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Bacteria that are resistant to uncouplers – what can they tell us?

Journal Club

Any successful theory concerning the nature of the energetic coupling between electron transport and ATP synthesis must give a satisfactory explanation of the mechanism of action of uncouplers. In this regard, the chemiosmotic theory predicted that uncouplers act by virtue of their ability, as lipophilic weak acids, to catalyse the electrogenic transfer of protons across the bilayer portions of energy coupling membranes. The good correlation between the protonophoric activity of a variety of structurally diverse compounds in black lipid membranes and their uncoupling potency in rat liver mitochondria thus lent compelling support to the notion that transmembrane proton translocation was indeed intimately involved in oxidative phosphorylation.

However, the description of high-affinity, proteinaceous binding sites for uncouplers in both mitochondria[1,2] and bacteria[3] has seriously questioned the view that the uncoupling ability of lipophilic weak acids during electron transport phosphorylation can be explained solely by their ability to conduct protons across phospholipid bilayers. Indeed, the existence of mutant strains of bacteria in which oxidative phosphorylation is rather resistant to uncouplers, compared with that observed in their wild-type parents, suggests that an interaction with one or more *proteins* in such coupling membranes is an important part of the uncoupling process.

Decker and Lang[4,5] described a strain of *Bacillus megaterium*, (strain C8) that was resistant to very high concentrations of the uncoupler carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP), and recently Guffanti, Blumenfeld and Krulwich[6] have carried out a more complete characterization of ATP synthesis in this organism. The mutation was apparently a single point mutation, as judged by the fairly high frequency of reversion to wild type (1 in 8×10^5), and Table I compares some of the bioenergetic properties of the wild-type and mutant strains[6]. The methods used were quite standard, the transmembrane pH difference and electrical potential difference being determined by the distribution of weak acids and permeant phosphonium salts. The apparent protonmotive force and the intracellular phosphorylation potential generated during malate respiration by the two strains is similar. However, the addition of 5 μM CCCP dramatically decreases the ability of the wild-type strain to maintain a substantial intracellular ATP pool whilst having relatively little effect upon the uncoupler-resistant mutant. The transmembrane electrical potential is greatly reduced in each case. Further differences between the two strains are found in their relative abilities to synthesize ATP in response to a K^+ diffusion potential, in their ATP hydrolase activities and in the respiration rates of intact cells.

What do these results tell us about the nature of the mutation in strain C8 and bacterial membrane energy coupling generally? The lowered rates of ATP hydrolase activity in the mutant strain suggested to the authors[6] that the lesion might lie in the ATPase itself. This is certainly an attractive possibility, but would not alone seem to explain the resistance of the strain to uncouplers. Other processes such as active transport would still, in principle, be subject to inhibition by the uncoupler, with potentially lethal consequences for the organism. The existence and nature of any high-affinity uncoupler-binding sites in *B. megaterium* is presently unknown, but is of obvious interest.

Since, in the presence of 5 μM CCCP,

TABLE I[a]

Assay	Wild type	Strain C8
$\Delta\bar{\mu}H^+$ at pH 5.5[b]	− 170 mV	− 192 mV
$\Delta\bar{\mu}H^+$ at pH 7.4	− 146 mV	− 156 mV
ΔG_P generated by malate respiration[c]	− 388 mV	− 398 mV
$\Delta\psi$ generated by malate respiration	− 90 to − 114 mV	− 98 to − 120 mV
ΔG_P generated by malate respiration with 5 μM CCCP	− 244 mV	− 356 mV
$\Delta\psi$ generated by malate respiration with 5 μM CCCP	− 33 to − 38 mV	− 45 to − 51 mV
[ATP] generated by a diffusion potential of − 200 mV	3.3 mM	2.1 mM
Respiration rate of intact cells	916 ng atom O/min/mg cell protein	1663 ng atom O/min/mg cell protein
ATP hydrolase activity of membranes	0.16 μmol/min/mg protein	0.04 μmol/min/mg protein

[a] after Ref. 9

[b] $\Delta\bar{\mu}H^+$ – apparent bulk-phase protonmotive force

[c] ΔG_P = intracellular phosphorylation potential = $\Delta G^{0'} + RT \ln \frac{[ATP]}{[ADP][P_i]}$

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the mutant strain could maintain a high intracellular phosphorylation potential in the absence of a significant bulk-phase electrochemical proton gradient (a phenomenon also observed by this group during studies of alkalophilic bacteria[7,8]) the authors inclined to the view that the 'form of energy that is directly coupled to ATP synthesis'[6] might be a relatively microscopic gradient of protons. Whatever the nature of the mutation in strain C8, such studies seem highly pertinent to our further understanding of electron transport phosphorylation.

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Spotting muscular dystrophy

Duchenne's muscular dystrophy is a wasting disease of muscle which is inherited as an X-linked recessive trait and affects approximately 30 in every 100,000 male live births. The muscle weakness usually leads to the diagnosis by 3–6 years of age, and survival beyond the age of 20 is unusual. As no treatment has been conclusively shown to be effective, attention has been focussed on detecting female carriers of the disease with a view to genetic counselling.

For the past 20 years one of the best available tests for carriers has been to measure the serum activity of creatine phosphokinase (CPK) as this is high in approximately two-thirds of female carriers. This is usually explained on the basis of the Lyon hypothesis; variable inactivation of the affected X-chromosome leads to a variable number of dystrophic fibres in the carrier muscle and hence variable leakage of muscle enzymes (indeed some Duchenne carrier females actually show clinical muscle weakness). However, a third of obligate carriers (e.g. women with one affected son and one other male relative affected) have serum CPK activities within the normal range. A further complication is that about one third of new cases occur in families with no previous history of the disease and no serum CPK elevation in the mother. It is unclear whether this is due to a high spontaneous mutation rate, or whether these 'isolated' cases are in fact being born to undetected carriers.

A possible new approach to this problem has been produced by Frearson, Taylor and Perry from the Department of Biochemistry at the University of Birmingham[1,2] who analysed the proteins of human urine by two-dimensional electrophoretic techniques (see *TIBS* (1981) *6*, 197–201). All of the 28 boys with Duchenne dystrophy examined showed a particular spot on their urine electrophoretograms which Frearson *et al.* have named 'protein C'. This protein has a mol. wt of 26,000 and an isoelectric point of 5.3 and could not be seen in the serum patterns of affected boys (unlike CPK which was visible on serum patterns but not on urine electrophoretograms). Protein C was also found in the urine of 5 of 9 sisters of the Duchenne sufferers and in 12 of 21 obligate female carriers of the disease, but in lesser amounts than in the affected boys. However, it appears unlikely that protein C is directly related to the primary defect in Duchenne dystrophy since it could also be found in some other genetically determined muscle diseases (the biochemical basis of these being equally obscure) and in a few per cent of the control urine samples analysed. It seems more likely that protein C is related to muscle damage *per se* rather than to the Duchenne dystrophic mechanism alone. The identity of protein C is unknown and attempts are now being made to isolate sufficient quantities for analysis. The potential clinical value of protein C lies in carrier detection and it needs to be clearly established whether protein C is a better marker than serum CPK in defining carrier status. This will entail the development of more sensitive ways of assaying the protein (probably radioimmunoassay) and the analysis of samples from a larger number of families at risk from Duchenne dystrophy.

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TIBS Journal Club

Twenty-two correspondents have agreed to contribute reports to **TIBS Journal Club.** Their names, addresses and fields of interest are listed below. Readers who wish to send reprints and preprints for consideration as topics for Journal Club should contact the appropriate correspondent.

A few additional correspondents will be appointed to cover topics not included on this list.

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