Cytochrome Oxidase[1]

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Separation and purification of the components of the cytochrome oxidase complex have literally presented an insoluble problem due to the difficulties experienced in attempting to dissolve the complex. Among the many attempts to obtain a solution of the enzyme has been that of Yamagutchi, Hiroshi and Ogura (*3*), who claimed a preparation from yeast and heart muscle that was active after filtration through a Seitz filter. Keilin (*5*) repeated the work and concluded that the enzymes were not in solution. Hogness, Altschul, and Abrams (*3*) reported a soluble preparation from yeast, but not all yeasts could be used, and the oxidase from any one source was not constant. More recently, Haas (*1*) has attempted to prepare a soluble oxidase from yeast by subjecting an insoluble complex to ultrasonic radiation. He succeeded only in reducing the size of the particle. Later he prepared a soluble component which is probably cytochrome A by incubating undiluted oxidase in boiling water for 3 minutes (*2*).

A report by Hopkins, Lutwak-Mann, and Morgan (*4*) on the use of bile salts in the preparation of succinic dehydrogenase suggested to us the use of sodium desoxycholate to dissolve the oxidase complex. By this method we have obtained a preparation which, when tested with the hydroquinone-cytochrome C system at pH 7.4, is 2.5 times as active as the Keilin and Hartree oxidase preparation. It retains its activity after centrifugation for 2 hours at 18,000 × g or after having been passed through a Seitz filter. Both preparations are clear and red-brown in color. A partial purification has been achieved by fractionating with sodium desoxycholate. The fractionated preparation is 4–6 times as active as the original Keilin and Hartree oxidase from which it is made.

All extracts and fractionations with desoxycholate were made on a Keilin and Hartree (*7*) oxidase (fresh lamb heart) that had been diluted 1:1 with 0.1 M Na_2HPO_4-KH_2PO_4 buffer of pH 7.4. Cytochrome C was made by the method of Keilin and Hartree (*6*), and the hydroquinone-cytochrome C test system was similar to the one used by Haas except that buffer of pH 7.4 was used instead of pH 7.1.

Table 1 summarizes the results of our first experiments. All of the enzyme preparations were diluted 1:25 with distilled water as soon as possible after having been obtained in final form. The control for the Keilin and Hartree oxidase consisted of 0.25 ml. of 0.1 M Na_2HPO_4-KH_2PO_4 buffer of pH 7.4, that for the two desoxycholate preparations of 0.25 ml., 2 per cent sodium desoxycholate. The 4 per cent desoxycholate extract was made by adding 80 mg. of desoxycholate to 2 ml. of the Keilin and Hartree oxidase, grinding in a cold mortar at 4° for 10 minutes, and centrifuging at 18,000 × g for 1 hour. The 2–4 per cent desoxycholate fraction was prepared by adding 40 mg. of desoxycholate to 2 ml. of the Keilin and Hartree oxidase, grinding and centrifuging as above, and then adding to the precipitate 2 ml. of 4 per cent desoxycholate made up in 0.1 M Na_2HPO_4-KH_2PO_4 buffer of pH 7.4. Following a grinding in a cold mortar for 5 minutes at 4°, the suspension was centrifuged for 1 hour at 18,000 × g and the supernatant tested immediately after being diluted.

The effect of 2 inhibitors on the activity of the desoxycholate–Keilin and Hartree oxidase is shown in Table 2. The enzyme preparation was made by adding 3 per cent of desoxycholate to a Keilin and Hartree oxidase, grinding in the cold for 10 minutes, and centrifuging for 1 hour at 20,000 × g. The complete inhibition by 1×10^{-3} M NaCN and NaN_3 suggests that one of the enzymes involved is probably cytochrome oxidase.

These sodium desoxycholate preparations were unable to mediate the oxidation of d-glucose by d-glucose dehydrogenase of mammalian liver, even though the diphosphopyridine nucleotide and cytochrome C were added. This observation agrees with the work of Hopkins, Lutwak-Mann, and Morgan (*4*), who were unable to catalyze the oxidation of succinate with their preparation. These reactions will be studied spectrophotometrically in the immediate future. For the present it appears that cytochrome oxidase should be a constituent of these sodium desoxycholate–Keilin and Hartree oxidase preparations. This conclusion is based on the inhibitory action of NaCN and NaN_3 and on the fact that the oxygen consumption is reduced by about 95 per cent in the absence of cytochrome C.

TABLE 1

Sample	Total protein (mg./0.25 ml.)	15-minute oxygen consumption ($mm.^3$/0.25 ml.)	QO_2 protein
Keilin and Hartree oxidase	0.202	27	400
Buffer control		7	
4 per cent desoxycholate extract	0.200	60	1,040
2–4 per cent desoxycholate fraction	0.029	26	2,480
Desoxycholate control		8	

TABLE 2

Sample	Final concentration of inhibitor	15-minute oxygen consumption ($mm.^3$/0.25 ml.)	QO_2 protein
3 per cent desoxycholate extract*		40	1,030
	1×10^{-3} M NaCN	2	0
	1×10^{-3} M NaN_3	6	0
Desoxycholate control		6	

* Total protein = 0.140 mg./0.25 ml. at 1:25 dilution.

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