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High-Level Mupirocin Resistance in *Staphylococcus aureus*: Evidence for Two Distinct Isoleucyl-tRNA Synthetases

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Mupirocin resistance in *Staphylococcus aureus* results from changes in the target enzyme, isoleucyl-tRNA synthetase (IRS). Twelve strains of *S. aureus* comprising four susceptible (MICs ≤ 4 μg/ml), four intermediate level-resistant (MICs between 8 and 256 μg/ml), and four highly resistant (MICs ≥ 512 μg/ml) isolates were examined for their IRS content and the presence of a gene known to encode high-level mupirocin resistance. Ion-exchange chromatography of cell extracts showed a single IRS active peak in mupirocin-susceptible strains, with 50% inhibitory concentrations (IC_{50}s) of 0.7 to 3.0 ng of mupirocin per ml. In strains showing intermediate mupirocin resistance, similar single IRS activity peaks were observed, but these were less sensitive to inhibition, and the mupirocin IC_{50}s for them were 19 to 43 ng/ml. Strains that were highly resistant to mupirocin displayed two distinct peaks; one was similar to that found with susceptible strains (IC_{50}, 0.9 to 2.5 ng/ml), but an additional peak with an IC_{50} of 7,000 to 10,000 ng/ml was also observed. A strain cured of the plasmid encoding high-level mupirocin resistance lacked the resistant IRS peak. Restriction digests, produced by endonuclease *Nco*I, of total bacterial DNA isolated from the highly resistant strains hybridized with a mupirocin resistance gene probe, whereas DNA isolated from the intermediate level-resistant and susceptible strains did not. These results demonstrate that two different IRS enzymes were present in highly mupirocin-resistant *S. aureus* strains. In strains expressing intermediate levels of resistance, only a chromosomally encoded IRS which was inhibited less by mupirocin than IRS from fully susceptible strains was detected.

Mupirocin (pseudomonic acid A) is a narrow-spectrum topical antibiotic active predominantly against gram-positive pathogens, particularly staphylococci, including methicillin-resistant *Staphylococcus aureus*, and streptococci (20). Previous studies (11, 13) have shown that mupirocin inhibits bacterial isoleucyl-tRNA synthetase (IRS), which charges the appropriate tRNA species with isoleucine. This inhibition decreases or abolishes bacterial protein synthesis by preventing the incorporation of isoleucine into nascent peptides. The epoxide-bearing end of the mupirocin molecule resembles the carbon skeleton of isoleucine and may compete for the active site of the IRS enzyme (11). Further studies, however, are necessary to elaborate this mechanism of action. Previous data from our laboratory (8) showed that inhibition of IRS by mupirocin was time dependent and irreversible, suggesting that the mupirocin-IRS enzyme complex was highly stable.

Since the introduction of mupirocin into clinical practice in 1985, there have been few reports of *S. aureus* strains exhibiting reduced susceptibility to the agent (17). These resistant strains can be divided arbitrarily into two distinct groups, those which exhibit intermediate levels of resistance (MICs, 8 to 256 μg/ml) and those which exhibit high levels of resistance (MICs, ≥512 μg/ml).

Staphylococcal strains exhibiting intermediate levels of resistance were first reported by Casewell and Hill (3), who found that expression of resistance was stable when isolates were subcultured on drug-free media. Such variants can also be selected in vitro after serial subculture in the presence of increasing concentrations of mupirocin (2, 3). The clinical incidence of this type of resistant variant is low and appears not to present a serious therapeutic problem (17). The molecular basis of this resistance is unclear but may arise from a mutation in the chromosomal gene resulting in a specific change at the active site of the IRS enzyme.

Clinical strains highly resistant to mupirocin were first reported in 1987 (15), and expression of resistance was unstable on subculture. Mupirocin resistance could be transferred between staphylococci at a low frequency by conjugation and was found to be associated with a large open circular (relaxed) plasmid. Later, Smith and Kennedy (18) isolated a strain of *S. aureus* which exhibited high-level resistance to mupirocin but appeared not to possess a plasmid. These findings were subsequently confirmed in our laboratories by in vitro transfer, curing studies, and pulsed-field gel electrophoresis to demonstrate the presence or absence of a large plasmid.

Despite the early reports of transmissible high-level mupirocin resistance, there have been few further reports of clinical isolates of staphylococci which exhibit mupirocin resistance. A large multicenter survey of in vitro mupirocin susceptibility in the United Kingdom (4) involving 8,220 strains of staphylococci (comprising 7,137 strains of *S. aureus* and 1,083 strains of coagulase-negative staphylococci) concluded that a small percentage (0.3%) of strains of *S. aureus* showed some resistance. Of these, only four isolates were highly resistant (0.06%); three of the four were from the same center and were likely to represent isolates of the same strain. The reported numbers of mupirocin-resistant strains among the coagulase-negative staphylococci tested were much higher (3%), although in this case the numbers of isolates highly resistant to mupirocin were not recorded. It is evident, however, that despite increasing worldwide usage, the reported incidence of mupirocin resistance, particularly plasmid-mediated high-level resistance, from all sources, remains extremely low and that the major-

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TABLE 1. Sources and antibiotic susceptibilities of and mupirocin IC_{50}s for *S. aureus* strains used in this study

Strain	Source	MIC (μg/ml) of[a]:				IC_{50} for IRS FPLC peaks (ng/ml)		
		MUP	FA	MEC	ERY	Unfractionated	First peak	Second Peak
RN2677	Reference strain	0.5	0.25	2	0.5	0.8	ND[b]	0.7
NCTC 6571	Reference strain	0.5	0.5	2	0.5	1.1	ND	2.0
NCTC 11561	Reference strain	0.5	0.5	4	1	2.9	ND	3.0
Ck	Skin isolate, Glasgow, United Kingdom	32	0.5	1,024	>1,024	110[c]	ND	25.8[c]
L5	Skin isolate, Liverpool, United Kingdom	32	256	32	>1,024	85	ND	18.7
C9	Skin isolate, New York, N.Y.	128	0.5	4	0.5	135	ND	21.1
C35	Skin isolate, New York, N.Y.	256	0.5	4	0.5	110	ND	43.3
RN2677(pJ2385)	Laboratory transconjugant	>4,096	0.5	4	0.5	10,000	8,200	2.5
F89	Skin isolate, Bristol, United Kingdom	>4,096	16	4	0.5	8,600	7,000	1.3
C1	Skin isolate, New York, N.Y.	>4,096	0.5	2	0.5	11,000	10,000	1.6
J2385 Mup^r	Skin isolate, London, United Kingdom	>4,096	0.5	4	>1,024	10,500	9,750	0.9
J2385 Mup^s	Cured variant of J2385	4	0.5	4	>1,024	2.5	ND	1.3

[a] MUP, mupirocin; FA, fusidate sodium; MEC, methicillin; ERY, erythromycin.
[b] ND, no activity detectable.
[c] IC_{50}s for the unfractionated extracts from intermediately resistant strains were higher than values for the active peaks, possibly as a result of differences in enzyme concentration.

ity of resistant isolates arise from long-term therapy of several weeks in duration (6).

Dyke et al. (7) mapped a 25-kb plasmid associated with high-level mupirocin resistance in *S. aureus*. The gene conferring resistance to mupirocin was subsequently cloned on a 4.05-kb *Eco*RI fragment into an *Escherichia coli-S. aureus* shuttle vector and designated pOX301. The DNA sequence of a small region of this gene was homologous with the sequence of the IRS gene from *E. coli*, providing evidence that high-level mupirocin resistance results from a modified IRS enzyme. This vector, incorporating the mupirocin resistance gene, was supplied by K. J. H. Dyke and used for hybridization work in the present study.

Previous studies (8) demonstrated a correlation between IRS enzyme inhibition by mupirocin and MICs for 21 *S. aureus* strains exhibiting various degrees of resistance to the antibiotic. The results showed that a reduction in the level of inhibition of the IRS enzyme by mupirocin correlated with a decrease in the susceptibilities of the strains investigated. The current study was undertaken to examine evidence for the existence of two possible mechanisms of mupirocin resistance.

MATERIALS AND METHODS

Bacterial strains. The strains of *S. aureus* used in this study and their sources and antibiotic susceptibilities are listed in Table 1. All strains were cultured on nutrient agar or tryptone soy broth unless otherwise stated.

Antibacterial compounds. Mupirocin lithium salt and methicillin sodium salt laboratory reference standards were prepared by SmithKline Beecham Pharmaceuticals (Worthing, United Kingdom). Fusidate sodium was purchased from Leo Laboratories Ltd. (Princes Risborough, Buckinghamshire, United Kingdom), and erythromycin (laboratory reference standard) was purchased from Sigma Chemical Co. (Poole, United Kingdom).

Susceptibility testing. The antibacterial agents, corrected for purity, were prepared in aqueous solution and serially diluted in Mueller-Hinton agar (BBL). The surface of the agar was inoculated with 1 μl of an undiluted overnight broth culture of the test bacteria to give a final inoculum of 5×10^5 CFU per spot (21).

Preparation of bacterial total DNA. Cells from 1.5 ml of overnight culture were washed in TES buffer (50 mM Tris [pH 8], 50 mM NaCl, 5 mM EDTA) before being resuspended in 200 μl of TES buffer containing 1 mg of lysostaphin per ml and 100 μg of RNase per ml and incubated at 37°C for 10 to 20 min. Addition of 40 μl of lysis buffer (0.5% sodium dodecyl sulfate [SDS], 50 mM Tris [pH 8], 500 mM EDTA, 1 mg of proteinase K per ml) and incubation at 50°C for 10 to 20 min were followed by phenol-chloroform extractions. DNA was precipitated as described by Sambrook et al. (16), resuspended in 50 μl of sterile water, and stored at −20°C.

DNA analysis. Restriction endonucleases were purchased from GIBCO-BRL (Paisley, United Kingdom) and used according to the manufacturer's recommended conditions. DNA fragments were separated by electrophoresis in 0.8% agarose gels and run in Tris-borate buffer (16).

Probe preparation. The mupirocin resistance gene of *S. aureus* has recently been fully sequenced (10). A region of this gene between the two *Nco*I sites was amplified by the polymerase chain reaction (PCR) to yield a 1.65-kb probe (Fig. 1). The oligonucleotide primers CAR II (5′-CCAT GCCTTACCAGTTGAATT-3′) and CAR III (5′-GGATC CCCGAGCACTATCCGA-3′) were synthesized with a Gene Assembler Plus (Pharmacia, Milton Keynes, United Kingdom). The amplification reaction mixture contained 10 ng of pOX301 plasmid DNA, 10 μl of 10× PCR buffer (supplied with Tub DNA polymerase by Amersham International, Amersham, United Kingdom), 100 pmol of each primer, deoxynucleoside triphosphates at final concentrations of 200 μM each, and 2.5 U of Tub DNA polymerase. The mixture was placed in a thermal cycler (Techne PHC-3) and denatured at 95°C for 5 min before being cycled 30 times at the following conditions: 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min.

Hybridization analysis. Filters for hybridization were prepared by the method of Southern (19) with nylon membranes (Hybond N$^+$; Amersham). DNA was fixed by treatment with alkali as recommended by the manufacturer. The filters were then probed with the 1.65-kb PCR-generated intragenic DNA fragment. The probes were purified through Centricon-30 microconcentrators (Amicon) and labeled by the Enhanced Chemiluminescence (ECL) gene detection system (Amersham).

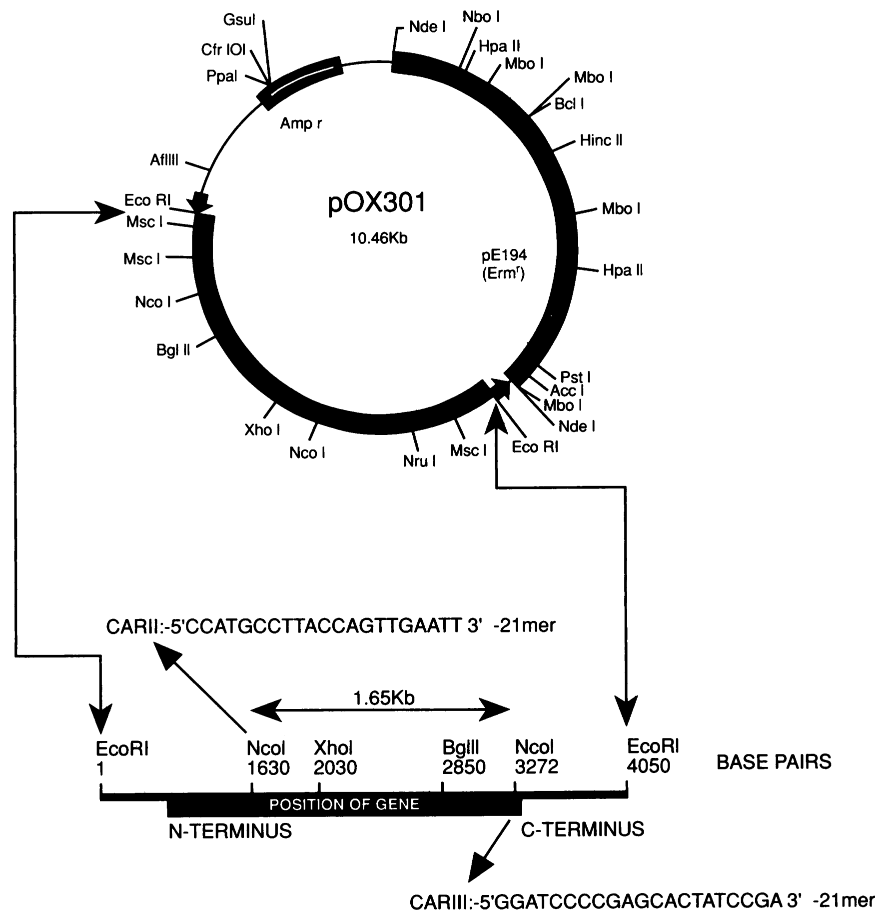

FIG. 1. Map of plasmid pOX301 showing the PCR-amplified *Eco*RI fragment bearing the mupirocin resistance gene (7). This plasmid formed the shuttle vector; the region between the two *Nco*I sites was amplified to make the hybridization probe.

Preparation of *S. aureus* cell IRS extracts. *S. aureus* strains were cultured by inoculation of 250-ml seed flasks containing 50 ml of nutrient broth no. 2 (Oxoid, Basingstoke, United Kingdom) with 50 μg of mupirocin per ml for highly resistant strains, 10 μg of mupirocin per ml for moderately resistant strains, or no mupirocin for sensitive strains and incubated overnight at 37°C while being shaken at 240 rpm. Five milliliters of each culture was used to inoculate 2-liter flasks containing 500 ml of nutrient broth no. 2 (all without mupirocin); these cultures were grown to the late log phase in a shaking incubator at 37°C for 6 h. Cells were harvested by centrifugation at 10,000 × g, and the pellets were resuspended in 40 ml of a buffer containing 0.1 M Tris (pH 7.9) (at 37°C), 10 mM $MgCl_2$, and 2 mM dithiothreitol, making a concentration of 25× the original bacterial cell density. Lysostaphin was added (10 μg/ml), and the suspensions were shaken at 240 rpm for 1 h at 37°C before being sonicated on ice for eight 30-s bursts with 15-s intervals for cooling (Soniprep 150; MSE). The cell debris was pelleted by centrifugation at 20,000 × *g* for 20 min, and the supernatant was centrifuged further at 120,000 × *g* for 2 h at 4°C. Ammonium sulfate was added to each supernatant to a 40% concentration, and the mixture was stirred on ice for 30 min. The resulting suspension was then centrifuged at 20,000 × *g*, and the precipitate was redissolved in BTP buffer (20 mM bis-Tris propane [pH 6.4], 10 mM $MgCl_2$, and 2 mM dithiothreitol) and dialyzed against the same buffer for 24 h with three changes of the dialysate. The protein concentrations in each extract were determined by the method of Bradford (1).

Fractionation of cell extracts by FPLC. Approximately 15-mg amounts of total protein (3 to 5 ml) of the IRS-containing extract were fractionated in each chromatographic run. The fast protein liquid chromatography (FPLC) system (Pharmacia) consisted of an LCC 500 Plus controller, two P-500 pumps connected to a mixing chamber, and a 10-ml injection loop. The column was a 1-ml HR 5/5 column containing Mono-Q Sepharose anion-exchange resin. This was serially connected to a UV-M detector set at 280 nm and a FRAC-100 fraction collector. The system was computer controlled with FPLC Manager software (Pharmacia). The flow rate throughout was 1 ml/min. Fifteen minutes after injection, a linear NaCl gradient (0 to 1 M NaCl; 50 min) was started in BTP buffer. The 1 M salt concentration was maintained for 5 min, and then the BTP buffer was returned to the original conditions for the final 5 min. Sixty 1-ml fractions were collected during each run.

IRS enzyme inhibition assays. The activity of IRS was determined by a radiometric assay modified from a previously described method (11). A reagent mixture (mix A) was prepared and stored frozen at −25°C in aliquots before use. This mixture contained 45 mM Tris-HCl (pH 7.9 determined at 37°C), 125 mM KCl, 18 mM magnesium acetate, 3.5 mM dithiothreitol, 2.5 mg of tRNA (Boehringer) per ml, 3.75 mg of ATP per ml, and 7.6 μM (92.4-kBq/ml)L-[U-^{14}C]isoleucine

(Amersham; supplied as 1.85 MBq/ml) or for more sensitive assays 0.54 μM (1.85-MBq/ml) L-[4,5-^{3}H]isoleucine (Amersham; supplied as 37.0 MBq/ml).

Threefold dilution series were prepared to give final concentrations in the following ranges: 100 mg/liter to 50 μg/liter for assay fractions and extracts from highly resistant strains, 3.7 mg/liter to 5 μg/liter for assay fractions and extracts from intermediately resistant strains, and 400 to 0.2 μg/liter for assay fractions and extracts from susceptible strains. An aliquot (50 μl) of unfractionated enzyme extract was added to 10 μl of a solution containing mupirocin in an appropriate range of concentrations in the wells of a 24-well microtiter plate and incubated at 37°C for 5 min. Next, 100 μl of reagent mix A was added to each well, and the plates were agitated to mix the enzyme and the substrate and then incubated at 37°C for a further 10 min. For IRS activity assay of the FPLC fractions, [^{3}H]isoleucine in place of [^{14}C]isoleucine was used to increase sensitivity and 100 μl of each fraction was mixed with 50 μl of reagent mix A. A 2-ml volume of 7% trichloroacetic acid solution was added to each well to terminate the reaction and precipitate tRNA. The plates were left on ice for 20 min before the samples were harvested onto filter mats (Pharmacia) with a Combi automatic sample-harvesting system (Skatron). The filter mats were sealed in bags with 12 ml of scintillant fluid and counted in a 1205 Betaplate liquid scintillation counter (Pharmacia). The IC_{50}s were determined from plots of inhibitor concentration versus percentage of control IRS activity (the IC_{50} is the concentration of mupirocin required to inhibit activity of the IRS enzyme by 50%).

Analysis of IRS-active FPLC fractions. Mupirocin IC_{50} determinations were performed on all the FPLC fractions which were determined to be IRS active. These assays used the ^{14}C-labelled isoleucine method as described above. The protein concentrations in these active fractions were measured with Pierce bicinchoninic acid protein assay reagent (Life Science Laboratories Ltd., Luton, United Kingdom).

RESULTS

MIC determinations. The MICs of mupirocin, fusidate sodium, methicillin, and erythromycin for all of the strains used in this study are given in Table 1. The data show that several of the clinical isolates were resistant to mupirocin but were not cross-resistant to the other antibiotics tested. The MICs of mupirocin obtained for the *S. aureus* isolates indicate various degrees of susceptibility; the MICs ranged from 0.5 to >4,096 μg/ml. Strains RN2677, NCTC 6571, NCTC 11561, and J2385 Mup^s were susceptible; strains Ck, L5, C9, and C35 were intermediately resistant; and strains RN2677(pJ2385), F89, C1, and J2385 Mup^r were highly resistant to mupirocin.

Hybridization analysis. The agarose gel separation of *Nco*I-digested total DNA from each of the strains is shown in Fig. 2A. It can be seen that although all of the isolates are *S. aureus* strains, digestion of DNA with the restriction enzyme produced a heterogeneous range of banding patterns. Figure 2B shows the results obtained when DNA extracts from these strains were hybridized with a gene probe specific for mupirocin resistance. Only those isolates for which the MIC of mupirocin is at least 512 μg/ml hybridized with the probe, producing a single band. However, *S. aureus* F89 (MIC > 4,096 μg/ml) was an exception, producing two bands. This strain could not be cured of its resistance and did not possess a transferable plasmid encoding this gene, unlike the other highly resistant isolates. Isolates for which the MICs are less than 256 μg of mupirocin per ml did not hybridize, even under conditions of low stringency.

FPLC separations of IRS cell extracts. Unfractionated cell extracts showed a general increase in the IC_{50}s for IRS with increasing MICs of mupirocin (Table 1), as previously reported (8). A representative anion-exchange chromatogram and IRS activity profile for a mupirocin-susceptible *S. aureus* strain are shown in Fig. 3A. Figure 3B shows the enzyme activity profile for an intermediately resistant strain, and Fig. 3C shows the profile for a highly resistant strain. SDS-polyacrylamide gel electrophoresis separation of the active fractions and the unfractionated extracts revealed that FPLC had partially purified the IRS enzyme, but no significant differences between resistant and sensitive fractions could be discerned. The protein concentration/IRS activity ratio was increased between 50- and 100-fold after FPLC fractionation. The FPLC protein profiles for all 12 strains in this study were similar and showed no identifiable differences between the three susceptibility groups. The activity profiles, however, did show differences. With the susceptible strains, only a single activity peak, eluting at 250 mM NaCl, was seen, and this peak was believed to represent normal chromosomally encoded IRS. These active fractions were sensitive to mupirocin (Table 1), with IC_{50}s ranging from 0.7 to 3.0 ng/ml. The levels of IRS activity and mupirocin inhibition in the unfractionated cell extracts were similar to those found in the active peaks. In the intermediately resistant strains, a single IRS active peak eluting at the same time as the sensitive type was seen. The active fractions of this peak were less sensitive to inhibition by mupirocin (IC_{50}, 18.7 to 43.3 ng/ml). Further attempts to separate different IRS enzyme components from the intermediately resistant strains by using a range of salt gradients consistently produced only a single activity peak. The highly resistant strains, however, possessed the mupirocin-sensitive enzyme and an additional enzyme eluting at 150 mM NaCl. This enzyme was inhibited less by mupirocin (IC_{50}s for this peak were 7,000 to 10,000 ng/ml).

S. aureus RN2677 was susceptible to mupirocin (MIC, 0.5 μg/ml) and possessed only one mupirocin-sensitive IRS enzyme. However, after acquisition of a plasmid following conjugation with a highly resistant strain, *S. aureus* J2385, it became highly mupirocin resistant (MIC, >4,096 μg/ml) and possessed the additional resistant enzyme.

DISCUSSION

The range of antibiotic susceptibilities of the 12 strains used in this study is typical of those seen in clinical settings and similar to those previously reported (20, 22). The results demonstrate that mupirocin resistance can be carried with other resistance determinants within the same strain, but to date it has not been linked with another antibiotic resistance determinant. However, mupirocin resistance has recently been reported to be associated with resistance to the disinfectant triclosan in a limited number of strains (5).

The variety of *Nco*I endonuclease digest patterns observed in this study demonstrates the considerable genetic diversity within staphylococcal populations despite their phenotypic similarity. These differences reflect considerable random mutations and insertions and have no effect on apparent resistance phenotypes but have proved valuable for epidemiological studies (9). The DNA hybridization data indicate the presence of a common gene, and thus the same additional enzyme is likely to operate in all the highly

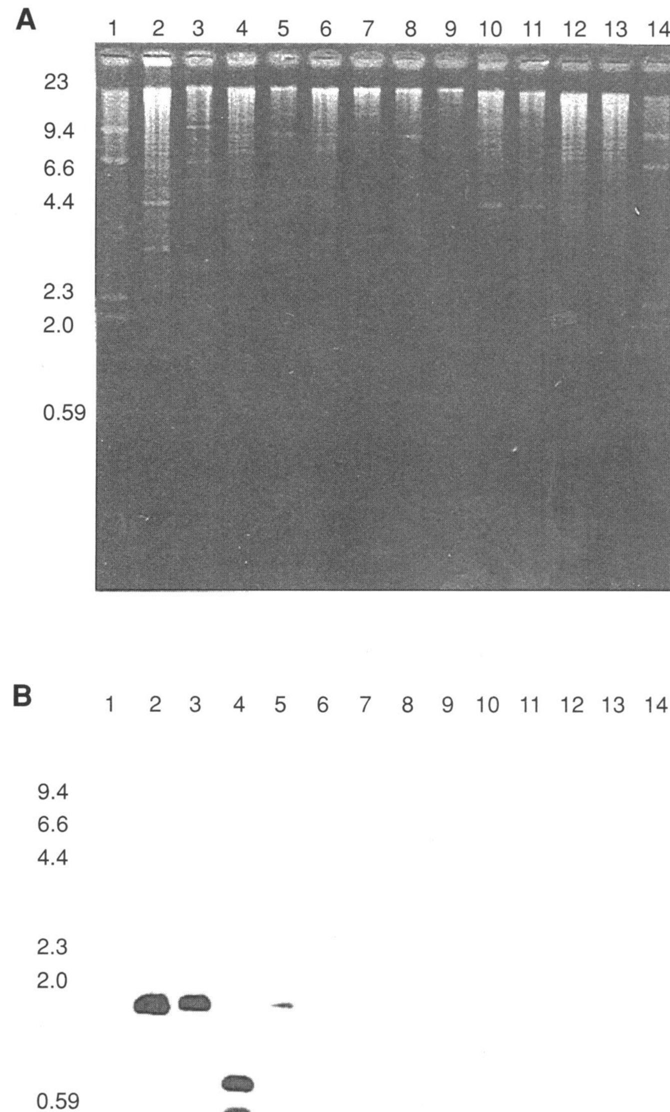

FIG. 2. (A) Agarose gel (0.8%) of *Nco*I digests of total DNA from each *S. aureus* strain used in this study. (B) Autoradiogram of the same gel showing which DNA fragments hybridized with the mupirocin resistance probe. Lanes (both panels): 1 and 14, λ *Hin*dIII-digested DNA (used as standard markers); 2, J2385 Mup^r; 3, C1; 4, F89; 5, RN2677(pJ2385); 6, C35; 7, C9; 8, L5; 9, Ck; 10, NCTC 11561; 11, J2385 Mup^s; 12, NCTC 6571; 13, RN2677.

resistant *S. aureus* strains (MICs, ≥512 μg/ml), including strain F89, in which the gene appears to have become incorporated into the chromosome. In the intermediately resistant strains (MICs, 8 to 256 μg/ml), this gene has not been detected, giving evidence that an altered or different IRS enzyme operates in these strains. The lack of DNA hybridization is unlikely to be due to poor detection, resulting from a low plasmid copy number or the presence of only a single gene copy, since this level of detection is within the limit of sensitivity of the nonradioactive hybridization system used in this study.

The FPLC activity profiles provide complementary evidence that an additional IRS enzyme, which is different from the original chromosomally encoded one, is present in the highly mupirocin-resistant strains. The clear resolution of the two enzyme peaks and the lack of hybridization with the resistance probe to DNA isolated from mupirocin-susceptible strains demonstrate that the two enzymes must be substantially different in structure. The mupirocin-resistant and -sensitive IRS enzymes are unlikely to have the same evolutionary origins, since their amino acid sequences are only 30% similar (10). This resistance may have been acquired in staphylococcal strains by transfer from other species in clinics or elsewhere. Cookson (4) suggested that acquisition of staphylococcal mupirocin resistance might have resulted from transfer of resistance genes from other bacteria such as *Enterococcus faecalis* or even *Pseudomonas* species, although further evidence is necessary to support this suggestion. In hybridization studies, DNA isolated from a mupirocin-producing strain, *Pseudomonas fluorescens* NCIB 10586, did not hybridize with the mupirocin resistance probe, indicating that common sequences are unlikely (14). In strains exhibiting intermediate resistance, however, only a single IRS activity peak was seen, but

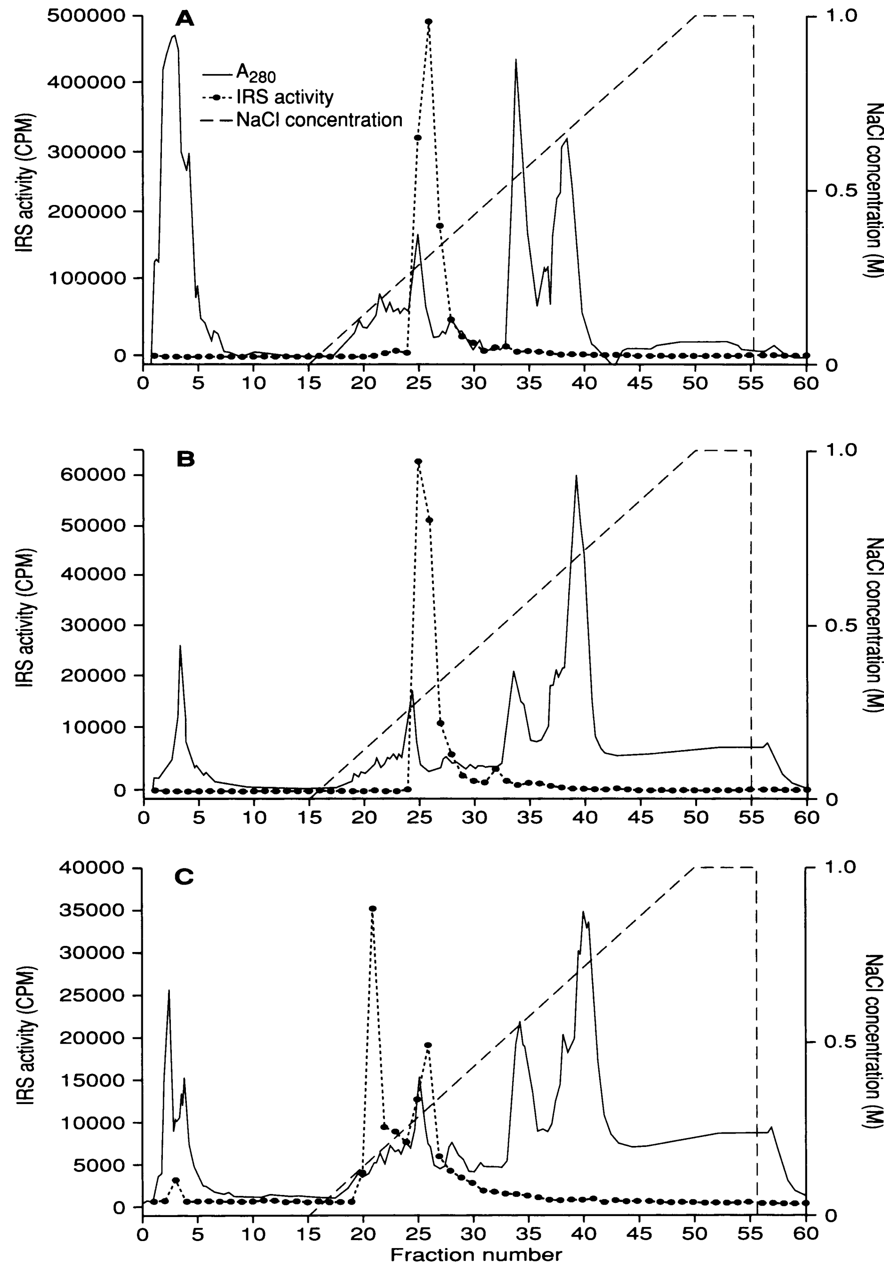

FIG. 3. Chromatograms of FPLC separation of crude *S. aureus* cell IRS extracts from three representative strains. Protein traces (A_{280}) are shown as solid lines, and IRS activity profiles are shown as dashed lines. (A) Mupirocin-sensitive strain RN2677. The salt gradient from 0 to 1 M NaCl, used in all separations to elute protein from the anion-exchange resin, is shown. (B) Intermediately resistant strain C35. (C) Highly resistant strain C1.

unlike the peaks from susceptible strains, the fractions from these strains required elevated mupirocin IC_{50}s (commensurate with those of the corresponding unfractionated extracts). Since, in these strains, a resistant enzyme could not be resolved from the sensitive one, it is possible that the original chromosomal enzyme has undergone a mutation at or near the active site, resulting in reduced inhibition by mupirocin. This altered IRS enzyme would thus replace the function of the normal mupirocin-sensitive type. A mutation involving a single amino acid substitution may not necessarily alter the chromatographic mobility in the system used. For example, a similar alteration has been reported for *Methanobacterium thermoautotrophicum* (12), in which the resistant enzyme had undergone a glycine-to-aspartic acid transition at a single point on the sequence, resulting in a 10-fold increase in the K_i for mupirocin. We propose that a similar single or multiple substitution has occurred in the IRS produced by *S. aureus* strains exhibiting intermediate mupirocin resistance (MICs, 8 to 256 μg/ml). These intermediately resistant strains are, however, approximately fivefold more common in clinical settings than highly resistant isolates (17), suggesting that such point mutations are relatively common events compared with the acquisition of a plasmid carrying the high-level resistance gene. Further work is in

progress to characterize the differences in IRS enzyme amino acid sequences between resistant and susceptible *S. aureus* strains. The findings of this study thus far clearly demonstrate that two separate IRS enzymes exist and that they contribute to different levels of mupirocin resistance observed among clinical strains.

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