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Activation and Inhibition of Mitochondrial Adenosine Triphosphatase by Various Anions and other Agents

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Abstract

The activating or inhibiting actions of a variety of anion species and of oligomycin, aurovertin and Dio-9 on the ATPase of a sonic particle preparation of rat liver mitochondria have been characterized by measurements of the relevant V_{max}, K_i and K_m values.

The normal V_{max} was increased by a factor near 7 by the anions: dichromate, chromate, pyrophosphate, orthophosphate, orthoarsenate and sulphate. The fully activating concentration varied from about 2 mM for dichromate to 150 mM for sulphate. The increase in V_{max} was accompanied by a time-dependent decrease in $(K_i)_{ADP}$, but there was no change in $(K_m)_{ATP}$. The increase in V_{max} by the activating anions was abolished by aurovertin; but in presence of oligomycin, the low V_{max} was increased by the activating anions by the same factor as the V_{max} in absence of oligomycin.

Certain anions, notably azide, decreased V_{max}, but did not affect $(K_i)_{ADP}$ or $(K_m)_{ATP}$. The decrease in V_{max} by azide and oligomycin were approximately additive. Even at high concentration, Dio-9 was without detectable effect on the ATPase, but it had a gramicidin-like effect on the intact mitochondria.

The specificity of the ATPase for ATP relative to GTP was found to be attributable to the high value of $(V_{max})_{ATP}$ compared with $(V_{max})_{GTP}$. The values of $(K_m)_{ATP}$ and $(K_m)_{GTP}$ were virtually the same.

Some rationalization of these and other supporting observations is attempted in terms of present knowledge of the constitution of the ATPase complex.

Introduction

The rate of hydrolysis of ATP by the coupling-factor component (F_1) of the ATPase of beef heart mitochondria was not apparently changed by 40 mM phosphate,[1] and a low (0·24 mM) phosphate concentration had little effect on the complete (oligomycin-sensitive) ATPase of rat liver mitochondria.[2] However, it was observed that physiologically normal levels of phosphate (20 mM) induced a seven-fold increase in V_{max} of the complete ATPase of rat liver mitochondria.[3] The low V_{max} of the ATPase in presence of oligomycin was likewise increased seven-fold by 20 mM phosphate; but the high V_{max} of the phosphate-activated ATPase was depressed to the phosphate-free level by aurovertin.[3]

In view of the participation of inorganic phosphate, both as an activator of the complete ATPase system and as a resultant of ATP hydrolysis, it was desirable to see whether any species of anion that would not be likely to participate directly in the hydrolytic reaction could act like phosphate in increasing V_{max}. The object of the work

described in this paper was therefore to observe the effect of a number of different anion species on the kinetic coefficients of the ATPase.

Materials and Methods

Rat liver mitochondria were isolated as described previously.[4] Sonic particles were prepared[2] from mitochondrial suspensions and were stored at 4° in 250 mM sucrose. As before,[2] the activity of the complete (oligomycin-sensitive) ATPase system was measured at 25° in 3·3 ml of medium near pH 7 containing 150 mM KCl, 3·3 mM glycylglycine, 5 mM $MgCl_2$ and sonic particles corresponding to 1·6 mg protein, using the pH method of Nishimura *et al.*,[5] in presence of 1 μM carbonylcyanide *p*-trifluoromethoxy phenylhydrazone (FCCP) to avoid possible effects of proton translocation. This method was previously shown to give identical results to a method depending on estimation of ATP by the luciferin–luciferase technique.[2] The ATP was added as a pulse (20 μl) of 150 mM KCl containing equimolar ATP and $MgCl_2$ adjusted to pH 7·05. For measurements of $(K_m)_{ATP}$ and V_{max} at a given concentration of an activating or inhibiting salt or antibiotic, the initial rate of the ATPase reaction was measured over a range of ATP concentrations between 30 and 300 μM and the results were analysed by means of Lineweaver–Burk plots, as previously described.[2,3] Similar measurements in presence of 60 μM ADP were used to obtain $(K_i)_{ADP}$ (see refs. 2 and 3). Analogous methods were employed to estimate K_m and K_i values for guanine nucleotides, and to estimate K_i values for GDP and ADP during ATP and GTP hydrolysis, respectively. When K_i underwent a time-dependent change initiated by adding nucleotide, the initial value and the value after 60 sec were estimated from Lineweaver–Burk plots of initial hydrolysis rates when the nucleoside diphosphate was added simultaneously with, or 60 sec in advance of, the nucleoside triphosphate, respectively, as described previously.[3]

Previous experiments,[3] employing atractyloside to inhibit adenine nucleotide translocation and *N*-ethylmaleimide to inhibit phosphate translocation, showed that translocation of ATP, ADP or phosphate was not rate-limiting under these experimental conditions.

Some measurements of the effective proton conductance of the cristae membrane of intact mitochondria were done by the respiratory pulse method;[6] and measurements of translocation of K^+ and of H^+ and/or OH^- were done by the osmotic swelling method.[7]

FCCP was a gift from Dr. P. G. Heytler of E.I. du Pont de Nemours and Co., Inc. (Wilmington, Delaware, U.S.A.) and Dio-9 was obtained from Royal Netherlands Fermentation Industries Ltd. (Delft, Holland). Aurovertin was generously provided by Dr. H. A. Lardy (Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin, U.S.A.) and by Dr. R. B. Beechey (Shell Research, Sittingbourne, Kent).

Simple organic and inorganic reagents were of Analar grade where available, or otherwise of the highest purity obtainable commercially.

Results and Discussion

Dependence of ATPase Activity on Ionic Strength and pH

Figure 1 shows that the initial rates of hydrolysis of 240 μM and 120 μM ATP by the ATPase in absence of ADP and of 120 μM ATP in presence of 60 μM ADP near

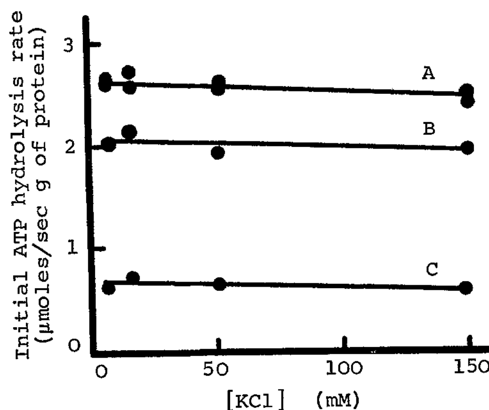

Figure 1. Effect of KCl concentration on the activity of the complete (oligomycin-sensitive) ATPase. Sonic particles (1·6 mg protein) were suspended in 3·3 ml of medium containing 5, 15, 50 or 150 mM KCl, 3·3 mM glycylglycine, 5 mM $MgCl_2$, 1 µM FCCP at pH 7·0 and at 25°. The osmotic pressure was maintained equivalent to 150 mM KCl by substituting 1·7 moles sucrose for 1 mole KCl. ATPase activity was initiated by pulses of ATP. Curves: A, 240 µM ATP; B, 120 µM ATP; C, 120 µM ATP + 60 µM ADP.

pH 7 were almost independent of KCl concentration between 5 mM and 150 mM, when the osmotic pressure was maintained equivalent to that of 150 mM KCl by substituting 1·7 moles sucrose for 1 mole KCl. Since the rate of the ATPase reaction was depressed by the same percentage over the range of KCl concentration from 5 to 150 mM in each case, it follows that V_{max} was depressed by this small amount (4%) and that $(K_m)_{ATP}/(K_i)_{ADP}$ did not change as the concentration of KCl was increased from 5 to 150 mM. Practically identical results were obtained by substituting Na^+ for K^+, but V_{max} values were depressed in the higher range of concentration when choline$^+$ was substituted for K^+. In 150 mM choline chloride, the value of V_{max} was 61% of that in 150 mM KCl.

Figure 2 shows that the ATPase activity in the routine 150 mM KCl medium was almost independent of pH between 6·5 and 8·0 in presence or absence of ADP or aurovertin. This is in contrast to the strong pH dependence of the isolated F_1 component of the ATPase of beef heart mitochondria.[1, 8]

The time-course of ATP hydrolysis in presence of aurovertin showed the same sigmoid characteristics at pH 6·5, 7·5 and 8·0 as previously described at pH 7·0 (see refs. 2 and 3), indicating time-dependent changes of $(K_m)_{ATP}$ and $(K_i)_{ADP}$.

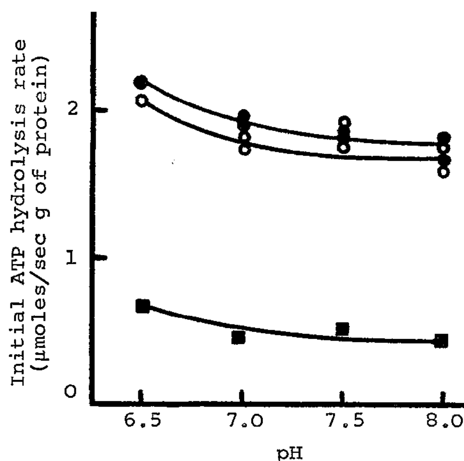

Figure 2. Effect of pH on the activity of the complete (oligomycin-sensitive) ATPase. Sonic particles (1·6 mg protein) were suspended in 3·3 ml of the routine KCl medium (150 mM KCl, 3·3 mM glycylglycine, 5 mM $MgCl_2$, 1 µM FCCP) at various pH values at 25°; and ATPase activity was initiated by adding ATP solutions which had been previously adjusted to the appropriate pH value. Because the Mg^{2+} salt of ATP tended to precipitate at alkaline pH values, $MgCl_2$ was omitted from ATP solutions at pH 7·5 and pH 8·0. Curves are identified as follows: ○, 120 µM ATP; ■, 120 µM ATP + 60 µM ADP; ●, 120 µM ATP added after particles had been preincubated for 10 min in presence of 12·5 mg aurovertin per gram of protein. Appropriate calibrations were used to allow the ATPase activity to be expressed as µmoles of ATP hydrolysed per second per gram of protein.

Dependence of ATPase Activity on Various Anion Species

Table I lists the ATPase activities near pH 7 observed in presence of various potassium salts at several concentrations in the routine KCl medium, the total concentration of anion being maintained constant at 150 mM. The activities are expressed as factors of the activity observed in a control consisting of the routine 150 mM KCl medium.

TABLE I. Effect of various anion species on the activity of the complete (oligomycin-sensitive) ATPase system

Anion	ATPase activity			
	0·5 mM	2 mM	5 mM	15 mM
Dichromate	4·0	7·5	7·0	
Chromate	3·8	6·8	6·4	5·8
Pyrophosphate		4·2	4·5	3·2
Orthophosphate	1·4	4·0	4·4	6·0
Orthoarsenate	1·3	1·6	3·1	5·5
Sulphate	1·1	1·5	2·4	4·9
Molybdate		0·9	1·1	1·6
Thiosulphate				1·04
Borate				0·90
Isethionate		1·0		0·91
Malonate				0·95
Citrate		0·80	0·91	
Oxalate		0·93	0·84	
β-Glycerophosphate		0·82		0·91
EDTA	0·80			
Nitrate				0·62
Chlorate		0·65		
Perchlorate		0·67	0·55	0·55
Thiocyanate				0·35

The ATPase activity of sonic particle preparations from rat liver mitochondria (1·6 mg protein) was estimated in presence of various anion species as the initial rate of hydrolysis of 150 μM ATP at pH 7·0 at 25°. The activity is expressed as a factor of the activity in control experiments in the routine KCl medium (3·3 ml 150 mM KCl, 3·3 mM glycylglycine, 5 mM $MgCl_2$, 1 μM FCCP) which gave a rate of hydrolysis of 2·0 μmoles ATP per second per gram of protein. When the listed anion species were included in the medium (as potassium salts at concentrations indicated), the KCl concentration was reduced to maintain the total anion concentration at 150 mM.

The anions shown in Table I are divided into three main classes: those that activated, those that had little or not effect, and those that inhibited. In the case of most of the anions that activated (dichromate, chromate, orthophosphate, orthoarsenate and sulphate), the maximal activation was by a factor near 7, as shown by the following activities of the ATPase (some relevant values at higher concentrations not being included in Table I): 2 mM dichromate, 7·5; 2 mM chromate, 6·9; 20 mM orthophosphate, 7·1; 20 mM orthoarsenate, 6·3; 150 mM sulphate, 5·9. In the case of activation by pyrophosphate, the lower value at 15 mM is attributable to depletion of Mg^{2+} required for the ATPase activity, since it was observed that a pH shift corresponding to

Mg^{2+} chelation occurred on adding the ATP (as Mg^{2+} salt) in 15 mM pyrophosphate, but not when the pyrophosphate concentration was 5 mM. This difficulty could not be overcome by adding more $MgCl_2$, because magnesium pyrophosphate precipitated under these circumstances.

Figure 3 shows that the time-course of ATP hydrolysis had the same relatively extended initial linear stage when activated by dichromate, chromate, pyrophosphate, orthoarsenate and sulphate as by orthophosphate. It was shown previously, in the case of orthophosphate,[3] that this was due to the fact that V_{max} was increased by the same factor as the initial rate of hydrolysis, that $(K_m)_{ATP}$ did not change, but there was a time-dependent fall of $(K_i)_{ADP}$, taking some 30 sec to complete, from an initial value

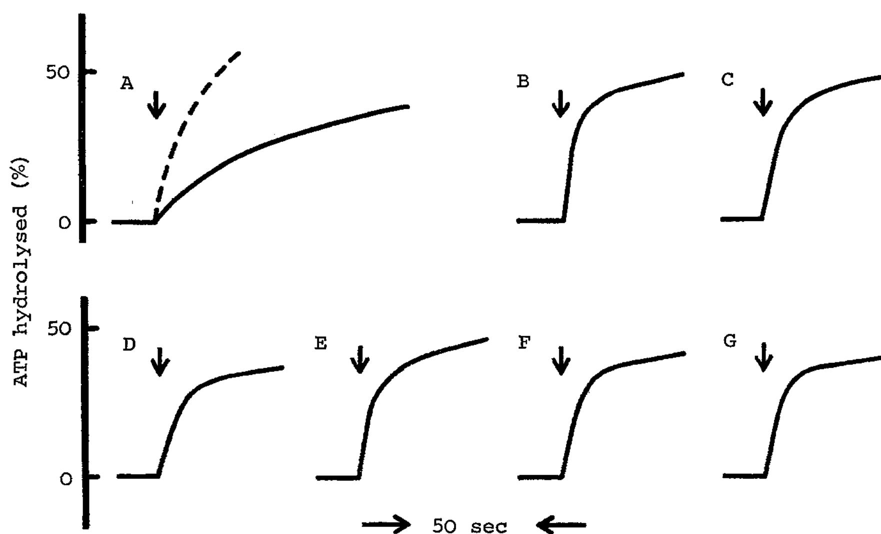

Figure 3. Time-course of ATP hydrolysis in presence and absence of activating anion species. Experimental conditions were as described in the legend to Table I. Curves: A, control; B, 2 mM dichromate; C, 2 mM chromate; D, 2 mM pyrophosphate; E, 20 mM orthophosphate; F, 20 mM orthoarsenate; G, 20 mM sulphate. The broken curve in A is the control time-course plotted with a time-scale contracted by a factor of seven to facilitate comparison of the shape with that in presence of the activating anion species. At the arrows, 150 μM ATP was added.

of about 50 μM to a final value of some 4·5 μM measured at 60 sec after addition of adenine nucleotide. The values of V_{max}, $(K_m)_{ATP}$ and $(K_i)_{ADP}$ for the ATPase activated by orthoarsenate and sulphate, described in Table II, were obtained from the usual data analysed as Lineweaver–Burk plots, and by measuring the initial rate of the ATPase reaction when ADP was added either simultaneously with ATP or 60 sec in advance, as before.[3] Thus, it was confirmed for orthoarsenate and sulphate that the activation of the ATPase, as given by the initial rate of ATP hydrolysis, could be quantitatively interpreted as an increase in V_{max}. The similar shapes of the curves representing the time-course of ATP hydrolysis in Fig. 3 indicate that the activation by dichromate, chromate and pyrophosphate may likewise be attributed to an increase in V_{max}.

The greatest increase of V_{max} obtained in presence of each of the activating anion species mentioned above was by a factor of about 7, but the concentration of the anion required to achieve this activation varied widely from some 2 mM for dichromate or

chromate to some 150 mM for sulphate. Molybdate appeared to be the least effective of the activating anion species, but it was not tested at a concentration above 15 mM.

Kinetic Coefficients for ATP and GTP Hydrolysis

The finding that, in contrast to the lack of effect on the F_1 component of the ATPase,[1] the activation of the complete (oligomycin-sensitive) ATPase by phosphate[3] was due to an increase in V_{max} appeared to be of interest in connection with observations on the relative rates of hydrolysis of ATP and GTP by F_1 and by the complete, reconstituted, ATPase, which indicated that the nucleotide specificity of F_1 favoured GTP hydrolysis, whereas that of the reconstituted ATPase favoured ATP hydrolysis.[9] Table II shows the results of measurements of V_{max}, and of the relevant K_m and K_i values during ATP and GTP hydrolysis in presence and absence of orthophosphate, and when ADP or GDP were added as inhibitors. In the absence of phosphate, $(K_m)_{ATP}$ and $(K_m)_{GTP}$ had the same value, but $(V_{max})_{GTP}$ was only 15% of $(V_{max})_{ATP}$. The hydrolysis of GTP was activated by phosphate to the same extent as ATP hydrolysis because both $(V_{max})_{ATP}$ and $(V_{max})_{GTP}$ were increased by the same factor, whereas $(K_m)_{ATP}$ and $(K_m)_{GTP}$ remained unchanged. As shown previously, the value of $(K_i)_{ADP}$ during ATP hydrolysis was changed in presence of phosphate and showed a time-dependent variation after addition

TABLE II. Kinetic coefficients of the complete (oligomycin-sensitive) ATPase system

		ATP hydrolysis	GTP hydrolysis
Control	$(K_m)_{ATP}$	106 μM	
	$(K_m)_{GTP}$		105 μM
	$(K_i)_{ADP}$	9 μM	
	V_{max}	3·4	0·51
Orthophosphate	$(K_m)_{ATP}$	(a) 106 μM	
(a) 2 mM		(b) 106 μM	
(b) 20 mM	$(K_m)_{GTP}$		(a) 110 μM
	$(K_i)_{ADP}$	(a) 4·5 μM*	(a) 4·0 μM*
		(b) 4·0 μM*	
	$(K_i)_{GDP}$	(a) 28 μM*	(a) 26 μM*
	V_{max}	(a) 13·6	(a) 2·1
		(b) 24·0	
Orthoarsenate, 5 mM	$(K_m)_{ATP}$	104 μM	
	$(K_i)_{ADP}$	4·5 μM*	
	V_{max}	10·5	
Sulphate, 20 mM	$(K_m)_{ATP}$	105 μM	
	$(K_i)_{ADP}$	3·0 μM*	
	V_{max}	18·3	

The ATPase activity of sonic particle preparations from rat liver mitochondria was measured at 25° at pH 7·0, either in the routine KCl medium (see legend to Table I) in absence of activating anion species (control), or in presence of the anions listed (at the concentrations indicated). In presence of activating anion species, $(K_i)_{ADP}$ and $(K_i)_{GDP}$ were time-dependent, and the values marked with asterisks are values obtained after incubation of the sonic particles with ADP or GDP for 60 sec before initiating ATP or GTP hydrolysis. The values of V_{max} are expressed as μmoles of ATP or GTP hydrolysed per second per gram of protein.

of nucleotide. The same type of time-dependent variation was seen in the case of $(K_i)_{ADP}$ and $(K_i)_{GDP}$ during both ATP and GTP hydrolysis in the phosphate-activated system. Nevertheless, as shown before[3] for inhibition of the ATPase reaction by ADP, inhibition of ATP or GTP hydrolysis by ADP or GDP was strictly competitive. It is noteworthy that in presence of phosphate, $(K_i)_{GDP}$ and $(K_i)_{ADP}$ had different values (Table II), but for each nucleoside diphosphate the K_i value was practically the same for ATP hydrolysis as for GTP hydrolysis.

In the case of the inhibitory anion species included in Table I, the time-course of ATP hydrolysis resembled that of the control in Fig. 3 and indicated that there was no time-dependent change in $(K_m)_{ATP}/(K_i)_{ADP}$. The inhibitory action of these ions can probably be attributed to a decrease of V_{max}, but could possibly be accounted for by an increase in $(K_m)_{ATP}$, accompanied by an equivalent increase in $(K_i)_{ADP}$.

Inhibition of ATPase Activity by Azide

Azide was found to have a much more potent inhibitory effect than the anion species listed in Table I, and this was studied in more detail.

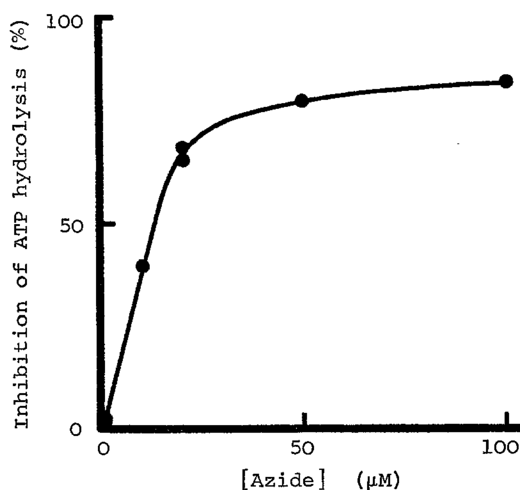

Figure 4. Inhibition of the complete (oligomycin-sensitive) ATPase by azide. Experimental conditions were as for the control experiments described in the legend to Table I, with the addition of azide (at various concentrations up to 100 μM) to the routine KCl medium. ATP was added at a concentration of 150 μM.

Figure 4 shows the dependence of inhibition of the ATPase on azide concentration. The extent of inhibition tended to a limiting value of some 90%. The Lineweaver–Burk plot in Fig. 5 shows that azide inhibition was strictly non-competitive with ATP, and was accounted for by a decrease of V_{max}. It is evident from Fig. 4 that, as in the case of inhibition by oligomycin,[2] V_{max} is not depressed much below some 10% of the normal value at high azide concentration. Half maximal inhibition occurred at 12 μM azide.

Meyerhof and Ohlmeyer[10] observed that half maximal inhibition of a soluble ATPase from yeast was given by 200 μM azide; and Pullman and others[1] found that half maximal inhibition of the F_1 component of the ATPase from beef heart mitochondria was given by less than 40 μM azide. Vigers and Ziegler[11] found that the concentration of azide required to inhibit soluble ATPases from beef heart and rat liver mitochondria was similar, but half maximal inhibition was observed at some 100 μM azide. The complete

(oligomycin-sensitive) ATPase studied here appears to be more sensitive to azide than these soluble ATPase preparations.

It was desirable to investigate whether the effects of azide and an activating anion species might be independent or mutually exclusive. It was observed in experiments similar to those of Table I that after 200 μM azide had depressed the ATPase activity to 0·09 of the control value, the addition of 20 mM sulphate raised it to 1·03 times the control value. The rate observed in presence of 20 mM sulphate alone was 5·1 times the control value, showing that azide, unlike aurovertin,[3] did not abolish the activating effect of sulphate. In this respect, azide resembled oligomycin.

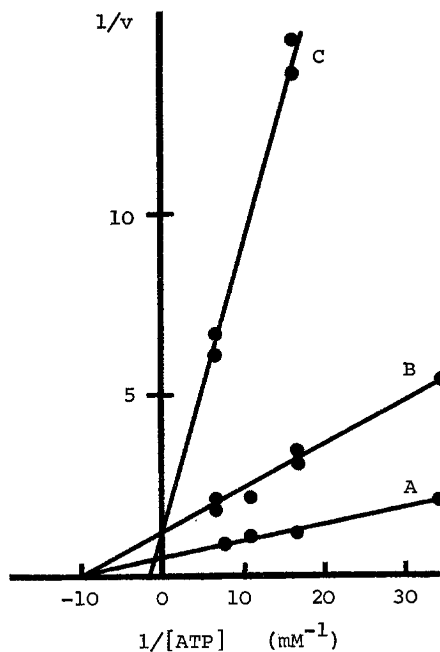

Figure 5. Lineweaver–Burk plot showing the effect of azide on the initial rate of ATP hydrolysis by the complete ATPase. Conditions were as for Fig. 4. Plots: A, no azide; B, in presence of 15 μM azide; C, in presence of 15 μM azide and with 60 μM ADP added simultaneously with the ATP pulse. The value of V_{max} in presence of 15 μM azide corresponds to the hydrolysis of 1·3 μmoles ATP per second per gram of protein.

Actions of Aurovertin, Oligomycin and Dio-9 on the ATPase

Since we previously observed that aurovertin prevented activation of the complete ATPase by phosphate, and Dio-9 was reported to have some properties rather similar to those of aurovertin (see ref. 12), it was desirable to examine the action of Dio-9 on the kinetic coefficients of the ATPase. There was no effect of Dio-9 (63 mg/g protein) on V_{max}, $(K_m)_{ATP}$ or $(K_i)_{ADP}$. When the ATPase was activated by 20 mM phosphate or 15 mM sulphate, Dio-9 likewise had no effect on the kinetic coefficients. The presence of Dio-9 (63 mg/g protein) did not affect the oligomycin-sensitivity of the ATPase.

At a concentration of 15 mg/g mitochondrial protein, Dio-9 was found to increase the normal effective proton conductance of the cristae membrane of rat liver mitochondria by a factor of about 5, using respiratory pulse methods similar to those described previously.[6] It was also observed by the use of Dio-9 to activate mitochondrial swelling in isotonic potassium phosphate, using similar methods to those described before,[7] that the action of Dio-9 resembled that of gramicidin or a mixture of FCCP and valinomycin. A careful survey of the literature[13–18] revealed that Dio-9 was found to inhibit

the ATPases of mitochondria, bacteria or chloroplast grana only when used at concentrations such that the weight of Dio-9 was comparable to the weight of protein in the ATPase preparation. We conclude that, at least in mitochondria, contrary to the widely accepted belief,[12] Dio-9 does not uniquely affect the ATPase, and probably influences ATP synthesis by an indirect mechanism similar to that of gramicidin.

As previously shown for the case of orthophosphate,[3] we observed that the presence of aurovertin (2·5 mg/g protein) abolished the activating effects of orthoarsenate and sulphate shown in Table II. Similarly, as previously shown for orthophosphate,[3] we observed that orthoarsenate and sulphate caused the low $(V_{max})_{ATP}$ of the ATPase in presence of oligomycin (6·8 mg/g protein) to be increased by the same factor as that in the absence of oligomycin, while $(K_m)_{ATP}$ and $(K_i)_{ADP}$ remained unchanged. It was also found that $(V_{max})_{GTP}$ was depressed by the same factor by oligomycin (6·8 mg/g protein) as $(V_{max})_{ATP}$, and that $(K_m)_{GTP}$ remained unchanged.

In experiments similar to those of Table I, we observed that after 200 μM azide had depressed the ATPase activity (for ATP hydrolysis) to 0·09 of the control value, the addition of oligomycin (6·8 mg/g protein) depressed it further to 0·023 of the control value, whereas the rate in presence of oligomycin alone was 0·08 of the control value. Thus, the low ATPase activity in presence of azide was mostly susceptible to inhibition by oligomycin; and in this sense the two inhibitors act almost additively.

Some Possible Interpretations of the Changes of V_{max}, K_m and K_i of the ATPase System

The original object of the studies in this paper was to compare the different anion species that activated the ATPase, in case some conclusions might be drawn as to whether activation involved direct participation of the activating ions in the hydrolytic reaction. The observations show that all the activating anion species carry two negative charges at pH 7, but this is not a sufficient condition for activation since thiosulphate, malonate and oxalate have no effect.

Some of the activating anions have the two negative charges associated with oxygen atoms bound to the same atom, as in sulphate, but others have the two negative charges associated with oxygen atoms bound to different atoms, as in dichromate. While it is evident that there are resemblances between phosphate and all the anion species found to activate the ATPase, and all the activating anion species also decrease $(K_i)_{ADP}$, so that it is possible that activation might involve direct participation of the anions in the hydrolytic reaction, the present evidence does not allow us to draw definite conclusions in this respect.

Selwyn[19] has attributed some activating effects of the 2,4-dinitrophenate anion on the ATPase activity of the isolated F_1 component of the ATPase of beef heart mitochondria to binding with Mg^{2+} involved in the ATPase reaction, and it is conceivable that some special binding relationship with Mg^{2+} could be involved in the activation of the complete ATPase by the divalent anions described here. It is relevant, however, that the divalent metal chelators, citrate, oxalate and EDTA, do not activate the ATPase.

We have observed that V_{max} and K_m (or K_i) may be independently varied by certain pairs of reagents, such as oligomycin and aurovertin respectively,[2] as would be expected

for a "classical" type of enzyme-catalysed reaction, and as indicated previously for the case when the ATPase complex catalyses ATP synthesis.[20] However, certain pairs of reagents, such as oligomycin and phosphate[3] (or other activating anion species) may independently decrease and increase V_{max} respectively, as though there are two independent rate-limiting parts of the ATPase complex. Also, certain pairs of reagents that might be expected to act independently because one affects V_{max} and the other affects K_m (or K_i), such as phosphate and aurovertin, respectively, are actually mutually exclusive, since, for example, aurovertin abolishes the increase of V_{max} induced by phosphate.[3] In this latter case, however, it must not be overlooked that the increase of V_{max} by the activating anion species, which is the predominant effect observed, is invariably accompanied by a time-dependent decrease in $(K_i)_{ADP}$ [or $(K_i)_{GDP}$].

Aurovertin has been shown to react stoichiometrically with the isolated F_1 component of the ATPase of beef heart mitochondria,[21] and the quantity of aurovertin bound by mitochondrial membranes is consistent with the estimated amount of F_1 present.[22] Recent work[23] on the relative sites of action of oligomycin and aurovertin on the ATPase system during ATP synthesis confirms that aurovertin interacts with a component of the system closer to the nucleotide-accepting and phosphate-accepting component (presumed to be F_1) than oligomycin. The fact that aurovertin abolishes the effects of phosphate, arsenate and sulphate on V_{max} and $(K_i)_{ADP}$, suggests that the activating anion species may exert their effects on the ATPase by interaction with the F_1 component. This would be consistent with the fact that oligomycin inhibition (decrease of V_{max}) is independent of anion activation (increase of V_{max}), since oligomycin is known[24] to react with a component of the ATPase complex other than F_1. It is also relevant that the specificity of the ATPase complex for ATP hydrolysis (compared with GTP), presumably determined by the nucleotide-accepting F_1 component, is dependent, not on a difference between $(K_m)_{ATP}$ and $(K_m)_{GTP}$, but on the relatively high value of $(V_{max})_{ATP}$ compared with $(V_{max})_{GTP}$.

According to the above interpretation, the overall V_{max} of the ATPase reaction would have to be determined by interaction between two different parts of the ATPase complex, one being F_1 and the other being the part that includes the oligomycin-reactive component. To explain the mutually independent contributions of F_1 and of the other part of the ATPase complex to the overall V_{max}, it would be necessary for the through-reaction to depend on both parts being simultaneously in the appropriate state, so that the frequency of reaction could depend on the coincidence-time during which both parts were independently reactive. This is, of course, only one possible explanation of the kinetic data.

The question obviously arises as to whether the oligomycin-insensitive ATPase activity might be attributed to F_1 that is dislocated (either for part of the time or permanently) from other components of the complex. The fact that the ATP/GTP nucleotide specificity is the same in presence and absence of oligomycin, whereas the isolated F_1 component of the ATPase (of beef heart mitochondria) is reported to have a higher specificity for GTP than the whole ATPase complex,[9] suggests that the F_1 component of the material or state of the complex catalysing the oligomycin-insensitive reaction is not, at all events, completely dislocated from the other components. The rather similar sensitivities of the normal and oligomycin-insensitive ATPase activities to inhibition by azide also suggest that the oligomycin-insensitive ATPase reaction may mainly be

attributed to the complete ATPase complex operating at a V_{max} value of only some 0·08 of the normal value.

A possible explanation for the almost additive inhibitory effects of azide and oligomycin might be that oligomycin affects only V_{max}, but azide acts as a competitive inhibitor for OH^- ions participating in the hydrolytic reaction.

Skye *et al.*,[25] noted an "unexplained stimulation of ATPase by addition of P_i" in the course of studies of oxygen and phosphate exchange reactions catalysed by the ATPase of chloroplasts from lettuce leaves. The observed activation by 2 mM phosphate was by less than a factor of 2, but it indicates that the ATPase of chloroplasts may possibly resemble that of mitochondria in being activated by phosphate and other anions.

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References

1. M. E. Pullman, H. S. Penefsky, A. Datta, and E. Racker, *J. Biol. Chem.*, **235** (1960) 3322.
2. P. Mitchell and J. Moyle, *FEBS Letters*, **6** (1970) 309.
3. P. Mitchell and J. Moyle, *FEBS Letters*, **9** (1970) 305.
4. P. Mitchell and J. Moyle, *Biochem. J.*, **104** (1967) 588.
5. M. Nishimura, T. Ito, and B. Chance, *Biochim. Biophys. Acta*, **59** (1962) 177.
6. P. Mitchell and J. Moyle, *Biochem. J.*, **105** (1967) 1147.
7. P. Mitchell and J. Moyle, *European J. Biochem.*, **9** (1969) 149.
8. M. J. Selwyn, *Biochem. J.*, **105** (1967) 279.
9. D. H. MacLennan and J. Asai, *Biochem. Biophys. Res. Commun.*, **33** (1968) 441.
10. O. Meyerhof and P. Ohlmeyer, *J. Biol. Chem.*, **195** (1952) 11.
11. G. A. Vigers and F. D. Ziegler, *Biochem. Biophys. Res. Commun.*, **30** (1968) 83.
12. E. C. Slater and H. F. Ter Welle, in: *Inhibitors, Tools in Cell Research*, Th. Bücher and H. Sies (eds.), Springer Verlag, Berlin, 1969, p. 258.
13. R. J. Guillory, *Biochim. Biophys. Acta*, **89** (1964) 197.
14. R. R. Fisher and R. J. Guillory, *Biochim. Biophys. Acta*, **162** (1968) 182.
15. G. Schatz, *J. Biol. Chem.*, **243** (1968) 2192.
16. F. M. Harold, J. R. Baarda, C. Baron, and A. Abrams, *Biochim. Biophys. Acta*, **183** (1969) 129.
17. R. E. McCarty, R. J. Guillory, and E. Racker, *J. Biol. Chem.*, **240** (1965) PC 4822.
18. R. E. McCarty and E. Racker, *J. Biol. Chem.*, **243** (1968) 129.
19. M. J. Selwyn, *FEBS Letters*, **1** (1968) 247.
20. C. P. Lee and L. Ernster, *European J. Biochem.*, **3** (1968) 391.
21. H. A. Lardy and S. M. Ferguson, *Ann. Rev. Biochem.*, **38** (1969) 991.
22. B. Chance, A. Azzi, I. Y. Lee, C. P. Lee, and L. Mela, *FEBS Symp.*, **17** (1969) 233.
23. R. L. Cross and J. H. Wang, *Biochem. Biophys. Res. Commun.*, **38** (1970) 848.
24. M. E. Pullman and G. Schatz, *Ann. Rev. Biochem.*, **36** (1967) 539.
25. G. E. Skye, N. Shavit, and P. D. Boyer, *Biochem. Biophys. Res. Commun.*, **28** (1967) 724.