the ~200,000 reported earlier [1]. Polyacrylamide gel electrophoresis under strongly denaturing conditions and high performance reverse phase liquid chromatography showed that cytochrome c_1aa_3 is composed of only two subunits in 1:1 ratio. Both polypeptides have blocked N-termini. The smaller subunit (~33,000) binds heme *c* and presumably no other metals. The larger subunit (~55,000) is thus thought to contain the elements of cytochrome aa_3 and therefore must be considered a single subunit cytochrome oxidase.

The bacterial cytochrome c_1aa_3 has been compared with beef heart cytochrome oxidase with a number of techniques including optical, EPR [1], Raman, MCD, and Mössbauer [2] spectroscopies. These experiments establish that the fundamental chemical properties of the redox centers are substantially similar in these two proteins.

Cytochrome c_{552} (from *Thermus*), horse heart cytochrome *c*, and tetramethylphenylenediamine greatly stimulate the ascorbate oxidase activity of cytochrome c_1aa_3. This enhancement is characterized by a 'high affinity' component which results in only a small velocity increase and a 'low affinity' component which gives a large velocity increase. Very similar behavior has been previously observed with mammalian cytochrome oxidase [3].

Preliminary experiments show that vesicularized c_1aa_3 is capable of proton pumping.

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I7

Aspects of the Chemistry of the Two Heme Centers of Cytochrome Oxidase

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Derivatives of heme *a* have been examined by optical, MCD and EPR spectroscopy [1]. Five- and six-coordinate high-spin ferric species exhibit optical spectra recently classified as 'Type a' by Quinn *et al.* [2] while a low-spin bis-imidazole ferric derivative exhibits a 'Type b' spectrum. On reduction the visible spectrum of the low-spin derivative intensifies markedly and exhibits a single maximum at 589 nm; the visible spectrum of the high-spin species changes shape but the intensity is only slightly changed. The ferric high-spin compounds exhibit a transition in the near-infrared which has absorbance and MCD characteristics similar to the 655 nm band [3] of the resting enzyme.

Composite spectra obtained by the addition of the individual spectra of the ferric high- and low-spin models and of the ferrous high- and low-spin models reproduce the essential features of the spectra of oxidized and reduced enzyme, respectively. The relative contributions of the high- and low-spin derivatives to the spectral changes at 589 nm produced by