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Purification of a 54-Kilodalton Protein (OprJ) Produced in NfxB Mutants of *Pseudomonas aeruginosa* and Production of a Monoclonal Antibody Specific to OprJ

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The 54-kDa outer membrane protein (designated OprJ) of a norfloxacin-resistant *nfxB* mutant of *Pseudomonas aeruginosa* PAO1 was purified by ion-exchange high-performance liquid chromatography. Mobility of OprJ in sodium dodecyl sulfate-polyacrylamide gel electrophoresis was not affected by reduction and heating. A murine monoclonal antibody (MAb) against OprJ was prepared to investigate existence of this protein in fluoroquinolone-susceptible and -resistant strains of *P. aeruginosa*. Western blot (immunoblot) analysis with this MAb revealed a single band at the position corresponding to OprJ in outer membrane proteins of NfxB mutants derived from clinical isolates. However, the MAb did not react with any outer membrane proteins of the respective parent strains. Complementation of the NfxB mutation by transformation with plasmid pNF111, which contained the wild-type *nfxB* gene, led to disappearance of the single band corresponding to OprJ. The existence of OprJ was associated with fluoroquinolone resistance. Furthermore, the MAb did not react with any outer membrane proteins of other fluoroquinolone-resistant *nalB* and *nfxC* mutants. These results suggest that OprJ is newly produced in NfxB mutants of *P. aeruginosa* and is involved in fluoroquinolone resistance specific to NfxB, and it appears that the MAb to OprJ should aid in detection of the NfxB mutation in *P. aeruginosa*.

Pseudomonas aeruginosa is an important pathogen in patients with infection because of its high level of intrinsic resistance to a number of structurally unrelated antimicrobial agents. Fluoroquinolones have been developed as one class of effective antimicrobial agents against *P. aeruginosa* and proven to be efficacious clinically (11). Recently, however, several investigators reported on the emergence of resistance to fluoroquinolones in clinical isolates of *P. aeruginosa* (1, 3).

In vitro studies on development of fluoroquinolone resistance in *P. aeruginosa* PAO have identified several loci in which mutations can produce resistance. Alterations in DNA gyrase (*gyrA* [*nfxA*] *gyrB*) or cell permeability (*nfxB nfxC nalB* [*cfxB*]) result in decreased susceptibility to fluoroquinolones (4, 7, 9, 20, 21, 23). The *gyrA* and *gyrB* mutations are associated with the altered subunit A and subunit B of DNA gyrase, respectively. The *nfxB*, *nfxC*, and *nalB* mutations are associated with the decreased outer membrane permeability to fluoroquinolones.

In these mutants with altered permeability, unusual proteins have appeared in their outer membranes. *nfxC* and *nalB* mutants have produced 50- and 49-kDa outer membrane proteins, respectively (4, 6). The *nfxB* mutation in *P. aeruginosa* PAO has been characterized by the appearance of a 54-kDa outer membrane protein (7). Okazaki et al. (17) indicated that the 54-kDa protein is associated with fluoroquinolone resistance in *P. aeruginosa* PAO, on the basis of complementation of the *nfxB* mutation with a plasmid bearing a wild-type *nfxB* gene. In contrast, Chamberland et al. (2) reported that the acquisition of the 54-kDa protein was not associated with fluoroquinolone resistance in clinical isolates.

We attempted to detect the appearance of the 54-kDa protein and evaluate the association of this protein with resistance to fluoroquinolones in various strains of *P. aeruginosa*. In this study, we have purified the 54-kDa outer membrane protein (designated OprJ by Masuda et al. [16]) from an NfxB mutant of *P. aeruginosa* PAO and prepared a murine monoclonal antibody (MAb) against this protein to evaluate its existence in fluoroquinolone-susceptible and -resistant *P. aeruginosa* strains.

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MATERIALS AND METHODS

Bacterial strains and plasmid. *P. aeruginosa* PAO1 (a wild-type strain) (8) and PAO4009 (FP5$^+$ *leu-9018 nir-9006*) (7) were kindly provided by H. Matsumoto. *P. aeruginosa* KH4023 (*nfxA* mutant of PAO4009) (7), KH4013E (*nfxB* mutant of PAO4009) (7), and PAO4014a (*nfxC* mutant of PAO4009) (4) were gifts from K. Hirai. *P. aeruginosa* PAO969 (*proC-130*) (20) and PAO6006 (*nalB* mutant of PAO969) (20) were kindly provided by D. Haas. *P. aeruginosa* KG1079D (OprD- and OprF-deficient strain) was an imipenem-resistant mutant isolated from *P. aeruginosa* KG1079 (OprF-deficient strain) by Gotoh et al. (5). *P. aeruginosa* R-13 and R-15 were NfxB mutants derived spontaneously from the PAO1 and KG1079D strains, respectively. Bacterial serotype was determined with Meiassay (Meiji Seika Kaisha, Ltd., Tokyo, Japan). Plasmid pNF111 carrying the *nfxB* gene was kindly provided by T. Okazaki (17). This plasmid has a wild-type DNA fragment of the *P. aeruginosa* PAO chromosome which complements the *nfxB* mutation and restores the susceptibility to fluoroquinolones, and it also contains a carbenicillin-resistant gene as a selective marker.

Antimicrobial agents. The antimicrobial agents used in this study were obtained as follows: norfloxacin, Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan; nalidixic acid, Daiichi Pharmaceutical Co., Tokyo, Japan; imipenem, Banyu Pharmaceutical Co., Tokyo, Japan; ceftazidime, Glaxo Japan, Tokyo, Japan; latamoxef and gentamicin, Shionogi Pharmaceutical Co., Osaka, Japan; carbenicillin, Fujisawa Pharmaceutical Co., Osaka, Japan. Chloramphenicol was obtained from a commercial source.

Susceptibility test. MICs were determined by the twofold agar dilution method with Mueller-Hinton medium (Eiken Chemical Co., Ltd., Tokyo, Japan). An inoculum of 10^4 to 10^5 CFU was used, and the MIC was defined as the lowest concentration that inhibited visible growth after 18 h of incubation at 37°C.

Isolation of fluoroquinolone-resistant mutants. Cells of *P. aeruginosa* grown in 10 ml of L broth at 37°C with shaking were centrifuged and suspended in 1 ml of L broth. A portion (0.1 ml) of the cell suspension was plated on L agar containing 1.56 or 3.13 μg of norfloxacin per ml and incubated for 48 h at 37°C.

Preparation of plasmid DNA and transformation. Plasmid DNA was prepared by the method described by Maniatis et al. (15). Transformation of *P. aeruginosa* was performed as follows. *P. aeruginosa* was incubated in L broth with shaking to an optical density of 1.5 at 550 nm. Bacterial culture (10 ml) was chilled on ice

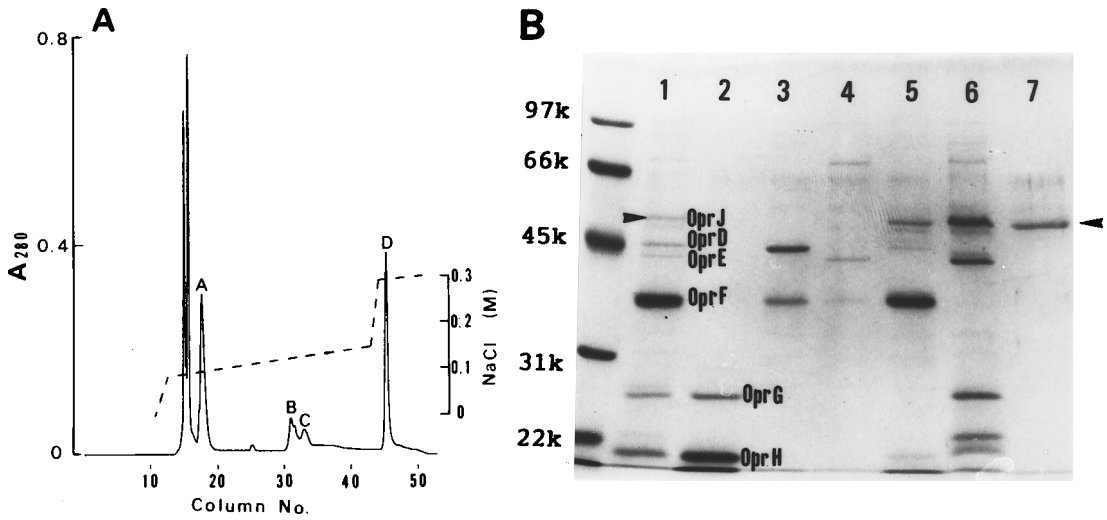

FIG. 1. (A) Elution profile of the outer membrane proteins of *P. aeruginosa* by the DEAE ion-exchange HPLC. The outer membrane protein of *P. aeruginosa* R-13 (10 mg of protein) was applied on a TSK gel DEAE-5PW column. The column was eluted in 1% β-*n*-octyl-D-glucoside–10 mM Tris-HCl (pH 8.0)–1 mM EDTA at a flow rate of 1 ml/min. Eluates were collected at a rate of 1 ml per fraction. (B) SDS-polyacrylamide gel electrophoresis of the outer membrane proteins. Lanes: 1, outer membrane of the R-13 strain; 2, fraction A of R-13; 3, fraction B of R-13; 4, fraction C of R-13; 5, fraction D of R-13; 6, outer membrane of the R-15 strain; 7, fraction D of R-15. The migration positions of standard proteins are shown on the left (in kilodaltons [k]). OprJ is indicated (arrowhead).

for 5 min. The cells were harvested and gently resuspended in 5 ml of 100 mM $MgCl_2$. After centrifugation, the cells were very gently resuspended in 2 ml of 100 mM $CaCl_2$. The suspension was chilled on ice for 30 min and centrifuged. The cells were very gently resuspended in 1 ml of 100 mM $CaCl_2$. A portion of the cell suspension (0.2 ml) was added to a chilled tube containing plasmid DNA, and the tube was further chilled on ice for 1 h. The operation described above was performed at 4°C or on ice. Then the cells were heated at 42°C for 5 min and plated on L agar containing 200 μg of carbenicillin per ml.

Preparation of outer membrane proteins. Outer membranes of *P. aeruginosa* were prepared by the method of Poxton et al. (19). Bacterial cells were grown, harvested, and suspended in 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.4). Cells were broken by oscillation with an ultrasonic disruptor. After unbroken cells were removed by centrifugation at 10,000 × *g* for 10 min, membranes were pelleted by centrifugation at 100,000 × *g* for 30 min and resuspended in 10 mM HEPES buffer (pH 7.4). The membrane suspension was then incubated with 2% (wt/vol) sodium *N*-lauroyl sarkosinate at room temperature for 1 h. Insoluble membrane proteins were pelleted by centrifugation at 100,000 × *g* for 30 min, suspended in distilled water, and used as outer membrane proteins.

Purification of OprJ by ion-exchange chromatography. OprJ was purified from the outer membrane of the R-15 strain. The outer membrane (10 mg of protein) of the R-15 strain was solubilized in 5 ml of 2% β-*n*-octyl-D-glucoside–10 mM Tris-HCl (pH 8.0)–1 mM EDTA. After centrifugation at 100,000 × *g* for 30 min the supernatant was applied to a DEAE ion-exchange high-performance liquid chromatography (HPLC) column (TSK-Gel DEAE-5PW; Tosoh, Tokyo, Japan) which had been equilibrated with the same buffer. The column was eluted with a gradient of 0 to 0.3 M NaCl in 1% β-*n*-octyl-D-glucoside–10 mM Tris-HCl buffer (pH 8.0)–1 mM EDTA. A_{280} was continuously monitored, and proteins were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.

SDS-polyacrylamide gel electrophoresis. SDS-polyacrylamide gel electrophoresis was performed on 10% acrylamide–0.26% bis(acrylamide)–SDS slab gels (13).

MAb. A hybridoma secreting a MAb specific to OprJ was isolated as described by Kohler and Milstein (12). Iscove's modified Dulbecco's medium (Life Technologies, Grand Island, N.Y.) was supplemented with sodium pyruvate, piperacillin, streptomycin, and 10% fetal bovine serum (Whittaker Bioproducts, Walkersville, Md.). Hypoxanthine, aminopterin, and thymidine were added as necessary. The myeloma cell line SP-2 was used to obtain the hybridoma. Hybrid cell lines were prepared as follows. Female BALB/c mice were injected three times intraperitoneally with 30 μg of OprJ on days 0, 7, and 14. Three days after the final injection, spleens were removed and splenocytes were collected. Splenocytes and SP-2 cells (10:1) were fused by polyethylene glycol. Fused cells were transferred into microtiter dishes at 3×10^4 splenocytes per well and maintained for 14 days in medium containing hypoxanthine, aminopterin, and thymidine. Hybridoma cell lines producing MAb to OprJ detectable by enzyme-linked immunosorbent assay were transferred to medium containing hypoxanthine and thymidine and cloned by limiting dilution.

Western blot (immunoblot). Outer membrane proteins were electrophoretically transferred from the SDS-polyacrylamide gel onto a nitrocellulose membrane, as described by Towbin et al. (22). After blotting, the nitrocellulose paper was washed with phosphate-buffered saline (PBS) (pH 7.4) and incubated with 5% skim milk in PBS for 1 h to block nonspecific binding of antibody. MAb to OprJ was added and incubated for 1 h with agitation. The nitrocellulose paper was washed five times with PBS and then reacted with a secondary antibody (horseradish peroxidase-conjugated fraction of a goat anti-mouse immunoglobulin) for 1 h with agitation. After the nitrocellulose paper was washed five times with PBS, color development was performed with the peroxidase substrate kit ABTS (Bio-Rad) as recommended by the manufacturer.

RESULTS

Purification of OprJ. To purify OprJ, the outer membrane proteins isolated from strain R-13 were applied to a DEAE

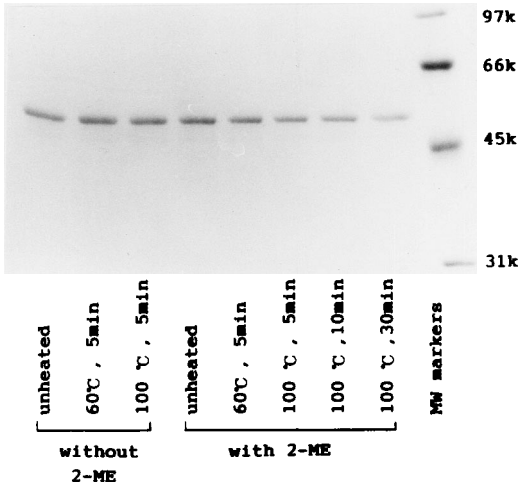

FIG. 2. Effects of heating and 2-mercaptoethanol (2-ME) on the mobility of OprJ in SDS-polyacrylamide gel electrophoresis. MW, molecular weight. The migration positions of standard proteins are shown on the right (in kilodaltons [k]).

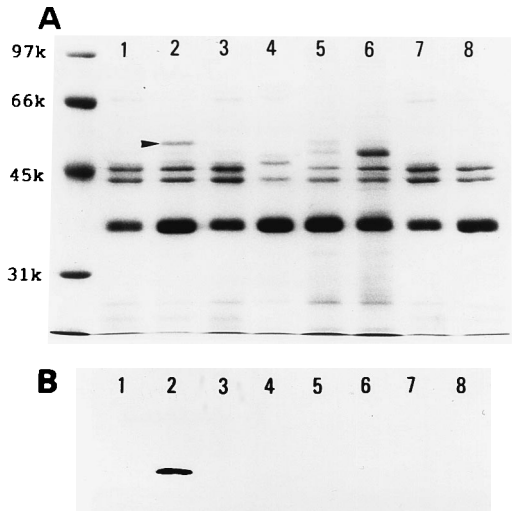

FIG. 3. (A) SDS-polyacrylamide gel electrophoresis of outer membrane proteins of quinolone-susceptible and quinolone-resistant *P. aeruginosa* strains. The migration positions of standard proteins are shown on the left (in kilodaltons [k]). OprJ is indicated (arrowhead). (B) Immunoblotting of outer membrane protein preparations probed with MAb to OprJ. Lanes: 1, PAO4009; 2, KH4013E (*nfxB*); 3, PAO4009; 4, KH4014a (*nfxC*); 5, PAO969; 6, PAO6006 (*nalB*); 7, PAO4009; 8, KH4023 (*nfxA*).

ion-exchange gel and eluted with a gradient of NaCl. Outer membrane proteins OprG and OprH were eluted together into peak A in Fig. 1A. Then, outer membrane protein OprD and a small amount of OprF were eluted at a concentration of NaCl around 0.1 M (peak B). OprJ was finally eluted with the majority of OprF and a small amount of OprD in peak D at an NaCl concentration at about 0.3 M.

Therefore, an NfxB-type mutant, R-15, was isolated from KG1079D, an OprD- and OprF-deficient strain of *P. aeruginosa* PAO1, to purify OprJ by the method as described above (Fig. 1B, lane 7). The mobility of this purified OprJ in SDS-polyacrylamide gel electrophoresis was not affected by addition of 2-mercaptoethanol and heating at 100°C (Fig. 2).

Production of MAb to OprJ. A hybridoma secreting MAb to OprJ was obtained. This murine MAb was classified in subtypes of immunoglobulin G2b.

The reactivities of the MAb with outer membrane proteins of fluoroquinolone-resistant mutants (*nfxA*, *nfxB*, *nfxC*, and *nalB*) derived from *P. aeruginosa* PAO are shown in Fig. 3. This MAb specifically detected the 54-kDa protein in KH4013E (*nfxB*), whereas no cross-reactivity to 49- and 50-kDa proteins produced in other fluoroquinolone-resistant strains, PAO6006 (*nalB*) and KH4014a (*nfxC*), was observed.

Reactivities of the MAb with outer membrane proteins of NfxB mutants derived from clinical isolates. To examine the production of OprJ in outer membrane proteins of NfxB mutants of *P. aeruginosa* strains other than PAO, NfxB mutants were isolated from clinical strains of norfloxacin-susceptible *P. aeruginosa* with different serotypes. Several NfxB-type mutants were selected and transformed with the recombinant plasmid pNF111, which contains a wild-type *nfxB* gene dominant to the mutant one. Transformants were obtained from six NfxB-type mutants. The reactivities of the MAb to OprJ with the outer membrane proteins of six sets of strains, with each set consisting of the parent strain and the NfxB mutant and its transformant, were examined by immunoblotting. The outer membrane protein profile and immunoblotting profile are shown in Fig. 4. The single band located at 54 kDa was detected for all NfxB mutants. No bands were observed for any parent strains or transformants of NfxB mutants in immunoblot analysis.

Detection of OprJ from fluoroquinolone-resistant clinical isolates. The existence of OprJ was evaluated for 34 strains of fluoroquinolone-resistant clinical isolates. OprJ protein was detected in two isolates (KY110 and KY135). Transformation of these resistant strains with plasmid pNF111 caused disappearance of OprJ (data not shown).

Drug susceptibility pattern. The drug susceptibilities of and the appearance of OprJ in six sets of parents, mutants, and transformants of clinical isolates are shown in Table 1. NfxB mutants showed a 16-fold increase in resistance to norfloxacin, compared with that of each parent strain. However, they tended to be hypersusceptible to β-lactams and aminoglycosides. Drug susceptibilities of the transformants were restored to almost the same levels as those of the parent strains, with the exception of that to carbenicillin. The appearance of OprJ correlated with resistance to fluoroquinolones.

Table 2 shows the drug susceptibilities of two strains of fluoroquinolone-resistant clinical isolates and their transformants. The MIC of norfloxacin against these two isolates was 25 μg/ml. Drug susceptibility patterns of these isolates were different from that of the NfxB mutant derived from *P. aeruginosa* PAO in that one strain was resistant to ceftazidime, carbenicillin, and chloramphenicol and the other was resistant to imipenem, carbenicillin, and chloramphenicol. Transformation with pNF111 made these isolates susceptible to norfloxacin. The OprJ protein disappeared in both transformants.

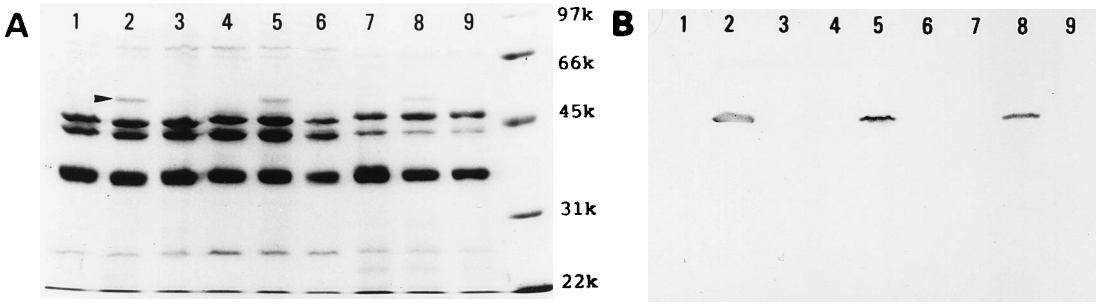

FIG. 4. (A) SDS-polyacrylamide gel electrophoresis of outer membrane proteins of quinolone-susceptible *P. aeruginosa* clinical isolates, their *nfxB* mutants, and strains transformed with plasmid pNF111. The migration positions of standard proteins are shown on the right (in kilodaltons [k]). OprJ is indicated (arrowhead). (B) Immunoblotting of outer membrane protein preparations probed with MAb to OprJ. Lanes: 1, KPA1 strain; 2, *nfxB* mutant (KPA1); 3, transformant (KPA1); 4, KPA2 strain; 5, *nfxB* mutant (KPA2); 6, transformant (KPA2); 7, KPA3 strain; 8, *nfxB* mutant (KPA3); 9, transformant (KPA3).

TABLE 1. Drug susceptibilities of NfxB mutants derived from clinical isolates and their reactivities with MAb to OprJ

P. aeruginosa strain (serotype)	MIC (μg/ml)[a]							OprJ
	NFLX	NA	IPM	CAZ	CBPC	GM	CP	
KPA1 (B)								
Parent	0.78	100	1.56	3.13	25	3.13	100	−
NfxB mutant	12.5	400	1.56	1.56	3.13	3.13	100	+
pNF111[b] transformant	1.56	100	1.56	3.13	800	3.13	100	−
KPA2 (E)								
Parent	0.78	100	0.39	1.56	25	3.13	100	−
NfxB mutant	12.5	800	0.20	0.78	12.5	1.56	100	+
pNF111 transformant	1.56	100	0.39	3.13	800	3.13	200	−
KPA3 (A)								
Parent	0.78	200	0.78	3.13	100	6.25	100	−
NfxB mutant	12.5	400	0.39	1.56	25	3.13	100	+
pNF111 transformant	0.78	200	0.78	6.25	>800	6.25	100	−
KPA4 (D)								
Parent	0.78	100	1.56	1.56	100	6.25	100	−
NfxB mutant	12.5	400	1.56	0.78	25	3.13	200	+
pNF111 transformant	0.78	100	1.56	3.13	>800	12.5	100	−
KPA5 (G)								
Parent	0.78	100	1.56	6.25	100	6.25	100	−
NfxB mutant	12.5	400	0.78	1.56	25	1.56	200	+
pNF111 transformant	0.78	100	1.56	6.25	>800	6.25	100	−
KPA6 (H)								
Parent	0.78	50	0.78	3.13	50	6.25	100	−
NfxB mutant	12.5	400	0.78	3.13	50	3.13	200	+
pNF111 transformant	0.78	50	0.78	3.13	>800	6.25	100	−

[a] Abbreviations: NFLX, norfloxacin; NA, nalidixic acid; IPM, imipenem; CAZ, ceftazidime; CBPC, carbenicillin; GM, gentamicin; CP, chloramphenicol.
[b] Plasmid pNF111 contains a carbenicillin resistance gene as a selective marker.

DISCUSSION

In this study, we purified OprJ from NfxB mutants of *P. aeruginosa* and prepared the murine MAb to investigate the existence of this protein in *P. aeruginosa*.

OprJ was eluted with OprD and OprF in DEAE ion-exchange chromatography. Therefore, we isolated an NfxB mutant from an OprD- and OprF-deficient strain to purify OprJ. Heating at 100°C and reduction by 2-mercaptoethanol did not affect the mobility of this OprJ protein in SDS-polyacrylamide gel electrophoresis. A murine MAb was prepared with this purified OprJ.

In immunoblot analysis, OprJ was not detected in outer membrane proteins of wild-type *P. aeruginosa* PAO or those of fluoroquinolone-resistant *nalB* and *nfxC* mutants, whose mutations were reported to be accompanied by the appearance of unusual outer membrane proteins with molecular masses of 49 and 50 kDa, respectively. This suggested that OprJ was newly synthesized in the *nfxB* mutant. Then, the NfxB mutants were isolated from fluoroquinolone-susceptible clinical strains with different serotypes. On the basis of reactivities of the MAb with outer membrane proteins of these mutants, it is suggested that OprJ might be produced in all NfxB mutants of *P. aeruginosa*. Furthermore, this OprJ was eliminated by transformation of NfxB mutants with recombinant plasmid pNF111 containing a wild-type *nfxB* gene. The NfxB mutants derived from clinical isolates showed the same drug susceptibility pattern as that of the *nfxB* mutant derived from PAO, while complementation of the NfxB mutation with plasmid pNF111 restored fluoroquinolone susceptibility to the level of the wild-type strain. Okazaki et al. (17) have previously reported that transformation with pNF225, which carries the wild-type *nfxB* gene, does not alter the drug susceptibilities of *nfxC* and *nalB* mutants or those of the parent strains. We have also confirmed these findings with pNF111. Our results suggested that the OprJ protein involved in fluoroquinolone resistance is specific to the NfxB mutation in *P. aeruginosa*.

Jakics et al. (10) reported that the drug susceptibility pattern and accumulation of norfloxacin in cells are consistent with an *nfxB*-type mutation in clinical isolates of *P. aeruginosa*. Our results indicated that OprJ appears in NfxB mutants of clinical isolates of *P. aeruginosa* as well as those of PAO strains. Chamberland et al. (2) reported that the 54-kDa outer membrane protein was not associated with fluoroquinolone resistance. However, our results suggest the association of this protein with resistance to fluoroquinolones in *P. aeruginosa*.

We detected OprJ in two clinical isolates (KY110 and KY135) of fluoroquinolone-resistant *P. aeruginosa*. These isolates were confirmed to be NfxB mutants by transformation with pNF111. The drug susceptibility pattern of these two isolates was different from that of *P. aeruginosa* KH4013E (*nfxB* mutant of the PAO strain). Besides the NfxB mutation,

TABLE 2. Drug susceptibilities of quinolone-resistant clinical isolates producing OprJ protein

P. aeruginosa strain	MIC (μg/ml)[a]						
	NFLX	NA	IPM	CAZ	CBPC	GM	CP
KY 110	25	>800	0.20	400	400	0.78	200
pNF111[b] transformant	0.78	400	0.39	400	>800	3.13	50
KY 135	25	>800	3.13	0.39	100	0.39	200
pNF111 transformant	0.78	200	6.25	0.39	>800	1.56	25
KH4013E	12.5	200	0.39	0.39	6.25	0.78	25

[a] Abbreviations: NFLX, norfloxacin; NA, nalidixic acid; IPM, imipenem; CAZ, ceftazidime; CBPC, carbenicillin; GM, gentamicin; CP, chloramphenicol.
[b] Plasmid pNF111 contains a carbenicillin resistance gene as a selective marker.

other resistance factors may have an effect on drug susceptibilities of these strains. Legakis et al. (14) reported the presence of the 54-kDa protein in a ciprofloxacin-resistant mutant which was resistant to β-lactams and aminoglycosides as well as fluoroquinolones, showing the drug susceptibility pattern of a NalB-type strain. The reliability of detection of OprJ by SDS-polyacrylamide gel electrophoresis for identification of the NfxB mutation is low because of the complicated outer membrane protein profile of fluoroquinolone-resistant *P. aeruginosa nfxC* and *nalB* mutants (4, 6). Therefore, it is difficult to identify the NfxB mutation on the basis of the drug susceptibility pattern and the presence of OprJ in the SDS-polyacrylamide gel. Our results indicated that the OprJ protein was newly produced in NfxB mutants of *P. aeruginosa* and might have a significant role in resistance to fluoroquinolones. We believe that the MAb specific to OprJ is a useful tool for detection of the NfxB mutation.

The mechanism of resistance to fluoroquinolones in NfxB mutants has been reported to be due to the decreased outer membrane permeability (7). However, the role of OprJ in NfxB mutants is yet unknown. Poole et al. (18) reported on the involvement of OprK, which has a role for secretion of the siderophore pyoverdine in *P. aeruginosa*, in the resistance to a number of antimicrobial agents including ciprofloxacin, tetracycline, and chloramphenicol. OprK is an outer membrane protein with a 50-kDa molecular mass and is homologous to a class of bacterial membrane efflux proteins. In this study, we have demonstrated that OprJ plays an important role in the resistance of *P. aeruginosa* to fluoroquinolones including ciprofloxacin. Characteristics common to OprK and OprJ may predict that OprJ plays an important role in the system responsible for antibiotic efflux.

The MAb to OprJ might be useful for analysis of the role of this protein. Further study is also necessary to clarify the mechanism of resistance to fluoroquinolones.

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