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ISOLATION OF MUTANTS OF *ESCHERICHIA COLI* UNCOUPLED IN OXIDATIVE PHOSPHORYLATION USING HYPERSENSITIVITY TO STREPTOMYCIN

MARIANNE E. MUIR and BRIAN J. WALLACE *

School of Microbiology, Faculty of Biological Sciences, University of New South Wales, Kensington, N.S.W. 2033 (Australia)

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Summary

Mutants of *Escherichia coli*, harbouring the *uncA401* or *uncB402* alleles, were found to take up streptomycin more rapidly than the coupled parent strains. The increased rate of uptake results in greater sensitivity of the uncoupled strains, compared to the parent strains, to low concentrations of streptomycin. Studies with unc^+ revertants showed that hypersensitivity to streptomycin is attributable to the mutation causing uncoupling. The uptake of streptomycin in an unc^- strain is abolished by addition of the chemical uncoupler carbonylcyanide *m*-chlorophenylhydrazone. The phenotype of hypersensitivity to streptomycin can be used as a selection procedure for the isolation of uncoupled strains. In an experiment reported here, nine out of 12 strains isolated as being sensitive to streptomycin (at 2.5 μg/ml), were found to be unable to grow on succinate as a sole source of carbon. Five of the nine Suc^- strains were found to be uncoupled in oxidative phosphorylation, and two of the five uncoupled strains lacked Mg^{2+}-ATPase activity. The mutations causing uncoupling were cotransducible with the *ilv* genes.

Introduction

Mutants of *Escherichia coli* in which oxidative phosphorylation is uncoupled from electron transport (unc^- mutants), have been isolated in several laboratories [1,2]. One selection procedure depends on the fact that, although

* To whom correspondence should be addressed.
Abbreviation: CCCP, carbonylcyanide *m*-chlorophenylhydrazone.

uncoupled strains grow aerobically on glucose, they fail to grow on reduced compounds such as succinate [3,4], D-lactate [5], or a mixture of acetate, malate and succinate [6], as sole sources of carbon. *Unc*$^-$ strains of *E. coli* have also been isolated by selecting for resistance to the aminoglycoside, neomycin [7,8]. In this case resistance appears to result, not from ribosomal alterations, but from a reduced capacity to take up aminoglycoside antibiotics [21]. However, uncoupling does not always lead to increased resistance to aminoglycosides. For example, Turnock [9] described a mutant which is more sensitive to streptomycin than the parent strain. The hypersensitivity in this case appeared to result from an increase in permeability since no differences in sensitivity to streptomycin of ribosomes from parent and mutant were detected. Later experiments revealed that the hypersensitive mutant had a reduced Mg^{2+}-ATPase activity [10], suggesting that uncoupling can lead to increased sensitivity to streptomycin.

In this paper we examine the effect of *unc* mutations on the rate of streptomycin uptake in streptomycin-sensitive strains of *E. coli*, and explore the possibility of using hypersensitivity to streptomycin as a means of isolating uncoupled mutants.

Materials and Methods

Media and growth of cells. (a) For streptomycin uptake and viability experiments. Cells were grown in nutrient broth (Oxoid) enriched with 0.5% brain heart infusion (Oxoid). For agar plates 2% agar (Difco) was added to brain heart infusion broth. Broth cultures were incubated at 37°C in a reciprocating shaking water bath (Model RW1812, Paton Industries, South Australia) at a speed setting of 100 oscillations per min. Cell growth was monitored by absorbance at 600 nm (A_{600}) using a Spectronic 20 spectrophotometer.

(b) For preparation of membranes. The mineral salts medium A described by Davis and Mingioli [12] was supplemented with glucose, 20 mM; thiamine-HCl, 10^{-6} M; L-isoleucine, 0.64 mM; L-valine, 0.71 mM; L-histidine, 0.5 mM; L-tryptophan, 0.2 mM, and casamino acids (Difco, vitamin free), 0.1%. 1 l cultures were incubated in 3-l flasks at 37°C and with vigorous shaking in a New Brunswick gyrotary shaker.

Streptomycin uptake experiments. The method of Bryan and Van Den Elzen [13] was used with the following modifications. 20 ml of bacterial culture (at A_{600} 0.2—0.25) was divided into two 10-ml aliquots. Unlabelled streptomycin (at a final concentration of either 2.5 μg/ml or 10 μg/ml) was added to one aliquot. This culture was used to monitor the effect of the antibiotic on growth. To the other aliquot a mixture of [^{3}H]dihydrostreptomycin and unlabelled streptomycin was added to a final concentration identical to that in the first aliquot, i.e. either 2.5 μg/ml or 10 μg/ml. The final specific activities were approximately 326 and 159 dpm/ng of streptomycin, respectively. It was assumed that the cellular transport mechanisms would not distinguish between streptomycin and dihydrostreptomycin. At various time intervals, samples (0.5 ml) were removed and filtered, the membrane filters (pore diameter 0.45 μM, Sartorious, Göttingen, F.R.G.), having been pretreated in streptomycin solution (2.5 mg/ml). The filters were washed with 20 ml of 3% NaCl,

then dried and counted in 5 ml of a toluene-based scintillation fluid (5 g PPO, Packard, U.S.A.; 0.3 g dimethyl POPOP, Packard, U.S.A.; per l of toluene) using a Packard Tricarb liquid scintillation counter. Tritium standards were run in parallel to monitor counter reproducibility and counting efficiency was 50.6%. Streptomycin accumulation is expressed as ng of streptomycin/mg dry wt. of bacteria.

Measurement of dry weights of bacteria. Cells grown in nutrient broth to an A_{600} of 1.25 were divided into aliquots which were then adjusted to A_{600} values between 0 and 1.0, using the same medium. Cells from 35 ml of each adjusted aliquot were collected by centrifugation in preweighed tubes and washed twice with medium A. The cell pellets were frozen then freeze-dried to constant weights. 1 ml of culture at A_{600} of 0.5 was equivalent to 0.2 mg dry weight of bacteria.

Determination of the effect of streptomycin on cell viability. Streptomycin was added at various concentrations to bacterial cultures (5 ml, A_{600} of 0.25). After incubation at 37°C for 1 h, 1 ml samples were serially diluted and duplicate 0.1 ml aliquots of the appropriate dilutions were spread onto brain heart infusion plates. Colonies were counted after incubation at 37°C overnight.

Preparation of membranes. Membranes were prepared as described previously [14].

Estimation of oxidases in membranes. The oxidase activities present in membrane preparations were measured as previously described [14].

Measurement of P/O ratios. P/O ratios were measured as previously described [14].

Measurement of Mg^{2+}-ATPase in membranes. Mg^{2+}-ATPase activity was measured by the method of Butlin et al. [4].

Estimation of protein. Protein concentrations were measured by the method of Lowry et al. [15] using bovine serum albumin as standard.

Transduction experiments. The generalised transducing bacteriophage Plkc was used for transduction experiments as described by Pittard [16].

Results

Construction and characterization of uncA⁻ and uncB⁻ mutants

Since the uptake of streptomycin by sensitive bacterial strains appears to be an energy-dependent process [13,17,18], we decided to investigate whether or not mutations which affect energy-transducing systems in the cytoplasmic membrane could influence the rate of entry of the antibiotic. The strains chosen for use were mutants of *E. coli* in which oxidative phosphorylation is uncoupled from electron transport due to mutations in the Mg^{2+}-ATPase complex. One such strain (AN120), harbouring an *uncA401* allele is affected in the F_1 portion of the Mg^{2+}-ATPase complex and lacks Mg^{2+}-ATPase activity, while strain AN283, with an *uncB402* allele, retains Mg^{2+}-ATPase activity but is uncoupled due to a lesion in the F_0 portion of the Mg^{2+}-ATPase complex (Ref. 19, see also Ref. 1).

Previous studies have shown that *unc* genes constitute an operon close to *ilv* [11]. Thus we were able to transfer the *uncA401* and *uncB402* alleles, originally in streptomycin-resistance strains, into JP2140, an *ilv⁻*, streptomycin-sen-

sitive strain, by cotransduction with *ilv*. *Unc*$^-$ transductants were selected by an inability to grown on succinate as sole source of carbon (Suc$^-$ phenotype). An *uncA*$^+$ and *uncB*$^+$ strain from each transduction were kept for comparison with the *unc*$^-$ strains. Table I lists the genotypes of the strains used.

The effect of *uncA401* and *uncB402* in strains NSW6 and NSW28, respectively, was demonstrated by measuring the specific activities of Mg^{2+}-ATPase and the P/O ratios in cytoplasmic membranes prepared from the mutants and comparing these values with those obtained for *unc*$^+$ strains. Specific activities for NADH, D-lactate and succinate oxidases were also determined. As expected from previous studies [3,4], membranes from strain NSW6 had very low Mg^{2+}-ATPase activity while the *unc*$^+$ strain (NSW5) retained full activity. Moreover membranes from strain NSW28 (*uncB402*), though possessing normal Mg^{2+}-ATPase activity, did not phosphorylate, while the *unc*$^+$ strain (NSW30) had a normal P/O ratio (Table II). It should be noted that the P/O ratio in the coupled strain was low (generally about 0.1), as expected for this type of membrane preparation.

Membranes from both *unc*$^+$ and *unc*$^-$ strains had specific activities for NADH, D-lactate and succinate oxidases within the normal ranges; however significant differences in the specific activities of D-lactate and succinate oxidases occurred between *unc*$^+$ and *unc*$^-$ strains. In particular both *uncA401* and *uncB402* membranes had 3—4-fold higher specific activities for D-lactate oxidase than membranes from the respective *unc*$^+$ strains (Table II).

Streptomycin uptake in uncA$^-$ *and uncB*$^-$ *mutants*

Fig. 1 shows that uncoupled strains accumulate streptomycin (at a final con-

TABLE I

STRAINS OF *E. COLI* K-12 USED

Genes coding for enzymes in biosynthetic pathways are denoted as follows: *thi*, thiamine; *ilv*, isoleucine-valine; *his*, histidine; *trp*, tryptophan; *arg*, arginine; *ent*, enterochelin. Streptomycin resistance is denoted by *str*^{R}; *unc* denotes uncoupling of oxidative phosphorylation from electron transport and *str*^{hs} denotes hypersensitivity to streptomycin.

Strain	Relevant genetic loci	Other information
JP2140	*ilv-1, his-29(am), trpA9605(am)*	Obtained from J. Pittard
AN120	*argE3, thi-1, str*^{R}, *uncA401*	Obtained from F. Gibson
AN283	*argHI, entA*$^-$, *str*^{R}, *uncB402*	Obtained from F. Gibson
NSW5	*his-29(am), trpA9605(am)*	Isolated after transduction with AN120 as donor and JP2140 as recipient
NSW6	*his-29(am), trpA9605(am), uncA401*	Isolated after tranduction with AN120 as donor and JP2140 as recipient
NSW28	*his-29(am), trpA9605(am), uncB402*	Isolated after transduction with AN283 as donor and JP2140 as recipient
NSW30	*his-29(am), trpA9605(am)*	Isolated after transduction with AN283 as donor and JP2140 as recipient
NSW13	*his-29(am), trpA9605(am), ilv-1, unc-900*	Strains NSW13 to NSW18 were derived from strain JP2140 after NTG treatment
NSW14	*his-29(am), trpA9605(am), ilv-1, unc-901*	
NSW15	*his-29(am), trpA9605(am), ilv-1, str*^{hs}*-4*	
NSW17	*his-29(am), trpA9605(am), ilv-1, unc-902*	
NSW18	*his-29(am), trpA9605(am), ilv-1, str*^{hs}*-7*	

TABLE II

SPECIFIC ACTIVITIES FOR OXIDASE SYSTEMS AND Mg^{2+}-ATPase, AND P/O RATIOS FOR *uncA401* AND *uncB403* RECOMBINANT STRAINS

The methods for the preparation of membranes and measurement of oxidase rates, Mg^{2+}-ATPase activity and P/O ratios are described in Materials and Methods. Membranes were prepared from cells grown in nutrient broth supplemented with brain heart infusion (0.5%). For the P/O ratios D-lactate was used as substrate; essentially the same results were obtained with succinate and NADH.

Membranes from	Rate of oxygen uptake (ngatoms O/min per mg protein)			Mg^{2+}-ATPase activity (nmol/min per mg protein)	P/O
	NADH	D-Lactate	Succinate		
NSW5 (*unc*$^+$)	412	23	187	428	
NSW6 (*uncA401*)	475	121	292	55	
NSW30 (*unc*$^+$)	416	46	300	373	0.12
NSW28 (*uncB402*)	497	143	350	277	<0.001

centration of 2.5 μg/ml) more rapidly than the corresponding coupled strains. With NSW5 (*unc*$^+$) a 40 min lag prior to the onset of uptake was observed. In contrast, an *uncA401* strain (NSW6) showed no lag and a rapid uptake rate was apparent 5 min after addition of the antibiotic (Fig. 1A). Moreover, in a 1 h period, NSW6 accumulated four times as much streptomycin as NSW5.

Similar results were obtained when accumulation of streptomycin by an *uncB402* mutant (NSW28) was compared to that in an otherwise isogenic *uncB*$^+$ strain, NSW30 (Fig. 1B). Thus with NSW28 accumulation began almost immediately after addition of streptomycin, whereas with NSW30 a 40 min lag preceded the onset of uptake, and the total amount of antibiotic accumulated by NSW30 (over 1 h) was 2.5-fold lower than for NSW28. Similar trends were obtained with streptomycin at 10 μg/ml, except that the duration of the lag prior to uptake with the coupled strains was reduced (see Fig. 2). Thus uncoupling oxidative phosphorylation by mutation at either *uncA* or *uncB* can lead to more rapid uptake of streptomycin compared to wild-type strains.

Chemical uncouplers, however, have the reverse effect to *unc*$^-$ on streptomycin uptake. Thus CCCP (at 10 μM final concentration) inhibited accumulation of streptomycin in both *unc*$^+$ (Fig. 2A) and *uncA401* (Fig. 2B) strains. Streptomycin uptake in the *uncA401* strain was more sensitive to inhibition by CCCP than that in the *unc*$^+$ strain. Moreover the difference in sensitivities was more pronounced at lower concentrations of CCCP (data not shown). The reason for greater sensitivity to CCCP of the *unc*$^-$ strain compared to the *unc*$^+$ strain is unknown.

Sensitivity of uncoupled mutants to streptomycin

The rapid uptake of streptomycin in uncoupled strains might result in an increased sensitivity to low concentrations of the antibiotic. We therefore compared the effects of various concentrations of streptomycin on cell viability in coupled and uncoupled strains. Fig. 3 shows that the percentage of cells of NSW6 (*uncA401*) which survived treatment with streptomycin (at concentrations up to 2.5 μg/ml) for 1 h was substantially less than for NSW5 (*unc*$^+$). However, the numbers of viable cells of both coupled and uncoupled strains

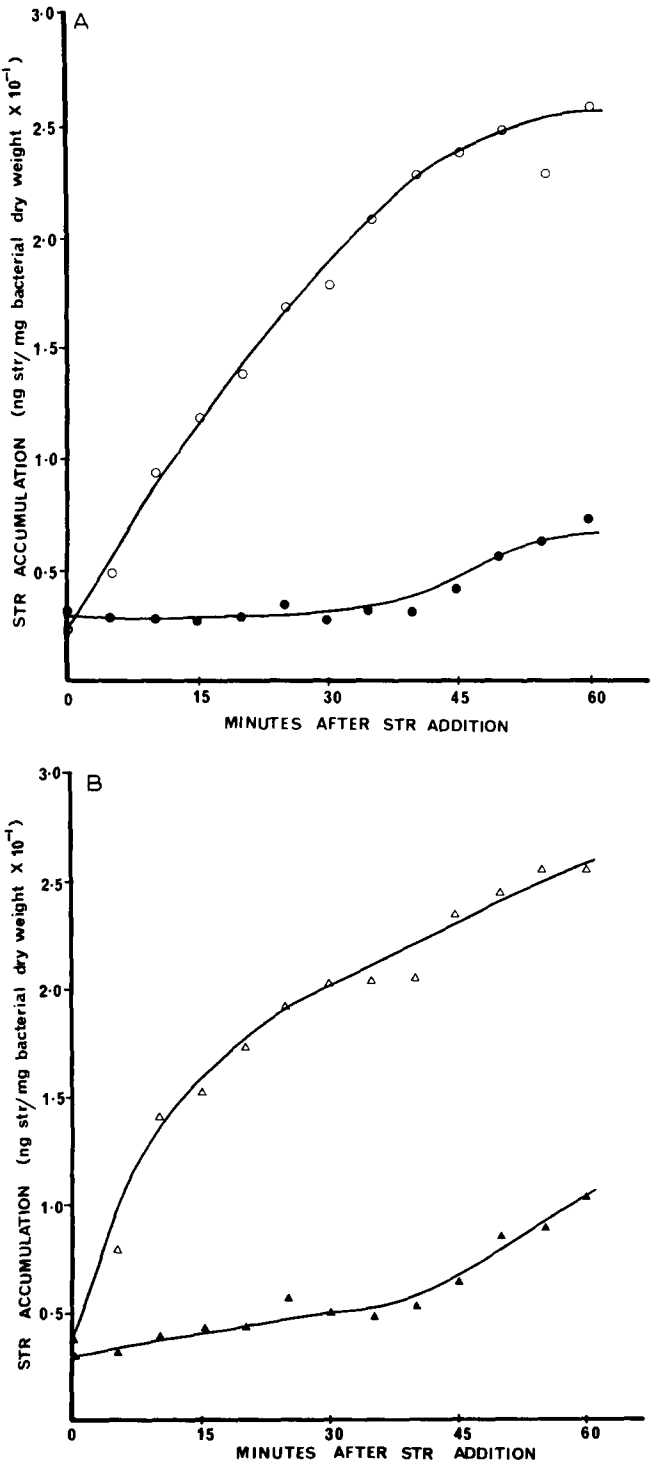

Fig. 1. Kinetics of streptomycin accumulation in coupled and uncoupled strains. (A) ●, NSW5 (*unc*$^+$), and ○, NSW6 (*uncA401*). (B) ▲, NSW30 (*unc*$^+$), and △, NSW28 (*uncB401*). The final concentration of streptomycin was 2.5 μg/ml.

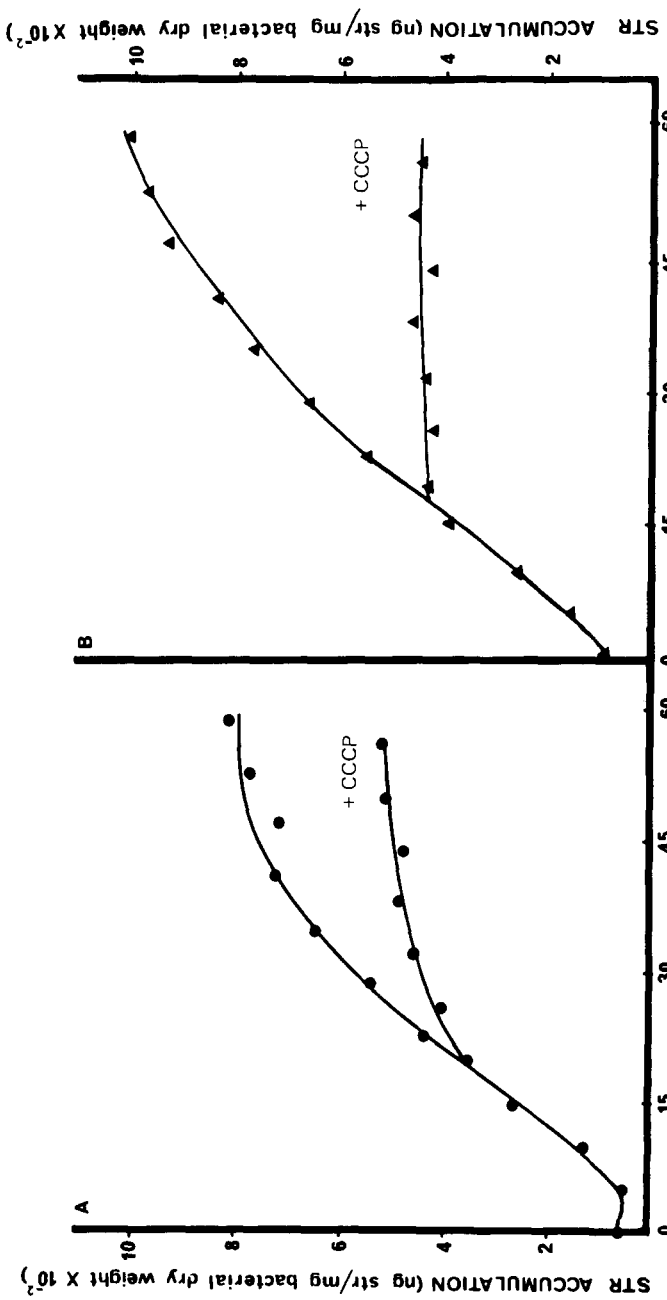

Fig. 2. Effect of CCCP on steptomycin accumulation in coupled and uncoupled strains. (A) ●, NSW5 (*unc*$^+$), and (B) ▲, NSW6 (*uncA401*). CCCP (10 μM) was added 15—20 min after streptomycin (10 μg/ml).

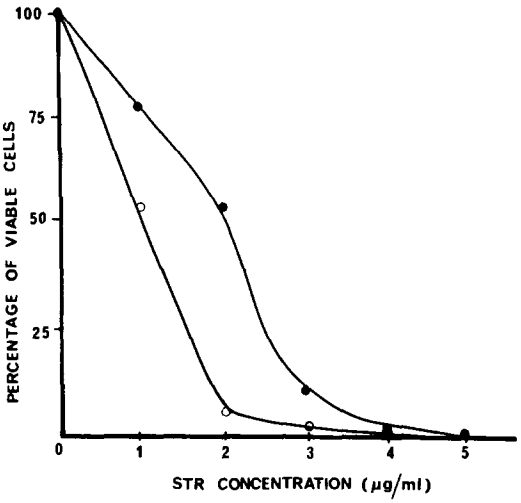

Fig. 3. Effect of streptomycin on viability of coupled and uncoupled strains. •, NSW5 (*unc*$^+$); ○, NSW6 (*uncA401*). Cultures were incubated at 37°C with various concentrations of streptomycin for 60 min prior to testing viability.

were reduced to less than 1% of those originally present following treatment at concentrations greater than 4 or 5 μg/ml. It is clear from Fig. 3 that if sensitivity to streptomycin is to be used to differentiate between coupled and uncoupled strains (see next section), careful attention should be given to the concentration used. For nutrient broth with 0.5% brain heart infusion (used in the experiment described in Fig. 3) a concentration of 2 μg/ml of streptomycin gave optimal differentiation between coupled and uncoupled strains. For brain heart infusion medium we found a concentration of 2.5 μg/ml to be most suitable.

The method of constructing strains NSW6 (*uncA401*) and NSW28 (*uncB402*) (i.e. by transduction of the *unc*$^-$ allele with *ilv*; see previous section) indicates that the gene responsible for hypersensitivity to streptomycin is either *uncA* (or *uncB*), or is a different gene situated close enough to *unc* and *ilv* to be cotransduced with *ilv*. To distinguish between these possibilities we isolated 12 *unc*$^+$ revertants of strain NSW6 (selected for ability to grow on succinate). All 12 *unc*$^+$ revertants grew well on brain heart infusion plates with 2.5 μg/ml streptomycin. (At this concentration of the antibiotic, growth of the *uncA401* strain is severely inhibited). Two of the 12 Suc$^+$ revertants were found to have Mg^{2+}-ATPase activities similar to that of NSW5. Moreover these two revertants accumulated streptomycin with kinetics similar to NSW5 (data not shown). Furthermore, ten Suc$^+$ revertants of strain NSW28 (*uncB402*) grew well on brain heart infusion plates with 2.5 μg/ml streptomycin, in contrast to the coupled parent strain whose growth was severely inhibited. Two of the ten revertants were found to have P/O ratios and streptomycin uptake kinetics similar to strain NSW30 (*unc*$^+$). These results indicate that the hypersensitivity to streptomycin observed in the uncoupled strains is due to the *uncA401* or *uncB402* alleles and not to other closely linked mutations.

It should be noted that when uncoupled (hypersensitive) strains were spread

on brain heart infusion plates with 2.5 μg/ml streptomycin, revertants, capable of growth, arose readily. These revertants were found to be uncoupled (Suc^-), suggesting that mutations (perhaps involving cell envelope components) can reverse the effect of uncoupling on streptomycin uptake and sensitivity.

Isolation of uncoupled mutants using hypersensitivity to streptomycin

The above results prompted us to attempt to isolate uncoupled mutants by selecting for hypersensitivity to streptomycin. Strain JP2140 was treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine as described by Adelberg et al. [20]. After phenotypic expression (overnight incubation at 37°C), cells were spread on brain heart infusion plates to give approximately 150 colonies/plate. These were then replicated to the same medium and to brain heart infusion plates with 2.5 μg/ml streptomycin. Colonies sensitive to this concentration of antibiotic were selected by inspection for absence of growth. In one experiment 12 streptomycin-hypersensitive strains were isolated. Of these, nine were found to be unable to grow on succinate as sole source of carbon. Cytoplasmic membranes were prepared from JP2140 and five of the streptomycin-hypersensitive strains, and assayed for Mg^{2+}-ATPase activity, the ability to oxidize NADH, D-lactate and succinate and for P/O ratios. All five strains had reduced P/O ratios compared to JP2140; strains NSW14, NSW15 and NSW18 retained some phosphorylation activity whereas the P/O ratios for strains NSW13 and NSW17 were less than 0.01 (Table III). Strains NSW14, NSW17 and NSW18 were found to contain Mg^{2+}-ATPase activities comparable to that of JP2140, whereas in strains NSW13 and NSW15, the Mg^{2+}-ATPase activities were substantially lower than that for JP2140.

Four of the streptomycin-hypersensitive strains contained NADH, D-lactate and succinate oxidase activities that were at least as high as those for JP2140. Whereas strains NSW14 and NSW15 had elevated NADH oxidase activities relative to JP2140, strain NSW17 contained somewhat lower activities of the three oxidases relative to JP2140 (Table III). The significance of the variations in oxidase rates in the hypersensitive mutants is not immediately evident.

TABLE III

SPECIFIC ACTIVITIES FOR OXIDASE SYSTEMS AND Mg^{2+}-ATPase, AND P/O RATIOS FOR STRAINS HYPERSENSITIVE TO STREPTOMYCIN

The methods for membrane preparation and measurement of oxidase rates, Mg^{2+}-ATPase activity and P/O ratios are described in Materials and Methods. For the P/O ratios D-lactate was used as substrate, however essentially the same results were obtained with NADH.

Membranes from	Rate of oxygen uptake (ngatoms O/min per mg protein)			Mg^{2+}-ATPase activity (nmol/min per mg protein)	P/O
	NADH	D-Lactate	Succinate		
JP2140	467	142	142	207	0.12
NSW13	575	156	74	35	<0.01
NSW14	758	214	80	250	0.03
NSW15	698	182	162	78	0.07
NSW17	388	81	56	384	<0.01
NSW18	562	206	234	204	0.04

Genetic analysis of strains NSW13, NSW14 and NSW17

The genes encoding the peptides for the Mg^{2+}-ATPase complex constitute an operon situated close to *ilv* on the bacterial chromosome [11]. In order to determine whether or not the mutations responsible for uncoupling in three of the streptomycin-hypersensitive strains mapped close to *ilv*, we introduced *ilv*$^+$ (by transduction) into strains NSW13, NSW14 and NSW17. Bacteriophage Plkc lysates prepared on these *ilv*$^+$ strains, were then used to transduce JP2140 to *ilv*$^+$. Forty *ilv*$^+$ transductants from each of the three crosses were tested for the ability to grow on succinate. A substantial number of *ilv*$^+$ recombinants from each cross were found to be unable to grow on succinate though giving almost normal growth on glucose.

Membranes from one Suc$^-$ and one Suc$^+$ recombinant strain (from each of the above transductions) were assayed for Mg^{2+}-ATPase activity, NADH, D-lactate and succinate oxidase activities, and for phosphorylation (P/O ratios). The results (not shown) were consistent with those of the original mutants, i.e. a Suc$^-$ transductant from NSW13 had a greatly reduced Mg^{2+}-ATPase activity compared to an isogenic Suc$^+$ transductant from the same cross, whereas Suc$^-$ transductants from strains NSW14 and NSW17 had Mg^{2+}-ATPase activities similar to those for isogenic Suc$^+$ strains but had very low P/O ratios (less than 0.01).

It is concluded that strains NSW13, NSW14 and NSW17 are uncoupled because of mutations in the *unc* genes (see Table I for allele numbers). No genetic mapping experiments have been carried out on the other uncoupled mutants, NSW15 and NSW18.

Discussion

The results presented in this paper indicate that alterations in a respiratory function of the cytoplasmic membrane can influence the rate of uptake of streptomycin in sensitive bacterial strains. Specifically, mutations in either the F_0 (*uncB402*) or F_1 (*uncA401*) components of the Mg^{2+}-ATPase lead to an increase in sensitivity (hypersensitivity) at concentrations of the antibiotic that are low enough to allow growth of wild-type strains (Fig. 3). Previous experiments [21] showed that the *uncA401* mutation increased the rate of uptake and the total amount of streptomycin accumulated in a streptomycin-resistant strain of *E. coli*.

Studies with Suc$^+$ revertants derived from either *uncA401* or *uncB402* mutants have confirmed that both uncoupling and hypersensitivity to streptomycin result from the mutations in the *unc* genes. In an earlier publication [10], a mutant of *E. coli* with reduced Mg^{2+}-ATPase activity, was found to be hypersensitive to streptomycin, but in this case no genetic evidence was presented to show that uncoupling and increased sensitivity to streptomycin resulted from a single mutation.

The reasons why some uncoupled strains take up streptomycin more readily than coupled strains are not yet understood. It is tempting to suggest that increased uptake results from the increased rates of electron flow from D-lactate to oxygen (and to a lesser extent from succinate and NADH to oxygen) which occur in uncoupled strains (see Table II). This suggestion is con-

sistent with the results of recent experiments which have shown that the entry of streptomycin into both streptomycin-sensitive and streptomycin-resistant bacterial strains is an energy-dependent process [13,18]. Inhibition of uptake under anaerobic conditions [22] or in the presence of respiratory chain inhibitors [13,17,18], and the reduction of uptake in certain respiratory mutants [21], suggest that a functional electron transport chain is required for entry of the antibiotic. Recent experiments in our laboratory, with strains containing low levels of ubiquinone (and no menaquinone), suggest that quinone concentrations rather than overall electron transport rates in membranes control the rates of streptomycin uptake (Muir, M., unpublished results; see also Ref. 21). The relationship between the energy requirements for streptomycin uptake and changes in the permeability of cytoplasmic membranes that occur in streptomycin-treated cells [23,24] is yet to be explained.

It is noteworthy that not all uncoupled mutants show an increased uptake rate for streptomycin. For example a neomycin-resistant mutant, NR-70, deficient in Mg^{2+}-ATPase, cannot phosphorylate, has impaired aerobic transport of several solutes [8] and has a reduced capacity to take up streptomycin and gentamicin [21]. It has been proposed that the *unc*$^-$ mutation in strain NR-70 results in a cytoplasmic membrane which is unable to maintain a transmembrane potential [2]. Moreover, chemical uncoupling agents such as CCCP (Fig. 2) and dinitrophenol (Muir, M., unpublished results; see also Refs. 13 and 18), which destroy the transmembrane proton gradient [25], reduce or abolish streptomycin uptake in *unc*$^+$ and *unc*$^-$ strains. These results suggest that the uptake of streptomycin depends on the existence of a proton gradient across the cytoplasmic membrane. Thus the selection of mutants which are resistant to aminoglycosides (e.g. neomycin), might generally yield strains in which the capacity to maintain a transmembrane potential has been reduced or abolished, while selection of mutants which are hypersensitive to aminoglycosides might yield strains which, although uncoupled, can maintain a transmembrane potential.

In an experiment described here we have shown that it is possible to isolate *unc*$^-$ strains by selecting for hypersensitivity to streptomycin. Thus nine of the 12 hypersensitive strains isolated were unable to grow on succinate as sole source of carbon. Experiments with respiratory membranes prepared from five of the Suc$^-$ strains showed that all had a reduced capacity to phosphorylate though not completely lacking in some cases (Table III). In addition, two of the five Suc$^-$ mutants had lower than wild-type Mg^{2+}-ATPase activities. A genetic analysis of three of the uncoupled mutants indicated that in each case the mutation was cotransducible with *ilv*, a result which is consistent with results from previous studies [11]. It is possible that the isolation of uncoupled strains by the selection procedure described here will aid in the identification of new classes of uncoupled strains.

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