# *Escherichia coli* aspartate receptor
## Oligomerization of the cytoplasmic fragment

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## INTRODUCTION

The aspartate receptor (Tar) is one member of a class of homologous, methylatable receptor proteins that are involved in transmembrane signaling in the chemotaxis system of *Escherichia coli.* Tar is composed of a 60-kD polypeptide with two transmembrane sequences, a periplasmic ligand-binding domain and a cytoplasmic region responsible for signaling and adaptation (reviewed in references 1–4). The Tar protein is well suited for biophysical studies of the mechanism of transmembrane signaling because it can be purified in the requisite quantities (5), and because genetic methods, which are well developed in *E. coli,* can be used to introduce specific labeling sites in the protein (6), to generate well defined fragments (7, 8) and to study the effects of mutations in vivo by gene replacement. We have studied a 31-kD cloned soluble cytoplasmic fragment of Tar (c-fragment) (see references 7 and 8). The wild-type fragment and nine point mutants of the c-fragments were studied. The latter were selected on the basis of the aberrant swimming behavior displayed by cells with a mutated *tar* gene (9). In effect, the mutations 'locked' the Tar protein into either a smooth-swimming state (smooth mutants) or a tumble signaling state (tumble mutants), while the wild-type Tar protein alternates between two states (9, 10). Here we report a striking correlation between the swimming phenotype produced by these mutations and changes in the properties of the c-fragment: mutations that induced smooth-swimming behavior in cells were found to induce oligomerization of the c-fragment and mutations that induced tumbling behavior in cells did not. In addition to the reversible oligomerization of the c-fragment, the results described below indicate that the Tar c-fragment is not a compact globular domain. These data also demonstrate the usefulness of mutant analysis in uncovering protein structure-function relationships, which may be applied generally to problems of protein function where suitable selection methods exist. In this particular context the results provide evidence that receptor clustering may be involved in the mechanism of transmembrane signaling.

## RESULTS AND DISCUSSION

The wild-type and mutant forms of the c-fragment were purified according to the protocol of Kaplan and Simon (8). As shown in Fig. 1, gel-filtration chromatography (GFC) could be used to detect oligomer formation and was observed in five of the six smooth mutant c-fragments studied. With the exception of the glutamate-301 to lysine mutant (E301K) all of the smooth mutant c-fragments displayed more than one peak by GFC. Apparent molecular weights (MW) of the different forms, determined by calibration with globular proteins standards, were found to be between 110 and 270 kD. In pH 7 buffer the wild-type and tumble mutant c-fragments displayed only the 110-kD peak, no other forms were detected. See legend to Fig. 1 for details. Evidence that these different MW forms did indeed represent oligomers was obtained from the dependence of the different forms on the protein concentration of the samples injected onto the GFC column. The fraction of protein in the low MW form increased as protein solutions became more dilute. This is illustrated in Fig. 2, which plots chromatograms of three of the smooth mutants (S461L, S325L, A370V) as a function of protein concentration. GFC was also used to establish that the different forms were interconvertible. When protein eluting in one MW fraction was collected and reinjected immediately, the majority of material eluting the second time ($\sim 90\%$) was observed to have the same apparent MW as the material collected. However, if reinjection of the collected fraction was delayed, all MW fractions were observed to elute the second time. For example, the middle peak of the A370V c-fragment was collected from the 10 mg/ml elution profile (Fig. 2 *C*), and was reinjected after an equilibration period. The subsequent elution profile, displayed in Fig. 2 *C* as the 0.5 mg/ml run, was observed to consist predominantly of the low MW form. This behavior has been observed for all of the

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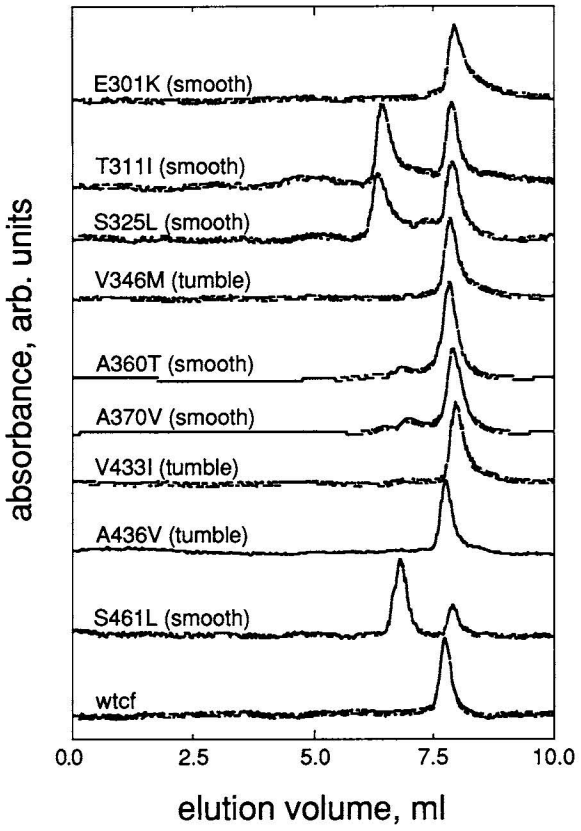


FIGURE 1 GFC of the wild-type and mutant Tar c-fragments was carried out on a TSK gel G3000SW$_{XL}$ column (0.8 × 30 cm). Absorbance was monitored at 280 or 214 nm. Material eluting from 6.0 to 6.5 ml and at 7.8 ml had apparent MWs of 270–220 and 110 kD, respectively, according to globular protein standards. The elution buffer was 20 mM K phosphate, 50 mM NaCl, pH 7.0. Swimming phenotypes and amino acid substitutions of the mutants are indicated with each trace at the left. The numbers indicating the location of the mutations correspond to the position in the intact receptor (9). Injection volumes were typically 100 μl. Flow rate: 0.5 ml · min$^{-1}$. Temperature: 4 °C. Protein concentrations of the samples were between 0.1 and 0.5 mg · ml$^{-1}$.

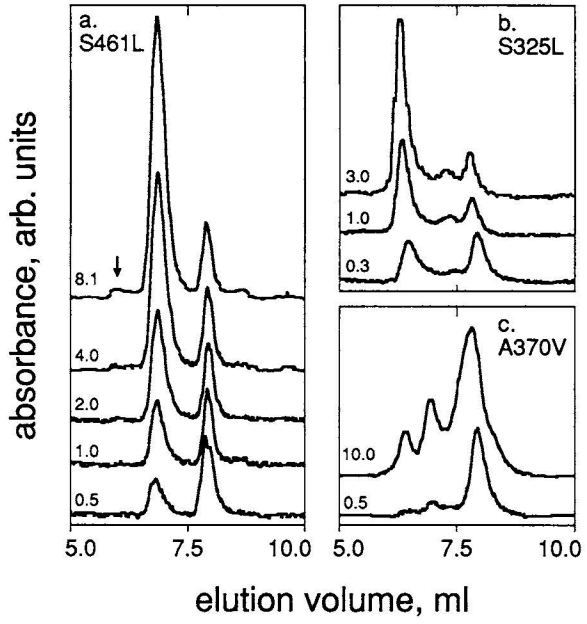


FIGURE 2 GFC of three oligomer-forming c-fragments as a function of protein concentration (mg · ml$^{-1}$; noted at the *left* near each trace). (*A*) S461L c-fragment: solutions were incubated and chromatographed at 20°C and eluted with a flow rate of 0.7 ml · min$^{-1}$. (*B*) S325L c-fragment: incubated and chromatographed at 4°C. Flow rate: 0.7 ml · min$^{-1}$. (*C*) A370V c-fragment: incubated and chromatographed at 4°C. Flow rate: 0.5 ml · min$^{-1}$.

oligomer-forming c-fragments we have studied, and is consistent with the known concentration dependence of association-dissociation processes.

The absolute MW of the low MW form observed in the GFC traces was found to be 31 kD by static light scattering experiments carried out with the wild-type c-fragment, which corresponded to the MW of a monomer based on the amino acid sequence (7, 8). The discrepancy between the apparent MW observed by GFC (110 kD) and the true MW is probably due to a nonglobular shape of the c-fragment. For example, the rod-shaped coiled-coil protein tropomyosin, which has a dimer MW of 60 kD, gives an apparent MW of 330 kD by GFC. Such shape-dependent excluded-volume effects on protein elution in GFC are well known, and can be accounted for by theory (11). The unusual elution volume of the c-fragment is consistent with the prediction of a coiled-coil structure on the basis of sequence analysis (1). Thus, the c-fragment might be expected to have an elongated shape.

The association-dissociation reaction was determined to occur between a monomer and a dimer in the case of one c-fragment (S461L). The weight-average MW of the protein, measured directly by static light scattering as a function of protein concentration, was in close agreement to the weight-average MW calculated from the GFC data (Fig. 2*A*) for a monomer-dimer equilibrium. The data are shown in Fig. 3. Also, the dimerization isotherm of the GFC data was found to be most consistent with a monomer-dimer equilibrium for this particular mutant (not shown). The dissociation constant was found to be ~20 μM at 20°C, and ~3 μM at 4°C. While it seems clear that the S461L c-fragment is involved in a reversible monomer-dimer equilibrium under these conditions, it is equally clear that other variants of the oligomer-forming c-fragments (S325L and A370V) have different behavior. The GFC data in Fig. 2 *B* and *C*, show the presence of three resolvable forms, suggesting strongly that these c-fragments form

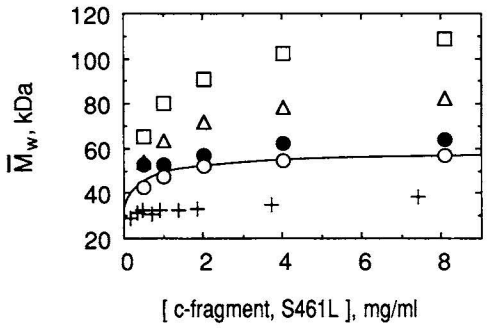


FIGURE 3 The weight-average MW ($MW_w$) of the S461L c-fragment was measured directly by static light scattering (SLS, ●) as a function of protein concentration on the same samples used in the GFC experiments of Fig. 2*A*. The fraction of the protein in the monomeric and oligomeric forms were determined from the GFC data, and $MW_w$ was determined by assuming either a monomer-dimer (○), monomer-trimer (△), or a monomer-tetramer (□) equilibrium. SLS data of the wild-type c-fragment (+) are plotted for comparison. The curve is given by $MW_w = 2M_1\sqrt{1 + 8c/M_1K_d}/(1 + \sqrt{1 + 8c/M_1K_d})$, where $K_d$ = 20 μM, $M_1$ = 31 kD, and $c$ is the concentration in mg/ml.

oligomers that are larger than dimers, perhaps trimers or tetramers.

The intact, detergent-solublized aspartate receptor has been found to have the MW of a dimer, and specific chemical cross-linking experiments have provided evidence for a dimeric state of the receptor in the membrane, leading to the suggestion that transmembrane signaling takes place within the receptor dimer (12). However, in view of the good correlation between receptor signaling state (inferred from swimming behavior) and oligomer formation, it is still reasonable to consider clustering as the mechanism of transmembrane signaling for the aspartate receptor, as has been proposed for growth factor receptors (13–15).

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