JOURNAL OF BACTERIOLOGY, Sept. 1994, p. 5639–5647
0021-9193/94/$04.00+0

Vol. 176, No. 18

Overexpression of *algE* in *Escherichia coli*: Subcellular Localization, Purification, and Ion Channel Properties

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Alginate-producing (mucoid) strains of *Pseudomonas aeruginosa* possess a 54-kDa outer membrane (OM) protein (AlgE) which is missing in nonmucoid bacteria. The coding region of the *algE* gene from mucoid *P. aeruginosa* CF3/M1 was subcloned in the expression vector pT7-7 and expressed in *Escherichia coli*. The level of expression of recombinant AlgE was seven times higher than that of the native protein in *P. aeruginosa*. Recombinant AlgE was found mainly in the OM. A putative precursor protein (56 kDa) of AlgE could be immunologically detected in the cytoplasmic membrane (CM). Surface exposition of AlgE in the OM of *E. coli* was indicated by labeling lysine residues with *N*-hydroxysuccinimide-biotin. Secondary-structure analysis suggested that AlgE is anchored in the OM by 18 membrane-spanning β-strands, probably forming a β-barrel. Recombinant AlgE was purified, and isoelectric focusing revealed a pI of 4.4. Recombinant AlgE was spontaneously incorporated into planar lipid bilayers, forming ion channels with a single-channel conductance of 0.76 nS in 1 M KCl and a mean lifetime of 0.7 ms. Single-channel current measurements in the presence of other salts as well as reversal potential measurements in salt gradients revealed that the AlgE channel was strongly anion selective. For chloride ions, a weak binding constant (K_m = 0.75 M) was calculated, suggesting that AlgE might constitute an ion channel specific for another particular anion, e.g., polymannuronic acid, which is a precursor of alginate. Consistent with this idea, the open-state probability of the channel decreased when GDP-mannuronic acid was added. The AlgE channel was inactivated when membrane voltages higher than +85 mV were applied. The electrophysiological characteristics of AlgE, including its rectifying properties, are quite different from those of typical porins.

Pseudomonas aeruginosa is an opportunistic human pathogen which infects particularly persons who are suffering from cystic fibrosis. The initial infection occurs mostly with nonmucoid bacteria, which convert to mucoid strains in an early stage of infection (13, 26). Mucoid bacteria produce copious amounts of alginate, which seems to be the most important virulence factor (12). The initial steps of the alginate biosynthesis, leading to the putative precursor GDP-mannuronic acid, are well-known (21). However, there is a lack of information about the final steps of the biosynthesis, i.e., the polymerization and export of alginate. Recently, the genes involved in acetylation (*algF*) and degradation (*algL*) of alginate were cloned and mapped in the alginate biosynthesis gene cluster (34 min) (10, 30). In addition, the gene product of *algG* (34 min) was purified and identified as an epimerase located in the periplasm that introduces guluronic acid residues into the alginate (9). We identified a 54-kDa protein (AlgE) in the outer membrane (OM) of *P. aeruginosa* which appears in mucoid strains only (15, 26). This protein was purified and the N-terminal amino acid sequence was determined (14). At the same time, the *algE* gene was mapped on the bacterial chromosome at 34 min and cloned and sequenced (7). The determined N-terminal amino acid sequence of the 54-kDa protein corresponded with that deduced from the nucleotide sequence of *algE* except for three positions (7, 14). Chu et al. (7) succeeded in expressing the cloned *algE* gene in nonmucoid strains of *P. aeruginosa*.

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For functional and structural analysis of AlgE, which represents a minor OM protein in *P. aeruginosa*, we have now established a heterologous overexpression system in *Escherichia coli*, and we have developed a procedure for purification of the recombinant AlgE. The recombinant protein was biochemically and biophysically characterized. After reconstitution of AlgE in planar lipid bilayer membranes, the protein revealed channel-forming activity.

MATERIALS AND METHODS

Strains and plasmids. *E. coli* JM109 [*e14*$^-$ (*mcrA*) *recA1 endA1 gyrA96 thi-1 hsdR17* (r_K^- m_K^+) *supE44 relA1* Δ(*lac-proAB*) (F′ *traD36 proAB lacI*^{q}*ZΔM15*)] was used for cloning and for the preparation of plasmid DNA (28). Strain *E. coli* K38(pGP1-2) (containing the T7 RNA polymerase gene under control of the λ P_L promoter) was employed for the expression studies (33). Mucoid strain CF3/M1 of *P. aeruginosa* was used for preparing native AlgE (36).

Plasmid pBluescript KS (Stratagene) served for cloning and DNA sequencing. Plasmid pKR22 was constructed by inserting the *algE* coding region in the *Hin*cII site of pBluescript KS. Plasmid pT7-7 was used as an expression vector (33). The *algE* expression plasmid pTR7-2 was constructed by insertion of a *Xho*I-*Hin*dIII DNA fragment from plasmid pKR22 into the *Nde*I site of plasmid pT7-7. DNA fragments were treated with mung bean nuclease to create blunt ends.

Media and genetic techniques. In general, bacteria were grown in Luria-Bertani broth or yeast extract-peptone medium. A medium containing 2% (wt/vol) tryptone, 1% (wt/vol) yeast extract, and additional salts was used to maximize protein production (33). When required, media were supplemented

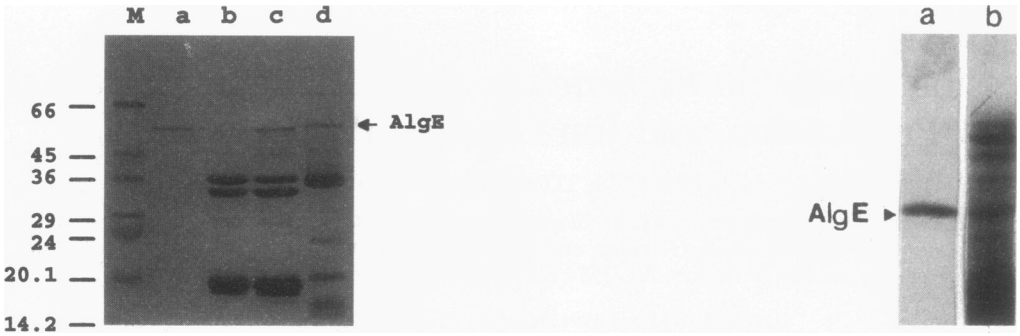

FIG. 1. SDS-PAGE of OM proteins demonstrating overexpression of *algE* in *E. coli*. Lane M, molecular mass markers (in kilodaltons); lane a, purified AlgE (control); lanes b and c, OMs from uninduced (30°C) and induced (42°C) *E. coli* K38(pGP1-2, pTR7-2), respectively; lane d, OM from mucoid *P. aeruginosa* CF3/M1. Proteins were stained with Coomassie brilliant blue R-250.

FIG. 3. NHS-biotin labeling of whole cells from *algE*-expressing *E. coli*. After the labeling, OMs were isolated and proteins of the OM were separated by SDS-PAGE, blotted, and then developed with either anti-AlgE antiserum (lane a) or avidin-HRP conjugate (lane b).

with antibiotics (ampicillin, 50 μg/ml; kanamycin, 40 μg/ml) in order to prevent plasmid segregation. Transformation of bacteria and all other genetic techniques were performed as described elsewhere (28). DNA was sequenced by the chain termination method (29), using an automatic laser fluorescence sequencer (Pharmacia). The PCR was performed, using the Vent DNA polymerase (Biozyme) and 30 cycles (2 min at 94°C, 2 min at 50°C, and 3 min at 72°C) with thermal cycler PHC-3 (Techne). Oligonucleotides (N terminus, 5′-dATGAA CAGCTCCCGTTCCGT-3′; C terminus, 5′-dTCAGCGCCG AGGGCTCGTCG-3′) were obtained from Appligene. Chromosomal DNA from *P. aeruginosa* CF3/M1 was isolated by the method of Mak and Ho (20) and used as target DNA in the PCR.

Subcellular localization and purification of recombinant AlgE. Cells were disrupted by ultrasonication. Cytoplasmic membranes (CMs) were prepared by sucrose gradient ultracentrifugation (2). OMs were prepared by the Sarkosyl method (25). The contamination of CM with OM was estimated from its 2-keto-3-desoxyoctonate (KDO) content and was given as a percentage of the total amount of KDO present in the CM and OM. AlgE was solubilized in 1% (wt/vol) *n*-octyl-β-D-glucopyranoside containing 150 mM KCl. AlgE was purified by immobilized metal ion affinity chromatography (14) followed by anion-exchange chromatography (MonoQ column, fast protein liquid chromatography apparatus; Pharmacia).

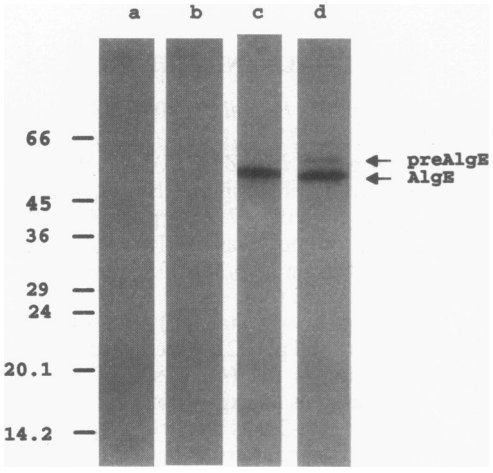

FIG. 2. Strip immunoblots from electrophoretically separated OM and CM proteins from induced and uninduced *E. coli* K38(pGP1-2, pTR7-2). The strips were treated with anti-AlgE antiserum diluted 1:40,000. Lanes a and b, uninduced OM and CM, respectively (controls); lanes c and d, induced OM and CM, respectively. AlgE, 54 kDa; preAlgE (precursor), 56 kDa. Molecular masses are indicated (in kilodaltons) on the left.

Gel electrophoresis and Western immunoblotting. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (19), protein bands were visualized by staining with Coomassie brilliant blue R-250 (0.025%, wt/vol; Bio-Rad).

Western blotting (35) of OM proteins was performed with polyvinylidene difluoride membranes (Millipore). AlgE was detected by using a polyclonal AlgE-specific rabbit antiserum (26) and a horse radish peroxidase (HRP)-antibody conjugate (Sigma) as a second antibody. Bound antibodies were detected by using a luminogenic HRP substrate and by film evaluation (ECL; Amersham).

Isoelectric focusing. Agarose gels (1%, wt/vol) containing 1% (wt/vol) *n*-octyl-β-D-glucopyranoside and one of three different ampholytes were used for isoelectric focusing. Protein bands were stained with Coomassie brilliant blue R-250 (see above).

Surface exposition of lysine residues. Whole cells of *E. coli* K38(pGP1-2, pTR7-2) overexpressing *algE* were labeled with *N*-hydroxysuccinimide (NHS)-biotin (Sigma) (6). Briefly, the bacteria were washed three times with phosphate-buffered saline (pH 8.0) and NHS-biotin was added to a final concentration of 5 mM. The labeling reaction was performed at 4°C for 5 min. Then OMs were prepared as described above, and labeled proteins were detected with an avidin-HRP conjugate. This type of protein labeling procedure attaches biotin only to surface-exposed lysine residues (6).

Chemical cross-linking of proteins. Dithio*bis*(succinimidyl-propionate) (DSP; Pierce) was used for cross-linking of AlgE under in vitro and in vivo conditions (1, 27). For treatment of purified AlgE, the concentration of DSP was 250 μg/ml. In experiments with whole cells, two- and threefold-higher concentrations were used as well. Treated proteins were analyzed by two-dimensional gel electrophoresis.

Lipid bilayer experiments. Lipid bilayer experiments were carried out by the methods of Mueller et al. (23). 1,2-Dioleoyl-glycero-3-phosphatidyl-ethanolamine (1,2-DOPE) and 1-palmitoyl-2-oleoyl-glycero-3-phosphatidylcholine (1,2-POPC) (Avanti, Alabaster, Ala.) were used in a 1:1 molar ratio in *n*-decane (12

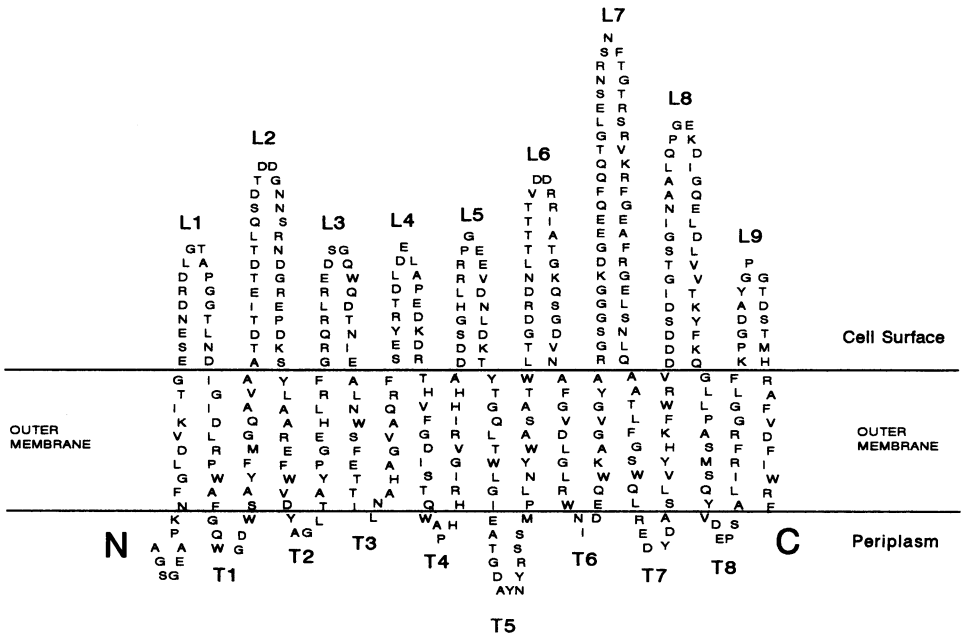

FIG. 4. Putative topological model of mature AlgE based on its amino acid sequence (single-letter code). The β-barrel structure shown consists of 18 antiparallel β-strands, nine loops (L1 to L9), and eight turns (T1 to T8). N, amino terminus; C, carboxy terminus.

mg/ml). All measurements were done after addition of 0.1 μg of AlgE per ml to the *cis*-side electrolyte of the setup at room temperature (21°C). The *trans*-side potential was grounded. Positive currents corresponded to cation transfer from *cis* to *trans*. Current fluctuations were stored on a DAT recorder (DTR 1200; Biologic). Data were filtered at 3 kHz, and single-channel conductances and mean lifetimes were determined by using the TAC program from Instrutech, Elmont, N.Y. The data were statistically treated by the method of Boheim (5). Zero-current (reversal) membrane potential measurements were done with a 10-fold KCl gradient (*cis*, 0.1 M; *trans*, 1 M). The AlgE channel was blocked by increasing the GDP-mannuronic acid concentration (1 to 8 μg/ml) at the *cis* side.

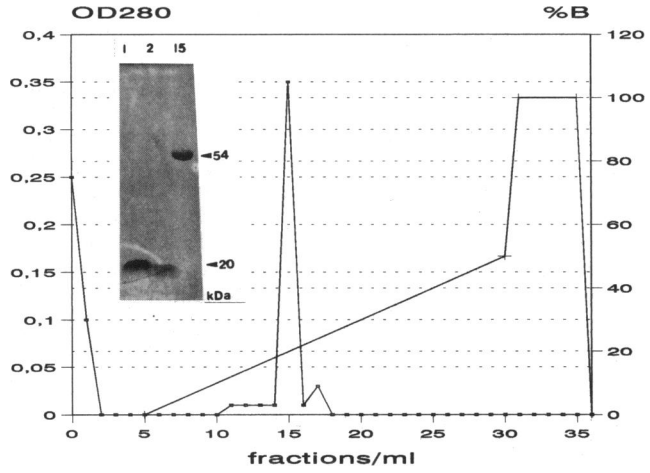

FIG. 5. Elution profile of anion-exchange chromatography used to isolate AlgE from *E. coli* K38(pGP1-2, pTR7-2). The column (monoQ) was loaded with pooled fractions from immobilized metal ion affinity chromatography. (Inset) SDS-PAGE of fractions 1, 2, and 15 (monoQ run). B, salt gradient; 100% is equal to a 1 M NaCl solution in 20 mM bis-Tris-propane (pH 6.5)–50 mM KCl–0.75% (wt/vol) methyl-6-0-(*N*-heptylcarbamoyl)-α-D-glucopyranoside. ■, optical density at 280 nm (OD_{280}); −⊢, percent B.

RESULTS AND DISCUSSION

Construction of an *algE* overexpression plasmid. The main aims of this study were to construct an overexpression system for *algE* in *E. coli* and to develop an efficient purification procedure to obtain high yields of AlgE. Therefore, we applied PCR, using oligonucleotides whose sequences corresponded to the ends of the *algE* coding region (7). Chromosomal DNA from *P. aeruginosa* CF3/M1 was the template DNA for amplification. The expected 1.5-kb DNA fragment was obtained and subsequently cloned in the *Hin*cII site of vector pBluescript KS, resulting in plasmid pKR22. DNA sequencing of both ends of the inserted 1.5-kb DNA fragment revealed that the entire *algE* gene was amplified and cloned. A *Xho*I-*Hin*dIII digest of pKR22 was performed to excise the 1.5-kb DNA fragment and to subclone it in the *Nde*I site of vector pT7-7, resulting in vector pTR7-2. Correct insertion was confirmed by DNA sequencing. In vector pTR7-2, *algE* was under control of the T7 promoter and vector pGP1-2 contained the T7 RNA

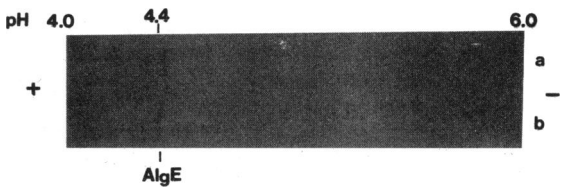

FIG. 6. Isoelectric focusing of purified native (a) and recombinant (b) AlgE. The proteins were focused in the presence of octylglucoside and then stained with Coomassie brilliant blue R-250. The ampholyte Servalyte pH 4–6 (Serva) was used to cover the indicated pH range. +, anode; −, cathode.

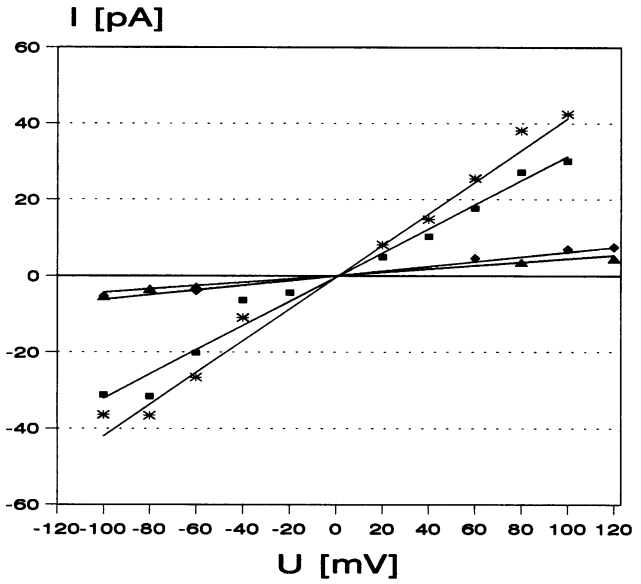

FIG. 7. Current-voltage relationships of recombinant AlgE incorporated into planar lipid bilayers with various salts. *cis* and *trans* sites contained the same salt concentration (0.5 M). ◆, K-acetate; ✱, KCl; ▲, K-gluconate; ■, NaCl.

polymerase gene, which was controlled by a thermoinducible promoter. *E. coli* K38(pGP1-2) containing pTR7-2 overexpressed *algE* when grown at 42°C (Fig. 1). The identity of the presumed AlgE band was confirmed by immunoblotting (data not shown).

Subcellular localization of recombinant AlgE and its precursor protein. The mature gene product of *algE* produced in *E. coli* was found mainly in the OM, but AlgE (54 kDa) could be immunologically detected in the CM as well. This was not due to contamination (10%) of the CM preparation with OM. A protein of 56 kDa, which likewise reacted with anti-AlgE antibodies and presumably represents the precursor of AlgE,

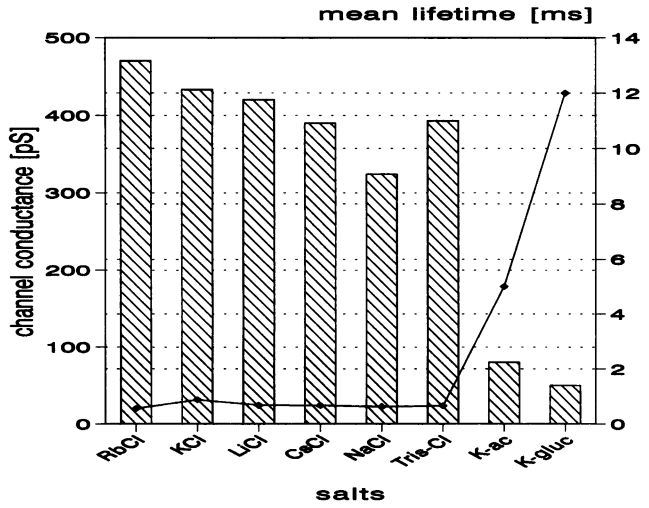

FIG. 8. Comparison of single-channel conductances (bars) and mean open-state lifetimes (diamonds) of recombinant AlgE in the presence of different salts. Recombinant AlgE was incorporated in planar lipid membranes. K-ac, K-acetate; K-gluc, K-gluconate.

FIG. 9. Single-channel current fluctuation traces of recombinant AlgE at 60 mV to demonstrate the effect of the type of anion on the mean open-state (o) lifetime, the mean closed-state (c) lifetime, and the single-channel conductance. (a) 0.5 M K-gluconate; (b) 0.5 M KCl.

was located exclusively in the CM and could not be detected in *E. coli* not expressing *algE* (Fig. 2). The cytosol contained neither AlgE nor its precursor (data not shown), suggesting a cotranslational export of AlgE across the CM.

Isolation and solubilization of AlgE from OM preparations required the simultaneous use of a nonionic detergent (for example, octylglucoside) and a high concentration of potassium chloride (150 mM). This suggests that AlgE is an integral OM protein. The following observations support this assumption.

(i) Treatment of whole cells of *E. coli* K38(pGP1-2, pTR7-2) with trypsin showed that AlgE was resistant to proteolysis, although the polypeptide is rich in lysine and arginine residues. AlgE was probably protected from enzymatic degradation by lipopolysaccharide (LPS) and phospholipid molecules partially surrounding the protein in the OM. However, AlgE became sensitive to trypsin when the cells were enzymatically treated in the presence of the chelating agent EDTA (2 mM). EDTA treatment results in the release of large quantities of LPS and destabilization of LPS by removing divalent cations. The resistance of various integral OM proteins, e.g., the porins, to proteolytic digestion is already well-known (8, 16, 22).

(ii) Insertion of purified recombinant AlgE into planar lipid bilayers revealed that it can function as an anion-specific channel (for details, see below). Transmembrane channel proteins are per definitionem integral membrane proteins.

(iii) NHS-biotinylation of whole cells from *algE*-expressing *E. coli*, causing a strong labeling of AlgE, supports the assumption that AlgE is in or at least associated with the OM and thus accessible to the reagent (Fig. 3).

The AlgE protein lacks hydrophobic sequences long enough to span the lipid bilayer. It was possible to develop a topological model of AlgE (Fig. 4) based on the amino acid sequence of AlgE (7) and the criteria used for the structural prediction of PhoE (34). According to this model, AlgE presented as a β-barrel, consisting of 18 antiparallel amphipathic β-strands spanning the OM, eight turns (T1 to T8) at the periplasmic site, and nine loops (L1 to L9) exposed to the cell surface. The loops generally correspond to the hydrophilic maxima in the protein, when analyzed by the method of Hopp and Woods (18). Furthermore, our suggestion that sequences L1 to L9 (Fig. 4) are loops is in good agreement with structural predictions of Paul and Rosenbusch (24) revealing the presence of β-turns in all these regions. The predicted transmembrane segments 4 and 5 each contain a charged residue of opposite charge, i.e., Arg and Glu, respectively, on the hydrophobic side of the β-strands. Possibly, these residues are involved in forming a salt bridge. On the other hand, single hydrophilic residues in the β-strands exposed to the external hydrophobic core of the putative AlgE structure could possibly be tolerated,

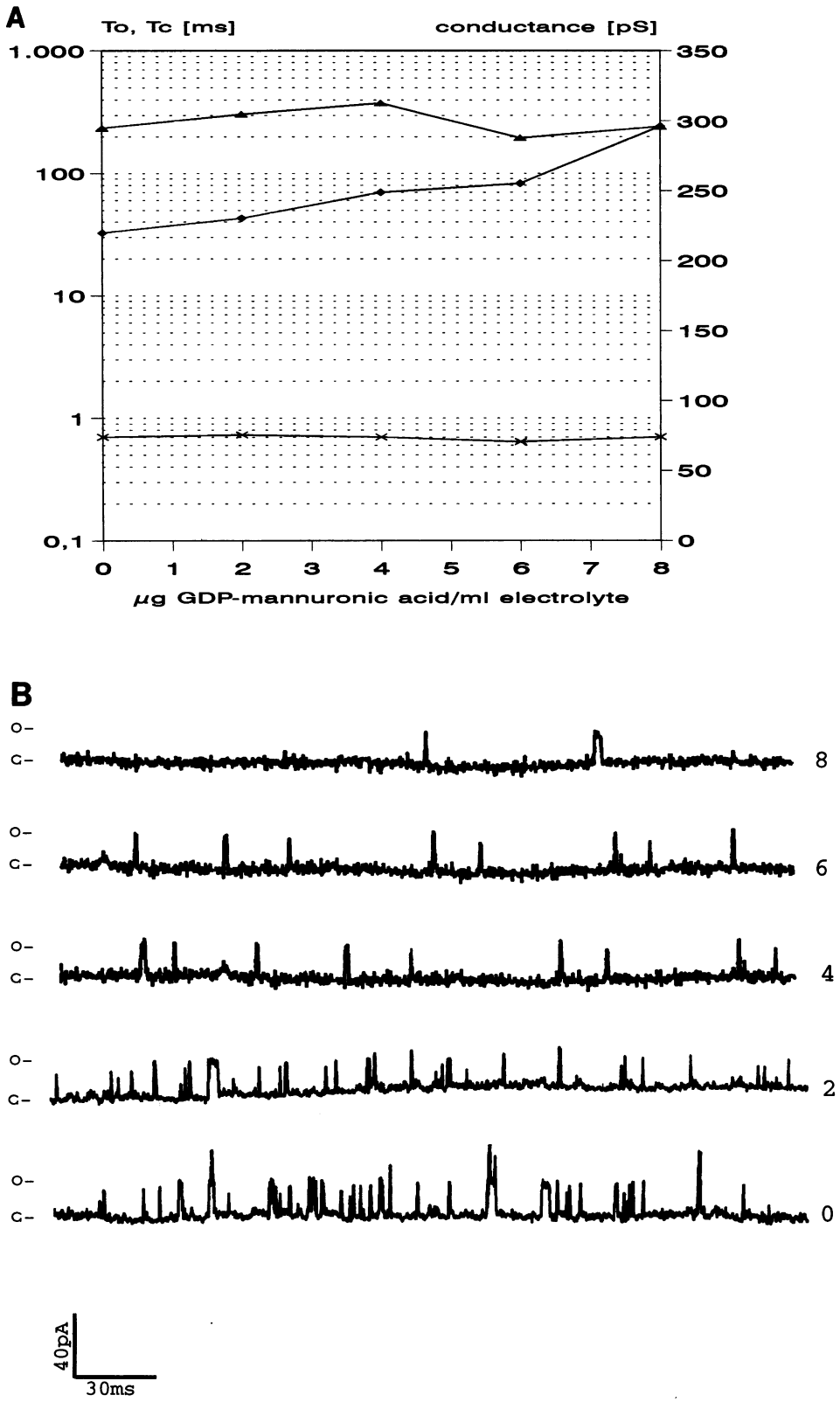

FIG. 10. (A) Effects of increasing GDP-mannuronic acid concentration on single-channel conductance (▲) and mean open-state (To) (×), and mean closed-state (Tc) (◆) lifetimes of recombinant AlgE. GDP-mannuronic acid was added to the *cis* side of the chamber. One thousand events were analyzed to determine To and Tc lifetimes at 60 mV as well as the single-channel conductance. Conductance and the To lifetime remained constant, whereas the Tc lifetime increased by a factor of about 8 after the addition of 8 μg of GDP-mannuronic acid per ml. (B) Single-channel current fluctuation traces at 60 mV demonstrating the effect of increasing GDP-mannuronic acid concentrations (indicated on the right in micrograms per milliliter) on To (o) and Tc (c). The electrolyte consisted of 0.5 M KCl–10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)-KOH (pH 7.0).

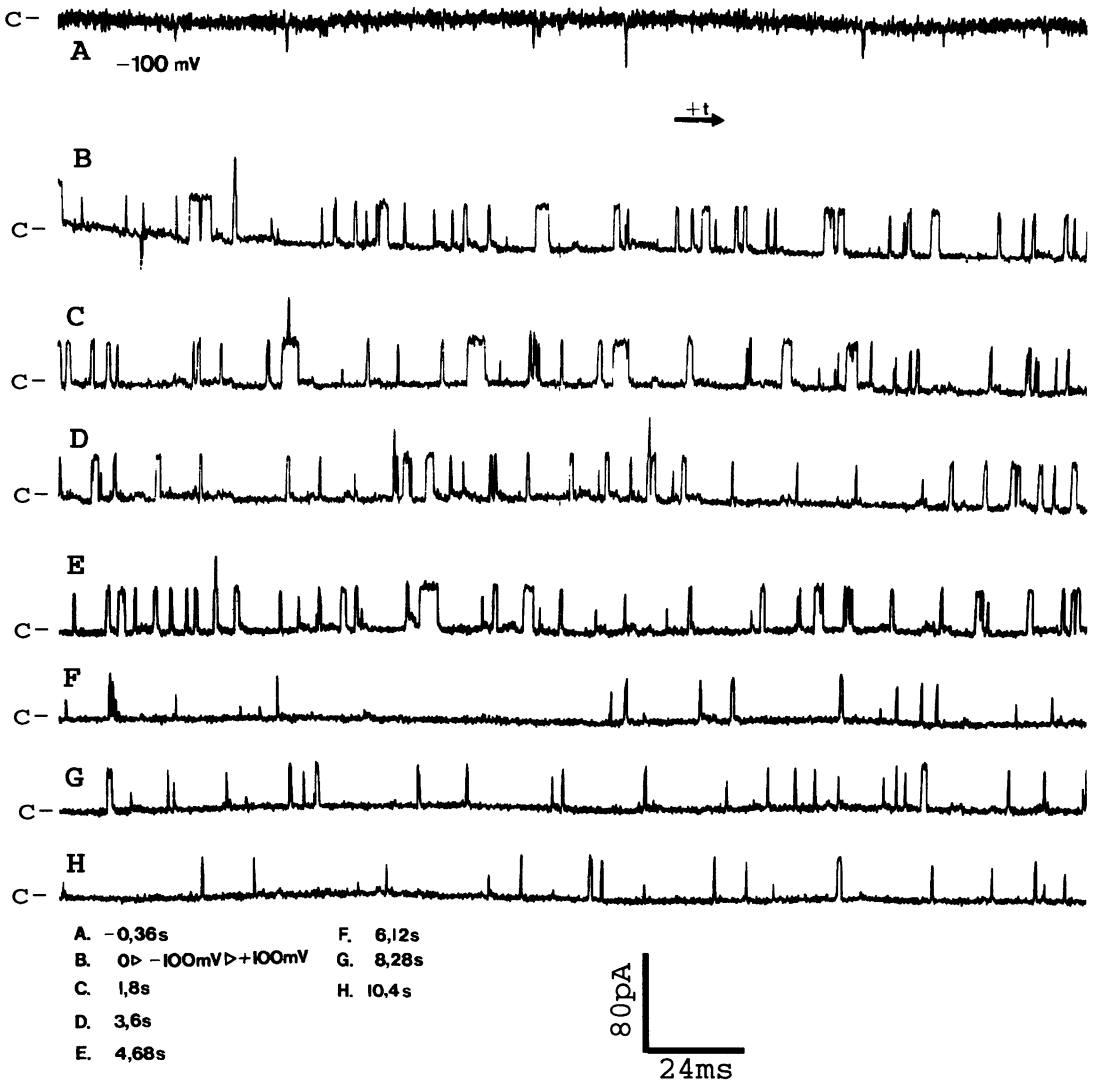

FIG. 11. Single-channel current fluctuation traces of recombinant AlgE as a function of time and voltage, demonstrating the inactivation behavior at +100 mV. Trace A shows single-channel current fluctuations at −100 mV. At B, the voltage was changed from −100 to +100 mV. The electrolyte consisted of 0.5 M KCl–10 mM HEPES-KOH (pH 7.0). c, zero-current level. Times are indicated at the bottom of the figure.

as was shown in the case of PhoE by mutagenesis (32). The topological model of AlgE (Fig. 4) is very similar to those already postulated and verified for some other integral OM proteins, e.g., porin PhoE from *E. coli* (8). The β-barrel model of AlgE predicts that hydrophilic parts of the polypeptide chain are exposed to the cell surface. Evidence for this structural prediction was obtained as follows. Whole cells of *E. coli* K38(pGP1-2, pTR7-2) were treated with NHS-biotin. This reagent is unlikely to penetrate cell membranes but is able to biotinylate exposed lysine residues of polypeptides. As shown in Fig. 3, AlgE was biotinylated even after a short reaction time (5 min). This agrees with the content of eight lysine residues in loops L1 to L9, i.e., potential targets for biotinylation. Our result was supported by the observation that AlgE could be iodinated by treating whole cells of *P. aeruginosa* CF3/M1 with Bolten-Hunter reagent (14). Furthermore, our result fits with the fact that cystic fibrosis patients whose airways are infected with mucoid *P. aeruginosa* organisms show an early and strong immune response to AlgE (26).

Purification and further characterization of recombinant AlgE. The OM of the *algE*-overexpressing strain *E. coli* K38(pGP1-2, pTR7-2) was isolated by the Sarkosyl method (25) and used to purify AlgE in large enough quantities for further characterizations. A homogeneous preparation of AlgE, as judged by SDS-PAGE, was obtained by immobilized metal ion affinity chromatography (14) followed by anion-exchange chromatography (Fig. 5). The isoelectric point of purified AlgE was determined to be 4.4 (Fig. 6). This value is significantly lower than that (pI = 5.6) calculated by Chu et al. (7) on the basis of the amino acid composition of AlgE. The pI of 4.4 was used to increase the efficiency of the AlgE purification procedure by reducing the pH to 6.5 for the anion-exchange chromatography.

Various channel proteins derived from the OM of gram-negative bacteria are often organized as oligomers (8). When whole cells of *E. coli* K38(pGP1-2, pTR7-2) were treated with the cross-linking agent DSP, the immunologically detected AlgE was monomeric. In contrast, DSP treatment of purified

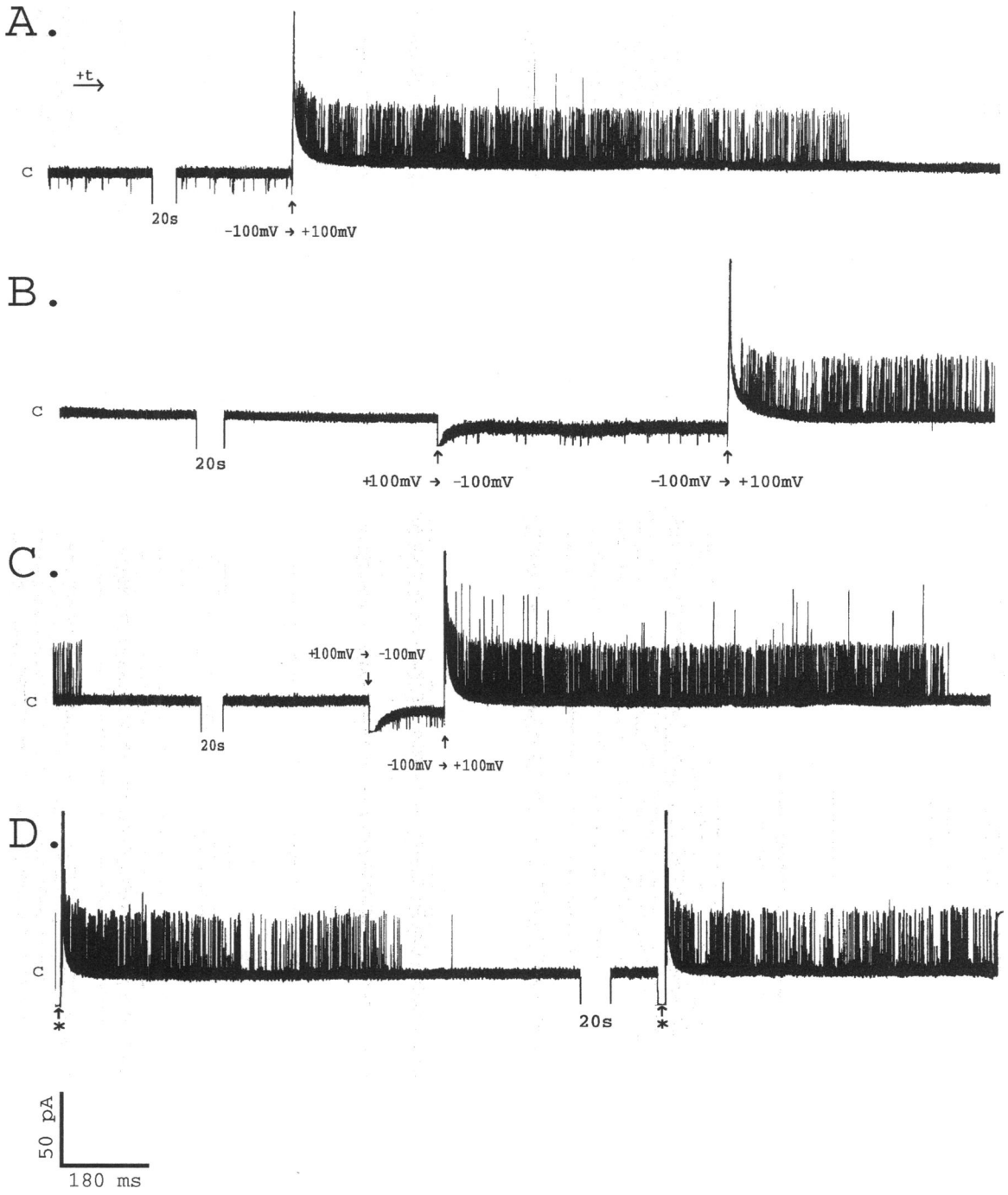

FIG. 12. Single-channel current fluctuation traces of recombinant AlgE demonstrating consistent behavior after recovery from inactivation at +100 mV and low steady-state activity at −100 mV. The same conditions as for Fig. 11 were used. Traces A to D show a continuous series of consistent inactivations and recoveries of channel activity following long and very short sign reversal times of the membrane potential. Trace D demonstrates that even very short reversal times (asterisks) led to the recovery of channel activity. Arrows indicate potential reversal points. c, zero-current level.

AlgE yielded two spots in the gel reacting with AlgE-specific antibodies. The molecular masses were 54 and 100 kDa (data not shown), which might indicate that AlgE is able to form dimers under certain conditions. In electrophysiological experiments, the cross-linked AlgE showed the same ion channel activity as the untreated AlgE. The fact that cross-linking occurred only when AlgE was treated with DSP under in vitro conditions is consistent with earlier cross-linking experiments with other OM proteins of *P. aeruginosa* (1).

Ion channel properties of recombinant AlgE. Purified recombinant AlgE incorporated spontaneously into planar lipid bilayers. This property was used to characterize its channel-

forming activity in detail. Single-channel current fluctuations measured in a standard electrophysiological setup (1 M KCl, +60 mV, and 21°C) and analyzed by computer assistance revealed a mean conductance of 0.76 nS and a mean open-state lifetime of 0.7 ms. These values differ significantly from the corresponding values obtained by studying, e.g., porin F of *P. aeruginosa* (4). In the latter case, the mean conductance is about seven times higher (approximately 5 nS) and the mean open-state lifetime is in the range of minutes. Typical porin channels such as OmpF and OmpC, with single-channel conductances of 1.9 and 1.5 nS, respectively, and mean lifetimes of minutes, behaved significantly differently from AlgE (3, 8). This means that AlgE seems not to function as a typical porin channel.

Current-voltage characteristics of the AlgE channel are shown in Fig. 7. The type of anion significantly influenced the channel conductance (Fig. 7 through 9). Chloride salts with Tris or various alkali cations revealed approximately the same conductance. Potassium salts of large anions, however, for example, acetate and gluconate, lowered the conductance about 10-fold (Fig. 8). Additionally, the mean open-state lifetime increased and the mean closed-state lifetime decreased when these anions were used (Fig. 9). These results indicate that the AlgE pores are anion selective. The anion selectivity of AlgE was confirmed by zero-current membrane potential measurements, which revealed a reversal potential of −53 mV when a 10-fold gradient of potassium chloride (0.1 versus 1.0 M) across the lipid membrane was applied. According to the Goldman-Hodgkin-Katz equation, we calculated a permeability ratio of $P_{cation}/P_{anion} = 0.025$.

The single-channel conductance of AlgE did not increase linearly with potassium chloride concentration but showed saturation behavior (data not shown). These results suggest that the AlgE channel contains a binding site for the chloride ion. Assuming Michaelis-Menten kinetics, the binding constant (K_m) of the AlgE channel for chloride ions was calculated to be 0.75 M. In comparison, the phosphate-specific OprP channel of *P. aeruginosa* revealed a K_m of 0.05 M for chloride ions (17). The weak binding of chloride ions by the AlgE channel might indicate that the in vivo function of AlgE is the transmembrane passage of another particular anion rather than that of chloride ions. In mucoid *P. aeruginosa*, AlgE might be involved in the translocation of polymannuronic acid across the OM. This polyanion is the putative precursor of alginate (21). This hypothesis was supported by the observation that GDP-mannuronic acid increased the mean closed-state lifetime of the AlgE channel with increasing concentrations, whereas the mean open-state lifetime remained constant (Fig. 10). Most likely, GDP-mannuronic acid caused the increase of mean closed-state lifetime by specific binding to AlgE, thereby blocking the translocation of permeable anions. Purified alginate (10, 20, 30, or 40 μg of electrolyte per ml) revealed no effect on the channel open-state probability, i.e., the mean open-state and mean closed-state lifetimes. As shown in the topological model of AlgE (Fig. 4), loops L1 to L9 contain a total of 27 basic amino acid residues (8 Lys and 19 Arg residues), some of which might be involved in recognition and/or binding of anions. In *Neisseria meningitidis*, the porin-like OM protein CtrA, which contains eight predicted β-strands, was described to be involved in capsule formation (11).

A further property of the AlgE channel is shown in Fig. 11 and 12. The application of voltages of >+85 mV caused inactivation of channel gating within seconds. The inactivation was due to a simultaneous decrease in channel opening rate and mean open-state lifetime (Fig. 11). A short sign reversal of the potential led immediately (delay of 5 to 10 ms) to recovery from inactivation, showing the previous channel opening rate (Fig. 12). Activation, inactivation, and recovery could be repeated several times and were consistent (Fig. 12). A similar effect was observed when OmpF from *E. coli* was studied (31).

Furthermore, at high negative voltages, we observed a low steady-state activity, i.e., a low mean open-state probability, whereas at high positive voltages the mean open-state probability is initially high (Fig. 11 and 12). This indicates that AlgE possesses another rectifying property besides inactivation.

Conclusions. Pairs of mucoid and nonmucoid strains of *P. aeruginosa* can be obtained in two ways: (i) isolation of spontaneous nonmucoid derivatives from clinical mucoid strains or (ii) selection of mucoid mutants from nonmucoid strains on carbenicillin agar (13). In each case, AlgE was found in the OMs of mucoid strains only (15, 26), suggesting that this protein is crucial for the bacterial ability to form and/or secrete alginate slime or a polymeric precursor. Our study provides a detailed biochemical and biophysical characterization of AlgE, focusing especially on the function of AlgE as an anion-specific channel. A technical prerequisite which was fulfilled was the construction of an *E. coli* strain which overexpressed the *algE* gene, allowing the purification of AlgE on a preparative scale. By the insertion of the *algE* coding region into the *Nde*I site of vector pT7-7, we could overexpress *algE* without creating a fusion protein. The electrophysiological analysis of AlgE will now be continued by studying *algE* mutants containing small deletions in the predicted loops in order to identify those amino acid sequences which are essential for the anion selectivity and the channel function. A number of short-deletion mutants have already been isolated. Insertion mutagenesis of *algE* will hopefully help to test our topological model. Another opportunity provided by the results presented here is screening of chemicals with low molecular weights for interference with the channel activity. Effective chemicals could be of therapeutic value, i.e., by inhibiting the formation of the virulence factor alginate.

ACKNOWLEDGMENTS

This research was supported by a grant from the Deutsche Forschungsgemeinschaft, by the Deutsche Fördergesellschaft der Mukoviszidose-Forschung e.V., and by the Minister für Wissenschaft und Forschung des Landes Nordrhein-Westfalen.

We thankfully acknowledge the skillful technical assistance of Blanka Kutscher.

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