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Overexpression of the *Escherichia coli sugE* Gene Confers Resistance to a Narrow Range of Quaternary Ammonium Compounds

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SugE of *Escherichia coli*, first identified as a suppressor of *groEL* mutations but a member of the small multidrug resistance family, has not previously been shown to confer a drug resistance phenotype. We show that high-level expression of *sugE* leads to resistance to a subset of toxic quaternary ammonium compounds.

Multidrug resistance in virulent bacteria is an increasingly important problem in treating infectious diseases in humans and animals (12). Five large ubiquitous superfamilies of drug efflux pumps include all currently recognized transporters responsible for multidrug resistance in bacteria (15). One of these superfamilies, the drug-metabolite transporter superfamily (TC 2.A.7) includes a prokaryotic-specific family known as the small multidrug resistance (SMR) family (TC 2.A.7.1) (8, 13). The members of this family are small (about 110 amino acids), exhibit four transmembrane segments, and can function as either homo- or heterodimeric systems (3, 7, 9). Based on the essentiality of a fully conserved glutamate residue in the EmrE pump of *Escherichia coli* and its homologues (10, 17), a simple model has been proposed for the coupling of H^+ import to substrate export (18). The model suggests time sharing of a single cation binding site presumed to be the conserved glutamate (18). The recently published low-resolution projection structure (7 Å) of EmrE reveals an asymmetric dimer with no apparent twofold-symmetry axis (16). This observation suggests that the two monomers have dissimilar structures and functions, leading to the proposal of a "half of sites"-type mechanism (14).

Several homooligomeric SMR family members in *E. coli* have recently been shown to confer a drug resistance phenotype (11). However, in spite of extensive efforts in this direction, no drug resistance phenotype for the distant SMR family homologues of the SugE subfamily of proteins from various gram-negative and gram-positive bacteria has been reported (8, 11, 13). The *sugE* gene was initially mapped to the 94-min region of the *E. coli* chromosome and was shown to phenotypically suppress a *groEL* mutation, mimicking the effect of *groE* overexpression when present on a multicopy plasmid (5). As demonstrated by Bishop et al. (1), a single gene, rather than the two-gene system proposed by Greener et al. (5), proved to be present at this locus.

We have cloned the *E. coli sugE* gene into two different vectors and here show that its overexpression in various *E. coli* strains confers a very specific and restricted phenotype: resistance to a subset of recognized antiseptics. Other compounds tested, including a variety of structurally related quaternary ammonium compounds as well as cationic dyes, did not appear to be substrates. Our results answer the long-standing question as to whether or not the product of the *sugE* gene, like several of its characterized distant homologues, is capable of functioning as a drug efflux pump. They lead to the suggestion that all members of the SMR family function in cationic drug export.

The *E. coli sugE* gene was cloned into the expression vectors pBAD24 and the pCR TA cloning vector, pCR2.1 (pCR2.1 TOPO; Invitrogen, Inc.). The procedure was as follows. The targeted gene was amplified by PCR with *Taq* polymerase using *E. coli* MG1655 chromosomal DNA as a template. For cloning in pBAD24, the primers (5′ to 3′) were CAGAATTC GATATGTCCTGGATTATCTTAG (sense) and CACTGCG CCTGGTAGTTAGTGAGTGC (antisense); for cloning in pCR2.1, the primers were CAGCGATAGTCACAAAGGTA ATAG (sense) and CACTGCAGCCTGGTAGTTAGTGAG TGC (antisense). The DNA was digested with the *Eco*RI and *Pst*I restriction enzymes (in pBAD24 [underlined]) with restriction sites flanking the target gene copied by PCR. The included genes were then cloned into the pBAD24 polylinker region. The pBAD24 ligation mixture was introduced into competent *E. coli* DH5α cells or another host. Finally, transformants were selected on the basis of ampicillin (60 μg/ml) resistance. The cloned gene(s) in pBAD24 was expressed either at low (basal) levels (in the absence of L-arabinose) or at high levels (in the presence of 0.2% L-arabinose). When cloning into pCR2.1, the amplified PCR product was designed to contain 134 bp upstream of the *sugE* gene. This DNA fragment was directly cloned into the vector following the instructions of the manufacturer. Recombinant plasmids were checked by restriction enzyme digestion and direct sequencing. In the case of the gene inserted into pCR2.1, the *sugE* gene proved to be oriented oppositely to the *lacZ* gene in the vector. Consequently, *sugE* is expressed under the control of its own promoter.

Drug assay plates were prepared with Luria-Bertani (LB) broth or M9 minimal agar, using glycerol instead of glucose as a carbon source, with or without 0.2% L-arabinose as an inducer, and various series of drug concentrations. *E. coli* strain DH5α and other hosts bearing the pBAD24 vector, the pCR2.1 vector, or bearing these plasmids with the gene of interest were grown overnight in LB broth with 60 μg of ampicillin/ml at 37°C with shaking (220 rpm). Subcultures were

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant genotype or phenotype	Source or reference
E. coli strains		
MG1655 DH5α	Wild type, donor strain F$^-$ ϕ80d *lacZΔM15* Δ(*lacZYA-argF*)*U169 deoR recA1 endA1 hsdR17*(r_K^- m_K^-) *gal-phoA supE44* λ$^-$ *thi-1 gyrA96 relA1*	
ABLE C	*lac*(lacZω$^-$) [Kan^r McrA$^-$ McrCB$^-$, McrF$^-$ Mrr$^-$ *hsdR*(r_K^- m_K^-)][F′ *proAB lacI^{q}ZΔM15* Tn*10*(Tet^r)]	Stratagene, Inc.
TOP10F′	F′[*lacI^q* Tn*10*(Tet^r)] *mcr*A Δ(*mrr-hsdRMS-mcrBC*) ϕ80 *lacZΔM15ΔlacX74 deo*R *recA1 araD139* Δ(*ara-leu*)*7697 galU galK rpsL*(Str^r) *endA1 nupG*	Invitrogen, Inc.
CG3014	W 3110 *galE*, resistant to phages T1, T5, carries Tn*10*(Tet^r), wild type	4
CG3010	As above, but *groEL44*, Tet^r	4
CG3013	As above, but *groES619*, Tet^r	4
Plasmids		
pBAD24	Amp^r, L-arabinose-inducible expression vector	6
pBAD24-*sugE*	Amp^r, pBAD24 carrying *sugE*	This study
pCR2.1 (pCR2.1 TOPO)	Amp^r, Kan^r, PCR cloning vector	Invitrogen, Inc.
pCR2.1-*sugE*	Amp^r, Kan^r, pCR 2.1 carrying the *sugE* gene plus 134-bp upstream promoter region of *sugE*	This study

grown to an A_{600} of 0.6 optical density units in LB broth with 60 μg of ampicillin per ml and 0.2% L-arabinose at 37°C with shaking. These cultures were diluted 10^0, 10^{-1}, and 10^{-2} in LB broth, and 5-μl samples of each transformant at each dilution were plated on the above-mentioned assay plates. When using LB, no inducer was required. The plates were incubated overnight at 37°C, and drug resistance was scored after 12, 18, and 24 h of growth.

Complementation experiments at various temperatures were performed by transforming *E. coli* strains CG3010, CG3013, and CG3014 with pCR2.1 and pCR2.1-*sugE*. Transformants were streaked onto LB plates containing 60 μg of ampicillin/ml, and the plates were incubated at 30, 37, or 43°C. Colony number, size, and appearance were monitored after 24 and 48 h.

The bacterial strains and plasmids used in this study are listed in Table 1. ABLE-C is an *E. coli* strain which reduces the copy number of a plasmid, allowing escape from toxic protein overexpression. TOP10F′ is a strain that allows tight regulation of plasmid gene expression. CG3010 and CG3013 are mutated in the *groEL* and *groES* genes, respectively, while CG3014 is the isogenic parental strain (4).

When the *sugE* gene was expressed using either the pBAD24 expression vector or the pCR2.1 vector, a resistance phenotype was observed regardless of whether the host *E. coli* strain was DH5α, TOP10F′, or ABLE-C (Table 2). Moreover, the phenotype was almost identical when the strains were examined on plates containing complex or minimal medium. Two of the several strain-medium combinations examined are presented in Table 2. The four compounds that exhibited diminished toxicity when *sugE* was expressed were the cetylpyridinium, cetyldimethylethyl ammonium, and cetrimide cations. They proved to be two to eight times less inhibitory when *sugE* was expressed. A resistance phenotype was not observed for the other quaternary ammonium compounds tested or for the cationic dyes examined (see footnote *b* to Table 2). The former compounds were not tested by Nishino and Yamaguchi (11), who reported the absence of a phenotype for strains expressing *sugE*.

E. coli strains mutant for GroEL or GroES do not grow well at 43°C (4). We therefore tested to see if *sugE* expression overcame the growth defect in either a *groEL* or a *groES* mutant strain. Following growth on LB plates containing 60 μg of ampicillin/ml, expression of the *sugE* gene in pCR2.1 did not correct the growth defect. We were therefore unable to observe a generalized suppression of the *groEL* or *groES* defect using this assay. It should be noted that Greener et al. (5) did not test for this phenotype. Moreover, their cloned *sugE* gene exhibits a sequencing difference compared with that reported by both Bishop et al. (1) and Blattner et al. (2), resulting in a longer transcript not found in the *E. coli* K-12 strains studied by Bishop et al. and by us. The larger gene reported by Greener et al. (5) overlaps the *encB* gene shown by Bishop et al. (1) to encode enterocidin B. We therefore suggest that the larger protein identified by Greener et al. (5) was artifactual and that the *E. coli* SugE is not a generalized suppressor of GroEL defects.

The results reported here establish that *sugE* gene expression can confer a highly specific drug resistance phenotype. Like most other members of the SMR family, the phenotype

TABLE 2. MICs of various quaternary ammonium compounds in *E. coli* strains expressing or not expressing *sugE*

Compound	MIC (μg/ml)[a]			
	TOP10F′ (pCR2.1)[b]	TOP10F′ (pCR2.1-*sugE*)[b]	DH5α (pBAD24)[b]	DH5α (pBAD24-*sugE*)[b]
Cetylpyridinium chloride ($C_{21}H_{38}NCl$)	20	160	20	160
Cetylpyridinium bromide ($C_{21}H_{38}NBr$)	40	160	40	180
Cetyldimethylethyl ammonium bromide ($C_{20}H_{42}NBr$)	60	120	60	120
Hexadecyltrimethyl ammonium bromide (cetrimide) ($C_{19}H_{42}NBr$)	80	120	60	120

[a] Assayed on LB plates using the pBAD24 and pCR2.1 vectors without L-arabinose. Inclusion of L-arabinose, promoting high-level expression when using the pBAD24 vector, proved toxic.

[b] Cells did not show increased resistance to other quaternary ammonium compounds (benzyl-dimethyl tetradecylammonium chloride, benzalkonium chloride) or to cationic dyes (tetraphenyl-arsonium chloride, pyronine Y, crystal violet, ethidium bromide).

apparently reflects specificity for cationic compounds. Unlike the other tested members of the family, however, SugE confers a resistance phenotype only to a small subset of structurally divergent quaternary ammonium disinfectants. No other SMR family member has been shown to transport such a limited range of compounds. The YvaE homologue of *Bacillus subtilis* confers resistance to these compounds as well as several others not transported by *E. coli* SugE (3), and YvaE was the first SMR pump in *B. subtilis* shown to export these antiseptics. Thus, the sequence-divergent SugE protein of *E. coli* exhibits a previously unrecognized narrow specificity for a very specific class of compounds. The same may be predicted for orthologous gene products from other bacteria. Whether or not these represent the only physiologically important substrates of the SugE subfamily members has yet to be determined.

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