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Piericidin A and inhibition of respiratory chain activity in *Escherichia coli* K12

Piericidin A, an insecticide, was first isolated by TAKAHASHI, SUZUKI AND TAMURA[1]. In view of the apparent structural relationship of this compound to ubiquinone, HALL *et al.*[2] investigated the effects of piericidin A on electron transport systems in beef heart mitochondria. They suggested that piericidin A had two inhibitory effects. At low levels (0.04 nmole/mg protein) it specifically reacted at a site related to NADH dehydrogenase which they suggested might be identical to the site of rotenone inhibition. At high levels (0.3 μmole/mg protein for 50% inhibition) it reacted in a reversible fashion in the region of ubiquinone in the succinoxidase system.

The isolation of a mutant strain of *Escherichia coli* K12 unable to form ubiquinone (COX, GIBSON AND PITTARD[3]) has provided an useful biological tool with which to investigate the function of ubiquinone (COX, SNOSWELL AND GIBSON[4]). Thus the relationship between piericidin A inhibition and ubiquinone function in respiratory systems of *E. coli* K12 using the ubiquinone deficient mutant and a revertant or normal strain was investigated.

The strains of *E. coli* K12 used were AB3285, a mutant strain unable to form ubiquinone and AB3290, a revertant strain of AB3285 (referred to here as the normal strain). These strains have been described in detail elsewhere[3]. The preparation of small respiratory particles, the measurement of oxygen uptakes and ubiquinone reduction have been described previously[4].

A comparison of the oxidation rates for various substrates catalysed by small particles from the normal strain of *E. coli* K12 and the mutant lacking ubiquinone is shown in Table I.

TABLE I

A COMPARISON OF THE OXIDATION RATES OF VARIOUS SUBSTRATES CATALYSED BY PARTICLES FROM NORMAL *E. coli* K12 AND A UBIQUINONE-DEFICIENT MUTANT AND THE INHIBITION BY PIERICIDIN A OF OXIDATIONS CATALYSED BY PARTICLES FROM NORMAL CELLS

Oxidation rates were determined polarographically. The percentage inhibition was determined by comparing the oxidation rate after the addition of the inhibitor to the rate obtained in the absence of inhibitor. The concentration of piericidin A used was 20 μM or 0.03–0.04 μmole/mg particle protein and that of ubiquinone-2 was 0.2 mM. Both piericidin A and ubiquinone-2 were added as solutions in absolute ethanol, 1–5 μl of solutions being added to a 2.0 ml reaction volume. Final substrate concentrations were 2 mM. The small-particle suspensions contained from 8.0 to 13.2 mg protein per ml and 0.1 ml fractions of these particle preparations were used in each experiment. The figures shown are an average of 5 experiments.

Substrate	*Oxidation rate (ngatoms O per min per mg protein)*		*Inhibition of oxidation rate of normal particles by piericidin A (%)*	
	Mutant	*Normal*	*−ubiquinone-2*	*+ubiquinone-2*
NADH	260	500	80	nil
L-Malate	51	141	54	nil
L-Lactate	156	225	65	6
α-Glycerophosphate	63	72	50	50
Dihydroorotate	45	42	100	100

It can be seen that the substrates NADH, malate and lactate are oxidised at a considerably slower rate by particles from the mutant cells compared with those from the normal cells. In contrast, the oxidation rates with α-glycerophosphate and dihydroorotate as substrates are virtually the same for particles from both the normal and mutant strains. Further, as shown in Table II endogenous ubiquinone-8 was not reduced in the presence of α-glycerophosphate and dihydroorotate, but was reduced in the presence of malate and lactate.

TABLE II

THE EFFECT OF PIERICIDIN A ON THE REDUCTION OF ENDOGENOUS UBIQUINONE-8 BY MALATE IN SMALL PARTICLES DERIVED FROM NORMAL *E. coli* K12 CELLS

0.5 ml of small-particle preparations containing 10.1–13.2 mg protein per ml were incubated at 25° for 2 min with 1.0 ml of buffer containing 30 mM phosphate buffer pH 7.4, 7.5 mM $MgCl_2$ *plus* 2 mM substrates and 20 μM piericidin A where indicated. The reaction was stopped by the addition of 5.0 ml of petroleum ether (b.p., 40–60°)–methanol (60:40, v/v) as described by KRÖGER AND KLINGENBERG[5] and oxidised and reduced ubiquinone estimated by the method of HOFFMANN *et al.*[6]. The figures shown are the average of 2 experiments.

Addition	*Reduction of endogenous ubiquinone-8* (%)
None	Nil
L-Malate	32
L-Malate + piericidin A	Nil
Piericidin A	Nil
L-Lactate	45
α-Glycerophosphate	Nil
Dihydroorotate	Nil

The above results suggest that ubiquinone is involved in the respiratory system associated with the oxidation of the substrates malate, lactate and NADH, and not in the system associated with the oxidation of α-glycerophosphate and dihydroorotate. This conclusion is supported by the observation that addition of ubiquinone-2 to particles from the mutant cells restores the oxidation rates with malate, lactate and NADH as substrates to the normal level. Addition of ubiquinone-2 at the same concentration to particles from normal cells had only a slight stimulatory effect on the oxidation rates of these substrates. Further, the cytochrome content of small particles from both mutant and normal cells is virtually the same[4], suggesting that the differences in respiratory rates described above are not due to an overall respiratory chain deficiency and indeed are due to a lack of ubiquinone.

Piericidin A (20 μM) substantially inhibited the oxidation of NADH, malate and lactate catalysed by small particles from the normal cells and this inhibition was completely reversed by the addition of ubiquinone-2 (Table I). The oxidation of α-glycerophosphate and dihydroorotate was also inhibited by piericidin A, but in contrast, this inhibition was not reversed by adding ubiquinone-2 (Table I).

The inhibition by piericidin A was examined more closely using malate as a substrate. The addition of ubiquinone-2 had little effect on the rate of malate oxidation

catalysed by particles from normal cells (Fig. 1a) but completely reversed the inhibition produced by the addition of piericidin A (Fig. 1b). Ubiquinone-1 was equally effective in reversing this inhibition although ubiquinone-6 only slightly reversed the inhibition. Menadione at a similar concentration to ubiquinone-2, *i.e.* 100 μM, only slightly reversed the piericidin A inhibition.

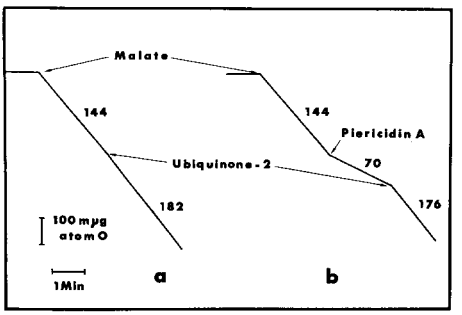

Fig. 1. The effect of piericidin A and ubiquinone-2 on the oxidation of malate catalysed by small particles from a normal strain of *E. coli* K12. Incubations were carried out at 25° in a volume of 2.0 ml containing small particles (equivalent to 1.0 mg protein), 30 mM phosphate pH 7.4 and 7.5 mM $MgCl_2$. 2 mM L-Malate, 100 μM ubiquinone-2 and 20 μM piericidin A were added at the points indicated. The figures shown indicate the rates of oxygen uptake in ngatoms O per min per mg protein.

The results shown in Table II indicate that no endogenous ubiquinone-8 is reduced in the absence of added substrate. However, when malate is added about 30 % of endogenous ubiquinone-8 is reduced (see also COX, SNOSWELL AND GIBSON[4]) and this reduction is completely prevented by the addition of piericidin A (Table II).

The results presented here indicate that piericidin A has two inhibitory effects on respiratory systems in *E. coli* K12. One effect is on respiratory systems not involving ubiquinone, such as that associated with the oxidation of α-glycerophosphate and dihydroorotate, and which is not reversed by added ubiquinone-2. The other inhibitory effect is seen with respiratory systems which do involve ubiquinone, such as those associated with the oxidation of NADH, malate and lactate, and this inhibition is completely reversed by the addition of ubiquinone-2. An important difference between these results and those obtained by HALL *et al.*[2] with beef heart mitochondrial systems is that in *E. coli* the two inhibitory effects are observed with the same level of piericidin A (*i.e.* 0.04 μmole/mg protein). JENG *et al.*[7] found that the NADH oxidase and succinoxidase systems in Azotobacter, unlike the mitochondrial systems are inhibited by the same level of piericidin A (1.5 μmoles/mg protein). Also it appears that high concentrations of piericidin A are generally required to inhibit bacterial respiratory systems[8].

The present results suggest that, in respiratory pathways in *E. coli* K12 which involve ubiquinone, piericidin A inhibits at or near the ubiquinone site. The site of the inhibitory action of piericidin A on respiratory pathways in *E. coli* K12 not involving ubiquinone is not yet known.

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