Cu_{a_3} OF CYTOCHROME *c* OXIDASE IS NOT A TYPE 1 (BLUE) COPPER

Gary W. BRUDVIG and Sunney I. CHAN*

A. A. Noyes Laboratory of Chemical Physics California, Institute of Technology, Pasadena, CA 91125, USA

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1. Introduction

Cytochrome *c* oxidase [1] contains two copper ions, both of which are unique among copper proteins. Cu_{a_3}, which along with cytochrome a_3 forms the oxygen binding site, is the only known example of a copper ion which is antiferromagnetically coupled to a heme in an enzyme. Cu_a, which is involved in electron transfer, exhibits an electron paramagnetic resonance (EPR) signal which is different from that of any other known copper protein [2]. In spite of the unique characteristics of these copper centers, there have been attempts to categorize them into one of the three groups of coppers which have been found in other proteins. It is becoming increasingly evident that Cu_a is unlike any of the types of copper found in other copper proteins [2,3]. On the other hand, it was recently proposed that Cu_{a_3} is a type 1 copper on the basis of X-ray absorption edge studies [4].

Type 1 copper proteins are characterized by a strong absorption near 600 nm ($\epsilon \simeq 5000\ cm^{-1}\ .M^{-1}$), a spectroscopic feature which gives these proteins their characteristic blue color [5]. This strong absorption band has been assigned to a thiolate sulfur → $d_{x^2-y^2}$ charge transfer transition [6] and, accordingly a cysteinate sulfur is a requisite ligand of a type 1 (blue) copper. Indeed, it is known that the A_{600} band in type 1 copper proteins disappears upon the addition of the sulfhydryl binding reagents Ag^+ or Hg^{2+} [5]. Since Cu_{a_3} is known to bind exogenous ligands [7], this site should be easily accessible to either Ag^+ or Hg^{2+}. Both of these sulfhydryl binding agents would decrease the absorption near 600 nm of oxidized cytochrome *c* oxidase if Cu_{a_3} is a type 1 copper. Therefore, the addition of Ag^+ or H^{2+} to oxidized cytochrome *c* oxidase should allow one to directly assess whether or not Cu_{a_3} is a type 1 copper.

2. Materials and methods

Cytochrome *c* oxidase was isolated from beef heart mitochondria by the method in [8] and was dissolved in 50 mM Tris + 0.5% Tween 20 which was brought to pH 7.4 with HNO_3. French bean plastocyanin was a gift of Professor H. B. Gray and was dissolved in 50 mM Tris/HNO_3 (pH 7.4). The concentration of cytochrome *c* oxidase was determined by the pyridine hemochromagen assay [9] and that of plastocyanin was determined optically at 597 nm using $\epsilon = 4500\ cm^{-1}\ .M^{-1}$ [10]. Solutions of $AgNO_3$ and $HgCl_2$ were added to the enzyme samples to yield a final Ag^+ or Hg^{2+} concentration that was 10-fold greater than the enzyme concentration.

The optical spectra were recorded at room temperature on a Beckman Acta CIII spectrophotometer.

3. Results and discussion

The addition of Ag^+ or Hg^{2+} to oxidized cytochrome *c* oxidase did not cause the 598 nm band of the enzyme to be significantly reduced in intensity (fig.1). We observed a slight decrease in A_{598} of 0.006 upon the addition of Ag^+ and 0.018 upon the addition of Hg^{2+} to the enzyme. However, small changes also occurred throughout the spectrum from 450–750 nm and these small optical changes are most

* To whom reprint requests should be sent

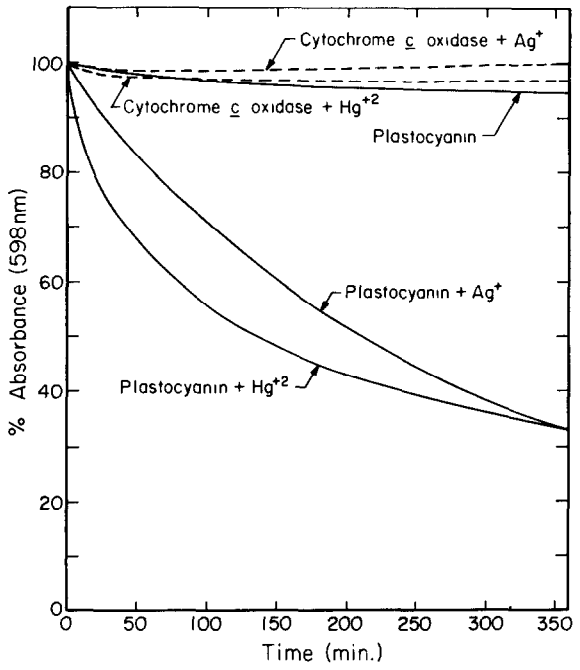

Fig.1. Changes in absorbance at 598 nm with time upon incubation of cytochrome *c* oxidase and plastocyanin with Ag^+, Hg^{2+}, or alone. The concentration of cytochrome *c* oxidased in these experiments was 2.5×10^{-5} M (based on 2 heme a/enzyme); that of French bean plastocyanin was 8.3×10^{-5} M.

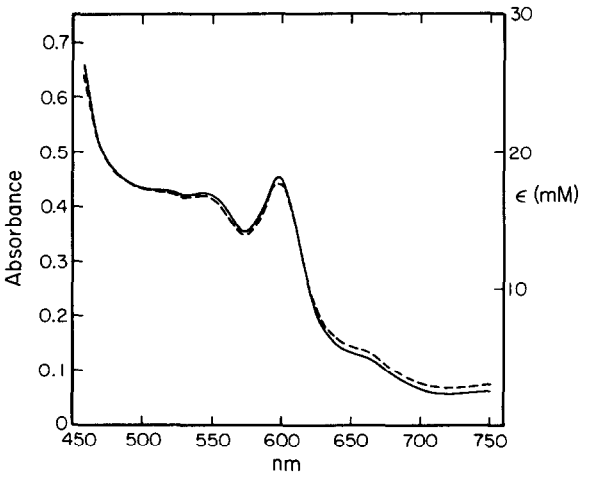

Fig.2. Absorption spectra of oxidized cytochrome *c* oxidase before (——) and after (- - - -) incubation of the enzyme for 6 h with $HgCl_2$.

likely due to a perturbation of the hemes by Ag^+ and Hg^{2+}. In neither case did we observe a decrease in absorbance near 600 nm of the magnitude expected if a type 1 copper absorption were bleached ($\Delta A = 0.12$ for $\Delta\epsilon = 4500\ cm^{-1}\ .M^{-1}$).

For comparison we repeated the experiments in [11] on the effect of Ag^+ and Hg^{2+} on plastocyanin (a type 1 copper protein). Our results confirm that both Ag^+ and Hg^{2+} do bleach the A_{597} band of this enzyme (fig.2). Our experiments on cytochrome *c* oxidase, therefore, indicate that Cu_{a_3} either is not a type 1 copper or is not accessible to Ag^+ or Hg^{2+} during the 6 h incubation of the enzyme in these experiments. However, Cu_{a_3} is known to bind externally added ligands [7], and it is presumably directly accessible to the solvent. Since oxidized cytochrome *c* oxidase does not have a large absorption near 600 nm which is sensitive to sulfhydryl binding reagents, we conclude, then, that Cu_{a_3} cannot be a type 1 copper. This conclusion is consistent with resonance Raman studies from this laboratory, which revealed no evidence of copper-associated Raman bands from cytochrome *c* oxidase when the excitation was carried out in the visible absorption bands of the protein near 600 nm [12].

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