effect by added deoxyribonucleosides. Alternatively, it has been postulated that the added deoxyribonucleosides are so rapidly cleaved to free bases as to be only partially effective in blocking the hydroxyurea effect (Young *et al.* 1967*a*). It seems unlikely, however, that all of the added deoxyribonucleoside was cleaved to the free base form, since the animals receiving the four deoxyribonucleosides but not hydroxyurea clearly showed a stimulation of the incorporation of phosphate into DNA that was significantly different from control values.

This study was in part supported by U.S. Public Health Research Grants CA-08344 and CA-08832 from the National Cancer Institute. The author acknowledges the technical assistance of Mrs Ellen Johnson.

Borenfreund, E., Krim, M. & Bendich, A. (1964). *J. nat. Cancer Inst.* **32**, 667.
Frenkel, E. P., Skinner, W. N. & Smiley, J. D. (1964). *Cancer Chemother. Rep.* **40**, 19.
Mohler, W. C. (1964). *Cancer Chemother. Rep.* **34**, 1.
Schwartz, H. S., Garofalo, M., Sternberg, S. S. & Philips, F. S. (1965). *Cancer Res.* **25**, 1867.
Vogler, W. R., Bain, J. A. & Huguley, C. M., jun. (1965). *Proc. Amer. Ass. Cancer Res.* **6**, 67.
Yarbro, J. W. (1966). *J. clin. Invest.* **45**, 1090.
Yarbro, J. W., Kennedy, B. J. & Barnum, C. P. (1965*a*). *Proc. nat. Acad. Sci., Wash.*, **53**, 1033.
Yarbro, J. W., Niehaus, W. G. & Barnum, C. P. (1965*b*). *Biochem. biophys. Res. Commun.* **19**, 592.
Young, C. W. & Hodas, S. (1964). *Science*, **146**, 1172.
Young, C. W., Schochetman, G., Hodas, S. & Balis, M. E. (1967*b*). *Cancer Res.* **27**, 535.
Young, C. W., Schochetman, G. & Karnofsky, D. A. (1967*a*). *Cancer Res.* **27**, 526.

Biochem. J. (1967) **104**, 54c

The Effect of Aurovertin on a Soluble Mitochondrial Adenosine Triphosphatase

By A. M. ROBERTON, R. B. BEECHEY, CAROLINE T. HOLLOWAY and I. G. KNIGHT
'Shell' Research Ltd., Milstead Laboratory of Chemical Enzymology, Sittingbourne, Kent

(*Received* 22 *June* 1967)

A soluble ATPase* can be prepared from ox heart mitochondria by homogenizing a suspension of an acetone-dried powder of mitochondria in an all-glass homogenizer (Selwyn, 1962, 1967). The properties of this ATPase are similar to those of the coupling factor F_1 described by Pullman, Penefsky, Datta & Racker (1960) with respect to metal ion activator requirements, activation by 2,4-dinitrophenol, substrate specificity and cold lability. The present communication provides further evidence for their identity. Selwyn's (1967) ATPase preparation is shown to behave similarly to factor F_1 with respect to its interaction with factor CF_0, the factor conferring oligomycin sensitivity on factor F_1 (Kagawa & Racker, 1966*b*). It is also shown that Selwyn's (1967) ATPase preparation is sensitive to aurovertin, a known inhibitor of oxidative phosphorylation and mitochondrial ATPase (Lardy, 1960; Kagawa & Racker, 1966*a*).

Methods. ATPase activity was measured by the method of Pullman *et al.* (1960). The soluble ATPase was preincubated at 30° for 3min. in a solution containing 250μmoles of sucrose, 25μmoles of tris–sulphate buffer, pH 7·4, 0·3μmole of NADH, 1μmole of KCN, 50μg. of lactate dehydrogenase and 25μg. of pyruvate kinase in a volume of 0·84ml. To start the reaction, a prewarmed solution containing 2μmoles of ATP, 2μmoles of $MgSO_4$, 2μmoles of phosphoenolpyruvate and 50μmoles of tris–sulphate buffer, pH 7·4, in 0·16ml. was added. The NADH oxidation was followed spectrophotometrically.

ATPase was prepared by the method of Selwyn (1967). It was purified to the stage of the second $(NH_4)_2SO_4$ fractionation, and stored in 50% glycerol at −20° until used. Factor CF_0 was prepared as described by Kagawa & Racker (1966*b*). Aurovertin was a generous gift from the Pitman–Moore Division of the Dow Chemical Co. (Zionsville, Ind., U.S.A.), and oligomycin A was given by Professor E. E. Van Tamelen. Oligomycin A and aurovertin were added to reactions as ethanolic solutions in volumes of 5μl. or less.

Results and discussion. The results of Expts. 1 and 5 (Table 1) show that the soluble ATPase is inhibited by factor CF_0. Addition of phospholipid to the ATPase + factor CF_0 mixture restimulates the ATPase activity, and this activity is sensitive to oligomycin A (Expts. 6 and 7). The soluble ATPase by

* Abbreviation: ATPase, adenosine triphosphatase.

Table 1. *Properties of soluble mitochondrial ATPase prepared by the method of Selwyn* (1967)

ATPase activities were measured as described in the Methods section. At 5min. before reactions were started, oligomycin A, aurovertin, 120mμmoles of Sigma soya-bean phospholipid in micellar form (Fleischer & Klouwen, 1961) and 200μg. of protein of factor CF_0 were added, where indicated. The soluble ATPase (32μg. of protein) was added 3min. before the reactions were started. Activities are expressed as mμmoles of ADP formed/min./32μg. of soluble ATPase protein.

Expt. no.	Additions	Activity
1	ATPase	55·7
2	ATPase+oligomycin A (0·2mμmole)	55·7
3	ATPase+ oligomycin A (5mμmoles)	54·4
4	ATPase+ oligomycin A (20mμmoles)	51·2
5	ATPase+CF_0	9·3
6	ATPase+CF_0+phospholipid	26·9
7	ATPase+CF_0+phospholipid+ oligomycin A (5mμmoles)	5·3
8	ATPase+aurovertin (50mμmoles)	18·2
9	ATPase+aurovertin (50mμmoles)+ CF_0	2·2
10	ATPase+aurovertin (50mμmoles)+ CF_0+phospholipid	4·5
11	ATPase+aurovertin (50mμmoles)+ CF_0+phospholipid+oligomycin A (5mμmoles)	2·6
12	ATPase+phospholipid+aurovertin (2mμmoles)	17·6
13	ATPase+aurovertin (5mμmoles)	17·6
14	ATPase+aurovertin (2mμmoles)	16·3
15	ATPase+aurovertin (0·2mμmole)	21·7
16	ATPase+aurovertin (0·1mμmole)	31·4
17	ATPase+aurovertin (0·05mμmole)	34·2
18	ATPase+aurovertin (0·025mμmole)	43·5

itself is not affected by oligomycin A (Expts. 1–4). These are all properties shown by factor F_1 (Kagawa & Racker, 1966*b*). The results support the suggestion that the two ATPases are identical.

Two alternative sites of action have been suggested for the inhibition of oxidative phosphorylation by aurovertin. Lardy, Connelly & Johnson (1964) have proposed that aurovertin acts on the respiratory-chain side of the oligomycin A-sensitive site, on the basis of the different quantitative actions of the two inhibitors against uncoupler-stimulated ATPase and swelling-agent-activated ATPase in mitochondria. Ernster, Lee & Janda (1966) have postulated, however, that aurovertin may act on the ATP side of the oligomycin A site, on the grounds that (1) ATP-utilizing reactions associated with oxidative phosphorylation are less sensitive to aurovertin than reactions leading to ATP synthesis, (2) aurovertin is unable to stimulate energy-linked reactions in 'EDTA particles', and (3) oligomycin but not aurovertin is able to inhibit the NADH oxidase activity in 'EDTA particles'. Also, H. A. Lardy & B. Chance (unpublished work cited by Ernster *et al.* 1966) have shown that aurovertin binds to and inhibits factor F_1. The terminal reaction of oxidative phosphorylation is suggested to be catalysed by factor F_1 (Penefsky, Pullman, Datta & Racker, 1960), and the oligomycin-sensitive site is on the factor CF_0 complex, the penultimate reaction in ATP synthesis (Kagawa & Racker, 1966*b*). It was therefore decided to determine whether aurovertin had an effect on the soluble ATPase or on factor CF_0. The results of Expts. 1, 8 and 13–18 (Table 1) show that the soluble ATPase was inhibited by more than 60% by 6·25mμmoles of aurovertin/mg. of protein of the soluble ATPase preparation (Expt. 15), and that 250 times this concentration of aurovertin caused no more than 70% inhibition (Expt. 8). Thus aurovertin inhibits the soluble ATPase, but this inhibition is incomplete at high concentrations, a situation that is also found in uncoupled mitochondria (Lardy, 1960). It is noteworthy that factor CF_0 is able to inhibit that part of the ATPase activity which is insensitive to aurovertin (Expts. 8–11).

The results suggest that the incomplete inhibition of ATPase activity by aurovertin in whole mitochondria may be a property of the terminal enzyme, i.e. factor F_1, rather than the result of a branched ATPase pathway as suggested by Lardy *et al.* (1964). The results support the conclusions of Ernster *et al.* (1966) that aurovertin acts on the ATP side of the oligomycin-sensitive site, and also confirm the results of Kagawa & Racker (1966*a*) and Lardy & Chance that aurovertin inhibits at a site located on the coupling factor F_1.

The authors thank Mrs L. A. Wilson for expert technical assistance, also Dr M. J. Selwyn for details of preparation of the ATPase before publication.

Ernster, L., Lee, C. P. & Janda, S. (1966). In *Colloq. Biochemistry of Mitochondria, 3rd Meeting Fed. Europ. biochem. Soc., Warsaw*, p. 29. Ed. by Slater, E. C. *et. al.*, London: Academic Press (Inc.) Ltd.

Fleischer, S. & Klouwen, H. (1961). *Biochem. biophys. Res. Commun.* **5**, 378.

Kagawa, Y. & Racker, E. (1966*a*) *J. biol. Chem.* **241**, 2461.

Kagawa, Y. & Racker, E. (1966*b*). *J. biol. Chem.* **241**, 2467.

Lardy, H. A. (1960). In *I.U.B./I.U.B.S. Symp. Biological Structure and Function, Stockholm*, vol. 2, p. 265. Ed. by Goodwin, T. W. & Lindberg, O. New York: Academic Press (Inc.) Ltd.

Lardy, H. A., Connelly, J. L. & Johnson, D. (1964). *Biochemistry*, **3**, 1961.

Penefsky, H. S., Pullman, M. E., Datta, A. & Racker, E. (1960). *J. biol. Chem.* **235**, 3330.

Pullman, M. E., Penefsky, H. S., Datta, A. & Racker, E. (1960). *J. biol. Chem.* **235**, 3322.

Selwyn, M. J. (1962). *Biochem. J.* **84**, 62P.

Selwyn, M. J. (1967). *Biochem. J.* (in the Press).