CHANGES IN INHIBITOR SENSITIVITY OF THE MITOCHONDRIAL ATPase ACTIVITY AFTER DETERGENT SOLUBILISATION

Paul E. LINNETT, A. David MITCHELL and R. Brian BEECHEY
Shell Research Limited, Woodstock Laboratory, Sittingbourne Research Centre, Sittingbourne, Kent ME9 8AG UK

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1. Introduction

Tzagoloff and Meagher have isolated a purified, oligomycin-sensitive form of the mitochondrial ATPase from yeast submitochondrial particles (SMP) with Triton X-100 [1]. In this paper we describe the properties of a similar preparation from beef heart SMP (Triton-ATPase). This ATPase activity is inhibited by oligomycin, peliomycin, ossamycin and aurovertin B. However, *N,N'*-dicyclohexylcarbodiimide (DCCD) is without effect and low concentrations of venturicidin A stimulate the ATPase activity in a markedly time-dependent fashion. The results suggest a functional separation of the sites of inhibitory action of DCCD, oligomycin and venturicidin A and reveal a novel venturicidin stimulatory mechanism.

2. Materials and methods

Beef heart SMP were prepared by the method of Hansen and Smith [2]. ATPase activities were measured at 30°C and pH 8.2 [3].

The Triton-ATPase was prepared as follows. SMP suspended in 0.25 M sucrose and 10 mM tris sulphate, pH 7.7, 0°C, at a concentration of about 15 mg of protein/ml were mixed with an appropriate volume of 10% (w/v) Triton X-100, adjusted to pH 7.7, to give 1 part of Triton to 2.5 parts of protein by weight [1]. The supernatant fraction obtained after centrifugation at 100 000 *g* for 1 hr was used directly. It could be stored for several days at 4°C or frozen at −15°C without apparent change in specific activity or response to inhibitors. Venturicidin A, aurovertin B and [^{14}C]DCCD were purified samples from this laboratory [4–6]. Peliomycin, ossamycin and triethyltin sulphate were the kind gifts of Drug Research and Chemotherapy, National Cancer Institute, USA and Dr D. E. Griffiths, University of Warwick, UK.

3. Results and discussion

The specific activity of the Triton-ATPase was lower than that of the starting SMP. This contrasts with the preparation from yeast at a similar stage of purification which had an an increased specific activity [1]. Whilst the beef heart Triton-ATPase was inhibited by low concentrations of oligomycin, it was somewhat less sensitive than the original SMP ATPase (see table 1) [7]. Peliomycin and ossamycin, which have a similar mode of action to oligomycin [8], and aurovertin B which inhibits the ATPase molecule directly [9], also inhibited the Triton-ATPase activity to a lesser extent than they inhibited the membrane-bound ATPase (table 1). The sensitivity to triethyltin sulphate was reduced in the Triton-ATPase in a somewhat variable manner. This inhibition by oligomycin, peliomycin and ossamycin implies that there is neither appreciable alternation in the binding site(s) for these inhibitors which is located on the membrane and not on the ATPase molecule itself [10], nor in the mechanism of the interaction between the binding site(s) and the ATPase molecule.

DCCD is an irreversible inhibitor of the membrane-bound ATPase [11] being covalently bound to a membrane proteolipid [12]. The specific radioactivity of the Triton-ATPase prepared from SMP previously inhibited with [^{14}C] DCCD showed that the DCCD-

Table 1
Sensitivity of the Triton-ATPase to inhibitors

Inhibitor	SMP			Triton-ATPase			
	ATPase activity (μmol · min^{-1} · mg of protein^{-1})	Inhibitor concentration (nmol · mg of protein^{-1})	% inhibition	ATPase activity (μmol · min^{-1} · mg of protein^{-1})	Inhibitor concentration (nmol · mg of protein)	% Inhibition	% Stimulation
None	3.04	–	–	1.14	–	–	–
Oligomycin	0.19	4.6	94	0.28	6.2	75	–
Peliomycin	0.34	3.1	89	0.22	6.2	81	–
Ossamycin	0.47	3.9	85	0.26	6.2	77	–
Triethyltin sulphate	0.27	34	91	0.55	17	52	–
Venturicidin A	0.17	3.4	95	2.97	31	–	162
Aurovertin B	0.44	29	86	0.40	35	65	–

Either alcoholic solutions of inhibitors or alcohol were added to the SMP or Triton-ATPase in the ATPase assay mix and preincubated for 5 min at 30°C. The assays were initiated by addition of ATP. It was assumed that the mol. wts of peliomycin and ossamycin were the same as for oligomycin B, 804 [19].

binding component was solubilised more efficiently than was the ATPase activity, which was no longer inhibited (table 2). Thus, Triton X-100 solubilisation of the ATPase disrupts the interaction of the ATPase molecule and the DCCD-binding site.

Venturicidin A is an inhibitor which has some functional similarities to oligomycin [8] but biochemical [4,8,13] and genetic studies [14,15] have suggested a different mechanism of action. It can be seen from table 1 that venturicidin A inhibited the SMP ATPase activity but relatively low concentrations stimulated the Triton-ATPase activity 2.5-fold (fig.1). This stimulation was time-dependent. Whereas oligomycin inhibition was complete by 2 min,

Table 2
The effect of Triton X-100 solubilisation on the ATPase activity of [^{14}C]DCCD-inhibited beef heart SMP.

Treatment	Control SMP	[^{14}C]DCCD-treated SMP	
	ATPase activity (μmole · min^{-1} · mg of protein^{-1})	ATPase activity (μmol · min^{-1} · mg of protein^{-1})	[^{14}C]DCCD bound (nmol · mg of protein^{-1})
Before detergent	3.3	0.1	1.0
Triton ATPase	0.6	0.8	1.7

Washed SMP were treated overnight at 0°C with methanol containing 1 nmol of DCCD · mg of protein^{-1} (3.1 Ci · mol^{-1}) or with methanol in the control. After centrifugation at 100 000 *g*, the pellets were resuspended in 0.25 M sucrose. 10 mM Tris sulphate buffer, pH 7.7, and their ATPase activities and radioactivity were measured. The Triton-ATPase preparations were made and their ATPase activities and radioactivity were measured.

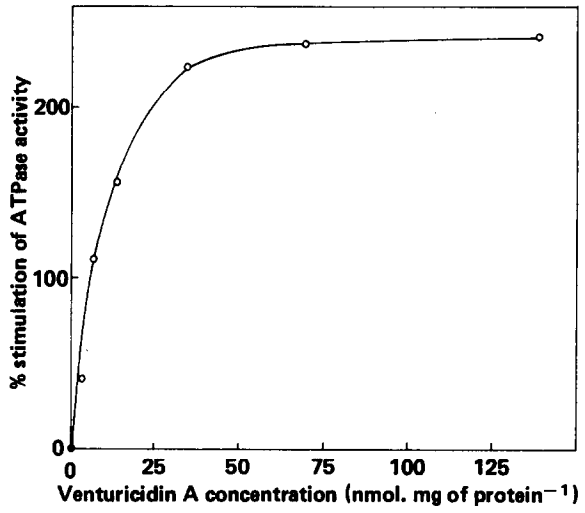

Fig.1. Stimulation of the Triton-ATPase by venturicidin A. Aliquots of venturicidin A in methanol (or methanol for controls) were preincubated with the Triton-ATPase in the ATPase assay medium for 5 min at 30°C. Initial ATPase activity was 0.23 μmol · min^{-1} · mg of protein^{-1}.

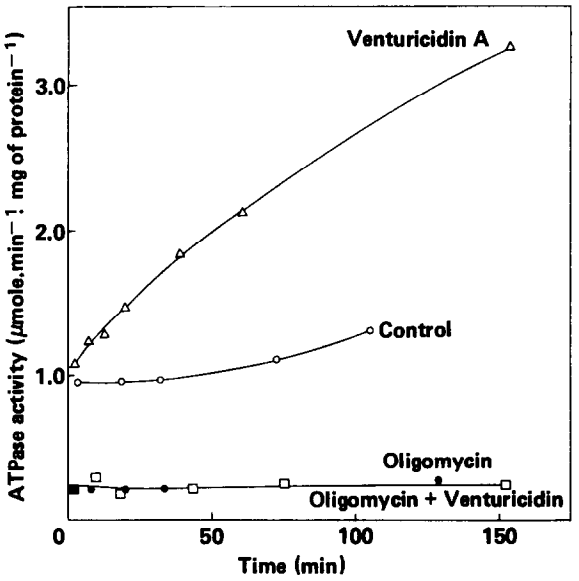

Fig.2. Time dependence of the effects of inhibitors on the Triton-ATPase. The Triton-ATPase (0.32 mg of protein in 200 μl) was mixed with (○) 3 μl of ethanol and 1 μl of methanol; (□) 3 μl of ethanolic oligomycin (1.0 mg · ml^{-1}); (△) 1 μl of methanolic venturicidin A (3.3 mg · ml^{-1}); (●) 4 μl of previously mixed 3:1 oligomycin and venturicidin A solutions, and incubated at 30°C. Aliquots (25 μl) were extracted and assayed for ATPase activity at the times specified.

the ATPase stimulation appeared to be increasing after 2.5 hr of incubation of the Triton-ATPase with venturicidin A (fig.2). Treatment of the Triton-ATPase with a mixture of oligomycin and venturicidin A caused a comparable inhibition to that with oligomycin alone, even after 1.5 hr (fig.2), which shows that venturicidin is unable to relieve oligomycin inhibition in this case. The explanation for this oligomycin 'dominance' is uncertain.

The Triton-ATPase from trypsin-treated SMP [16] which are deficient in the endogenous inhibitor [17] was still stimulated by venturicidin A (data not shown). This excludes a mechanism for the stimulation due to the displacement of the inhibitor protein by venturicidin. Venturicidin A in the presence of Triton X-100 had no effect on the activity of the soluble ATPase prepared by chloroform treatment of beef heart SMP [18] even after prolonged incubation at 30°C, which rules out a direct interaction with the ATPase molecule itself.

The present results show that Triton treatment of the mitochondrial membrane-bound ATPase modifies its reponse to some inhibitory compounds. Thus, DCCD covalently bound to its site of action does not exert an inhibitory effect on the ATPase in the presence of Triton. The venturicidin site(s) is also modified such that the functional effect of venturicidin is to stimulate rather than to inhibit the Triton-ATPase. This could be due to either; (1) a complete dissociation of the interaction of the inhibitory venturicidin site with the ATPase, enabling a stimulatory venturicidin site to manifest itself, or (2) the conversion of an inhibitory venturicidin binding site to a stimulatory site, or (3) an effect of Triton on some other hypothetical component which normally mediates the inhibitory effects of venturicidin, such that the binding of venturicidin causes a stimulation rather than inhibition.

It is now evident that mitochondrial ATPase activity can be both stimulated (present results) and inhibited by the interaction of small molecules with membrane components, which can then interact with the ATPase molecule. This could be the basis for a

fine control mechanism of the ATPase activity, assuming that there are naturally occuring effectors in the membrane with effects analogous to those of the antibiotics reported here. Such a mechanism would be additional to the postulated regularory role of the inhibitor protein [17].

The results emphasise differences both between the Triton-ATPase preparations from beef heart and from yeast SMP, and the Triton-solubilised and the membrane-bound ATPases.

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