Forms of cytochrome oxidase

OKUNUKI and his colleagues[1,2] have recently reported that the spectrum of a cytochrome oxidase preparation shows some differences according to whether it is oxidized by $K_3Fe(CN)_6$, or by O_2, and have suggested that the spectrum obtained with oxygen is due to formation of an O_2 complex of the same type as oxyhaemoglobin. As already communicated to the Fourth International Congress of Biochemistry in September, 1958 we have also observed a difference between the two spectra but our studies make it doubtful that the compound formed with O_2 is a complex with molecular O_2.

The SMITH AND STOTZ[3] "type II" preparation of cytochrome oxidase was used. It contained cytochromes *a* and a_3, and was free from spectroscopically identifiable amounts of cytochromes *b*, *c* and c_1, and of mitochrome[4].

Fig. 1 shows the absorption spectra between 576 and 641 mμ under four different conditions. Curve 1 is the spectrum of the preparation in the presence of air. Curve 2 was obtained 18 h after evacuation of a solution. Under these conditions, cytochromes *a* and a_3 are slowly and simultaneously reduced, the degree of reduction reaching 95 % of that obtained with $Na_2S_2O_4$ (curve 3). When the evacuated solution was opened to air and O_2 bubbled through, the spectrum shown in curve 1 was slowly restored.

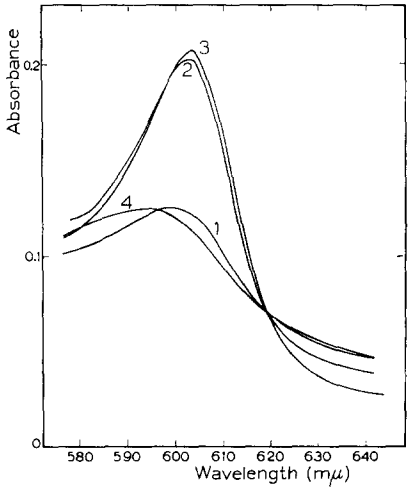


Fig. 1. Spectra of cytochrome oxidase. Curve 1 was obtained in air, curve 2 after evacuation of a solution, curve 3 with $Na_2S_2O_4$ in N_2, and curve 4 by addition of $K_3Fe(CN)_6$ $2.4 \cdot 10^{-4}$ *M* or $2.4 \cdot 10^{-3}$ *M*) to the evacuated solution.

Curve 4 shows the spectrum obtained by addition of $K_3Fe(CN)_6$. The same spectrum was obtained whether the $K_3Fe(CN)_6$ was added in the presence of air or anaerobically to a preparation reduced spontaneously by evacuation. Dialysis of the $K_3Fe(CN)_6$-treated preparation did not change the spectrum. The cytochrome oxidase activity (measured manometrically with cytochrome *c* and ascorbic acid) was not affected by treatment with $K_3Fe(CN)_6$, followed by dialysis.

Calculations showed that the spectrum of curve 1 could not be obtained by addition of curves 3 and 4 in various proportions. Thus, it cannot be due to the

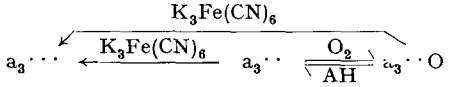
Abbreviations: $a \cdot \cdot \cdot$, $a_3 \cdot \cdot \cdot$, ferricytochrome *a* and a_3; $a \cdot \cdot$, $a_3 \cdot \cdot$, ferrocytochrome *a* and a_3.

presence of an inactive cytochrome *a* or a_3 which is oxidizable by $K_3Fe(CN)_6$ but not by O_2, as suggested by CHANCE[1] to explain OKUNUKI's results. A compound not represented in curves 3 and 4 must be at least partly responsible for the spectrum in curve 1.

The positions of the peaks of the absorption bands (in air, 420 and 598 mμ; with $Na_2S_2O_4$, 444 and 605 mμ; with $K_3Fe(CN)_6$, 424 and 591–3 mμ) differ in some respects from those given by OKUNUKI[1,2], but they agree in showing that the spectrum of cytochrome $(a + a_3)$, oxidized with $K_3Fe(CN)_6$, is not the same as that obtained with O_2. It seems probable that ferricyanide gives the combined spectrum of $a\cdot\cdot\cdot$ and $a_3\cdot\cdot\cdot$. Since O_2 reacts with $a_3\cdot\cdot$, curve 1 probably represents the combined spectrum of $a\cdot\cdot\cdot$ and a second oxidized form of cytochrome a_3. Our experiments make it unlikely that the latter is a complex of molecular O_2 and $a_3\cdot\cdot$, since both the formation of the spectrum of $(a\cdot\cdot + a_3\cdot\cdot)$ on evacuation, and its disappearance on re-oxygenation, were very slow reactions. It is possible, however, that the second oxidized form contains an oxygen atom bound to the ferrous iron atom, as in ferryl compounds[5].

Although it is not yet known whether both forms of oxidized cytochrome a_3 are concerned in the enzymic reaction, it is significant that both are equally active. Since it is likely that $a_3\cdot\cdot$ is concerned in the enzymic reaction, both forms of oxidized cytochrome a_3 must be reducible in the presence of ascorbic acid and cytochrome *c*.

In summary, it is suggested that curves 3, 4 and 1 given in Fig. 1 represent the spectra of $a\cdot\cdot + a_3\cdot\cdot$, $a\cdot\cdot\cdot + a_3\cdot\cdot\cdot$ and $a\cdot\cdot\cdot$ and $a_3\cdot\cdot O$ (or another form of oxidized cytochrome a_3), respectively. The interconversions of the three forms of cytochrome a_3 are possibly described by the following scheme



where AH represents reducing compounds present in the enzyme preparation. The presence of ascorbic acid and cytochrome *c* in the manometric method of measuring the cytochrome oxidase reaction may cause an increase in the rate of some of these reactions. Additional reactions may also be introduced (*cf.* SLATER[6]). In this connection, the report of SEKUZU *et al.*[2] that, in the presence of cytochrome *c*, O_2 gives the same compound as obtained with ferricyanide (i.e. $a_3\cdot\cdot\cdot$ in the above scheme) is of great interest.

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