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Purification and Characterization of the Wild-Type and Mutant Carboxy-Terminal Domains of the *Escherichia coli* Tar Chemoreceptor

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The carboxy-terminal half of the *Escherichia coli* Tar chemoreceptor protein was cloned into an overproducing plasmid with the transcription of the insert under the control of the strong hybrid *tac* promoter. Two dominant mutations in the *tar* gene, which result in "tumble-only" (*tar-526*) or "swim-only" (*tar-529*) phenotypes and which are postulated to produce proteins locked in specific signalling modes, were introduced separately onto the overproducing plasmid. After induction with isopropyl-β-D-thiogalactopyranoside, cells containing the plasmids produced about 10% of their soluble cellular protein as the carboxy-terminal fragments. A scheme to purify the overproduced fragments was developed. Typical yields of pure fragment were 5, 30, and 20 mg per liter of induced culture for the wild type, 526 mutant, and 529 mutant, respectively. Fast-protein liquid chromatography-gel filtration analysis of the pure fragments showed that they all existed as oligomers (ca. 103,000 daltons), possibly trimers or tetramers (monomer size is 31,000 daltons). However, the 529 mutant fragment showed an additional oligomeric form (240,000 daltons) corresponding approximately to an octamer. When chromatographed in the presence of 1% octylglucoside, all three fragments showed an identical single oligomeric size of about 135,000 daltons. Further differences between the fragments such as ion-exchange behavior and susceptibility to degradation were found. Taken together, these results suggest that conformational differences between the 529 mutant fragment and the other fragments exist and that these differences may correlate with the phenotypic effects of the *tar-529* mutation.

Bacterial cells respond to changes in concentrations of chemicals in their environment by biasing their swimming behavior (for reviews, see references 12, 18, and 26). They can reverse the direction of rotation of their flagellar filaments, causing the cell to "tumble" and leading to a change in the direction of movement (17). Regulation of the frequency of flagellar rotation reversals affects the net movement of the cell. The frequency of reversals is controlled by a family of receptor-transducer proteins that reside in the cytoplasmic membrane (18). These proteins interact directly or via other binding proteins with a variety of ligands, and some of these act as chemoattractants. In the presence of increasing concentrations of attractant molecules, a signal is generated that results in the suppression of flagellar rotation reversal. On the other hand, when the concentration of an attractant decreases, the frequency of tumbles is increased. Four different chemotaxis receptor-transducer proteins have been identified and sequenced in *Escherichia coli* and *Salmonella typhimurium* (3, 4, 14, 28). They vary in their specificities toward different chemoeffectors. However, they all appear to interact with the same cytoplasmic chemotaxis proteins to generate and transmit signals to change the direction of flagellar rotation.

Genetic analysis of several of the *E. coli* chemotaxis receptor proteins (23, 24, 27) combined with a comparison of their amino acid sequences has revealed the presence of distinct functional domains that can be associated with specific regions of the protein molecule. In particular, the N-terminal region, whose sequences are highly diverse between the various chemoreceptors, appears to be associated with ligand binding and hence receptor specificity. In addition, two membrane-spanning regions and a cytoplasmic carboxy-terminal region have been delineated. Large portions of the cytoplasmic region are highly conserved and are probably responsible for signalling, i.e., interacting with other components of the chemotaxis system resulting in the transmission of a signal to the bacterial flagellar motor. Other portions of the carboxy-terminal region of the receptor proteins have been implicated in controlling the sensitivity of signal transduction, i.e., the process of adaptation. Adaptation is correlated with methylation and demethylation of several glutamic acid residues in the carboxy-terminal regions of the receptor. These modifications, catalyzed by the CheR (a methyltransferase) and CheB (a demethylase) proteins, are postulated to regulate the sensitivity of the receptor-transducer (10, 30).

The biochemical study of the chemotaxis receptor proteins, and of membrane proteins in general, is hampered by their insolubility in aqueous environments. Difficulties both in isolating the proteins and in reconstituting their native activities arise as a consequence of having to release the proteins from a lipid environment into an aqueous one. One way to overcome these problems is to study a soluble portion of the protein that possesses a known activity. In this paper we report the molecular cloning, purification, and partial characterization of the carboxy-terminal domain of the chemotaxis Tar receptor protein of wild-type *E. coli* and two behavioral mutants of *E. coli*. Both of the behavioral mutations map within the highly conserved region of the carboxy-terminal domain (23) that is thought to be involved in signalling. These mutations, termed *tar-526* (alanine residue 436 changed to valine) and *tar-529* (serine residue 461 changed to leucine), are dominant when present on plasmids in a wild-type background (23), conferring the mutant phenotype in the presence of the wild-type gene. The *tar-526* mutation causes tumble-only movement, whereas the pres-

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ence of the *tar-529* mutation results in an unusually low frequency of tumbles. Since these mutations result in a dominant phenotype, it has been suggested that they are "fixed" in a specific signalling form. Thus, a comparative study of the wild-type and mutant carboxy-terminal fragments may provide further insight into the mechanism of signalling in the bacterial chemotaxis system.

MATERIALS AND METHODS

Materials. Restriction endonuclease *Asp*718 was purchased from Boehringer Mannheim Biochemicals. All other restriction enzymes were obtained from Bethesda Research Laboratories, Inc., Boehringer, or New England Biochemicals Inc. T4 DNA ligase and DNA polymerase I Klenow fragment were from Bethesda Research Laboratories, Inc. The *tac* promoter Geneblock (*Hin*dIII-*Bam*HI fragment) was obtained from Pharmacia Fine Chemicals. 1-*o*-*n*-Octyl-β-D-glucopyranoside (octylglucoside), isopropyl-β-D-thiogalactopyranoside (IPTG), and 5-bromo-4-$^{+}$chloro-3-$^{+}$indolyl-$^{+}$β-D-galactopyranoside (X-gal) were from Boehringer. Protein standards for gel permeation chromatography were purchased from Sigma Chemical Co. DEAE Sepharose CL-4B was from Pharmacia, and Affi-Gel Blue (Cibacron Blue F3GA) was from Bio-Rad Laboratories.

General techniques. Recombinant DNA techniques including DNA isolation, digestion, ligation, and transformation were performed essentially by the method of Maniatis et al. (19). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 12.5% acrylamide (0.33% bisacrylamide) mini-slab gels (0.075 by 7 by 8 cm) was performed by the method of Laemmli (16) with a Hoefer Scientific Instruments (San Francisco, Calif.) Mighty Small II apparatus. Western immunoblotting was performed with a Bio-Rad Immun-Blot assay kit and previously described polyclonal anti-Tar antibodies (23) as recommended by the manufacturer. Protein concentrations were estimated by the method of Bradford (5) with a Bio-Rad protein assay kit with bovine serum albumin as a standard.

Plasmids, strains and growth conditions. Plasmids pNC189, pNC189V10, and pNC189L3 were constructed during the course of this study. Plasmids pUC9 (31) and pAK101 (13) have been described previously. Plasmid pNM46N526 is the same as pNM46 (25), except that it carries the *tar-526* mutation (23). Plasmid pAK101RI is the same as pAK101 except that the *Ava*I site in the beginning of the *tap* gene has been changed to an *Eco*RI site (A. Krikos, unpublished results). Plasmid pAK101N529 is the same as pAK101, except that it carries the *tar-529* mutation (23).

E. coli JM103 [relevant genotype Δ(*lac pro*) F′ *lacI*^{q} (21)] was used in all experiments. Cells were grown in L broth (19) supplemented with 100 μg of ampicillin per ml when appropriate. For routine growth and induction experiments, overnight cultures of JM103 transformed with the appropriate plasmid were inoculated (0.1 ml) into 125-ml flasks containing 10 ml of medium. Flasks were incubated at 37°C with shaking. Cell growth was monitored by measuring A_{600}. When the culture reached an A_{600} of ca. 1, IPTG was added to 1 mM and incubation was continued for an additional 4 to 5 h. The cultures were then put on ice, harvested at 12,000 × *g* for 10 min, and then suspended to an A_{600} of about 10 in SDS-PAGE sample buffer for electrophoretic analysis.

Construction of plasmids that overproduce the Tar carboxy-terminal fragments. Construction of an overproducing plasmid containing the wild-type Tar carboxy-terminal fragment is summarized in Fig. 1. A 1.1-kilobase-pair *Nde*I-*Eco*RI fragment consisting of the carboxy-terminal half of the *tar* gene and a small 5′-coding region of the *tap* gene was cut out of plasmid pAK101RI, which contains a 3.2-kilobase-pair insert, including the *tar* gene (13). The cohesive ends of the *Nde*I-*Eco*RI fragment were filled in, and the fragment was ligated into the *Sma*I site of plasmid pUC9 to produce plasmid pNC18. Plasmids were selected, and inserts were screened for by transforming *E. coli* JM103 cells with the constructs and plating them on L-agar plates containing 50 μg of ampicillin per ml, 50 μg of X-gal per ml, and 50 μM IPTG. The insert direction was determined by restriction enzyme analysis. The strong hybrid *tac* promoter consisting of the p*trp* −35 and p*lacUV5* −10 consensus sequences (7) was then inserted as a 91-base-pair *Hin*dIII-*Bam*HI fragment directly upstream of the *tar* insert by exchange with the *Hin*dIII-*Bam*HI pNC18 fragment to produce plasmid pNC189; the presence of the promoter was verified by restriction enzyme mapping.

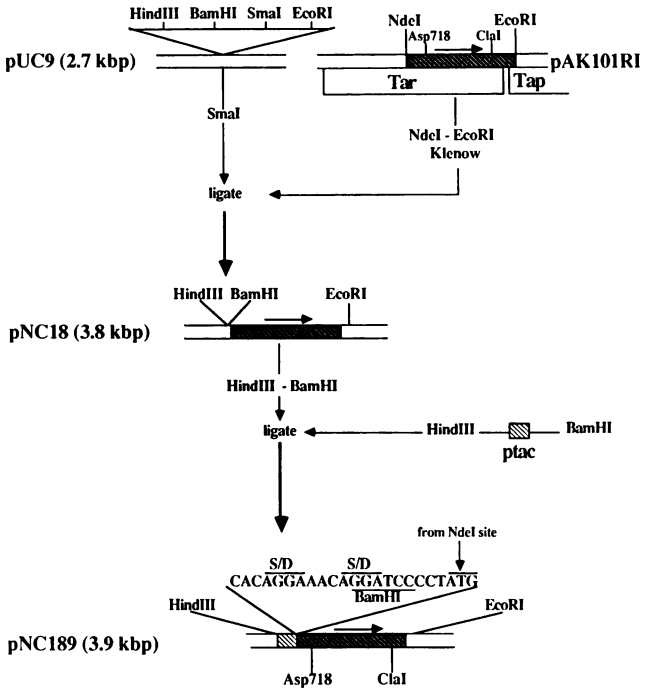

FIG. 1. Construction of a plasmid that overproduces the Tar carboxy-terminal fragment. The coding region of the carboxy-terminal half of Tar was inserted into plasmid pUC9 to produce plasmid pNC18, and a p*tac* fragment was then inserted directly upstream of the *tar* insert to produce plasmid pNC189 as described in Materials and Methods.

DNAs of plasmids pNC189 (wild-type Tar fragment, Fig. 1), pNM46N526 (carrying mutation *tar-526*) and pAK101 N529 (carrying mutation *tar-529*) were transformed into and reisolated from *E. coli* JM101 *dam* (20, 21). These DNAs were then cut with the enzymes *Asp*718 (recognizes the same sequence as *Kpn*I) and *Cla*I (sensitive to *dam* methylation) at the *tar* sites depicted in Fig. 1. The mutant *Asp*718-*Cla*I fragments were then ligated into pNC189 in place of the wild-type fragment to produce plasmids pNC189V10 (526 mutant fragment) and pNC189L3 (529 mutant fragment).

Purification of Tar carboxy-terminal fragments. Overnight cultures of *E. coli* JM103 carrying one of the plasmids pNC189, pNC189V10, or pNC189L3 were inoculated (5 ml) into 500 ml of L broth supplemented with 100 μg of ampi-

cillin per ml in four 2.8-liter flasks and incubated with vigorous shaking at 37°C. After 2 to 2.5 h of incubation, IPTG was added to a final concentration of 1 mM; the cultures were incubated for an additional 4.5 h and then put on ice for 10 min. All of the following procedures were performed at 4°C. All buffers used contained 20 mM Tris, pH 8.0 (at 4°C, buffer A), with the addition of 50 mM NaCl (buffer B), 80 mM NaCl (buffer C), or 1.0 M NaCl (buffer D). The cells were collected by centrifugation at 8,000 × *g* for 15 min, washed with 400 ml of buffer B, and finally suspended in 60 ml of buffer B. The cell suspension was passed twice through a French press (30,000 lb/in^2) and centrifuged at 5,000 × *g* for 5 min to remove whole cells and large debris. The supernatant fluid was then centrifuged at 100,000 × *g* for 1 h to precipitate membranes. Solid ammonium sulfate was added to the final supernatant fluid to reach 40% saturation. The mixture was stirred on ice for 30 min and then centrifuged at 17,000 × *g* for 10 min. The ammonium sulfate-precipitated pellet was dissolved in and diluted with buffer B (to ca. 200 ml) until its conductivity was equal to that of buffer C (4.4 mS) and applied directly to a DEAE Sepharose CL-4B column (2.6 by 29 cm) at a linear flow rate of 17 cm/h. The column was then washed with ca. 200 ml of buffer C, developed with a 600-ml gradient of 80 to 250 mM NaCl in buffer A, and washed finally with 200 ml of buffer D at the above flow rate. Fractions (10 ml) collected during the gradient application were assayed for protein content (see above) and for NaCl content by measuring conductivity with NaCl in buffer A as a standard. Fractions containing the carboxy-terminal fragment as determined by SDS-PAGE were pooled and then concentrated and desalted by ultrafiltration over a YM10 membrane (Amicon Corp.) to 7 mg of protein per ml (7 to 15 ml) and 15 mM NaCl, respectively. The YM10 concentrate was applied to an Affi-Gel Blue column (2 by 17 cm) at a flow rate of 12 cm/h; the flow was then stopped for 30 min to allow for maximal equilibration. The column was then washed with 100 ml of buffer A. Fractions (5 ml) collected during this wash that contained the fragment (as determined by SDS-PAGE) were pooled, brought to 40% saturation ammonium sulfate by adding 0.67 volumes of saturated ammonium sulfate in buffer A, and stored at 4°C.

Fast-protein liquid chromatography and gel filtration. Gel filtration was performed with a Pharmacia fast-protein liquid chromatography system at 22°C, unless stated otherwise. Protein samples (100 μl) in 20 mM Tris–100 mM NaCl (pH 7.5) at a concentration of 0.5 to 2 mg/ml were injected onto a Superose 12 column (1 by 30 cm) and eluted at a flow rate of 30 cm/h. The eluent was monitored continuously at 280 nm; fractions (0.5 ml) were collected and assayed by SDS-PAGE to verify the identity of peaks. A molecular mass calibration curve was constructed by determining the void volume with dextran blue and the elution volumes of the following proteins run separately: thyroglobulin (669,000 daltons [Da]), apoferritin (443,000 Da), β amylase (200,000 Da), alcohol dehydrogenase (150,000 Da), bovine serum albumin (66,000 Da), carbonic anhydrase (29,000 Da), and cytochrome *c* (12,400 Da). The total included volume and column packing were monitored by injecting acetone at 5 mg/ml.

RESULTS

Construction of overproducing plasmids. The first step in the study of the carboxy-terminal domain of the *E. coli* Tar protein was construction of overproducing plasmids containing the fragments of interest (Fig. 1; see Methods and Materials). The availability of a unique *Nde*I site at the appropriate position in the *tar* sequence and of the strong consensus *tac* promoter enabled the formation of a construct that transcribed the DNA of interest under the inducible control of a strong promoter and had two ribosome-binding sites 13 and 6 base pairs from the start codon. The final construct had two ribosome-binding sites derived from the p*tac* fragment, an ATG start codon that originated from the *tar Nde*I site, and the correct open reading frame to code for the 296 carboxy-terminal amino acids of Tar (Fig. 1). The dominant mutations *tar-526* and *tar-529* causing tumble-only and swim-only phenotypes, respectively (23), were introduced onto plasmid pNC189 to produce plasmids pNC189 V10 and pNC189L3 (as described in Materials and Methods).

Induction of plasmids and expression of C-terminal fragments. *E. coli* JM103 cells transformed with each of the three above plasmids were grown in L broth in the presence of 1 mM IPTG. When whole cells were subjected to SDS-PAGE, and the gels were stained with Coomassie blue they showed a protein band appearing in transformed cells but absent from uninduced cells and from control cells containing plasmid pNC02 that has the same insert but in the anti-sense direction (Fig. 2A). This band appeared to be one of the major proteins in the cell, and had an apparent molecular mass of 30,000 Da. The molecular mass of the Tar carboxy-terminal fragment calculated from its amino acid sequence (14) is 31,100 Da. The identity of the 30,000-Da band was further verified by performing an immunoblot of the same gel with polyclonal anti-Tar antibodies (23) as the probe (Fig. 2B). These antibodies identified a band of the same mobility as the Coomassie blue-stained 30,000-Da band.

Examination of the induction kinetics of the overproducing plasmids revealed the following. When cultures were induced in the midlog phase (A_{600} of 0.7 to 1.4) the carboxy-terminal fragments were detected by Coomassie blue staining of whole cells separated by SDS-PAGE approximately 1 h after induction (data not shown). However, Western immunoblotting of the same gel (Fig. 2B) showed the presence of the fragments even before induction, indicating that the *tac* promoter is leaky even in the presence of the *lacI*^{q} mutation carried by the host strain. After prolonged induction, in vivo degradation of the fragments occurred, as evidenced by the ladder pattern observed in the Western blots (Fig. 2B). Interestingly, this degradation was more pronounced in the wild-type and 529 fragments, whereas the 526 fragment was relatively resistant. Therefore, for routine production experiments, cells were induced in the midlog phase (A_{600} of ca. 1) for 4 to 6 h to maximize overproduction but limit proteolysis.

Purification of the carboxy-terminal fragments. A procedure to purify the overproduced proteins was developed. The same procedure was used for all three fragments. As an example, the purification of the protein produced by plasmid pNC189V10 (526 fragment) is summarized in Fig. 3. Typically, cells from an induced *E. coli* JM103 culture containing the overproducing plasmids were broken by passage through a French press, and the membranes were precipitated by ultracentrifugation. The carboxy-terminal fragment was found exclusively in the cytoplasmic fraction and could be completely precipitated in 40% saturation ammonium sulfate. Due to the acidic nature of the fragment, it bound to a DEAE-Sepharose column at low ionic strength at pH 8.0. It was then separated from the major contaminants by elution with a shallow NaCl gradient (Fig. 4). Interesting differences

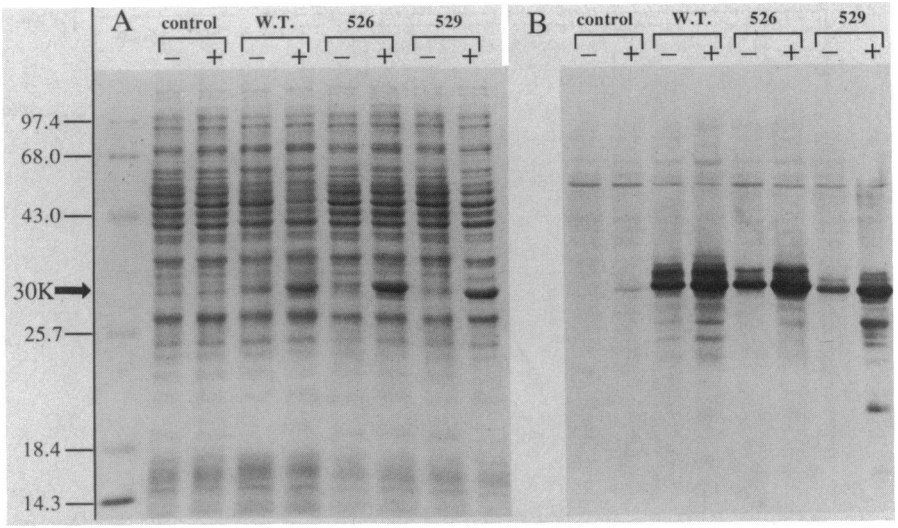

FIG. 2. SDS-PAGE of *E. coli* cells transformed with the overproducing plasmids. *E. coli* JM103 cells containing plasmid pNC02 (control, see text), pNC189 (wild-type fragment), pNC189V10 (526 mutant fragment), or pNC189L3 (529 mutant fragment) were grown in the presence (+) or absence (−) of IPTG as described in Materials and Methods. Whole cell samples were subjected to SDS-PAGE analysis on 12.5% acrylamide gels. Duplicate gels were stained with Coomassie blue (A) or electroblotted onto nitrocellulose paper, which was then subjected to Western immunoblotting with anti-Tar antibodies (B). Numbers on the left represent molecular masses in kilodaltons of the protein markers.

between the ion-exchange behavior of the three fragments were found; the wild-type fragment eluted at a salt concentration of 150 mM, whereas the 526 and 529 fragments eluted at 160 and 180 mM, respectively. By this stage, the fragments were relatively pure, and they were finally purified by passage through an Affi-Gel Blue column. Under loading conditions of relatively low ionic strength (ca. 15 mM NaCl) and elution with no salt, the contaminating proteins bound to the column, whereas the fragment flowed right through and was recovered in a homogeneous form as evidenced by SDS-PAGE (Fig. 3). Typical yields of pure fragment per liter of induced culture were 5, 30, and 20 mg for the wild-type, 526, and 529 fragments, respectively.

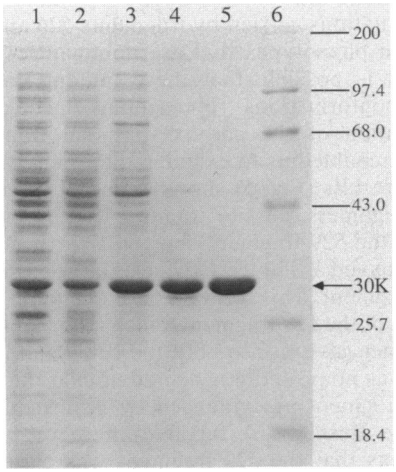

FIG. 3. Purification of the 526 mutant Tar carboxy-terminal fragment. Samples collected during the purification procedure described in Materials and Methods were subjected to SDS-PAGE and stained with Coomassie blue. Electrophoresis was performed on 12.5% acrylamide mini-gels (0.075 by 7 by 8 cm). The following fractions (including amounts of protein) were applied to each lane: 1, whole cells (10 μg); 2, cytoplasmic fraction (100,000 × *g* supernatant) (9 μg); 3, 40% saturation ammonium sulfate precipitate (3 μg); 4, pooled DEAE Sepharose CL-4B peak (3 μg); 5, pooled Affi-Gel Blue peak (2 μg); 6, molecular size standards in kilodaltons.

To verify the identity of the purified carboxy-terminal fragments, they were subjected to N-terminal amino acid sequence analysis. The amino acid sequences found for the first 15 amino acids of both the wild-type and mutant fragments were identical to the sequence predicted from the DNA sequence of the *tar* gene (14).

Gel filtration behavior of the pure fragments. To characterize the aggregation states of the pure fragments and to further pursue the presence of possible differences between the wild-type and mutant fragments, a study of their molecular weights under native conditions was performed. When subjected to gel permeation chromatography over a Superose-12 fast-protein liquid chromatography column in 20 mM Tris–100 mM NaCl (pH 7.5), all three fragments showed similar elution positions (12.6 to 12.7 ml), corresponding to molecular masses of 101,000 to 106,000 Da (Fig. 5, lines II), indicating that under these conditions the fragments are aggregated, possibly as dimers, trimers, or tetramers. However, the 529 mutant fragment showed an additional aggregation form that eluted at 11.2 ml, corresponding to a molecular mass of 240,000 Da, suggesting a form larger than a tetramer. This high-molecular-mass form was in equilibrium with the smaller 101,000-Da form, since when either of the two 529 forms was isolated and reinjected onto the column, it gave rise to both the 240,000- and 101,000-Da forms (data not shown).

All three fragments showed the aggregation behavior when chromatographed in the above buffer in the presence or absence of 1 mM EDTA and at 4 or 22°C. However, when chromatography was performed in the presence of 1% octylglucoside, the 240,000-Da form of the 529 fragment was abolished, and the fragment eluted as a single aggregation species that was identical to the wild-type and 526 fragments (Fig. 5, lines I). In the presence of octylglucoside, all fragments eluted at 12.1 to 12.2 ml, corresponding to a

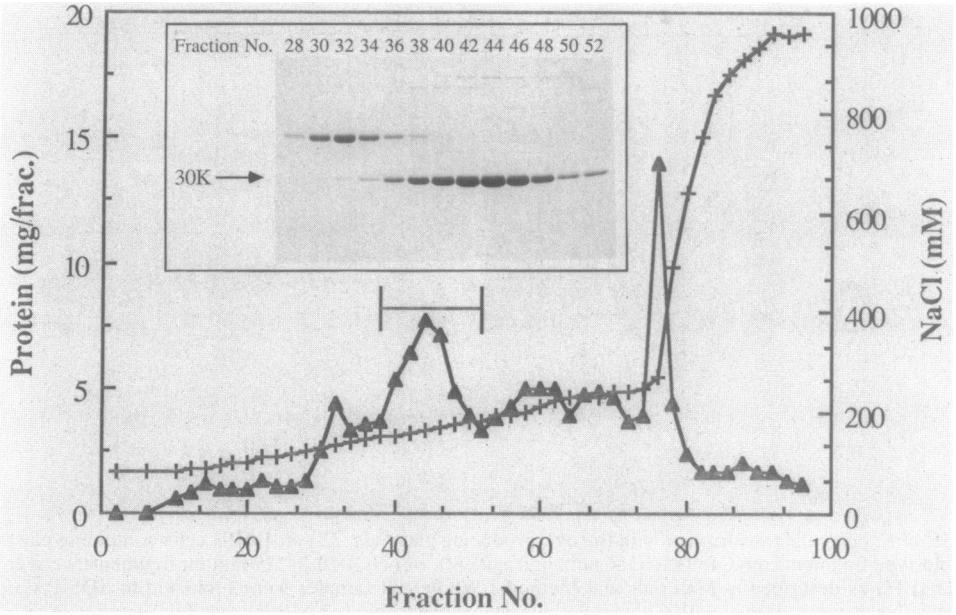

FIG. 4. Anion-exchange chromatography of the 526 mutant Tar carboxy-terminal fragment. The 40% saturation ammonium sulfate pellet was applied to and eluted from a DEAE Sepharose CL-4B column as described in Materials and Methods. Fractions collected during application of the NaCl gradient were assayed for protein content (Δ) and for NaCl concentration (+). Inset shows SDS-PAGE analysis (Coomassie blue stain) of the indicated fractions. Fractions 39 through 51 were pooled as indicated by the bar (see Fig. 3, lane 4).

molecular mass of 135,000 to 139,000 Da. This reproducible aggregation behavior suggests that there may be differences in the structures of the 529 fragment and the other fragments.

DISCUSSION

To perform an in vitro study of the postulated signalling portion of the *E. coli* Tar chemoreceptor, we cloned the cytoplasmic, carboxy-terminal half of the Tar protein onto an overproducing plasmid. In addition, we introduced onto the plasmid two behavioral mutations that are dominant over the wild-type gene (23), thus enabling the comparison of different fixed states of the receptor-transducer molecule. Cells transformed with the overproducing plasmids contained about 10% of their total protein as the carboxy-terminal fragment after induction with IPTG. This resulted in a twofold advantage: (i) purification was greatly facilitated since relatively few steps were required to obtain the proteins in a pure form, and (ii) production of protein in amounts sufficient for biochemical studies was possible without having to resort to the use of large-volume fermentors.

Fractionation of plasmid containing cells after IPTG induction revealed that the overproduced fragments were found in the cytoplasmic fraction and were not precipitated at 100,000 × *g*. Purification was then achieved by ammonium sulfate precipitation followed by conventional liquid chromatography. The main purification step was anion-exchange chromatography (Fig. 3 and 4). The acidic carboxy-terminal fragments (net charge of −10 based on amino acid composition) bound to a DEAE-Sepharose column and were eluted at 150 to 180 mM NaCl after application of a NaCl gradient. Impurities were then removed by passing the fragments over an Affi-Gel Blue column.

The yields of pure carboxy-terminal fragments per liter of induced culture were 5, 30, and 20 mg for the wild-type, 526, and 529 fragments, respectively. The differences in the yields of the wild-type and mutant fragments are consistent with their different susceptibilities to in vivo degradation as evidenced by immunoblots of whole-cell extracts after SDS-PAGE (Fig. 2B). The tumble-only 526 mutant fragment that showed almost no degradation was isolated in the greatest amounts, whereas the wild-type and swim-only mutant 529 fragments, which were both degraded to different extents, were isolated in lower amounts.

The differential degradation might be indicative of different conformations of the wild-type and mutant fragments. Since the mutations carried by fragments 526 and 529 result in behavioral phenotypes that are dominant over the wild type (23), it is possible that these mutant fragments are locked in conformations representing specific signalling forms. Isolation of the carboxy-terminal fragments in a soluble form enabled us to examine their native aggregation states. The results (Fig. 5) showed that all three fragments existed as oligomers, possibly dimers, trimers, or tetramers. In addition, the 529 fragment showed a further aggregation form that behaved as if it were twice as large as the 526 or the wild-type fragment. This form was not due to an irreversible modification of the 529 fragment, since rechromatography of either oligomer gave rise to both the small and large forms. Also there was no proteolytic degradation at the N terminus of the 529 fragment as evidenced by N-terminal sequence analysis (see Results). A further difference between the fragments was that the 529 fragment was eluted from the ion-exchange column at a higher salt concentration than were the wild-type and 526 fragments. All three fragments were produced in the same strain (*E. coli* JM103), which does not have a functional *cheR cheB* modification system (F. W. Dahlquist, personal communication); therefore, there should be no net charge or methylation differences between the fragments. Therefore, the above differences in chromatographic behavior and proteolytic susceptibility should reflect differences in the tertiary structure of the fragments.

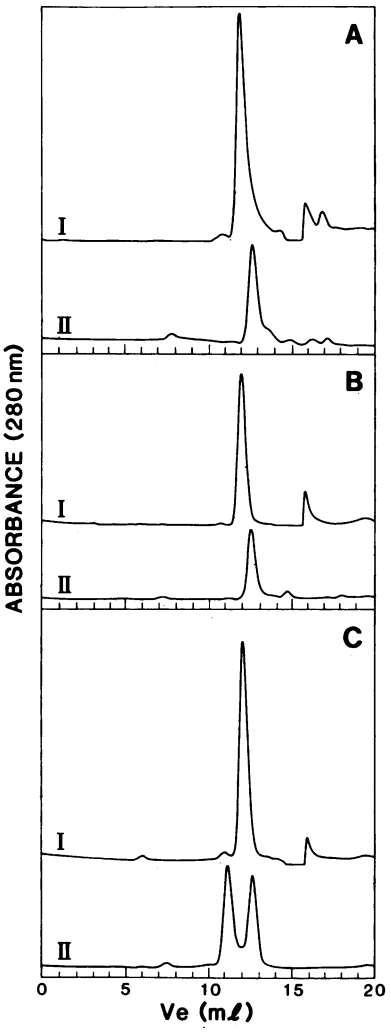

FIG. 5. Fast-protein liquid chromatography gel filtration analysis of the Tar carboxy-terminal fragments. Protein samples of the wild-type (A), 526 mutant (B), and 529 mutant (C) fragments were applied to a Superose 12 column as described in Materials and Methods in the presence (I) or absence (II) of 1% octylglucoside. Elution volumes (Ve) calculated by a Pharmacia LCC-500 Liquid Chromatography Controller were reproducible to ±0.05 ml between multiple runs.

It is interesting that a similar Tar carboxy-terminal fragment has been isolated from *S. typhimurium* (22). This fragment was produced by a single proteolytic cleavage of the receptor protein. The cleavage site at amino acid 259 of the *S. typhimurium* Tar protein produced a fragment shorter by three amino acids at the N terminus than the *E. coli* fragments reported here. A comparison of the *S. typhimurium* (28) and *E. coli* (14) Tar carboxy-terminal amino acid sequences shows an average homology of 90%, with long stretches (up to 93 amino acids) of 100% homology. The *S. typhimurium* fragment showed a similar ion-exchange behaviour (eluted at 140 mM NaCl) as the *E. coli* fragments and was also found to exist in an oligomeric form. Interestingly, the gel filtration behavior of the *S. typhimurium* fragment was similar to that of the 529 mutant fragment in that it showed two oligomeric forms (135,000 and 225,000 Da) in the absence of octylglucoside, and only one form (100,000 Da) in the presence of 1.25% octylglucoside.

The effect of octylglucoside on the chromatographic behavior of the carboxy-terminal fragments was twofold: (i) it caused a decrease in the mobility of all three fragments on the sizing column, and (ii) it abolished the high-molecular-weight form of the 529 mutant fragment. The apparent increase in molecular size observed in the presence of octylglucoside (Fig. 5) could be due to either an actual shift in aggregation size (i.e., trimer to tetramer) or to a change to a more extended conformation (1, 2), e.g., as a consequence of octylglucoside binding. The interaction of the fragments with octylglucoside suggests that the cytoplasmic domain of the Tar protein participates in a hydrophobic interaction, possibly with the membrane or with another protein. The 529 swim-only mutant fragment might have an alteration in this domain that enhances multimerization.

The data presented in this paper are consistent with in vivo cross-linking studies with *E. coli* Tsr and Tar chemoreceptors (6) and with in vitro results obtained for the *S. typhimurium* purified whole Tar receptor (8, 9) and its proteolytic N-terminal and C-terminal domains (22) that show that they exist as oligomers. The significance of these observations is still unclear, although a role for receptor multimerization in signal transduction is possible. On the other hand, it has been shown that the *S. typhimurium* multimeric Tar protein probably undergoes a conformational change after binding of the chemoattractant aspartate (8). Thus, transmembrane transmission of information via the receptor may be mediated by the stabilization of a signalling configuration of a single Tar multimer. The results we have presented indicate that there might exist conformational differences between the carboxy-terminal domains of two dominant mutants. In this regard, comparative circular dichroism spectroscopy of the 526 and 529 mutant fragments (J. S. Remington and N. Kaplan, unpublished data) showed that the two fragments had different peak maxima, further indicating that these fragments may exist in different conformations. Studies directed toward obtaining the three-dimensional crystallographic structures of the wild-type and mutant fragments are currently in progress.

It is interesting that when the mutant Tar carboxy-terminal fragments are present in the cytoplasm of *E. coli* cells deleted for all four chemoreceptors, they still exhibit some functional properties relevant to the whole Tar protein (25). Thus, the 526 mutant fragment can elicit a behavioral change (i.e., decrease in tumble bias) in response to a decrease in the intracellular pH. Furthermore, the 526 mutant fragment but not the 529 mutant fragment was modified in vivo by both the *cheR* and *cheB* methylating and demethylating enzymes. Therefore, at least in the case of the 526 mutant fragment, in vitro studies on the purified signalling half of a chemoreceptor protein may be relevant to its natural activities. The use of these isolated fragments together with the other purified chemotaxis proteins may allow us to reconstitute the signal transduction pathway.

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LITERATURE CITED

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