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An Escherichia coli mutant exhibiting temperature-sensitive ATP synthesis

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Summary; A mutant strain SM434 (ttr-3) of Escherichia coli that exhibits a temperature-sensitive Unc(succinate-nonutilizing) phenotype was characterized. The mutant allele ttr-3 was not linked to the ilvA gene, but was complemented by F111 carrying 81 min - 91 min of the E. coli chromosome. The mutant strain SM434 exhibited resistance to N,N'-dicyclohexylcarbodiimide (DCCD) and a temperature-sensitive phenotype at the level of ATP synthesis, compatible with that of cell growth. These findings indicate that the mutant strain SM434 could carry a mutation (ttr-3) in an unknown gene responsible for the energy-transduction system.

Adenosine triphosphate (ATP) formation by oxidative phosphorylation has been observed in many membrane system. According to Mitchell's chemiosmotic hypothesis, the membrane gradient of the electrochemical potential formed by translocation of protons across the membrane is the motive force of ATP formation (1). In fact, the proton gradient has been shown to be formed through the proton-pumps of complex I, III and IV of the respiratory chain of mitochondria (2,3), and H^+-ATPase has been found to catalize ATP synthesis dependent upon the proton gradient (4).

ABBREVIATION: DCCD, N,N'-dicyclohexylcarbodiimide.

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However, it is still unknown how the proton gradient formed through the respiratory chain is related to H^+-ATPase for ATP synthesis. One method for studying the mechanism of proton-localization is to isolate mutant strains defective in components responsible for the energy-transduction system. Ito et. al. isolated mutant strains of E. coli that showed a temperature-sensitive Unc phenotype (5,6,7). In this work, one of these mutants, strain SM434 (ttr-3), which exhibited a phenotype of resistance to the proton-pump-binding agent N,N'-dicyclohexylcarbodiimide (DCCD), was characterized to obtain information on the mechanism of ATP formation.

MATERIALS AND METHODS

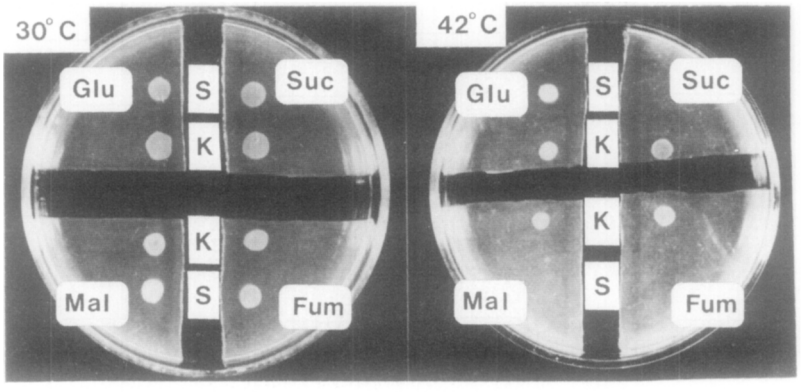
The wild type strain KH434 (thy,gal) and mutant strain SM434 (thy, gal, ttr-3) were used. Strain KL728 (F111/leu,his,arg,met,lac,gal,xyl, mtl,mal,recA,ton,tsx,str) was used as a donor strain in mating experiments, and strain IM5 (ilvA,rel,tonA,bgl,tna,$T2^r$) was used as a recipient strain in P1-transduction experiments. M63 medium consisting of 100 mM KH_2PO_4, 15 mM $(NH_4)_2SO_4$ and 0.2 μM $FeSO_4$(pH7.0), was used as minimal medium. Mating experiments and P1-transduction experiments were carried out as described by Miller (8).

Cell suspensions (0.5 mg protein) were incubated for 4 min with 20 mM succinate or 0.2 % (w/v) glucose to allow ATP production. The reaction was then stopped by adding $HClO_4$, the solution was neutralized, and ATP content was determined by measuring the fluorescence at 570 nm with luciferin-luciferase (9) in a fluorence-spectrometer.

RESULTS

The wild type strain KH434 and the mutant strain SM434 were grown on plates containing glucose, succinate, fumarate or malate. Strain SM434 exhibited the temperature- sensitive Unc phenotype (Fig. 1). The wild type strain KH434 grew on plates containing any of the four carbon sources at both 30°C and 42°C, whereas at 42°C, the mutant strain SM434 could grow on plate containing glucose, but not on those containing succinate, fumarate or malate.

This temperature-sensitive Unc phenotype suggested that the mutant allele ttr-3 might be located in one of the unc genes coding for protein subunits of H^+-ATPase. Since unc genes are located at 84 min on the E. coli chromosome (10), strain SM434 was mated with



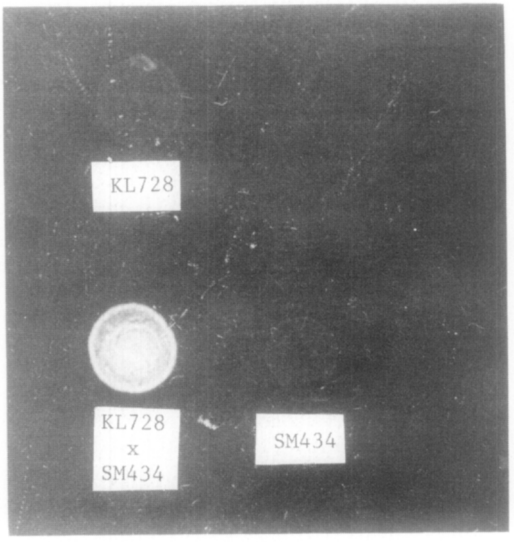
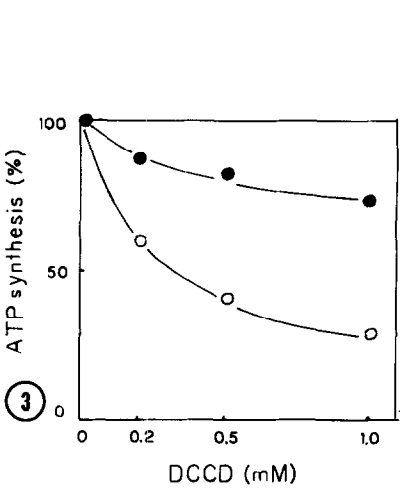


Fig. 1. Temperature-sensitive Unc phenotype of strain SM434. Strains KH434 (K) and SM434 (S) were incubated onto plates containing of 0.4 % (w/v) glucose, 40 mM succinate, 40 mM fumarate or 40 mM malate and incubated for 2 days at 30°C or 42°C.

Fig. 2. Complementation experiment with F111. Strain SM434 was mated with strain KL728 harboring F111 by the method of Miller (7). Samples of each strain and mated cells were incubated onto a plate containing 40 mM succinate and incubated for 2 days at 42°C.

strain KL728 harboring F111. The temperature-sensitive Unc phenotype of strain SM434 might be complememted by F111 because the latter carries a partial chromosome (81 min - 91 min) of *E. coli* (11). The mated cells grew on a selective plate containing succinate at 42°C, indicating that the mutant allele *ttr-3* was complemented by F111 (Fig. 2). Thus, the *ttr-3* allele is located on the chromosome between 81 min and 91 min. The linkage between the *ttr-3* allele and *ilvA* gene was then examined in



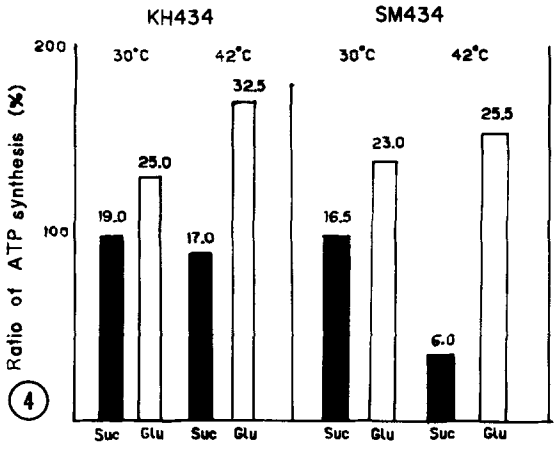


Fig. 3. DCCD-resistant ATP synthesis in strain SM434. Strain KH434 (○) and strain SM434 (●) were incubated with the indicated amount of DCCD for 2 min. Then, ATP synthesis in 4 min with 20 mM succinate were measured.

Fig. 4. Temperature-sensitive ATP synthesis in strain SM434. Cells were pre-incubated for 2 min at 30°C or 42°C. Then, 0.2 %(w/v) glucose (glu) or 20 mM succinate (suc) was added to initiate ATP synthesis. After 4 min, the reaction was stopped by the addition of $HClO_4$. The amount of ATP produced is shown as a percentage of that with succinate at 30°C in each strain. The amount of ATP produced in n moles/mg protein is shown at the top of each bar.

a P1-transduction experiment. P1 phage grown in strain SM434 was infected into strain IM5 defective in the ilvA gene and 100 ilv^+ transductants were examined. None of the ilv^+ transductants exhibited the temperature-sensitive Unc phenotype. Thus, the ttr-3 allele was not localized near the ilvA gene. Since unc genes show high linkage (about 30 %) with ilv genes (12), the mutant allele ttr-3 does not lie in the region of unc genes. The above mating and P1-transduction experiments suggested that strain SM434 might carry a mutation (ttr-3) in an unknown gene responsible for the energy-transduction system.

ATP synthesis of strain SM434 was examined to determine whether this strain carries a mutation in a component responsible for the energy-transduction system, Strain SM434 exhibits DCCD-resistant growth with succinate (5). Strain SM434 produced more ATP than the wild type strain KH434 with succinate in the presence of DCCD at concentrations of 0 - 1 mM DCCD (Fig. 3). Then, we measured ATP

production with glucose or succinate in the absence of DCCD to examine the temperature-sensitivity at the level of ATP synthesis. Fig. 4 shows that ATP synthesis by strain SM434 with succinate was markedly reduced by temperature shift to 42°C. The temperature-sensitivity of ATP synthesis in strain SM434 is compatible with the result on its growth shown in Fig. 1.

DISCUSSION

In this work we examined the characteristic of strain SM434 (*ttr-3*), which exhibits the temperature-sensitive Unc phenotype. The complementation of this strain by F111 showed that the allele *ttr-3* is located at 81 min - 91 min on the *E. coli* chromosome. However, a P1-transduction experiment showed that the *ttr-3* allele is not linked to the *ilvA* gene. Strain SM434 also exhibited DCCD-resistance and temperature-sensitivity at the level of ATP synthesis, which were compatible with results on its cell growth. These results show that strain SM434 carries a mutation in a component responsible for the energy-transduction system. Two groups of genes in the region of 81 min - 91 min, *unc* genes and the *ecfB* gene, are probably related to the energy-transduction system. Unc genes are linked to *ilvA* gene (12) but the *ttr-3* allele is not. Therefore, the mutant allele *ttr-3* does not seem to be located in the region of *unc* genes. The *ecfB* gene is related to active transport (13) and does not seem to be directly responsible for ATP synthesis. In contrast, the mutant allele *ttr-3* is directly responsible for ATP synthesis because strain SM434 exhibits temperature-sensitive ATP synthesis. Therefore, the mutant allele *ttr-3* does not seem to be located in *ecfB* gene. We conclude that strain SM434 carries a mutation (*ttr-3*) in an unknown gene. Further analysis of strain SM434 should help in understanding the energy-transduction system.

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