Biochem. J. (1976) **160**, 335–342
Printed in Great Britain

Mitochondrial Adenosine Triphosphatase of the Fission Yeast, *Schizosaccharomyces pombe* 972h⁻

CHANGES IN ACTIVITY AND INHIBITOR-SENSITIVITY IN RESPONSE TO CATABOLITE REPRESSION

By DAVID LLOYD and STEVEN W. EDWARDS
Department of Microbiology, University College, Newport Road, Cardiff CF2 1TA, Wales, U.K.

(*Received* 8 *June* 1976)

1. The specific activity of mitochondrial ATPase (adenosine triphosphatase) in extracts of *Schizosaccharomyces pombe* decreased 2.5-fold as the glucose concentration in the growth medium decreased from 50mM to 15mM. 2. During the late exponential phase of growth, ATPase activity doubled. 3. Sensitivity to oligomycin and Dio-9 as measured by values for I_{50} (μg of inhibitor/mg of protein giving 50% inhibition) at pH 6.8 increased sixfold and ninefold respectively during the initial decrease in ATPase activity, and this degree of sensitivity was maintained for the remainder of the growth cycle. 4. Increased sensitivity to *NN'*-dicyclohexylcarbodi-imide, triethyltin and venturicidin was also observed during the early stage of glucose de-repression. 5. Smaller increases in sensitivity to efrapeptin, aurovertin, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole, quercetin and spegazzinine also occurred. 6. The ATPase of glycerol-grown cells was less sensitive to inhibitors than that of glucose-repressed cells; change in values for I_{50} were not so marked during the growth cycle of cells growing with glycerol. 7. When submitochondrial particles from glycerol-grown cells were treated by passage through Sephadex G-50, a fourfold increase in activity was accompanied by increased inhibitor resistance. 8. Gel filtration of submitochondrial particles from glucose-de-repressed cells gave similar results, whereas loss of ATPase occurred in submitochondrial particles from glucose-repressed cells. 9. It is proposed that alterations in sensitivity to inhibitors at different stages of glucose de-repression may be partly controlled by a naturally occurring inhibitor of ATPase. 10. The inhibitors tested may be classified into two groups on the basis of alterations of sensitivity of the ATPase during physiological modification: (a) oligomycin, Dio-9, *NN'*-dicyclohexylcarbodi-imide, venturicidin and triethyltin, and (b) efrapeptin, aurovertin, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole, quercetin and spegazzinine.

The mitochondrial ATPase* of the fission yeast *Schizosaccharomyces pombe* (Goffeau *et al.*, 1972, 1973; Landry & Goffeau, 1972) has similar properties to the mammalian enzyme complex and to that from *Saccharomyces cerevisiae* (Kagawa & Racker, 1966; Schatz *et al.*, 1967; Tzagoloff & Meagher, 1971). The ATPase of glycerol-grown *S. pombe* was present at twice the activity of that of glucose-grown cells, and differences in stoicheiometry of the five subunit polypeptides of the soluble ATPases (F_1-ATPases) from glucose- and glycerol-grown cells were evident on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Goffeau *et al.*, 1973).

Gel filtration of submitochondrial particles from ox heart results in activation of ATPase (Racker & Horstman, 1967); a similar effect has been shown for particles from baker's yeast (Tzagoloff, 1969), but only when the cells were grown under conditions of glucose de-repression. An ATPase inhibitor has been characterized in mitochondria from *Candida utilis*; purification and amino acid analysis of this peptide (Satre *et al.*, 1975) indicates that it is different from the analogous peptide from ox heart (Pullman & Monroy, 1963; Brooks & Senior, 1971). When *C. utilis* is grown in the presence of high concentrations of a fermentable substrate the activity of the ATPase-inhibitor peptide is markedly decreased (Satre *et al.*, 1975).

S. pombe has a more fermentative pattern of metabolism than that of *C. utilis* (Lloyd, 1974). Glucose de-repression of some mitochondrial activities in the fission yeast (e.g. succinate dehydrogenase) occurs during exponential growth on glucose when the concentration of the carbon source falls to below 35mM; cytochrome *c* oxidase is de-repressed at 15mM-glucose (Poole & Lloyd, 1973). The catabolite de-repressed state may also be studied in glycerol-grown cells (Poole & Lloyd, 1974). In the present study we

* Abbreviations: ATPase, adenosine triphosphatase; Nbd-Cl, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole.

report that complex changes in specific activities of mitochondrial ATPase, and in its sensitivity to nine different inhibitors, occur during glucose de-repression and are correlated with the activity of a naturally occurring ATPase inhibitor. The changing responses to inhibitors also provide an insight into the probable sites of inhibitor action within the mitochondrial ATP synthetase complex.

Experimental

Maintenance, growth and harvesting of the organism

S. pombe 972h$^-$ was maintained on 3% (w/v) malt-extract agar and grown with forced aeration in minimal media containing either 1% glucose or 1% glycerol, exactly as described previously (Poole *et al.*, 1973; Poole & Lloyd, 1974). Organisms were counted in a Thoma haemocytometer slide (Hawkesley, Lancing, Sussex, U.K.). Harvesting of cultures was at 4°C by acceleration to 6000rev./min followed by immediate deceleration in the 6×250ml rotor of an MSE 18 centrifuge. Organisms were washed once by resuspending in 50mM-Tris/H_2SO_4 buffer (pH8.6) and then repeating the harvesting procedure.

Preparation of cell-free extracts

Disruption of organisms in 50mM-Tris/H_2SO_4 buffer, pH8.6, was by one slow passage through a chilled French press (Milner *et al.*, 1950) at 107MPa (15600lb/in^2). The resulting suspension was centrifuged at 4000rev./min (200*g*; $r_{av.}$ 7.6cm) for 5min in the 16×15ml rotor of an MSE 18 centrifuge to remove unbroken cells and cell-wall fragments; the supernatant (termed the 'cell-free extract') was decanted, care being taken to avoid disturbance of the lower layers.

Preparation of submitochondrial particles

Cell-free extracts were centrifuged at 18000rev./min (38000*g*; $r_{av.}$ 7.6cm) for 20min in the 8×50ml rotor of an MSE centrifuge to sediment mitochondrial fragments. After resuspension of the pellets in 5vol. of disruption buffer, treatment for 15s with ultrasound (20kHz, 500W MSE sonicator, power setting 5) gave a suspension of submitochondrial particles.

Gel filtration of submitochondrial particles

Gel filtration of submitochondrial particles was on a Sephadex G-50 column (0.9cm×15cm). Elution was with 50mM-Tris/H_2SO_4 buffer; the particles were eluted in the void volume (3.6ml).

Enzyme assays

ATPase (EC 3.6.1.3) was assayed by two methods. (*a*) Proton release was measured on adding extract to a stirred medium containing 10mM-Tris/H_2SO_4 buffer, 4mM-ATP and 6mM-$MgSO_4$, at pH8.6 or at 6.8. A trace of carbonic anhydrase prevented drift of pH caused by absorption of atmospheric CO_2. An EIL combination pH-electrode connected to a Pye model 79 pH-meter monitored the decreasing pH in the reaction mixture (2.0ml total volume). The output of the pH-meter was connected via an amplifier to a 50mV potentiometric recorder. Calibration was by adding known volumes of 100mM-HCl. (*b*) Extract was incubated in a medium containing 50mM-Tris/H_2SO_4 buffer, 4mM-ATP and 6mM-$MgSO_4$ at pH8.6 or at 6.8 in a total volume of 1ml. After the reaction was stopped by addition of 0.1ml of 50% (w/v) trichloroacetic acid, the protein precipitate was centrifuged off at 10^4*g*-min, and supernatant was assayed for P_i by the method of Fiske & Subba-Row (1925). Corrections for enzyme and substrate blanks were done as a routine.

All enzyme assays were performed within 6h of preparation of the extracts. Enzyme units are expressed as nmol of product formed/min at 30°C.

Other determinations

Protein. Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Glucose. Glucose was determined by using Sigma kit 510.

Chemicals

ATP, carbonic anhydrase and quercetin (3,3′,4′,5,7-pentahydroxyflavone) were from Sigma (London) Chemical Co. Ltd., Norbiton Station Yard, Kingston-upon-Thames, Surrey, U.K. Bovine serum albumin was from Armour Pharmaceutical Co., Eastbourne, Sussex, U.K. Sephadex G-50 was from Pharmacia Fine Chemicals, Uppsala, Sweden. *NN′*-Dicyclohexylcarbodi-imide and venturicidin were from British Drug Houses, Poole, Dorset, U.K.; this venturicidin is probably an approx. 4:1 mixture of venturicidin A and B. Oligomycin used was from Sigma and is a 17:3 mixture of oligomycin B and oligomycin A. Dio-9 was from Koninklijke Nederlandsche Gist and Spiritus fabriek, Delft, The Netherlands. A solution of Dio-9 containing 1mg/ml gives a $\Delta E_{303-400} = 0.1$ (Guillory, 1964); on this basis the commercial product was found to be only 40% pure. Nbd-Cl (7-chloro-4-nitrobenzo-2-oxa-1,3-diazole) was from Aldrich Chemical Co., Wembley, Middx., U.K. Triethyltin sulphate, efrapeptin (antibiotic A23871) and aurovertin were gifts from Dr.

D. E. Griffiths and Dr. T. G. Cartledge, Department of Molecular Sciences, University of Warwick, Coventry, U.K. Spegazzinine, a dihydroindole alkaloid, was a gift from the Division de Quimica Orgánica Superior, Facultad de Ciencias Exactas, Universidad Nacional de la Plata, La Plata, Argentina. All inhibitors were dissolved in dimethylformamide.

Results

Effect of pH on ATPase activity

ATPase assays performed at various pH values by using a cell-free extract of glucose-grown cells indicated the presence of three distinct pH optima. At the lowest of these (pH 6.2), little or no oligomycin-sensitivity was observed; that this enzyme activity is non-mitochondrial has been confirmed by Delhez & Goffeau (1975). The optima at both pH 6.8 and 8.6 are due to oligomycin-sensitive ATPase, and these were the pH values used in all further work.

Changes in ATPase activity and inhibitor-sensitivities during growth in the presence of glucose

In a medium that initially contained 1% glucose, exponential growth continued until a stationary-phase population (6.5×10^7 cells/ml) was attained (Fig. 1*a*). The mean generation time was 2.65h throughout the period of exponential growth. Total protein in the culture closely paralleled cellular growth and reached a maximum in the stationary phase of growth of 0.4mg/ml. Glucose, the limiting nutrient in these cultures, was utilized rapidly until 18h after inoculation, when the growth rate began to decline. At this stage the cells become glucose-de-repressed; the actual glucose concentration at which catabolite de-repression for different enzymes occurs shows considerable variation (Poole & Lloyd, 1973; Lloyd, 1974). Comparison of changes in ATPase activity (Fig. 1*b* and 1*c*) with those previously observed for cytochrome *c* oxidase, succinate dehydrogenase and malate dehydrogenase (Poole & Lloyd, 1973) indicates that the control of activity of this enzyme is more complex. At both pH 6.8 and 8.6 ATPase activity is high in the presence of repressing concentrations of glucose. As growth proceeds and glucose is utilized, ATPase activity declines. An increase in specific enzyme activity then occurs, perhaps owing to glucose de-repression of the biosynthesis of the enzyme complex, followed by a transient decrease as the growth rate falls and the cells enter the stationary phase of growth. Resolution of the total ATPase into its oligomycin- and Dio-9-sensitive and -insensitive components indicates that at both pH values the changes in total ATPase are closely similar to those seen for the inhibitor-sensitive enzyme. The most pronounced discrepancy between the effects of these two inhibitors is in the glucose-repressed organisms after 14h of growth. Changes in activities of the inhibitor-insensitive components were less pronounced (Figs. 1*b* and 1*c*); although an initial decrease in activity was evident between 14

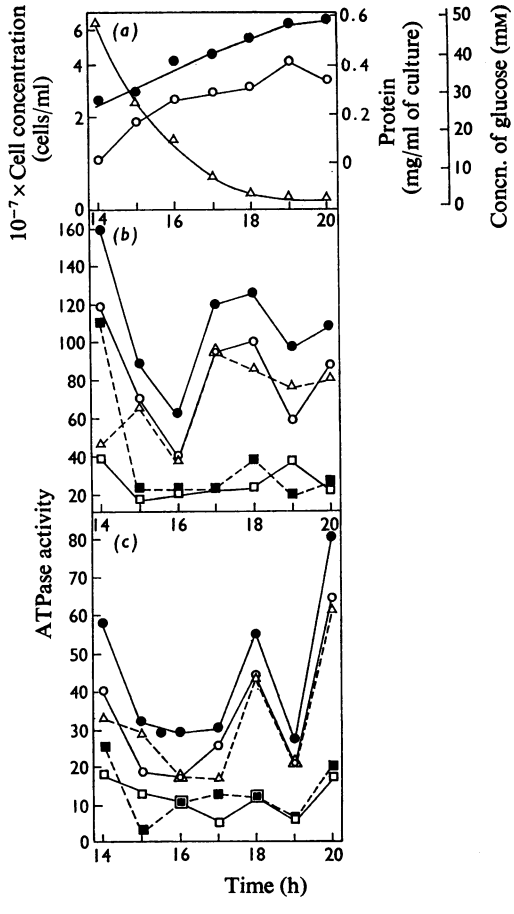

Fig. 1. *Changes in cell population, glucose and protein concentrations in the medium and specific activities of ATPase in cell-free extracts from S. pombe during growth in glucose-containing medium*

(*a*) Cell concentration (cells/ml; ●), glucose concentration (△) and protein concentration in the medium (○). (*b*) Total ATPase (●), oligomycin-sensitive ATPase (○), Dio-9-sensitive ATPase (△), oligomycin-insensitive ATPase (□) and Dio-9-insensitive ATPase (■) all measured by the proton-release method at pH 8.6. (*c*) Total ATPase (●), oligomycin-sensitive ATPase (○), Dio-9-sensitive ATPase (△), oligomycin-insensitive ATPase (□) and Dio-9-insensitive ATPase (■), all measured by proton-release method at pH 6.8. The enzyme activities are all expressed as specific activities (nmol/min per mg of protein). Inhibition data were corrected for the small degree of inhibition (<11%) produced by dimethylformamide.

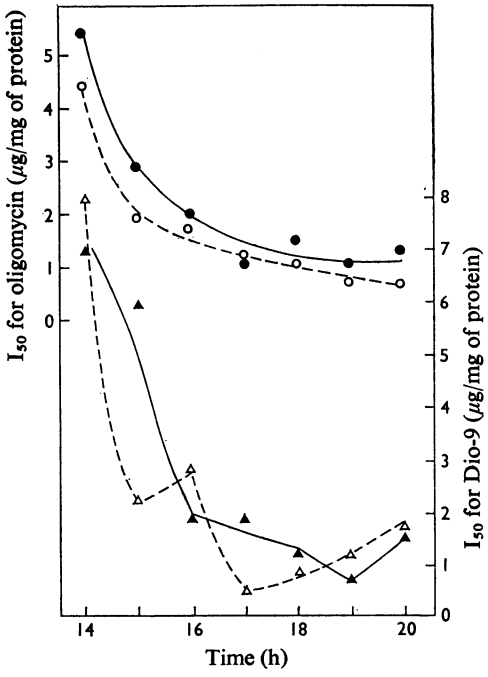

Fig. 2. *Changes in sensitivity to oligomycin and Dio-9 of ATPase in extracts from S. pombe during growth in glucose-containing medium*

Values are shown for I_{50} (μg/mg of protein) for oligomycin at pH6.8 (●) and at pH8.6 (○) and for Dio-9 at pH6.8 (▲), and at pH8.6 (△). Inhibition was corrected for the small degree of inhibition (<11%) produced by dimethylformamide.

and 15h, no clear glucose-de-repression effect was observed.

Measurement of amounts of inhibitors necessary to produce 50% inhibition (I_{50} values) indicates a marked decrease in inhibitor resistance between the glucose-repressed and -de-repressed conditions at both pH values (Fig. 2). Thus oligomycin-sensitivity increased sixfold, whereas Dio-9-sensitivity increased up to ninefold at pH6.8. The most marked increases in sensitivity to both inhibitors occurred before the glucose concentration in the medium decreased to 15mM, i.e. at glucose concentrations still high enough to repress many mitochondrial oxidative activities (Poole & Lloyd, 1973).

Table 1 summarizes the I_{50} values obtained for ten different inhibitors of mitochondrial ATPase. At both pH values used, between 70 and 100% of the total ATPase activity of the cell-free extracts may be inhibited at high inhibitor concentrations, and in most cases pronounced differences in the proportions of inhibitor-insensitive activity between glucose-repressed and de-repressed cells are not evident. However, some alteration in this respect is with Dio-9 or triethyltin at pH8.6 and efrapeptin at pH6.8, where inhibition of the ATPase of extracts of glucose-de-repressed cells was more complete. A wide range of values for I_{50} is indicated, e.g. at pH6.8 in extracts of glucose-repressed cells ATPase was on a molar basis, approx. 1600-fold more resistant to *NN'*-dicyclohexylcarbodi-imide than to venturicidin. The values for I_{50} of all inhibitors tested were less in extracts of glucose-de-repressed cells than in those pre-

Table 1. *Values for I_{50} of inhibitors of mitochondrial ATPase in extracts of glucose-repressed and glucose-de-repressed S. pombe and the percentage of total ATPase activity which was inhibitor-sensitive*

Glucose-repressed cells were harvested at a concentration of 1.8×10^7 cells/ml, and glucose-de-repessed cells at a concentration of 4.2×10^7 cells/ml. Titration curves for each inhibitor were obtained by adding small known volumes of inhibitor solutions (in dimethylformamide) to the ATPase reaction mixture (proton-release method). After correction for inhibition by solvent at each addition, values for I_{50} were calculated and expressed as μg of inhibitor/mg of protein necessary to give 50% inhibition. Total inhibitor-sensitive ATPase was determined by addition of excess of inhibitor and is expressed as a percentage of total ATPase.

	ATPase in extracts of							
	Glucose-repressed cells				Glucose-de-repressed cells			
	at pH6.8		at pH8.6		at pH6.8		at pH8.6	
Inhibitor	I_{50}	Maximum inhibition (%)	I_{50}	Maximum inhibition (%)	I_{50}	Maximum inhibition (%)	I_{50}	Maximum inhibition (%)
Oligomycin	5.0	70	3.4	75	0.8	80	0.6	80
NN'-Dicyclohexylcarbodi-imide	9.2	90	8.5	90	2.7	80	2.5	80
Triethyltin	0.065	90	0.058	55	0.012	90	0.020	80
Venturicidin	0.021	70	0.028	60	0.004	90	0.008	71
Dio-9	8.7	55	5.2	30	0.90	75	1.20	80
Efrapeptin	0.02	46	0.018	73	0.018	80	0.011	75
Aurovertin	0.80	95	0.75	65	0.85	90	0.45	58
Nbd-Cl	0.80	100	2.8	55	2.7	80	2.0	55
Quercetin	1.80	95	4.9	90	3.4	71	4.4	90
Spegazzinine	18.5	90	18.5	80	24.0	90	12.5	90

Table 2. *Changes in values of I_{50} for inhibitors of mitochondrial ATPase of S. pombe in cell-free extracts of glucose-repressed and de-repressed cells*

Values were calculated from the data shown in Table 1.

Inhibitor	Ratio $\frac{I_{50}\text{ at pH6.8}}{I_{50}\text{ at pH8.6}}$ for ATPase in extracts of: Glucose-repressed cells	Glucose-de-repressed cells	Ratio $\frac{I_{50}\text{ at pH8.6 (glucose-repressed)}}{I_{50}\text{ at pH8.6 (glucose-de-repressed)}}$
Oligomycin	1.47	1.34	5.67
NN'-Dicyclohexylcarbodi-imide	1.08	1.08	3.40
Triethyltin	1.12	0.60	2.90
Venturicidin	0.75	0.50	3.50
Dio-9	1.67	0.75	4.34
Efrapeptin	1.11	1.64	1.64
Aurovertin	1.06	1.89	1.66
Nbd-Cl	0.29	1.35	1.40
Quercetin	0.36	0.77	1.11
Spegazzinine	1.0	1.92	1.48

pared from glucose-repressed cells. Two classes of inhibitors can be distinguished on the basis of the extent of decreased I_{50} values during this transition (Table 2); oligomycin, *NN'*-dicyclohexylcarbodi-imide, triethyltin, venturicidin and Dio-9 all show a decrease greater than 2.9-fold, whereas efrapeptin, aurovertin, Nbd-Cl, quercetin and spegazzinine show a less than 1.66-fold change. A similar classification of inhibitors is evident on examination of the change in ratio I_{50} (pH6.8)/I_{50} (pH8.6) between ATPase of glucose-repressed and de-repressed cells (Table 2). This ratio declines for oligomycin, triethyltin, venturicidin and Dio-9; for *NN'*-dicyclohexylcarbodi-imide it remains constant, but increases for efrapeptin, aurovertin, Nbd-Cl and quercetin and spegazzinine.

Changes in ATPase activity and inhibitor-sensitivities during growth in the presence of glycerol

Exponential growth in the presence of glycerol continued until a stationary-phase population (7×10^7 cells/ml) was attained (Fig. 3*a*); the mean generation time was 5h throughout. Previous work has shown that the glycerol is still present (concn. approx. 40mM) when growth ceases (Poole & Lloyd, 1974). The specific activity of ATPase is high in the mid-exponential growth phase, about twice that of glucose-grown cells at the same stage (Fig. 3*b* and 3*c*). A transient decrease in specific activity is followed in the late exponential growth phase by a maximum, and then the activity declines as the growth rate falls. As the cells progress from exponential growth into the stationary phase of growth, increased inhibitor-sensitivity (as reflected by decreased values for I_{50}) was observed for oligomycin, Dio-9, efrapeptin and Nbd-Cl, whereas decreased inhibitor-sensitivity was observed for all five of the other inhibitors tested (Table 3). However, in general the ATPase of glycerol-grown cells is more inhibitor-resistant at all stages of growth than even the most inhibitor-resistant ATPase from glucose-grown cells, i.e. that from glucose-repressed cells; the only exceptions to this were for efrapeptin and triethyltin.

Gel filtration of submitochondrial particles

Table 4 shows the effects of gel filtration of submitochondrial particles on ATPase activity and inhibitor-sensitivity. Whereas particles from glucose-repressed cells show decreased ATPase activity, those from cells in the early stage of glucose de-repression and from glycerol-grown cells show marked increases in activity accompanied by decreased sensitivity to oligomycin, aurovertin and Dio-9.

Discussion

Glucose de-repression of mitochondrial ATPase in *S. pombe* occurs in two distinct phases. The initial stage occurs at a higher glucose concentration than that previously shown to de-repress mitochondrial oxidative activities (Poole & Lloyd, 1973), and involves both a decrease in activity and an increase in inhibitor-sensitivity. Gel filtration of submitochondrial particles indicates that the action of a natural inhibitor of ATPase activity becomes manifest during this growth phase. These changes are followed by a second phase of glucose de-repression which leads to increased enzyme activity but little alteration in inhibitor-sensitivity.

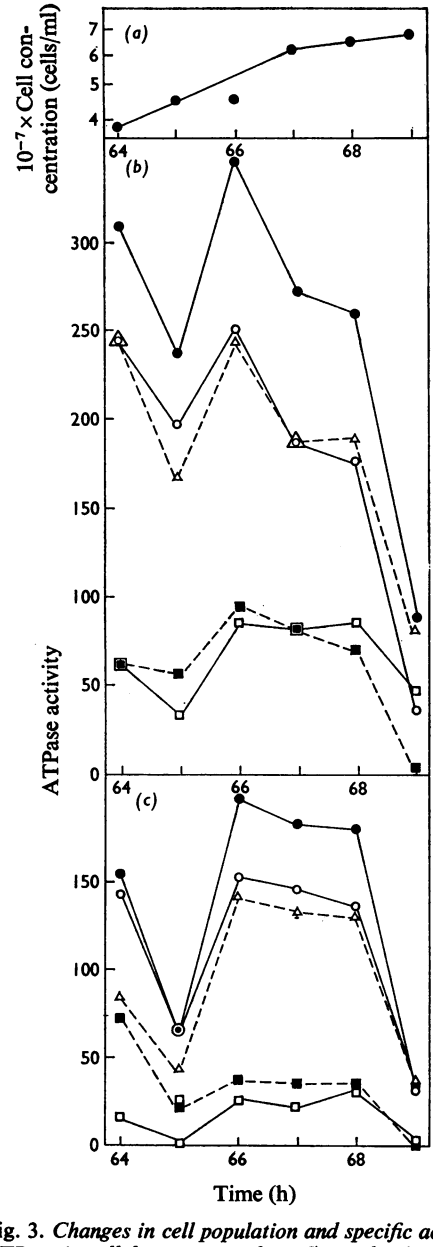

Fig. 3. *Changes in cell population and specific activities of ATPase in cell-free extracts from S. pombe during growth in glycerol-containing medium*

(*a*) Cell concentration (cells/ml). (*b*) Total ATPase (●), oligomycin-sensitive ATPase (○), Dio-9-sensitive ATPase (△), oligomycin-insensitive ATPase (□) and Dio-9-insensitive ATPase (■), all at pH 8.6. (*c*) Total ATPase (●), oligomycin-sensitive ATPase (○), Dio-9-sensitive ATPase (△), oligomycin-insensitive ATPase (□) and Dio-9-insensitive ATPase (■), all at pH 6.8. Inhibition data were corrected for the small degree of inhibition (<11%) produced by dimethylformamide. The enzyme activities are expressed as specific activities (nmol/min per mg of protein).

The mechanism of action of inhibitors used in studies of mitochondrial energy-conservation reactions and the exact location of inhibitor-binding sites within the ATP synthetase complex has not been elucidated. Oligomycin inhibits the terminal phosphate transfer that occurs during oxidative phosphorylation; it also inhibits ATPase in submitochondrial particles and in purified preparations of oligomycin-sensitive ATPase but not the activity of F_1-ATPase (Lardy *et al.*, 1964). The locus of the site of action of oligomycin is assumed to be at one or more of the four hydrophobic subunits which comprise the membrane factor of the oligomycin-sensitive ATPase. Other inhibitors acting on the membrane factor include venturicidin (Walter *et al.*, 1967), *NN'*-dicyclohexylcarbodi-imide (Bulos & Racker, 1968; Beechey *et al.*, 1967) and trialkyltin salts (Kagawa & Racker, 1966; Williamson & Metcalf, 1967; Stockdale *et al.*, 1970). Differences in the genetic loci determining resistance of mutants of *Sacc. cerevisiae* to these inhibitors suggest strongly that the inhibitor-binding sites for oligomycin are associated with two or possibly three of the mitochondrially synthesized subunits of the membrane factor (Avner & Griffiths, 1973; Griffiths & Houghton, 1974). Two independent cistrons located on mitochondrial DNA are involved in venturicidin resistance; one of these also determines an oligomycin-sensitivity site and the other is a determinant of a triethyltin-sensitivity site (Lancashire & Griffiths, 1975; Griffiths *et al.*, 1975). The reaction of *NN'*-dicyclohexylcarbodi-imide with yeast submitochondrial particles is not identical with that in mammalian systems, as in the yeast system the inhibitor does not become covalently bound (D. E. Griffiths, personal communication). Dio-9 and aurovertin inhibit F_1-ATPase (Lardy *et al.*, 1964; Roberton *et al.*, 1967; Slater & Ter Welle, 1969; Chang & Penefsky, 1973), as do efrapeptin (Lardy *et al.*, 1975), Nbd-Cl (Ferguson *et al.*, 1974), quercetin (Lang & Racker, 1974) and spegazzinine (Roveri & Vallejos, 1974). Thus the site(s) of action of these five inhibitors is quite different from the other four inhibitors used in this study.

The transition from the glucose-repressed to the glucose-de-repressed state is accompanied by large increases in sensitivity to oligomycin, *NN'*-dicyclohexylcarbodi-imide, triethyltin, venturicidin and Dio-9, but only small increases in sensitivity to the other four inhibitors. A similar sorting into two groups is evident on comparison of the changes in ratios of I_{50} at the two pH values between repressed and de-repressed states. Thus on the basis of previous evidence the behaviour of Dio-9 is unexpected; the present results suggest that it is acting more like those inhibitors that bind to the membrane factor rather than those reacting with F_1-ATPase itself. Previous work suggests that the action of Dio-9 in yeast may

Table 3. *Values for I_{50} for inhibitors of mitochondrial ATPase in cell-free extracts of glycerol-grown S. pombe and comparison with those for glucose-repressed cells*

Glycerol-grown cells were harvested at concentrations of 2.6×10^7 cells/ml (mid-exponential phase of growth) and at 8×10^7 cells/ml (stationary phase of growth). Inhibitor-sensitivities were determined as in Table 1.

	ATPase (pH8.6) in extracts of glycerol grown cells from					
	Mid-exponential phase		Stationary phase			
Inhibitor	I_{50} (A)	Maximum inhibition (%)	I_{50} (B)	Maximum inhibition (%)	Ratio A/B	Ratio $\frac{I_{50}\text{ (A)}}{I_{50}\text{ (pH8.6) (glucose-de-repressed)}}$
Oligomycin	14.0	67	5.0	45	2.8	23.3
NN'-Dicyclohexylcarbodi-imide	11.0	90	26.0	70	0.42	4.4
Triethyltin	0.048	80	0.044	90	1.09	2.4
Venturicidin	0.04	80	0.057	95	0.70	5.0
Dio-9	20.0	67	5.0	95	4.0	16.6
Efrapeptin	0.017	80	0.010	95	1.7	1.5
Aurovertin	2.0	68	12.50	70	0.16	4.4
Nbd-Cl	10.0	80	6.70	70	1.5	5.0
Quercetin	5.7	85	13.20	50	0.43	1.3

Table 4. *Changes in specific activities and inhibitor-sensitivities of ATPase in submitochondrial particles from glucose- and glycerol-grown S. pombe on treatment with Sephadex G-50*

Details of gel filtration are given in the Experimental section.

ATPase in submitochondrial particles from cells grown with ...	Glucose						Glycerol	
Harvested at a cell population of ...	2.2×10^7 cells/ml		2.7×10^7 cells/ml		4.2×10^7 cells/ml		2.6×10^7 cells/ml	
Sephadex G-50 treatment	−	+	−	+	−	+	−	+
Specific activity (nmol/min per mg of protein)	2546	1935	975	1450	1295	880	240	950
I_{50} (μg of inhibitor/mg of protein) for								
Oligomycin	3.2	5.0	4.5	4.8	2.2	3.8	6.4	12.5
Efrapeptin	0.42	0.14	0.26	0.28	0.28	0.33	0.01	0.19
Aurovertin	3.4	6.0	4.1	9.5	7.8	11.5	7.0	12.0
Dio-9	53.0	15.3	22.0	30.0	22.0	30.0	37.0	60.0

be different from that on heart F_1-ATPase (Schatz *et al.*, 1967).

Increased sensitivity to inhibition of the ATPase of submitochondrial particles after glucose de-repression accompanies the binding of a natural ATPase inhibitor to the ATP synthetase complex and may be reversed by gel filtration of the particles. Binding of the natural inhibitor produced only a small increase in sensitivity to those inhibitors that bind to F_1-ATPase, but a large increase in sensitivity to those reacting with the membrane factor. The functions of the natural inhibitor may be similar to those proposed for the F_1-ATPase-inhibitor peptide characterized from mammalian sources (Asami *et al.*, 1970; Racker & Horstman, 1972; Van de Stadt *et al.*, 1973), i.e. to control ATP-driven functions, to control ATPase activity in response to substrate oxidation rates and ATP/ADP ratios, or to prevent undesirable hydrolysis of ATP. The present system is ideal for further investigations of these alternatives.

The low sensitivity of mitochondrial ATPase in glycerol-grown cells to all the inhibitors studied requires further study. The marked differences between this ATPase and the enzyme in glucose-grown cells with respect to all the inhibitors other than venturicidin and triethyltin may arise in at least four different ways: (*a*) modifications of subunit structure or subunit complement of the ATP synthetase; (*b*) altered conformation of the complex or of the inner mitochondrial membrane; (*c*) the presence of compounds non-specifically adsorbing or inactivating the inhibitors, (*d*) alteration of the gross composition

of the inner mitochondrial membrane with respect to sterol and/or fatty acid composition. The last alternative seems unlikely, in view of the findings of Watson *et al.* (1975). The extent of changes in inhibitor-sensitivities of the ATPase of the wild-type organism under different physiological conditions of growth suggest possible non-specific mechanisms for increased inhibitor resistances in mutants that have been described [see Lloyd (1974) for a short review] and emphasize the necessity for defining growth conditions when ATPase preparations from these mutant organisms are characterized.

We acknowledge the technical assistance of Mrs. L. John and helpful discussions with Dr. D. E. Griffiths and Dr. T. G. Cartledge.

References

Asami, K., Juntii, K. & Ernster, L. (1970) *Biochim. Biophys. Acta* **205**, 307–311
Avner, P. R. & Griffiths, D. E. (1973) *Eur. J. Biochem.* **32**, 301–311
Beechey, R. B., Williams, V., Holloway, C. T. & Knight, I. G. (1967) *Biochemistry* **6**, 3867–3872
Brooks, J. C. & Senior, A. E. (1971) *Arch. Biochem. Biophys.* **147**, 467–470
Bulos, B. & Racker, E. (1968) *J. Biol. Chem.* **243**, 3891–3900
Chang, T.-M. & Penefsky, H. S. (1973) *J. Biol. Chem.* **248**, 2746–2754
Delhez, J. & Goffeau, A. (1975) *Abstr. FEBS Meet. 10th*, Abstr. no. 1191
Ferguson, S. J., Lloyd, W. J. & Radda, G. K. (1974) *FEBS Lett.* **38**, 234–236
Fiske, C. H. & SubbaRow, Y. (1925) *J. Biol. Chem.* **66**, 375–382
Goffeau, A., Colson, A.-M., Landry, Y. & Foury, F. (1972) *Biochem. Biophys. Res. Commun.* **48**, 1448–1454
Goffeau, A., Landry, Y., Foury, F., Briquet, M. & Colson, A.-M. (1973) *J. Biol. Chem.* **248**, 7097–7105
Griffiths, D. E. & Houghton, R. L. (1974) *Eur. J. Biochem.* **46**, 157–167
Griffiths, D. E., Houghton, R. L., Lancashire, W. E. & Meadows, P. A. (1975) *Eur. J. Biochem.* **51**, 393–402
Guillory, R. J. (1964) *Biochim. Biophys. Acta* **89**, 197–207
Kagawa, Y. & Racker, E. (1966) *J. Biol. Chem.* **241**, 2461–2466
Lancashire, W. E. & Griffiths, D. E. (1975) *Eur. J. Biochem.* **51**, 377–392
Landry, Y. & Goffeau, A. (1972) *Arch. Int. Physiol. Biochim.* **80**, 604–606
Lang, D. R. & Racker, E. (1974) *Biochim. Biophys. Acta* **333**, 180–186
Lardy, H. A., Connelly, J. L. & Johnson, D. (1964) *Biochemistry* **3**, 1961–1968
Lardy, H. A., Reed, P. & Lin, C.-H. C. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 1707–1710
Lloyd, D. (1974) *Mitochondria of Micro-organisms*, p. 553, Academic Press, London and New York
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
Milner, H. W., Lawrence, N. S. & French, C. S. (1950) *Science* **111**, 633–634
Poole, R. K. & Lloyd, D. (1973) *Biochem. J.* **136**, 195–207
Poole, R. K. & Lloyd, D. (1974) *Biochem. J.* **144**, 141–148
Poole, R. K., Lloyd, D. & Kemp, R. B. (1973) *J. Gen. Microbiol.* **77**, 209–220
Pullman, M. E. & Monroy, G. C. (1963) *J. Biol. Chem.* **238**, 3762–3769
Racker, E. & Horstman, L. L. (1967) *J. Biol. Chem.* **242**, 2527–2551
Racker, E. & Horstman, L. L. (1972) in *Energy Metabolism and Regulation of Metabolic Processes in Mitochondria* (Mehlman, M. A. & Hanson, R. W., eds.), pp. 1–25, Academic Press, New York
Roberton, A. M., Beechey, R. B., Holloway, C. T. & Knight, I. G. (1967) *Biochem. J.* **104**, 54c
Roveri, O. A. & Vallejos, R. H. (1974) *Biochim. Biophys. Acta* **333**, 187–194
Satre, M., De Jerphanion, M.-B., Huet, J. & Vignais, P. V. (1975) *Biochim. Biophys. Acta* **387**, 241–255
Schatz, G., Penefsky, H. S. & Racker, E. (1967) *J. Biol. Chem.* **242**, 2552–2560
Slater, E. C. & Ter Welle, H. F. (1969) in *Inhibitors, Tools in Cell Research* (Bucher, Th. & Sies, H., eds.), pp. 258–278, Springer-Verlag, Berlin
Stockdale, M., Dawson, A. P. & Selwyn, M. J. (1970) *Eur. J. Biochem.* **15**, 342–351
Tzagoloff, A. (1969) *J. Biol. Chem.* **244**, 5027–5033
Tzagoloff, A. & Meagher, P. (1971) *J. Biol. Chem.* **246**, 7328–7336
Van de Stadt, R. J., De Boer, B. L. & Van Dam, K. (1973) *Biochim. Biophys. Acta* **292**, 338–349
Walter, P., Lardy, H. A. & Johnson, D. (1967) *J. Biol. Chem.* **242**, 5014–5018
Watson, K., Houghton, R. L., Bertoli, E. & Griffiths, D. E. (1975) *Biochem. J.* **146**, 409–416
Williamson, R. L. & Metcalf, R. L. (1967) *Science* **158**, 1964–1966