Three-dimensional structural model of the serine receptor ligand-binding domain

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Abstract

Computer-based homology modeling techniques were used to construct a three-dimensional model of the *Escherichia coli* serine receptor ligand-binding domain based on the crystal structure of the *Salmonella typhimurium* aspartate receptor and the sequence homology between the two receptors. Residues that have been found in mutagenesis studies to be necessary for serine binding are located in a proposed serine-binding site. Several other mutations that affect swimming behavior require relatively small shifts in α -carbon positions in the model to give a minimized structure, suggesting that small changes in receptor conformation can affect the signaling state of the receptor.

Keywords: *Escherichia coli*; ligand-binding domain; serine receptor; three-dimensional structural model

Only a few structures of membrane receptors have been determined, due to the difficulty in crystallizing membrane proteins, and the fact that many receptor sizes are too large for solving structures using NMR. To date there is one X-ray structure of each of the following classes of receptors: channels (porin) (Weiss et al., 1991), receptors with one transmembrane domain (the human growth hormone receptor extracellular domain) (de Vos et al., 1992), receptors with a few transmembrane domains (the aspartate receptor ligand-binding domain) (Milburn et al., 1991), and receptors with many transmembrane domains (the bacterial photoreaction center) (Deisenhofer & Michel, 1989).

Proteins that have high sequence homology and similar functions often have similar three-dimensional structures, as is seen in such cases as trypsin and chymotrypsin and myoglobin and hemoglobin. This similarity can be used to predict the structure of a second protein from the known crystal structure of its homologous analogue. Thus, the structure of human immunodeficiency virus (HIV) protease was predicted based on its sequence homology to the protease from Rous sarcoma virus (Weber et al., 1989). The predicted structure agreed closely with the subsequently determined crystal structure (to 2.1 Å rms [root mean square], except for the positions of a flex-

ible flap and six residues at the amino-terminus) (Weber, 1990).

The recent determination of the crystal structure of the aspartate receptor ligand-binding domain makes these techniques applicable to other chemotaxis receptors, which will facilitate interpretation of their biological properties. Specifically, sequence homology with the *Escherichia coli* serine receptor indicates that the structure of the aspartate receptor is likely to be a good foundation on which to predict the structure of that receptor (Boyd et al., 1983). The serine receptor encoded by the *E. coli* *tsr* gene enables the cells to respond to the attractant serine and the repellents leucine and indole (Springer et al., 1977; Reader et al., 1979). Its ligand-binding domain has 39% sequence identity to the aspartate receptor ligand-binding domain. The sequence homology was therefore used to construct a model of the serine receptor ligand-binding domain based on the known structure of the aspartate receptor ligand-binding domain.

Results

Structure proposed

A model of the *E. coli* serine receptor ligand-binding domain was constructed, based on the crystal structure of the *Salmonella typhimurium* aspartate receptor and the sequence similarities between the two receptors. The aspartate receptor ligand-binding domain is a dimer of four-helix bundles (Milburn et al., 1991). Based on sequence

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homology, the serine receptor was predicted to have the same number of residues in the helical regions of each four-helix bundle (Fig. 1). The loops between helices one and two and between helices two and three of each subunit were predicted to be one residue longer in the serine receptor than in the aspartate receptor, so the serine receptor model was first built without residues 78 and 112 in those loops.

The Homology program from Quanta was used to replace each residue in the aspartate receptor structure with the corresponding serine receptor residue according to the sequence alignment shown in Figure 1. When the two residues were different, each side-chain atom that corresponds to a side-chain atom in the aspartate receptor residue was added, and the positions of additional atoms in each residue were then determined from a set of residue templates (*Protein Package Reference Manual, Quanta 3.0*, June 1990, Polygen Corporation, Waltham, Massachusetts). The Homology program was then used to insert residue 78 between residues 77 and 79, and residue 112 between residues 111 and 113 in each subunit. The model was then subjected to 200 cycles of Powell minimization using Xplor.

In the aspartate receptor, helices one through three of each subunit are made up of residues 32–76, 86–109, and 116–144, respectively. The corresponding residues in the serine receptor were predicted to form the helices in the four-helix bundle, but after energy minimization, some of these residues were not found to be in an α -helical conformation. In the minimized serine receptor model, helices one through three of each subunit are made up of residues 35–75, 90–110, and 119–145, respectively (Fig. 2A; Kinemage 1). Due to a conserved proline, "helix four" in the model is actually two shorter helices consisting of residues 150–153 and 157–179, connected by a "proline bulge," as is found in helix four of the aspartate receptor, which consists of a similar proline bulge and two short helices.

The residues at the beginning of helix one and at the end of helix four are probably nonhelical in the model due to the absence of the residues before residue 34 and after residue 182. The insertion of a residue between helices two

and three probably caused the change in the ends of those two helices. Other changes in the ends of the helices were probably due to the minimization method used.

To compare the crystal structure of the aspartate receptor to the serine receptor model, the α -carbon chains of the two structures were superposed (Fig. 2A), and then the distances the peptide backbone had to shift to accommodate the changes in sequence were determined. The distance between corresponding α -carbons is plotted in Figure 2B. The rms deviation for the distances was only 0.496 Å, which suggests the new sequence is compatible with the four-helix bundle structure of the aspartate receptor. The largest deviations occur at the loops between helices one and two and helices two and three and are caused by the insertion of residues there. There are also peaks around residue 36 and residue 130. The aspartate receptor crystal structure contained a cysteine cross-link at residue 36 that is not present in the serine receptor model. The absence of this cross-link probably caused the difference in α -carbon positions around residue 36. The reason for the difference in α -carbon positions around residue 130 is not clear.

Predicted serine-binding site

Aspartate and serine bind directly to their receptors (Wang & Koshland, 1980) with binding constants of 5 μ M (Clarke & Koshland, 1979). To predict where serine binds to the receptor, the Autodock docking simulation algorithm (Goodsell & Olson, 1990) was used with serine as the ligand and the serine receptor model as the target molecule.

Sixteen docking simulation runs resulted in 12 independent docking solutions. The lowest energy serine-docking solution placed serine in a pocket corresponding to the aspartate-binding site in the aspartate receptor crystal structure. Six of the other 11 solutions also placed serine in this site or its symmetrically identical site. While these seven serine-docking solutions were calculated to be within 3 kcal docking energy, the orientation of the serine ligand varied relative to the receptor side chains that line the pocket. Most of the remaining docking solution sites were

Fig. 1. Sequence alignment of the tsr and tar_S N-terminal domains. The ligand-binding domains of tsr (residues 34–182) and tar_S (residues 34–180) were aligned using the Needleman–Wunsch algorithm, and an alignment was chosen in which the two insertions are located in the loops between the helices. | = conserved residues. The residues that are located in the helices of the aspartate receptor structure are underlined. The corresponding residues in the serine receptor were predicted also to be in an α -helical conformation. The residues that are located in the helices in the minimized serine receptor model are underlined.

Fig. 2. Superposition of the serine receptor ligand-binding domain model on the aspartate receptor crystal structure. **A:** The α -carbons of the residues comprising the helices of the serine receptor model (thick lines) were superposed onto the corresponding α -carbons of the aspartate receptor crystal structure (thin lines), using a least-squares minimization. This superposition shows the small distance the residues moved in order to accommodate the new sequence. The residues that are found at the ends of the helices are numbered. **B:** The distances between the corresponding α -carbons in **A** were plotted against residue number to determine locations of the most movement (thin lines). The locations of the helices in the serine receptor model are indicated (thick line), where helix is denoted by 1.5, and turn or loop is denoted by 1.3. The locations of residues that make up the predicted serine-binding site are indicated by diamonds.

located in the loops at the top of the four-helix bundle, probably due to an artifact of being at the edge of the target lattices.

The lowest energy docking solution was combined with the serine receptor model, and the receptor/ligand complex model was subjected to energy minimization. The serine-docking site in the minimized complex is compared to the aspartate-binding site in the aspartate receptor crystal structure in Figure 3. The main docking pocket contains two residues (Arg 64 and Thr 156) that when mutated affect serine binding and the response to serine (Hedblom & Adler, 1980; Lee et al., 1988), as well as several residues that are conserved among the three chemotaxis receptors that bind amino acid ligands (the *S. typhimurium* aspartate receptor, the *E. coli* aspartate receptor, and the serine receptor), but not in the two receptors that bind only periplasmic proteins (tap and trg). This finding supports the identification of the pocket as the serine-binding site.

The side chains of four residues that interact with the aspartate ligand in the aspartate receptor crystal structure have exact counterparts in the serine receptor model (Fig. 3C). In the aspartate receptor (1) the guanidinium group of Arg 64 forms hydrogen bonds to the α -carboxyl group of aspartate, (2) the hydroxyl group of Thr 154 hydrogen bonds to the α -amino group, and (3) the guanidinium groups of Arg 73 and Arg 69 make hydrogen bonds to the side-chain carboxyl group. These residues are conserved in the serine receptor and are found in positions at which they could make similar contacts with serine. (1) The guanidinium group of Arg 64 is in a position where it could make contacts with the α -carboxyl group of serine similar to Arg 64 in the aspartate receptor. (2) The hydroxyl group of Thr 156 could make contacts with the α -amino group. (3) The guanidinium groups of Arg 73 and Arg 69 could make hydrogen bonds to the hydroxyl group of serine. The guanidinium group of Arg 69 in the aspartate receptor also forms a hydrogen bond to the α -

Fig. 3. Proposed serine-binding site in the serine receptor model compared to the aspartate-binding site in the aspartate receptor crystal structure. **A:** The lowest energy serine-docking solution and the serine receptor model structure complex were energy minimized together with 200 steps of Powell minimization. The serine ligand (thick lines) and the receptor side chains that make up the docking site (thin lines) are shown. **B:** The aspartate-binding site from the crystal structure is shown for comparison. The aspartate ligand (thick lines) makes hydrogen bonds with three conserved arginines and the "proline bulge" (thin lines). **C:** The predicted hydrogen bonds between the serine receptor and the serine ligand are shown (top) based on the hydrogen bonds between the aspartate receptor and the aspartate ligand (bottom) and sequence conservation between the two receptors.

carboxyl group of aspartate, and the guanidinium group of Arg 69 of the serine receptor model could make a similar hydrogen bond to the α -carboxyl group of serine.

There is one notable difference between the residues that contact the ligands in the two receptors. The side-chain hydroxyl group of Tyr 149 in the aspartate receptor makes contacts with aspartate by forming hydrogen bonds with the side-chain carboxyl and, through water, the α -carboxyl group. The side chain of the corresponding residue in the serine receptor, Phe 151, cannot make hydrogen bonds. This residue may be important in substrate specificity, because serine and aspartate do not bind to each other's receptor (Clarke & Koshland, 1979).

Several conserved residues form a "proline bulge" along one side of the aspartate-binding site of the aspartate receptor. Thr 154, Pro 153, Gln 152, Ala 151, and Phe 150 of the aspartate receptor are conserved in the serine re-

ceptor as Thr 156, Pro 155, Gln 154, Asp 153, and Phe 152, which are predicted to form a similar bulge. Tyr 149 is located at the N-terminal end of the bulge in the aspartate receptor and becomes Phe 151 in the serine receptor. The backbone carbonyl groups of Gln 152 and Tyr 149 make hydrogen bonds to the α -amino group of aspartate in the crystal structure, which has been called the "nitro cation hole" (Lynch & Koshland, 1992). The backbone carbonyl groups of Gln 154 and Phe 151 could make similar hydrogen bonds to the α -amino group of serine in the predicted structure.

Locations of mutant residues in tsr

The serine receptor ligand-binding domain model helps provide an explanation of the behavior of mutants in the

serine receptor. Mutations that replaced Arg 64 with cysteine or histidine or Thr 156 with isoleucine or proline reduced the response to serine of cells expressing mutant receptors in behavioral assays (Hedblom & Adler, 1980) and decreased the binding of serine to membranes that contain the serine receptor (Lee et al., 1988; Lee & Imae, 1990). The location of Arg 64 and Thr 156 in the predicted serine-binding site and the interaction of the corresponding residues in the aspartate receptor with aspartate support the prediction that these side chains interact directly with the serine ligand and that mutation of these residues prevents normal serine binding.

Parkinson and coworkers discovered eight pseudorevertant serine receptors that were capable of correcting the swimming mode of strains that were too smooth or too tumbling because they lacked one of the enzymes that modify the receptor (Ames et al., 1988). The swimming state of strains that lack the methylesterase (*cheB*) and that have overmethylated receptors can be corrected by the mutations T63M or M171I. Conversely, strains that lack the methyltransferase (*cheR*) and that undermethylate their receptors can be corrected by receptors with one of the following mutations: A70V, R73H, A101T, A135T, D179N, or A181V.

To study how the mutations found by Parkinson and coworkers (Ames et al., 1988) could affect receptor signaling, each mutant residue was introduced into the serine receptor periplasmic domain model, and each resulting model was then minimized by 200 steps of Powell minimization.

The mutant residues are found in three regions of the receptor (Fig. 4). The side chains of the substitutions A70V, T63M, A101T, and A135T point toward the other helices in the four-helix bundle of each subunit and involve an increase in side-chain volume that may push the helices apart and distort the structure. In addition, A70V and T63M are also located, along with R73H, at or near the residues that are proposed to form the serine-binding site, and R73H replaces one of the conserved arginine residues with a histidine. M171I, D179N, and A181V are located in helix four near the beginning of the second transmembrane domain. The side chains of these three residues point toward the other helices in a "pseudo-four-helix bundle" formed by helices 1, 1', 4, and 4', and may have steric consequences for transmembrane signaling.

Each minimized model was superposed on the wild-type serine receptor model. The distances between the corresponding α -carbons were determined, as well as the rms deviation for all the α -carbons. In a second comparison, helix one of each pseudorevertant model was superposed on helix one of the wild-type model and the locations of helix four in the same subunit and helices one and four in the other subunit were studied. In all eight models, the residue substitution caused a significant shift in α -carbon position at nearby residues and only a very small shift in α -carbon positions at residues further away.

Fig. 4. The α -carbon chain of the serine receptor model with the locations of the pseudorevertant mutations are indicated as follows: R73H (A, A'), A70V (B, B'), T63M (C, C'), A101T (D, D'), A135T (E, E'), M171I (F, F'), D179N (G, G'), and A181V (H, H').

Discussion

Proposed structure

A model of the *E. coli* serine receptor periplasmic ligand-binding domain was constructed by using computer homology modeling techniques and the crystal structure of the *S. typhimurium* aspartate receptor periplasmic ligand-binding domain. The two receptors share 39% sequence identity in this region, and 40 of the 106 residues that make up the helices are conserved. Of these, 33 face into the four-helix bundle or make up the dimer interface, which suggests that the serine receptor folds into a similar dimer of four-helix bundles. That the serine receptor sequence is compatible with the four-helix bundle structure of the aspartate receptor subunits is also demonstrated by the fact that only a small movement of backbone α -carbons is needed to accommodate the sequence changes and produce the structure with the lowest energy.

In a study of homologous proteins, Chothia and Lesk (1986) found that the rms deviation of the main-chain atom positions, Δ , is related to the fraction of substituted residues, H , by the expression: $\Delta (\text{\AA}) = 0.40e^{187H}$. Two four-helix bundles that share 39% sequence identity would thus be predicted to have an rms deviation of main-chain atom positions of 1.199 $\text{\AA}$. The lower calculated rms between the aspartate receptor crystal structure and

the serine receptor model suggests the model is more similar to the aspartate receptor structure than the actual serine receptor structure may be, but the model is likely to be a good estimate of the serine receptor ligand-binding domain structure.

The high conservation of the residues that make up the predicted serine-binding site described below indicates the overall structure of the modeled serine-binding site should be fairly accurate although the conformations of side chains may differ.

In homologous proteins, the most structure differences are usually found in the loops that connect secondary structural elements. The loops between the helices in the aspartate receptor structure and serine receptor model vary in both length and sequence. These loop regions are likely to be the regions of highest modeling errors in the model.

Serine-binding site

Serine-docking simulations identified a pocket that is formed by residues that are conserved between the aspartate receptor and the serine receptor. Several of these residues are known to make hydrogen bonds to aspartate in the aspartate receptor crystal structure and are in positions in the serine receptor model where they could make similar contacts to serine. Two of these residues, Arg 73 and Arg 69, are conserved in the chemotaxis receptors that bind amino acid ligands (tar, tsr), but not in those receptors that do not bind amino acids (trg, tap). Their forming hydrogen bonds to the α -carboxyl and α -nitrogen of aspartate and serine is consistent with their being conserved in receptors that bind amino acid ligands but not in those that do not.

Tyr 149 becomes Phe 151 in the serine receptor and may be important in ligand specificity of the receptor, since the hydroxyl side chain of serine is less polar than the γ -carboxyl group of aspartate.

The predicted binding site also explains how mutations at two positions, Thr 156 and Arg 64, reduce serine binding to membranes containing the serine receptor (Hedblom & Adler, 1980; Lee et al., 1988). Wang and Koshland (1980) demonstrated that serine binds directly to the serine receptor. In the model described here, both of these residues may interact directly with the serine ligand, and their replacement may prevent the receptor from making as many contacts with serine.

Locations of other mutant residues

Parkinson and coworkers found pseudorevertant serine receptor mutations in the ligand-binding domain that compensate for an overmethylated or undermethylated receptor. The positions of these side chains suggest a reason for the mutations affecting the signaling state of the receptor, although it is not clear why T63M and M171I can correct an overmethylated receptor, but the others can

correct an undermethylated receptor. The side chains of A70V, T63M, A101T, and A135T face the other helices that make up the four-helix bundle of each subunit and therefore could disrupt transmembrane signaling if a relative motion of the helices within the four-helix bundle is important, as has been predicted (Lynch & Koshland, 1992). T63M, A70V, and R73H are located near the residues that are identified with serine binding. R73H replaces an arginine that may make contacts with the serine ligand and thus would be expected to alter serine binding.

The final three mutations—M171I, D179N, and A181V—are located in helix four, near the beginning of the second transmembrane domain. The side chains of these residues point toward the other helices in the dimer. A model of the aspartate receptor transmembrane structure (Milburn et al., 1991) predicted that helices one and four of each subunit extend through the membrane to become the four transmembrane helices. Proposed models of transmembrane signaling in the chemotaxis receptors involve a relative motion of the transmembrane helices (Lynch & Koshland, 1992; Stoddard et al., 1993). The substitutions M171I, D179N, and A181V may alter the locations of the transmembrane helices by changing the packing of helices one and four in each subunit. These changes could affect motion between the transmembrane helices.

The small change in side-chain volume caused by the substitutions A70V, A101T, and A135T suggests that a very small change in the packing of the side chains within a four-helix bundle of the subunit can affect signaling. This is consistent with the minimal effect each mutation had on the positions of corresponding helices when the mutant models were compared to the wild-type model, although an alternative possibility for the absence of a large movement is that the minimization method used to construct the pseudorevertant models allows movement of side chains and less movement of helices.

The small shift in α -carbon position caused by the introduction of mutant residues at positions M171I, D179N, and A181V may be due in part to the absence of the full receptor structure. The absence of the transmembrane sequences, the cytoplasmic domain, and the lipid bilayer may prevent the ends of the receptor fragment from adopting the exact native conformation in the crystal structure. Without the transmembrane domains, the fragment may be closer to the aspartate-bound form in the same way the structure of myoglobin is closer to the oxygen-bound form of hemoglobin than to deoxy-hemoglobin. This could explain the fact that Milburn and coworkers did not find large differences in the conformations of the aspartate-bound and apo forms of the aspartate receptor crystal structures, whereas Falke and Koshland (1987) observed a global conformational change in the full receptor.

Because the structures of transmembrane proteins are very difficult to obtain, the extrapolation of a known

structure by homology modeling can be particularly valuable.

Methods

Sequence alignments

The sequences for tar_s and tsr (Boyd et al., 1983; Russo & Koshland, 1983; Lee et al., 1988) were aligned using the Needleman-Wunsch algorithm (Needleman & Wunsch, 1970) with an identity score of 1.0 and a gap-open penalty of 5.0. The highest-scoring alignment placed the two inserted residues near the ends of helices in the four-helix bundle. Because insertions and deletions in homologous proteins are more likely to occur in the loops between helices, an alignment that scored slightly lower was used so that the two inserted residues were in the loops (Fig. 1). Because the residues from Ser 25 to Leu 33 and Gln 181 to Arg 188 were nonhelical in the crystal structure, only the regions corresponding to the four-helix bundle portion of the aspartate receptor periplasmic domain were modeled. This region includes Gln 34 to Phe 180 in the aspartate receptor and Lys 34 to Val 182 in the serine receptor. This includes 149 residues for the periplasmic domain of each subunit and 298 residues for the dimer of the periplasmic domain. In this region, the aspartate receptor and the serine receptor are 39% identical with 47 identical residues, 17 conservative changes, 57 nonconservative changes, and 2 inserted residues per subunit.

Homology modeling

An initial model of the serine receptor periplasmic domain was constructed, using the Homology program of the Quanta 3.0 program package on a Silicon Graphics Iris Workstation and the X-ray crystal structure of the *S. typhimurium* aspartate receptor that had been solved to 2.4 Å resolution with an *R*-factor of 0.206 (Milburn et al., 1991). The initial structure was minimized, using the Powell algorithm from XPLOR (Brunger, 1991, 1992) with 200 steps of minimization. During the minimization calculations, the total energy of the structure decreased from 9.8×10^7 kcal/mol to -8,600 kcal/mol. To compare the serine receptor model to the aspartate receptor structure, the α -carbon chain of the serine receptor model structure was superposed on the α -carbon chain of the aspartate receptor crystal structure, and the distances between corresponding α -carbons were calculated.

Construction of models of pseudorevertant receptors

A model of the serine receptor periplasmic domain was constructed for each of the pseudorevertant mutations by introducing the mutant residue in place of the wild-type residue in the serine receptor model. Each mutant model

was then subjected to Powell minimization with 200 steps of minimization. During the minimization calculations, the total energy of each structure reached approximately -9,000 kcal/mol. Each pseudorevertant model was compared to the wild-type serine receptor model by two methods: (1) The α -carbon chain of each mutant model was superposed on the wild-type serine receptor model, using a least-squares superposition, and the distances between corresponding α -carbons were determined. (2) Helix one of each mutant model was superposed on helix one of the wild-type serine receptor model, and the relative positions of the three other helices in each model were compared.

Serine-docking simulations

Target grid

The serine receptor ligand-binding domain model was used as the target molecule. The QUANTA program was used to calculate partial charges for all the atoms in the target molecule. Cubic lattice grids (30 Å × 30 Å × 30 Å) of interaction potentials for carbon, nitrogen, oxygen, and sulfur and an electrostatic grid were calculated with a 0.5-Å spacing. The grids were centered on ND2 of N68 and enclosed most of the dimer of four-helix bundles. N68 is located on helix 1 and points across the dimer interface toward helix 1'.

Ligand

The partial charge on each serine atom was determined with the program QUANTA. The serine ligand was inserted into the center of the interaction grids for the target molecule.

Simulated annealing parameters

Each simulation consisted of 300–500 cycles with up to 30,000 accepted or 30,000 rejected steps per cycle. Initial temperatures were between 100 and 125. The temperature reduction factors were 0.9–1.125. The maximum translation step was 2.0 Å, and the maximum rotation and torsion steps were 5°. The dielectric constant was 40.

Minimizations

The serine ligand/serine receptor model complex from the lowest energy serine-docking solution was minimized with 200 steps of Powell minimization on a Cray computer.

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